Online Data Supplement

Linoleic acid-derived lipid mediators increase in a female-dominated subphenotype of COPD

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Supplemental materials and methods

Subjects and study design

The chest X-ray as well as the clinical examination did not reveal any other significant disease than COPD. Clinical data concerning symptoms, medications (including oral contraceptives or estrogen replacement), smoking history, and additional exposures relevant for COPD were recorded by means of a questionnaire. Based upon patient questionnaire, no females consumed any potential lipid mediator-modifying medication prior to sampling (*e.g.,* NSAIDs). Two male COPD smokers reported taking Trombyl® (acetylsalicylic acid). The study was approved by the Stockholm Regional Ethical Board (Case number 2006/959-31/1) and all volunteers gave informed written consent.

Sample collection

Airway epithelial brushings as well as bronchoalveolar lavage (BAL) samples were collected by means of fiberoptic bronchoscopy as previously described (1-3). BAL samples were collected from the middle-lobe bronchus using 5 x 50 mL of phosphate buffered saline (PBS, 37°C). The combined aspirates were filtered and centrifuged at 400 *g* for 5 min at 4°C. Supernatant was isolated and 2 mL aliquots were kept in -80ºC until analysis.

Chemicals

All lipid mediator standards were purchased from Cayman Chemical (Ann Arbor, MI), with the exception of 12(13)-EpODE, 9,10,13-TriHOME and 9,12,13-TriHOME, which were obtained from Larodan (Solna, Sweden) and LTB_5 from Enzo Life Sciences (Farmingdale, NY). Milli-Q ultrapure deionized water was used (Millipore Corp., Billerica, MA). Isopropanol was purchased from Sigma-Aldrich. Methanol and acetonitrile were from Rathburn (Peeblesshire, U.K.). Acetic and formic acid were obtained from Fisher Scientific (Waltham, MA).

Lipid mediator measurements (except for cysteinyl leukotrienes and isoprostanes)

Extraction of analytes was performed with 3cc/60 mg HLB Oasis SPE cartridges (Waters Corp., Milford, MA). Cartridges were conditioned with 3 mL of methanol and 3 mL of water/methanol 95:5 with 0.1% of acetic acid, followed by 3.3 mL of bronchoalveolar lavage fluid (BALF)

mixed with 10 µL of internal standards in concentrations according Table E2. Cartridges were washed with 3 mL of water/methanol 95:5 with 0.1% of acetic acid and then dried under vacuum-induced air stream for 30 min. Analytes were eluted from the columns with 1 mL of methanol and 2 mL of ethyl acetate in cryotubes (Corning Inc.; Corning, NY) containing 6 µL of glycerol 30% in methanol. After evaporation of the eluates under vacuum, samples were resuspended in 100 µL of methanol containing 441 nM 12-([cyclohexylamino]carbonyl)amino-dodecanoic acid (CUDA), filtered by centrifugation in Amicon Ultrafree-MC, PVDF 0.1 µm (Millipore) and transferred to autosampler vials with inserts prior to injection.

Liquid chromatography was performed on an Acquity UPLC BEH column (C18, 2.1x150 mm, 1.7µm, Waters). The mobile phase was a gradient of solvents A (water with 0.1% of acetic acid) and B (acetonitrile/isopropanol 90:10) with flow rate of 0.5 mL min^{-1}. The gradient initiated with 90% of A, and changed linearly to 65% in min 3.5, to 60% in min 5.5, to 58% in min 7, to 50% in min 9 and to 35% in min 15. A XevoTQ mass spectrometer (Waters) was used to detect all compounds using electrospray ionization (ESI) in negative mode. The general MS parameters were set as follows: desolvation temperature: 600˚C; capillary voltage: 2 kV; desolvation gas (L/hr): 1000. Lipid mediators were quantified using internal and external standards (Table E1). Calibration curves were calculated by least-squares linear regression with 1/x weighting. Chromatographic retention time, mass spectrometric transitions and linear range of the calibration curve are provided in Table E2.

Analysis of cysteinyl leukotrienes and isoprostanes

Attempts were made to analyze cysteinyl leukotrienes and isoprostanes by UPLC-MS/MS. However, when methods described in (4) were applied, strong matrix effects were found to suppress the signal of the internal standards and analytes. The volume of sample extracted was optimized in order to control matrix effects on internal standards, with 1 mL of BALF the optimum. However, LTC_4, LTD_4, LTE_4, EXC_4, EXD_4, EXE_4, 8-iso$PGF_{2\alpha}$ and 8,12-iso-i$PF_{2\alpha}$-VI were under the limit of detection (LOD) of the method. Accordingly, cysteinyl leukotrienes were analyzed by enzyme immunoassay with the CysLT Express kit from Cayman Chemical (Ann Arbor, MI). Concentration was expressed as ng mL^{-1}.

mRNA analysis

RNA from BAL cells was extracted into two fractions containing small RNAs (including miRNAs) and large RNAs (containing mRNA) using the NucleoSpin® miRNA kit according to the manufacturer's instructions (Macherey-Nagel; Düren, Germany). RNA quality and quantity was assessed for concentration and purity by determining UV 260/280 and 230/260 absorbance ratios obtained by the Nanodrop ND-1000 spectrophotometer (Nanodrop; Wilmington, DE). RNA integrity and size distribution was examined by gel electrophoresis on RNA Pico LabChips (Agilent Technologies; Palo Alto, CA) processed on the Agilent 2100 Bioanalyzer. RNA was amplified using the Low Input Quick Amplification Kit (Agilent Technologies) according to the manufacturer's protocol, and subsequent Cy3-CTP labeling was performed by using one-color labeling kits (Agilent Technologies). Clean-up of the labeled and amplified probe was performed (Zymo Research Corporation; Irvine, CA). The size distribution and quantity of the amplified product was assessed by Nanodrop. Equal amounts of Cy3-labeled target were hybridized to Agilent human whole-genome 4x44K Ink-jet arrays containing a total of 41,000 probes corresponding to 19,596 entrez genes. Hybridizations were performed at 65°C for 17 hours at a rotation of 10 rpm. Arrays were scanned by using the Agilent microarray G2565BA scanner (Agilent Technologies) with Scan region: Agilent HD (61x21.6) and a resolution of 5µm, TIFF: 16 bit, XDR: 0.10. Raw signal intensities were extracted with Feature Extraction v10.1 software (Agilent Technologies). Flagged outliers were not included in any subsequent analyses. Microarray datasets were normalized using the *quantile* normalization method according to Bolstad (5) using the R package *limma* in *Bioconductor* (6, 7). No background subtraction was performed, and the median feature pixel intensity was used as the raw signal before normalization. Univariate and multivariate statistical analyses were performed on a selected subset of 13 probes representing 7 genes (EPHX1, EPHX2, CYP2C, CYP1A2, CYP2J, CYP3A4, and CYP2E1) involved in the biosynthesis of the EpOMEs and DiHOMEs.

AB-PAS staining and quantitation of airway epithelial goblet cells

Cytospin glasses were prepared according standard methods from airway epithelial brushings collected during bronchoscopy, and stored in -70°C until use. Cytospin specimen were fixed with cold acetone (-70°C) for 10 min, followed by 3% acetic acid for 3 min. Samples were then incubated in Alcian Blue- acetic acid solution for 15 min, then washed under running water.

Subsequently, cytospin glasses were incubation in 0.5% periodic acid was for 10 min, washed, then placed in to Schiff's reagent for 15 min. Samples were then counterstained with Mayer haematoxylin, and sealed with Immu-mount glue (Thermo Scientific, MI, USA) and coverslips. The number of AB-PAS positive goblet cells as well as the total number of epithelial cells were calculated per visual field with 400x magnification (Leica DM3000, hcx fl plan 40x/0.65). Two to 10 visual fields were counted depending of the cell density of the specific sample, and the results are presented as percent AB-PAS positive cells out of total cells. All samples were analysed by an experienced researcher in a blinded fashion. All chemicals were from Sigma-Aldrich (Steinheim, Germany) unless otherwise stated. Of the 11 female COPD smoker samples, 2 of the cytospins were of insufficient quality to produce accurate counts. Accordingly, Figure 4D displays the results of the remaining n=9 individuals.

