

Supplemental Methods

Study design and participants

Adult severe asthma patients 18 years or older that met prior American Thoracic Society criteria for the definition of severe asthma (1) were included in the study. Briefly, subjects were on either controller treatment with high-dose inhaled corticosteroids (ICS>800mcg fluticasone or equivalent) or treatment with oral steroids >50% of the year. In addition, two of the following minor criteria were met 1) requirement for additional daily treatment with other controller medication (long-acting β -agonist, theophylline, Leukotriene receptor antagonists, etc.), 2) asthma symptoms requiring short-acting β -agonist on daily basis, 3) persistent airway obstruction (FEV1<80%, PEFr>20%), 4) one or more urgent care visits per year, 5) three or more oral steroid bursts per year, 6) near-fatal asthma events in the past. Participants needed to have evidence of uncontrolled asthma with either a history of recent exacerbations (≥ 2 in past 2 months) or an Asthma Control Test (ACT) score less than 20. The exclusion criteria included an age less than 18 years of age, baseline FEV1 <30% predicted, pregnancy or nursing women, and current smokers or subjects with >15 pack-year history.

HILIC-QEHF Analysis

20 μ L of plasma was extracted with 225 μ L of methanol at -20°C containing an internal standard mixture (2) and 750 μ L of MTBE (methyl tertiary butyl ether) (Sigma Aldrich) at -20°C . Samples were shaken for 6 min at 4°C with an Orbital Mixing Chilling/Heating

Plate (Torrey Pines Scientific Instruments). Then 188 μL of LC-MS grade water (Fisher) was added. Samples were then vortexed, centrifuged and the bottom (polar) layer was collected in two aliquots of 125 μL and evaporated to dryness. The polar layer was resuspended in 60 μL of an acetonitrile: water (4:1, v/v) mixture with 5 $\mu\text{g}/\text{ml}$ Val-Try-Val, CUDA (Sigma), D9-Caffeine, D4-Acetylcholine, D3-Creatinine, D9-Choline, D9-TMAO, D3-1-Methylnicotinamide, D9-Betaine, D3-Histamine, N-methyl-, D9-Butyrobetaine, D3-L-Carnitine, D9-Crotonobetaine, D3-Creatine, D3DL-Alanine, D5-L-Glutamine, D3-DL-Glutamic acid, D3DL-Aspartic acid, 15N2-L-Arginine (Cambridge Isotope Laboratories). Samples were vortexed, sonicated for 5 min and centrifuged then transferred to LC-MS vials. Method blanks and pooled human plasma (BioreclamationIVT) were included as quality control samples.

Five microliters of sample resuspended in 60 μL of 80:20 ACN: H_2O was analyzed used chromatographic conditions as follows: ACQUITY UPLC BEH Amide column (130 \AA ; 1.7 μm ; 2.1 mm internal diameter \times 150 mm length; Waters, product number 186004802) with a ACQUITY UPLC BEH Amide pre-column (130 \AA ; 1.7 μm ; 2.1 mm internal diameter \times 5 mm length; Waters, product number 186004799). The column compartment was maintained at 45°C. The mobile phases were prepared with 10 mM ammonium formate and 0.125% formic acid (Sigma–Aldrich) in either 100% LC-MS grade water for mobile phase (A) or 95:5 v/v acetonitrile: water for mobile phase (B). Gradient elution was performed from 100% (B) at 0-2 min to 70% (B) at 7.7 min, 40% (B) at 9.5 min, 30% (B) at 10.25 min, 100% (B) at 12.75 min, isocratic until 16.75 min with a column flow of 0.4 mL/min. Three mass spectrometers and UHPLC systems were used for acquisition with the following parameters in both ESI (+) and ESI (--) modes.

Analyses were performed on a Vanquish UHPLC system (Thermo Scientific) coupled to a Q Exactive HF (Thermo Fisher) a quadrupole/orbital ion trap mass spectrometer Q Exactive HF with a HESI-II ion source (Thermo Fisher Scientific). Simultaneous MS¹ and MS/MS (data-dependent MS/MS) acquisition was used. The parameters were ESI polarity, positive or negative; sheath gas pressure, 60 psi; aux gas flow, 25 arbitrary units; sweep gas flow, 2 arbitrary units; spray voltage, ± 3.6 kV; capillary temperature, 300°C; aux gas heater temperature, 370°C; MS¹ mass range, m/z 60–900; MS¹ resolving power, 30,000 FWHM (m/z 200); number of data-dependent scans per cycle: 3; MS² resolving power, 15,000 FWHM (m/z 200); acquisition speed: 2 MS¹ spectra/s; normalized collision energy, 20, 30, 40%.

GC-TOFMS Analysis

30 μ L of plasma was extracted with 1 ml of degassed acetonitrile:isopropanol: water (3:3:2, v/v/v) mixture (Fisher) at -20°C and centrifuged at 14,000 rcf. All supernatants were removed and evaporated to dryness using a CentrVap. To remove membrane lipids and triglycerides, dried samples were resuspended with 0.5 mL of acetonitrile: water (1:1, v/v) mixture, decanted and evaporated to dryness using a CentrVap. Samples were derivatized with 10 μ L of methoxyamine hydrochloride in pyridine (40 mg/mL) by shaking at 30°C for 90 min followed by trimethylsilylation with 90 μ L of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, Sigma-Aldrich) by shaking at 37°C for 30 min containing C8–C30 fatty acid methyl esters (FAMES) as internal standards. Derivatized samples were subsequently submitted for analysis by GC-TOFMS (0.5 μ L injection).