Outlier detection in specific correlations

With regards to the PLS correlation between the EpOMEs and DiHOMEs with lung function of female smoking COPD patients (Figure 5A), one subject (3207) was found to be a significant outlier, with a significantly higher mast cell count (Dixon Q-test $p<0.05$) compared to the other COPD subjects. The subject was excluded from the results presented in the main manuscript; however, the correlation remained significant also when including the confirmed outlier ($r=0.72$, $p=0.01$).

Supplemental Results

Correlation with clinical data and cell composition

Correlations were examined for those lipid mediator and BALF cell pairs with known metabolic associations: LTB_4 *vs.* neutrophils and 15-HETE *vs.* eosinophils. The only significant correlation was LTB_4 *vs.* neutrophils ($r=0.56$; $p=3.4x10^{-10}$), which associated with smoking status. No other correlations with clinical parameters or cell data were observed.

A weak correlation was found between the 5 linoleates (excluding 9,10,13-TriHOME) *vs*. neutrophil abundance ($r=0.57$, $p=0.03$) in the male COPD group. However, this correlation was completely driven by one subject, which was a confirmed to be an outlier in terms of neutrophil abundance (Dixon's Q $p<0.05$). Exclusion of the confirmed outlier resulted in a non-significant correlation ($r=0.44$, $p=0.13$).

Table E1. Nomenclature for lipid mediators. Compounds are categorized by their parent polyunsaturated fatty acid (PUFA): arachidonic acid (AA), linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and mead acid (MA). In addition, compounds are provided by their analytical use as internal standards (IS) or technical standards (TS).

PUFA	Abbreviation	Common name	Systematic name
AA	PGD_2	prostaglandin D_2	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid
	11-β-$PGF_{2\alpha}$	9α,11β-prostaglandin $F_{2\alpha}$	9α,11β,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid
	PGJ_2	prostaglandin J_2	11-oxo-15S-hydroxy-prosta-5Z,9,13E-trien-1-oic acid
	15-deoxy-$\Delta^{12,14}$-PGJ_2	15-deoxy-$\Delta^{12,14}$-prostaglandin J_2	11-oxo-prosta-5Z,9,12E,14E-tetraen-1-oic acid
	PGE_2	prostaglandin E_2; dinoprostone	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid
	$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$; dinoprost	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid
	6-keto-$PGF_{1\alpha}$	6-keto-prostaglandin $F_{1\alpha}$	6-oxo-9α,11α,15S-trihydroxy-prost-13E-en-1-oic acid
	TxB_2	thromboxane B_2	9α,11,15S-trihydroxythromba-5Z,13E-dien-1-oic acid
	12-HHTrE	12-HHT	12-hydroxy-5Z,8E,10E-heptadecatrienoic acid
	PGB_2	prostaglandin B_2	9-oxo-15S-hydroxy-prosta-5Z,8(12),13E-trien-1-oic acid
	8-isoPGE_2	8-iso-prostaglandin E_2	9-oxo-11α,15S-dihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid
	LTB_4	leukotriene B_4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid
	6-*trans*-LTB_4	6-*trans*-leukotriene B_4	5S,12R-dihydroxy-6E,8E,10E,14Z-eicosatetraenoic acid
	20-hydroxy-LTB_4	20-hydroxy-leukotriene B_4	5S,12R,20-trihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid
	20-carboxy-LTB_4	20-carboxy-leukotriene B_4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraene-1,20-dioic acid
	5-HETE	5-HETE	(±)5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
	8-HETE	8-HETE	(±)8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid
	9-HETE	9-HETE	(±)-9-hydroxy-5Z,7E,11Z,14Z-eicosatetraenoic acid
	11-HETE	11-HETE	(±)11-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid
	12-HETE	12-HETE	(±)12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid
	15-HETE	15-HETE	(±)15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
	20-HETE	20-HETE	(±)20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid

PUFA	Abbreviation	Common name	Systematic name
AA	5-KETE	5-oxoETE	5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
	12-KETE	12-oxoETE	12-oxo-5Z,8Z,10E,14Z-eicosatetraenoic acid
	15-KETE	15-oxoETE	15-oxo-5Z,8Z,11Z,13E-eicosatetraenoic acid
	5(6)-EpETrE	5(6)-EET	(±)5(6)-epoxy-8Z,11Z,14Z-eicosatrienoic acid
	8(9)-EpETrE	8(9)-EET	(±)8(9)-epoxy-5Z,11Z,14Z-eicosatrienoic acid
	11(12)-EpETrE	11(12)-EET	(±)11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid
	14(15)-EpETrE	14(15)-EET	(±)14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid
	5,6-DiHETrE	5,6-DHET	(±)5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid
	8,9-DiHETrE	8,9-DHET	(±)8,9-dihydroxy-5Z,11Z,14Z-eicosatrienoic acid
	11,12-DiHETrE	11,12-DHET	(±)11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid
	14,15-DiHETrE	14,15-DHET	(±)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid
	5,6-DiHETE	5,6-DiHETE	5,6-dihydroxy-8Z,11Z,14Z,17Z-eicosatetraenoic acid
	5,15-DiHETE	5,15-DiHETE	5,15-dihydroxy-6E,8Z,10Z,13E-eicosatetraenoic acid
	8,15-DiHETE	8,15-DiHETE	8,15-dihydroxy-5Z,9E,11Z,13E-eicosatetraenoic acid
	LXA_4	5(S),6(R)-lipoxin A_4	5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid
	LXB_4	5(S),14(R)-lipoxin B_4	5S,14R,15S-trihydroxy-6E,8Z,10E,12E-eicosatetraenoic acid
LA	9-HODE	9-HODE	(±)-9-hydroxy-10E,12Z-octadecadienoic acid
	13-HODE	13-HODE	(±)-13-hydroxy-9Z,11E-octadecadienoic acid
	9-KODE	9-oxoODE	9-oxo-10E,12Z-octadecadienoic acid
	13-KODE	13-oxoODE	13-oxo-9Z,11E-octadecadienoic acid
	9,10,13-TriHOME	9,10,13-TriHOME	9,10,13-trihydroxy-11E-octadecenoic acid
	9,12,13-TriHOME	9,12,13-TriHOME	9,12,13-trihydroxy-10E-octadecenoic acid
	EKODE	*trans*-EKODE-(E)-Ib	9-oxo-11-(3-pentyl-2-oxiranyl)-10E-undecenoic acid
	9(10)-EpOME	leukotoxin	(±)9(10)-epoxy-12Z-octadecenoic acid
	12(13)-EpOME	iso-leukotoxin	(±)12(13)epoxy-9Z-octadecenoic acid
	9,10-DiHOME	leukotoxin diol	(±)9(10)-dihydroxy-12Z-octadecenoic acid
	12,13-DiHOME	isoleukotoxin diol	12,13-dihydroxy-9Z-octadecenoic acid
	12(13)-EpODE	12(13)-EpODE	(±)-cis-12,13-epoxy-9Z,15Z-octadecadienoic acid

PUFA	Abbreviation	Common name	Systematic name
DGLA	PGE_1	prostaglandin E_1	9-oxo-11α,15S-dihydroxy-prost-13E-en-1-oic acid
	8-HETrE	8-HETrE	8S-hydroxy-9E,11Z,14Z-eicosatrienoic acid
	15-HETrE	15-HETrE	15S-hydroxy-8Z,11Z,13E-eicosatrienoic acid
ALA	9-HOTrE	9-HOTE	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid
	13-HOTrE	13-HOTE	13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid
	9-KOTrE	9-KOTE	9-oxo-10E,12Z,15Z-octadecatrienoic acid
EPA	PGD_3	prostaglandin D_3	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E,17Z-trien-1-oic acid
	PGE_3	prostaglandin E_3	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E,17Z-trien-1-oic acid
	LTB_5	leukotriene B_5	5S,12S-dihydroxy-6Z,8E,14Z,17Z-eicosapentanenoic acid
	5-HEPE	5-HEPE	(±)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid
	8-HEPE	8-HEPE	(±)-8-hydroxy-5Z,9E,11Z,14Z,17Z-eicosapentaenoic acid
	9-HEPE	9-HEPE	(±)-9-hydroxy-5Z,7E,11Z,14Z,17Z-eicosapentaenoic acid
	11-HEPE	11-HEPE	(±)-11-hydroxy-5Z,8Z,12E,14Z,17Z-eicosapentaenoic acid
	12-HEPE	12-HEPE	(±)-12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid
	15-HEPE	15-HEPE	(±)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid
	18-HEPE	18-HEPE	(±)-18-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid
	14(15)-EpETE	14,15-epoxy eicosatetraenoic acid	(±)14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid
	17(18)-EpETE	17,18-epoxy eicosatetraenoic acid	(±)17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
	14,15-DiHETE	14,15-DiHETE	(±)14,15-dihydroxy-eicosa-5,8,11,17-tetraenoic acid
	17,18-DiHETE	17,18-DiHETE	17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
	LXA_5	lipoxin A_5	5S,6R,15S-trihydroxy-7E,9E,11Z,13E,17Z-eicosapentaenoic acid
DHA	8-HDoHE	8-HDoHE	(±)8-hydroxy-4Z,6E,10Z,13Z,16Z,19Z-docosahexaenoic acid
	11-HDoHE	11-HDoHE	(±)11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid
	14-HDoHE	14-HDoHE	(±)14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid
	17-HDoHE	17-HDoHE	(±)17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid
	16(17)-EpDPE	16(17)-EpDPE	(±)16(17)-epoxy-4Z,7Z,10Z,13Z,19Z-docosapentaenoic acid

PUFA	Abbreviation	Common name	Systematic name
DHA	19(20)-EpDPE	19(20)-EpDPE	(±)19(20)-epoxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
	10,17-DiHDoHE	protectin D_1	10(S),17(S)-dihydroxy-4Z,7Z,11E,13Z,15E,19Z-docosahexaenoic acid
	19,20-DiHDPA	19,20-DiHDPA	(±)19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
	RvD_1	resolvin D_1	7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid
	17(R)-RvD_1	17(R)-resolvin D1	7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid
	RvD_2	resolvin D_2	7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid
	RvE_1	resolvin E_1	5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid
MA	LTB_3	leukotriene B_3	5S,12R-dihydroxy-6Z,8E,10E-eicosatrienoic acid
	5-HETrE	5-HETrE	5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid
IS	[$^2H_4$]-PGD_2	prostaglandin D_2-d_4	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic-3,3,4,4-d4 acid
	[$^2H_4$]-PGE_2	prostaglandin E_2-d_4	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic-3,3,4,4-d4 acid
	[$^2H_4$]-6-keto-$PGF_{1\alpha}$	6-keto-prostaglandin $F_{1\alpha}$-d_4	6-oxo-9α,11α,15S-trihydroxy-prost-13E-en-1-oic-3,3,4,4-d4 acid
	[$^2H_4$]-PGB_2	prostaglandin B_2-d_4	9-oxo-15S-hydroxy-prosta-5Z,8(12),13E-trien-1-oic-3,3,4,4-d4 acid
	[$^2H_4$]-TxB_2	thromboxane B_2-d_4	9α,11,15S-trihydroxy-thromba-5Z,13E-dien-1-oic-3,3,4,4-d4 acid
	[$^2H_4$]-LTB_4	leukotriene B_4-d_4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic-6,7,14,15-d4 acid
	[$^2H_8$]-5-HETE	5(S)-HETE-d_8	5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic-5,6,8,9,11,12,14,15-d8 acid
	[$^2H_8$]-15-HETE	15(S)-HETE-d_8	15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic-5,6,8,9,11,12,14,15-d8 acid
	[$^2H_6$]-20-HETE	20-HETE-d_6	20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic-16,16,17,17,18,18-d6 acid
	[$^2H_7$]-5-KETE	5-OxoETE-d_7	5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic-6,8,9,11,12,14,15-d7 acid
	[$^2H_{11}$]-11(12)-EpETrE	11(12)-EET-d_{11}	(±)11(12)-epoxy-5Z,8Z,14Z-eicosatrienoic-16,16,17,17,18,18,19,19,20,20,20-d11 acid
	[$^2H_{11}$]-14,15-DiHETrE	14,15-DHET-d_{11}	(±)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic-16,16,17,17,18,18,19,19,20,20,20-d11 acid
	[$^2H_4$]-9-HODE	9(S)-HODE-d_4	9S-hydroxy-10E,12Z-octadecadienoic-9,10,12,13-d4 acid
	[$^2H_4$]-9(10)-EpOME	9(10)-EpOME-d_4	(±)9(10)epoxy-12Z-octadecenoic 9,10,12,13-d4 acid
	[$^2H_4$]-9,10-DiHOME	9,10-DiHOME-d_4	(±)9,10-dihydroxy-12Z-octadecenoic-9,10,12,13-d4 acid
TS	CUDA	CUDA	12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid

Table E2. Calibration levels of the lipid mediators analyzed. Analytical external standards are categorized by their parent polyunsaturated fatty acid (PUFA): arachidonic acid (AA), linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and mead acid (MA). In addition, compounds are provided by their analytical use as internal standards (IS) or technical standards (TS). Chromatographic retention time (RT), parent ion, product ion, the internal standard (IS) assigned to each lipid mediator and the on-column injected linear range are reported.