Primary metabolite data was collected using a Leco Pegasus IV time-of-flight (TOF) MS (Leco Corporation) coupled to an Agilent 6890 GC (Agilent Technologies) equipped with a 30 m long 0.25 mm id Rtx-5Sil MS column (0.25 μ m film thickness) and a Gerstel MPS2 automatic liner exchange system (Gerstel GMBH & Co. KG). The chromatographic gradient used a constant flow of 1 ml/min with the following gradient: 50°C (1 min), 20°C/min to 330°C, hold 5 min. Mass spectrometry data were collected using 1525 V detector voltage at m/z 85–500 with 17 spectra/s, electron ionization at –70 eV and an ion source temperature of 250°C. QC injections, blanks and pooled human plasma were used for quality assurance throughout the run. Data was processed by ChromaTOF for deconvolution, peak picking, and BinBase (3) for metabolite identifications.

Clinical trial analysis

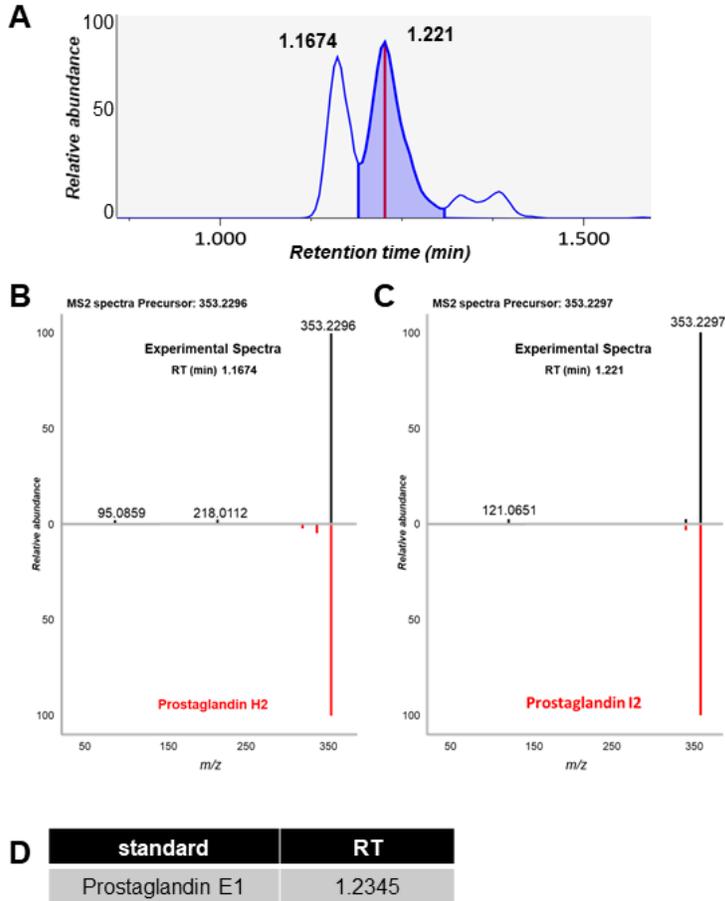
For the sample size calculation, we used a conservative power estimate based on a t-test analysis of 2x2 cross-over design (4). For a sample size of 50 people, we would have 95% power to detect a 0.6 standard deviation change in the mean lung function at $\alpha = 0.025$ and accounting for a 10% attrition rate. Improvements of this magnitude are consistent with those reported in recent crossover trials in asthma with either FEV1 (5), symptoms scores (6), or morning PEF (7) as outcomes.

For analyses in Tables 2 and 3, we relied on statistical modeling to properly handle these complex parameters, as per above. For example, for secondary outcome: FEV1 change (in liters) $FEV1 \text{ change} = \text{Random Intercept (i)} - \text{Treatment} * 0.13 - \text{FeNO} * 0.08 + \text{Treatment} * \text{FeNO} * 0.23$. For subject i who is in FeNO high group during his treatment period, his FEV1 change would be: $FEV1 \text{ change} = \text{Random intercept for}$

subject i - $1 \cdot 0.13 - 1 \cdot 0.08 + 1 \cdot 1 \cdot 0.23$. For subject i who is in FeNO high group during his placebo period, his FEV1 change would be: FEV1 change = Random intercept for subject i - $0 \cdot 0.13 - 1 \cdot 0.08 + 0 \cdot 1 \cdot 0.23$.

Supplemental Figures

Supplemental Figure 1: Peaks and spectra associated with Tier 2 prostaglandin annotations. A) Retention time and peaks of prostaglandin annotations in the aligned clinical plasma samples. B & C) ESI (+) MS/MS spectra of each annotated peak. D) Retention time on the HILIC method used of an authentic prostaglandin standard.



References

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