PUFA	Compound	Calibration level concentration (nM)									
		10	9	8	7	6	5	4	3	2	1
AA	PGD_2	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	11-β-$PGF_{2\alpha}$	$6.30\ 10^{2}$	$2.80\ 10^{2}$	$5.60\ 10^{1}$	$2.80\ 10^{1}$	$5.60\ 10^{0}$	$1.40\ 10^{0}$	$7.00\ 10^{-1}$	$3.50\ 10^{-1}$	$1.75\ 10^{-1}$	$7.00\ 10^{-2}$
	PGJ_2	$9.04\ 10^{2}$	$4.02\ 10^{2}$	$8.04\ 10^{1}$	$4.02\ 10^{1}$	$8.04\ 10^{0}$	$2.01\ 10^{0}$	$1.00\ 10^{-1}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.00\ 10^{-1}$
	15-deoxy-$\Delta^{12,14}$- PGJ_2	$6.26\ 10^{2}$	$2.78\ 10^{2}$	$5.56\ 10^{1}$	$2.78\ 10^{1}$	$5.56\ 10^{0}$	$1.39\ 10^{0}$	$6.95\ 10^{-1}$	$3.48\ 10^{-1}$	$1.74\ 10^{-1}$	$6.95\ 10^{-2}$
	PGE_2	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	$PGF_{2\alpha}$	$6.30\ 10^{2}$	$2.80\ 10^{2}$	$5.60\ 10^{1}$	$2.80\ 10^{1}$	$5.60\ 10^{0}$	$1.40\ 10^{0}$	$7.00\ 10^{-1}$	$3.50\ 10^{-1}$	$1.75\ 10^{-1}$	$7.00\ 10^{-2}$
	6-keto-$PGF_{1\alpha}$	$6.32\ 10^{2}$	$2.81\ 10^{2}$	$5.61\ 10^{1}$	$2.81\ 10^{1}$	$5.61\ 10^{0}$	$1.40\ 10^{0}$	$7.02\ 10^{-1}$	$3.51\ 10^{-1}$	$1.75\ 10^{-1}$	$7.02\ 10^{-2}$
	TxB_2	$1.12\ 10^{3}$	$4.97\ 10^{2}$	$9.93\ 10^{1}$	$4.97\ 10^{1}$	$9.93\ 10^{0}$	$2.48\ 10^{0}$	$1.24\ 10^{0}$	$6.21\ 10^{-1}$	$3.10\ 10^{-1}$	$1.24\ 10^{-1}$
	12-HHTrE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	PGB_2	$2.15\ 10^{3}$	$9.57\ 10^{2}$	$1.91\ 10^{2}$	$9.57\ 10^{1}$	$1.91\ 10^{1}$	$4.78\ 10^{0}$	$2.39\ 10^{0}$	$1.20\ 10^{0}$	$5.98\ 10^{-1}$	$2.39\ 10^{-1}$
	8-isoPGE_2	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	LTB_4	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	6-*trans*-LTB_4	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	20-hydroxy-LTB_4	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	20-carboxy-LTB_4	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$
	5-HETE	$2.12\ 10^{3}$	$9.44\ 10^{2}$	$1.89\ 10^{2}$	$9.44\ 10^{1}$	$1.89\ 10^{1}$	$4.72\ 10^{0}$	$2.36\ 10^{0}$	$1.18\ 10^{0}$	$5.90\ 10^{-1}$	$2.36\ 10^{-1}$
	8-HETE	$2.15\ 10^{3}$	$9.54\ 10^{2}$	$1.91\ 10^{2}$	$9.54\ 10^{1}$	$1.91\ 10^{1}$	$4.77\ 10^{0}$	$2.38\ 10^{0}$	$1.19\ 10^{0}$	$5.96\ 10^{-1}$	$2.38\ 10^{-1}$
	9-HETE	$2.13\ 10^{3}$	$9.49\ 10^{2}$	$1.90\ 10^{2}$	$9.49\ 10^{1}$	$1.90\ 10^{1}$	$4.74\ 10^{0}$	$2.37\ 10^{0}$	$1.19\ 10^{0}$	$5.93\ 10^{-1}$	$2.37\ 10^{-1}$
	11-HETE	$2.15\ 10^{3}$	$9.54\ 10^{2}$	$1.91\ 10^{2}$	$9.54\ 10^{1}$	$1.91\ 10^{1}$	$4.77\ 10^{0}$	$2.38\ 10^{0}$	$1.19\ 10^{0}$	$5.96\ 10^{-1}$	$2.38\ 10^{-1}$
	12-HETE	$2.15\ 10^{3}$	$9.54\ 10^{2}$	$1.91\ 10^{2}$	$9.54\ 10^{1}$	$1.91\ 10^{1}$	$4.77\ 10^{0}$	$2.38\ 10^{0}$	$1.19\ 10^{0}$	$5.96\ 10^{-1}$	$2.38\ 10^{-1}$
	15-HETE	$2.19\ 10^{3}$	$9.73\ 10^{2}$	$1.95\ 10^{2}$	$9.73\ 10^{1}$	$1.95\ 10^{1}$	$4.87\ 10^{0}$	$2.43\ 10^{0}$	$1.22\ 10^{0}$	$6.08\ 10^{-1}$	$2.43\ 10^{-1}$
	20-HETE	$6.29\ 10^{2}$	$2.80\ 10^{2}$	$5.59\ 10^{1}$	$2.80\ 10^{1}$	$5.59\ 10^{0}$	$1.40\ 10^{0}$	$6.99\ 10^{-1}$	$3.49\ 10^{-1}$	$1.75\ 10^{-1}$	$6.99\ 10^{-2}$
	5-KETE	$2.26\ 10^{2}$	$1.00\ 10^{2}$	$2.01\ 10^{1}$	$1.00\ 10^{1}$	$2.01\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	12-KETE	$2.26\ 10^{2}$	$1.00\ 10^{2}$	$2.01\ 10^{1}$	$1.00\ 10^{1}$	$2.01\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	15-KETE	$2.26\ 10^{2}$	$1.00\ 10^{2}$	$2.01\ 10^{1}$	$1.00\ 10^{1}$	$2.01\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	5(6)-EpETrE	$2.25\ 10^{2}$	$9.98\ 10^{1}$	$2.00\ 10^{1}$	$9.98\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$

PUFA	Compound	Calibration level concentration (nM)									
		10	9	8	7	6	5	4	3	2	1
AA	8(9)-EpETrE	$2.25\ 10^{2}$	$9.98\ 10^{1}$	$2.00\ 10^{1}$	$9.98\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	11(12)-EpETrE	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.98\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	14(15)-EpETrE	$2.25\ 10^{2}$	$9.98\ 10^{1}$	$2.00\ 10^{1}$	$9.98\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	5,6-DiHETrE	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$
	8,9-DiHETrE	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	11,12-DiHETrE	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$
	14,15-DiHETrE	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$
	5,6-DiHETE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	5,15-DiHETE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	8,15-DiHETE	$1.34\ 10^{2}$	$5.94\ 10^{1}$	$1.19\ 10^{1}$	$5.94\ 10^{0}$	$1.19\ 10^{0}$	$2.97\ 10^{-1}$	$1.49\ 10^{-1}$	$7.43\ 10^{-2}$	$3.71\ 10^{-2}$	$1.49\ 10^{-2}$
	LXA_4	$1.33\ 10^{2}$	$5.90\ 10^{1}$	$1.18\ 10^{1}$	$5.90\ 10^{0}$	$1.18\ 10^{0}$	$2.95\ 10^{-1}$	$1.48\ 10^{-1}$	$7.38\ 10^{-2}$	$3.69\ 10^{-2}$	$1.48\ 10^{-2}$
	LXB_4	$1.34\ 10^{2}$	$5.94\ 10^{1}$	$1.19\ 10^{1}$	$5.94\ 10^{0}$	$1.19\ 10^{0}$	$2.97\ 10^{-1}$	$1.49\ 10^{-1}$	$7.43\ 10^{-2}$	$3.71\ 10^{-2}$	$1.49\ 10^{-2}$
LA	9-HODE	$4.49\ 10^{3}$	$2.00\ 10^{3}$	$3.99\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$9.98\ 10^{0}$	$4.99\ 10^{0}$	$2.50\ 10^{0}$	$1.25\ 10^{0}$	$4.99\ 10^{-1}$
	13-HODE	$4.49\ 10^{3}$	$2.00\ 10^{3}$	$3.99\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$9.98\ 10^{0}$	$4.99\ 10^{0}$	$2.50\ 10^{0}$	$1.25\ 10^{0}$	$4.99\ 10^{-1}$
	9-KODE	$2.26\ 10^{2}$	$1.01\ 10^{2}$	$2.01\ 10^{1}$	$1.01\ 10^{1}$	$2.01\ 10^{0}$	$5.03\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	13-KODE	$2.26\ 10^{2}$	$1.01\ 10^{2}$	$2.01\ 10^{1}$	$1.01\ 10^{1}$	$2.01\ 10^{0}$	$5.03\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	9,10,13-TriHOME	$2.07\ 10^{3}$	$9.20\ 10^{2}$	$1.84\ 10^{2}$	$9.20\ 10^{1}$	$1.84\ 10^{1}$	$4.60\ 10^{0}$	$2.30\ 10^{0}$	$1.15\ 10^{0}$	$5.75\ 10^{-1}$	$2.30\ 10^{-1}$
	9,12,13-TriHOME	$2.05\ 10^{3}$	$9.10\ 10^{2}$	$1.82\ 10^{2}$	$9.10\ 10^{1}$	$1.82\ 10^{1}$	$4.55\ 10^{0}$	$2.28\ 10^{0}$	$1.14\ 10^{0}$	$5.69\ 10^{-1}$	$2.28\ 10^{-1}$
	EKODE	$2.26\ 10^{2}$	$1.01\ 10^{2}$	$2.01\ 10^{1}$	$1.01\ 10^{1}$	$2.01\ 10^{0}$	$5.03\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.29\ 10^{-2}$	$2.51\ 10^{-2}$
	9(10)-EpOME	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.98\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	12(13)-EpOME	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.98\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	9,10-DiHOME	$2.16\ 10^{3}$	$9.62\ 10^{2}$	$1.92\ 10^{2}$	$9.62\ 10^{1}$	$1.92\ 10^{1}$	$4.81\ 10^{0}$	$2.40\ 10^{0}$	$1.20\ 10^{0}$	$6.01\ 10^{-1}$	$2.40\ 10^{-1}$
	12,13-DiHOME	$2.16\ 10^{3}$	$9.62\ 10^{2}$	$1.92\ 10^{2}$	$9.62\ 10^{1}$	$1.92\ 10^{1}$	$4.81\ 10^{0}$	$2.40\ 10^{0}$	$1.20\ 10^{0}$	$6.01\ 10^{-1}$	$2.40\ 10^{-1}$
	12(13)-EpODE	$2.20\ 10^{2}$	$9.79\ 10^{1}$	$1.96\ 10^{1}$	$9.79\ 10^{0}$	$1.96\ 10^{0}$	$4.89\ 10^{-1}$	$2.45\ 10^{-1}$	$1.22\ 10^{-1}$	$6.12\ 10^{-2}$	$2.45\ 10^{-2}$
DGLA	PGD_1	$4.47\ 10^{2}$	$1.99\ 10^{2}$	$3.97\ 10^{1}$	$1.99\ 10^{1}$	$3.97\ 10^{0}$	$9.93\ 10^{-1}$	$4.96\ 10^{-1}$	$2.48\ 10^{-1}$	$1.24\ 10^{-1}$	$4.96\ 10^{-2}$
	PGE_1	$6.30\ 10^{2}$	$2.80\ 10^{2}$	$5.60\ 10^{1}$	$2.80\ 10^{1}$	$5.60\ 10^{0}$	$1.40\ 10^{0}$	$7.00\ 10^{-1}$	$3.50\ 10^{-1}$	$1.75\ 10^{-1}$	$7.00\ 10^{-2}$
	8-HETrE	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.98\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	15-HETrE	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$
ALA	9-HOTrE	$2.26\ 10^{2}$	$1.01\ 10^{2}$	$2.01\ 10^{1}$	$1.01\ 10^{1}$	$2.01\ 10^{0}$	$5.03\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	13-HOTrE	$1.35\ 10^{2}$	$5.98\ 10^{1}$	$1.20\ 10^{1}$	$5.98\ 10^{0}$	$1.20\ 10^{0}$	$2.99\ 10^{-1}$	$1.49\ 10^{-1}$	$7.47\ 10^{-2}$	$3.74\ 10^{-2}$	$1.49\ 10^{-2}$
	9-KOTrE	$2.28\ 10^{2}$	$1.01\ 10^{2}$	$2.02\ 10^{1}$	$1.01\ 10^{1}$	$2.02\ 10^{0}$	$5.06\ 10^{-1}$	$2.53\ 10^{-1}$	$1.27\ 10^{-1}$	$6.33\ 10^{-2}$	$2.53\ 10^{-2}$

PUFA	Compound	Calibration level concentration (nM)									
		10	9	8	7	6	5	4	3	2	1
EPA	PGD_3	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	PGE_3	$2.72\ 10^{2}$	$1.21\ 10^{2}$	$2.42\ 10^{1}$	$1.21\ 10^{1}$	$2.42\ 10^{0}$	$6.05\ 10^{-1}$	$3.02\ 10^{-1}$	$1.51\ 10^{-1}$	$7.56\ 10^{-2}$	$3.02\ 10^{-2}$
	LTB_5	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	5-HEPE	$6.33\ 10^{2}$	$2.81\ 10^{2}$	$5.63\ 10^{1}$	$2.81\ 10^{1}$	$5.63\ 10^{0}$	$1.41\ 10^{0}$	$7.03\ 10^{-1}$	$3.52\ 10^{-1}$	$1.76\ 10^{-1}$	$7.03\ 10^{-2}$
	8-HEPE	$1.07\ 10^{3}$	$4.75\ 10^{2}$	$9.49\ 10^{1}$	$4.75\ 10^{1}$	$9.49\ 10^{0}$	$2.37\ 10^{0}$	$1.19\ 10^{0}$	$5.93\ 10^{-1}$	$2.97\ 10^{-1}$	$1.19\ 10^{-1}$
	9-HEPE	$1.07\ 10^{3}$	$4.77\ 10^{2}$	$9.54\ 10^{1}$	$4.77\ 10^{1}$	$9.54\ 10^{0}$	$2.39\ 10^{0}$	$1.19\ 10^{0}$	$5.97\ 10^{-1}$	$2.98\ 10^{-1}$	$1.19\ 10^{-1}$
	11-HEPE	$1.03\ 10^{3}$	$4.57\ 10^{2}$	$9.14\ 10^{1}$	$4.57\ 10^{1}$	$9.14\ 10^{0}$	$2.29\ 10^{0}$	$1.14\ 10^{0}$	$5.71\ 10^{-1}$	$2.86\ 10^{-1}$	$1.14\ 10^{-1}$
	12-HEPE	$1.04\ 10^{3}$	$4.62\ 10^{2}$	$9.24\ 10^{1}$	$4.62\ 10^{1}$	$9.24\ 10^{0}$	$2.31\ 10^{0}$	$1.16\ 10^{0}$	$5.78\ 10^{-1}$	$2.89\ 10^{-1}$	$1.16\ 10^{-1}$
	15-HEPE	$1.02\ 10^{3}$	$4.52\ 10^{2}$	$9.04\ 10^{1}$	$4.52\ 10^{1}$	$9.04\ 10^{0}$	$2.26\ 10^{0}$	$1.13\ 10^{0}$	$5.65\ 10^{-1}$	$2.83\ 10^{-1}$	$1.13\ 10^{-1}$
	18-HEPE	$1.02\ 10^{3}$	$4.52\ 10^{2}$	$9.04\ 10^{1}$	$4.52\ 10^{1}$	$9.04\ 10^{0}$	$2.26\ 10^{0}$	$1.13\ 10^{0}$	$5.65\ 10^{-1}$	$2.83\ 10^{-1}$	$1.13\ 10^{-1}$
	14(15)-EpETE	$2.26\ 10^{2}$	$1.00\ 10^{2}$	$2.01\ 10^{1}$	$1.00\ 10^{1}$	$2.01\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	17(18)-EpETE	$2.26\ 10^{2}$	$1.00\ 10^{2}$	$2.01\ 10^{1}$	$1.00\ 10^{1}$	$2.01\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$6.28\ 10^{-2}$	$2.51\ 10^{-2}$
	14,15-DiHETE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	17,18-DiHETE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	LXA_5	$1.34\ 10^{2}$	$5.93\ 10^{1}$	$1.19\ 10^{1}$	$5.93\ 10^{0}$	$1.19\ 10^{0}$	$2.97\ 10^{-1}$	$1.48\ 10^{-1}$	$7.42\ 10^{-2}$	$3.71\ 10^{-2}$	$1.48\ 10^{-2}$
DHA	8-HDoHE	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	11-HDoHE	$2.69\ 10^{2}$	$1.20\ 10^{2}$	$2.39\ 10^{1}$	$1.20\ 10^{1}$	$2.39\ 10^{0}$	$5.98\ 10^{-1}$	$2.99\ 10^{-1}$	$1.49\ 10^{-1}$	$7.47\ 10^{-2}$	$2.99\ 10^{-2}$
	14-HDoHE	$2.69\ 10^{2}$	$1.20\ 10^{2}$	$2.39\ 10^{1}$	$1.20\ 10^{1}$	$2.39\ 10^{0}$	$5.98\ 10^{-1}$	$2.99\ 10^{-1}$	$1.49\ 10^{-1}$	$7.47\ 10^{-2}$	$2.99\ 10^{-2}$
	17-HDoHE	$2.69\ 10^{2}$	$1.20\ 10^{2}$	$2.39\ 10^{1}$	$1.20\ 10^{1}$	$2.39\ 10^{0}$	$5.98\ 10^{-1}$	$2.99\ 10^{-1}$	$1.49\ 10^{-1}$	$7.47\ 10^{-2}$	$2.99\ 10^{-2}$
	16(17)-EpDPE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	19(20)-EpDPE	$2.25\ 10^{2}$	$9.99\ 10^{1}$	$2.00\ 10^{1}$	$9.99\ 10^{0}$	$2.00\ 10^{0}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$6.24\ 10^{-2}$	$2.50\ 10^{-2}$
	10,17-DiHDoHE	$2.70\ 10^{2}$	$1.20\ 10^{2}$	$2.40\ 10^{1}$	$1.20\ 10^{1}$	$2.40\ 10^{0}$	$5.99\ 10^{-1}$	$3.00\ 10^{-1}$	$1.50\ 10^{-1}$	$7.49\ 10^{-2}$	$3.00\ 10^{-2}$
	19,20-DiHDPA	$6.31\ 10^{2}$	$2.80\ 10^{2}$	$5.61\ 10^{1}$	$2.80\ 10^{1}$	$5.61\ 10^{0}$	$1.40\ 10^{0}$	$7.01\ 10^{-1}$	$3.50\ 10^{-1}$	$1.75\ 10^{-1}$	$7.01\ 10^{-2}$
	RvD_1	$2.70\ 10^{2}$	$1.20\ 10^{2}$	$2.40\ 10^{1}$	$1.20\ 10^{1}$	$2.40\ 10^{0}$	$6.00\ 10^{-1}$	$3.00\ 10^{-1}$	$1.50\ 10^{-1}$	$7.50\ 10^{-2}$	$3.00\ 10^{-2}$
	17(R)-RvD_1	$2.70\ 10^{2}$	$1.20\ 10^{2}$	$2.40\ 10^{1}$	$1.20\ 10^{1}$	$2.40\ 10^{0}$	$6.00\ 10^{-1}$	$3.00\ 10^{-1}$	$1.50\ 10^{-1}$	$7.50\ 10^{-2}$	$3.00\ 10^{-2}$
	RvD_2	$2.70\ 10^{2}$	$1.20\ 10^{2}$	$2.40\ 10^{1}$	$1.20\ 10^{1}$	$2.40\ 10^{0}$	$6.00\ 10^{-1}$	$3.00\ 10^{-1}$	$1.50\ 10^{-1}$	$7.50\ 10^{-2}$	$3.00\ 10^{-2}$
	RvE_1	$2.70\ 10^{2}$	$1.20\ 10^{2}$	$2.40\ 10^{1}$	$1.20\ 10^{1}$	$2.40\ 10^{0}$	$5.99\ 10^{-1}$	$3.00\ 10^{-1}$	$1.50\ 10^{-1}$	$7.49\ 10^{-2}$	$3.00\ 10^{-2}$
MA	LTB_3	$4.49\ 10^{2}$	$2.00\ 10^{2}$	$3.99\ 10^{1}$	$2.00\ 10^{1}$	$3.99\ 10^{0}$	$9.99\ 10^{-1}$	$4.99\ 10^{-1}$	$2.50\ 10^{-1}$	$1.25\ 10^{-1}$	$4.99\ 10^{-2}$
	5-HETrE	$4.52\ 10^{2}$	$2.01\ 10^{2}$	$4.02\ 10^{1}$	$2.01\ 10^{1}$	$4.02\ 10^{0}$	$1.00\ 10^{0}$	$5.02\ 10^{-1}$	$2.51\ 10^{-1}$	$1.26\ 10^{-1}$	$5.02\ 10^{-2}$

PUFA	Compound	Calibration level concentration (nM)									
		10	9	8	7	6	5	4	3	2	1
IS	$[^2H_4]$-PGD_2					$2.01\ 10^2$					
	$[^2H_4]$-PGE_2					$2.01\ 10^2$					
	$[^2H_4]$-6-keto-$PGF_{1\alpha}$					$2.00\ 10^2$					
	$[^2H_4]$-PGB_2					$2.01\ 10^2$					
	$[^2H_4]$-TxB_2					$2.00\ 10^2$					
	$[^2H_4]$-LTB_4					$2.00\ 10^2$					
	$[^2H_8]$-5-HETE					$2.01\ 10^2$					
	$[^2H_8]$-15-HETE					$2.01\ 10^2$					
	$[^2H_6]$-20-HETE					$1.99\ 10^2$					
	$[^2H_7]$-5-KETE					$2.00\ 10^2$					
	$[^2H_{11}]$-11(12)-EpETrE					$1.99\ 10^2$					
	$[^2H_{11}]$-14,15-DiHETrE					$2.00\ 10^2$					
	$[^2H_4]$-9-HODE					$2.00\ 10^2$					
	$[^2H_4]$-9(10)-EpOME					$2.00\ 10^2$					
	$[^2H_4]$-9,10-DiHOME					$2.01\ 10^2$					
TS	CUDA					$4.41\ 10^2$					

Table E3. Analytical characterization of the standards employed in the lipid mediator mass spectrometry platform. Analytical external standards are categorized by their parent polyunsaturated fatty acid (PUFA): arachidonic acid (AA), linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and mead acid (MA). In addition, compounds are provided by their analytical use as internal standards (IS) or technical standards (TS). Chromatographic retention time (RT), parent ion, product ion, the internal standard (IS) assigned to each lipid mediator and the on-column injected linear range are reported.

PUFA	Lipid mediator	RT (min)	Parent ion $[M-H]^-$ (m/z)	Product ion (m/z)	IS	Linear range (fmol)
AA	PGD_2	6.10	351.1	271	$[^2H_4]$-PGD_2	0.98-3750
	11-β-$PGF_{2\alpha}$	5.35	353.3	193	$[^2H_4]$-PGD_2	2.6-2050
	PGJ_2	8.00	333	189	$[^2H_4]$-PGE_2	0.75-3000
	15-deoxy-$\Delta^{12,14}$- PGJ_2	11.55	315.2	271	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	0.53-4650
	PGE_2	5.85	351.2	271	$[^2H_4]$-PGE_2	1.9-3750
	$PGF_{2\alpha}$	5.75	353.3	193	$[^2H_4]$-PGD_2	1.4-410
	6-keto-$PGF_{1\alpha}$	4.35	369.2	163	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	2.6-2050
	TxB_2	5.20 (broad)	373.2	173	$[^2H_4]$-TxB_2	0.98-750
	12-HHTrE	10.55	279.2	179	$[^2H_4]$-9-HODE	3.8-150
	PGB_2	8.00	333.2	175	$[^2H_4]$-PGB_2	1.8-7125
	8-isoPGE_2	5.70	351.2	271	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	0.98-150
	LTB_4	9.65	335.2	195	$[^2H_4]$-LTB_4	1.9-3375
	6-trans-LTB_4	9.40	335.2	195	$[^2H_4]$-LTB_4	3.8-1500
	20-hydroxy-LTB_4	4.50	351.3	195	$[^2H_4]$-LTB_4	3.8-1500
	20-carboxy-LTB_4	4.35	365.2	195	$[^2H_4]$-LTB_4	1.9-300
	5-HETE	14.25	319.1	115	$[^2H_8]$-5-HETE	1.8-1425
	8-HETE	13.70	319.1	155	$[^2H_7]$-5-KETE	4.5-15500
	9-HETE	13.90	319.2	179	$[^2H_8]$-15-HETE	4.5-1425
	11-HETE	13.40	319.1	167	$[^2H_8]$-15-HETE	1.8-7125
	12-HETE	13.60	319.1	179	$[^2H_8]$-15-HETE	1.8-7125
	15-HETE	12.97	319.1	175	$[^2H_8]$-15-HETE	1.8-7310
	20-HETE	11.85	319.3	275	$[^2H_6]$-20-HETE	42-4725
	5-KETE	15.00	317.2	203	$[^2H_7]$-5-KETE	0.98-1675
	12-KETE	13.85	317.2	153	$[^2H_7]$-5-KETE	15-750
	15-KETE	13.25	317.2	113	$[^2H_7]$-5-KETE	0.98-1675
	5(6)-EpETrE	15.55	319.2	191	$[^2H_{11}]$-11(12)-EpETrE	1.9-1675
	8(9)-EpETrE	15.30	319.3	155	$[^2H_{11}]$-11(12)-EpETrE	3.8-150
	11(12)-EpETrE	15.05	319.2	167	$[^2H_{11}]$-11(12)-EpETrE	0.38-150
	14(15)-EpETrE	14.50	319.3	257	$[^2H_{11}]$-11(12)-EpETrE	3.8-1675
	5,6-DiHETrE	12.15	337.3	145	$[^2H_{11}]$-14,15-DiHETrE	0.38-3375
	8,9-DiHETrE	11.50	337.2	127	$[^2H_{11}]$-14,15-DiHETrE	0.98-3375
	11,12-DiHETrE	11.00	337.3	167	$[^2H_{11}]$-14,15-DiHETrE	0.38-300
	14,15-DiHETrE	10.50	337.3	207	$[^2H_{11}]$-14,15-DiHETrE	0.98-3375
	5,6-DiHETE	11.85	335.2	115	$[^2H_{11}]$-14,15-DiHETrE	1.9-750
	5,15-DiHETE	9.28	335.3	255	$[^2H_{11}]$-14,15-DiHETrE	1.9-750
	8,15-DiHETE	8.94	335.3	155	$[^2H_{11}]$-14,15-DiHETrE	2.3-975
	LXA_4	6.85	351.3	115	$[^2H_8]$-5-HETE	0.30-1010
	LXB_4	6.10	351.3	221	$[^2H_4]$-9,10-DiHOME	9.0-975

	Lipid mediator	RT (min)	Parent ion $[M-H]^-$ (m/z)	Product ion (m/z)	IS	Linear range (fmol)
LA	9-HODE	12.75	295.3	171	$[^2H_4]$-9-HODE	3.8-1500
	13-HODE	12.60	295.2	195	$[^2H_4]$-9-HODE	3.8-1500
	9-KODE	13.40	293.2	185	$[^2H_4]$-9(10)-EpOME	1.9-150
	13-KODE	13.00	293.2	113	$[^2H_4]$-9(10)-EpOME	0.98-150
	9,10,13-TriHOME	5.75	329.1	139	$[^2H_4]$-PGE_2	4.5-135
	9,12,13-TriHOME	5.65	329.1	211	$[^2H_4]$-PGE_2	1.8-135
	EKODE	11.20	309.2	291	$[^2H_4]$-9(10)-EpOME	0.98-1675
	9(10)-EpOME	14.60	295.2	171	$[^2H_4]$-9(10)-EpOME	1.9-3375
	12(13)-EpOME	14.35	295.2	195	$[^2H_4]$-9(10)-EpOME	3.8-3375
	9,10-DiHOME	10.25	313.2	201	$[^2H_4]$-9,10-DiHOME	1.8-712.5
	12,13-DiHOME	9.85	313.1	183	$[^2H_4]$-9,10-DiHOME	1.8-7200
	12(13)-EpODE	13.10	293.2	183	$[^2H_4]$-9(10)-EpOME	1.8-1650
DGLA	PGD_1	6.15	353.3	317.3	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	3.75-3375
	PGE_1	6.05	353.3	317.3	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	1.35-4725
	8-HETrE	14.29	321.3	157	$[^2H_8]$-5-HETE	3.75-300
	15-HETrE	13.85	321.3	221	$[^2H_8]$-15-HETE	1.9-1500
ALA	9-HOTrE	11.25	293.2	171	$[^2H_{11}]$-14,15-DiHETrE	0.19-150
	13-HOTrE	11.40	293.2	195	$[^2H_{11}]$-14,15-DiHETrE	9.0-90
	9-KOTrE	11.88	291.2	185	$[^2H_4]$-9(10)-EpOME	3.8-1675
EPA	PGD_3	5.25	349.3	269	$[^2H_4]$-PGD_2	3.75-3375
	PGE_3	5.09	349.3	269	$[^2H_4]$-PGD_2	0.56-2025
	LTB_5	8.25	333.2	195	$[^2H_4]$-LTB_4	0.98-3375
	5-HEPE	12.65	317.3	115	$[^2H_8]$-15-HETE	2.6-2100
	8-HEPE	12.15	317.1	155	$[^2H_8]$-15-HETE	2.3-3550
	9-HEPE	12.30	317	149	$[^2H_8]$-15-HETE	2.3-3550
	11-HEPE	11.91	317	167	$[^2H_8]$-15-HETE	2.3-3375
	12-HEPE	12.15	317	179	$[^2H_8]$-15-HETE	2.3-3450
	15-HEPE	11.80	317.1	219	$[^2H_8]$-15-HETE	2.3-3375
	18-HEPE	11.40	317.1	215	$[^2H_8]$-15-HETE	2.3-3375
	14(15)-EpETE	13.30	317.3	207	$[^2H_7]$-5-KETE	1.9-1675
	17(18)-EpETE	12.90	317.3	255	$[^2H_7]$-5-KETE	1.9-1675
	14,15-DiHETE	9.51	335.2	207	$[^2H_{11}]$-14,15-DiHETrE	15-1675
	17,18-DiHETE	9.20	335.2	247	$[^2H_{11}]$-14,15-DiHETrE	15-1675
	LXA_5	5.80	349.1	115	$[^2H_8]$-5-HETE	1.2-975
DHA	8-HDoHE	14.00	343.2	189	$[^2H_8]$-5-HETE	7.5-1500
	11-HDoHE	13.65	343.3	149	$[^2H_8]$-5-HETE	2.3-2025
	14-HDoHE	13.40	343.2	281	$[^2H_8]$-5-HETE	4.5-2025
	17-HDoHE	13.10	343.2	281	$[^2H_7]$-5-KETE	4.5-900
	16(17)-EpDPE	14.75	343.3	281	$[^2H_{11}]$-11(12)-EpETrE	3.8-750
	19(20)-EpDPE	14.30	343.2	281	$[^2H_{11}]$-11(12)-EpETrE	1.9-150
	10,17-DiHDoHE	9.20	359.2	153	$[^2H_{11}]$-14,15-DiHETrE	2.3-2050
	19,20-DiHDPA	10.53	361.1	229	$[^2H_{11}]$-14,15-DiHETrE	1.4-4750
	RvD_1	6.75	375.1	141	$[^2H_{11}]$-14,15-DiHETrE	2.3-2050
	17(R)-RvD_1	6.85	375.1	141	$[^2H_{11}]$-14,15-DiHETrE	4.5-2050
	RvD_2	6.15	375.2	175	$[^2H_{11}]$-14,15-DiHETrE	18-2050
	RvE_1	4.40	349.1	195	$[^2H_{11}]$-14,15-DiHETrE	4.5-2050
MA	LTB_3	11.00	337.2	195	$[^2H_4]$-LTB_4	1.9-3375
	5-HETrE	16.05	321.3	205	$[^2H_8]$-5-HETE	0.98-300

	Lipid mediator	RT (min)	Parent ion $[M-H]^-$ (m/z)	Product ion (m/z)	IS	Linear range (fmol)
IS	$[^2H_4]$-PGD_2	6.10	355.3	193		
	$[^2H_4]$-PGE_2	5.85	355.3	275		
	$[^2H_4]$-6-keto-$PGF_{1\alpha}$	4.35	373.2	167		
	$[^2H_4]$-PGB_2	8.00	337.2	179		
	$[^2H_4]$-TxB_2	5.22 (broad)	373.2	173		
	$[^2H_4]$-LTB_4	9.60	339.3	153		
	$[^2H_8]$-5-HETE	14.15	327.2	116		
	$[^2H_8]$-15-HETE	12.85	327.3	182		
	$[^2H_6]$-20-HETE	11.80	325.3	281		
	$[^2H_7]$-5-KETE	14.95	324.2	210		
	$[^2H_{11}]$-11(12)-EpETrE	14.95	330.3	167		
	$[^2H_{11}]$-14,15-DiHETrE	10.40	348.3	207		
	$[^2H_4]$-9-HODE	12.70	299.3	172		
	$[^2H_4]$-9(10)-EpOME	14.55	299.1	172		
	$[^2H_4]$-9,10-DiHOME	10.17	317.2	203		
TS	CUDA	9.70	339.3	214		

Table E4. Average concentration ± standard deviation of lipid mediators in BALF. Compounds are categorized by their parent polyunsaturated fatty acid (PUFA): arachidonic acid (AA), linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and mead acid (MA). The synthetic pathway is provided with the color-coding scheme shown in Figure S1. Units are in pM, except for CysLTs (ng/mL). LOD indicates that values were below the method limit of detection.

Lipid mediator (pM)	PUFA	Pathway	Male				Female			
			Healthy	Smokers	COPD	COPD ExS	Healthy	Smokers	COPD	COPD ExS
PGD2	AA	COX	12.3±8.61	79.1±60.3	82.6±46.4	50.9±21.9	14.0±12.4	84.5±117	109±88.4	56.6±70.3
11-β-PGF2α	AA	COX	13.6±6.10	22.1±10.5	16.7±10.1	LOD	12.9±3.74	20.2±10.5	17.0±5.13	LOD
PGJ2	AA	COX	9.14±6.86	6.47±3.08	6.98±3.37	LOD	7.03±1.65	5.53±2.06	7.56±4.97	7.81±4.28
PGE2	AA	COX	9.71±4.41	43.8±31.1	51.7±39.7	26.8±20.4	15.1±6.23	36.7±43.7	42.2±25.5	17.2±14.0
PGF2α	AA	COX	LOD	48.7±32.7	47.8±34.0	30.3±5.29	21.8±1.62	37.7±26.2	52.1±36.0	LOD
TxB2	AA	COX	31.4±27.8	268±252	276±198	133±130	55.6±55.4	183±145	384±276	75.2±67.9
12-HHTrE	AA	COX	35.0±21.6	153±153	158±135	158±110	62.2±62.7	108±91.8	261±181	60.9±42.3
LTB4	AA	5-LOX	40.0±33.3	518±508	622±569	107±121	81.1±95.6	449±498	425±357	60.0±33.2
6-*trans*-LTB4	AA	5-LOX	19.4±10.6	132±105	166±145	54.0±43.0	51.2±47.7	126±118	146±115	24.9±19.6
CysLTs	AA	5-LOX	53.4±19.5	64.6±29.7	62.9±24.0	55.9±9.69	55.8±16.8	69.7±26.3	56.9±13.2	52.2±17.1
5-HETE	AA	5-LOX	89.8±60.4	1040±987	1361±1540	421±502	235±330	852±958	1310±1390	207±160
5-KETE	AA	5-LOX	23.6±19.7	90.6±80.9	122±110	42.1±30.7	39.8±51.3	57.4±33.5	128±99.1	34.6±23.1
11-HETE	AA	ROS	7.59±8.09	13.5±11.9	13.5±9.78	8.09±3.57	6.27±3.81	12.1±11.02	19.0±7.23	7.49±1.05
12-HETE	AA	12-LOX	68.2±65.5	139±132	92.7±82.6	188±185	137±123	139±199	165±180	87.6±107
15-HETE	AA	15-LOX	440±424	1140±917	1020±963	1300±1090	732±562	1040±1330	1480±1400	521±474
15-KETE	AA	15-LOX	100±71.7	329±203	320±223	235±180	163±102	396±602	275±140	107±77.2
5(6)-EpETrE	AA	CYP	11.7±5.01	28.4±19.5	30.4±17.9	18.3±3.89	12.8±5.81	22.0±9.84	23.1±9.23	15.8±10.0
11(12)-EpETrE	AA	CYP	6.96±3.08	19.4±10.1	19.8±11.3	7.75±1.90	7.43±3.75	13.7±6.01	14.5±8.47	6.21±2.19
11,12-DiHETrE	AA	EH	3.13±0.150	7.38±5.16	5.10±1.11	LOD	3.25±0.460	6.29±2.27	5.11±2.01	2.98±0.180
14,15-DiHETrE	AA	EH	6.11±0.910	9.5±3.53	8.03±0.890	LOD	6.81±1.63	7.45±1.69	8.42±1.82	6.54±1.68
5,6-DiHETE	AA	EH	8.04±2.12	29.3±21.5	30.8±23.6	11.7±4.28	12.0±8.25	26.4±28.4	29.4±22.7	7.06±0.580
13-HODE	LA	15-LOX	650±571	1250±1610	911±769	1410±1120	942±634	1090±970	1010±853	599±383
13-KODE	LA	15-LOX	277±176	291±170	284±174	286±26.3	344±283	295±146	207±56.3	250±143
9-KODE	LA	NA	92.4±86.0	62.0±53.8	90.6±91.3	96.3±48.7	93.8±109	56.6±39.8	74.5±29.8	92.9±65.6
9,10,13-TriHOME	LA	NA	105±59.8	67.5±40.0	110±62.3	188±54.3	106±53.2	54.6±16.9	127±30.5	125±60.6

Lipid mediator (pM)	PUFA	Pathway	Male				Female			
			Healthy	Smokers	COPD	COPD ExS	Healthy	Smokers	COPD	COPD ExS
EKODE	LA	ROS	197±164	129±160	166±196	223±172	197±197	79.8±57.8	106±63.5	217±200
9(10)-EpOME	LA	CYP	2570±1230	1910±866	2280±811	3070±1020	2500±1230	1340±717	2950±1190	2360±846
12(13)-EpOME	LA	CYP	2350±1090	2050±872	2310±669	2800±870	2320±1080	1470±709	2940±1040	2180±764
9,10-DiHOME	LA	EH	337±174	491±243	461±161	516±250	384±251	378±208	547±195	287±112
12,13-DiHOME	LA	EH	313±180	422±210	412±163	500±273	359±278	330±219	499±239	266±127
PGE1	DGLA	COX	3.63±1.32	4.59±2.33	4.97±2.08	2.97±1.29	3.73±1.17	4.41±1.73	5.15±2.14	3.01±1.44
15-HETrE	DGLA	15-LOX	49.4±50.8	94.8±91.0	86.7±94.7	175±201	85.9±60.7	85.1±96.8	153±185	55.3±35.8
13-HOTrE	ALA	15-LOX	64.8±53.7	68.0±77.3	47.9±23.3	73.0±51.0	52.1±39.9	73.5±125	48.6±23.4	44.5±19.7
LTB5	EPA	5-LOX	3.17±2.45	15.8±13.7	19.2±18.9	3.61±2.84	5.34±3.77	18.2±19.1	24.6±47.4	2.94±1.11
5-HEPE	EPA	5-LOX	18.0±4.63	89.8±67.8	97.2±97.1	20.9±8.18	45.7±31.3	93.0±95.2	186±408	43.1±13.8
12-HEPE	EPA	12-LOX	16.4±6.65	24.9±14.9	14.3±4.18	25.2±21.6	26.3±23.0	22.4±27.6	36.1±40.6	18.7±14.2
15-HEPE	EPA	15-LOX	93.9±53.1	132±124	95.6±66.8	170±112	151±112	159±177	224±279	92.3±82.7
14-HDoHE	DHA	ROS	67.7±62.6	82.4±71.5	69.9±43.6	90.2±63.2	113±83.3	117±152	120±127	62.1±62.6
17-HDoHE	DHA	15-LOX	413±207	440±351	400±226	547±340	471±302	728±895	573±489	371±255
5-HETrE	MA	5-LOX	11.4±4.52	70.7±57.7	118±122	36.5±28.0	15.6±12.4	74.0±98.1	80.1±62.0	10.8±6.89

Table E5. Average concentration ± standard deviation of lipid mediators in serum. Compounds are categorized by their parent polyunsaturated fatty acid (PUFA): arachidonic acid (AA), linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), α-linoleic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and mead acid (MA). In addition, the synthetic pathway is provided with the color-coding scheme shown in Figure S1. LOD indicates that values were below the method limit of detection.

Lipid mediator (nM)	PUFA	Pathway	Male				Female			
			Healthy	Smokers	COPD	COPD ExS	Healthy	Smokers	COPD	COPD ExS
PGD_2	AA	COX	0.159±0.168	0.240±0.200	0.181±0.151	0.297±0.253	0.227±0.212	0.133±0.119	0.253±0.334	0.084±0.040
PGE_2	AA	COX	0.200±0.161	0.291±0.287	0.229±0.209	0.364±0.290	0.205±0.147	0.174±0.149	0.234±0.302	0.056±0.044
$PGF_{2\alpha}$	AA	COX	0.129±0.125	0.158±0.116	0.152±0.104	0.185±0.171	0.173±0.101	0.123±0.124	0.209±0.236	0.111±0.068
11-β-$PGF_{2\alpha}$	AA	COX	0.110±0.047	0.217±0.394	0.133±0.072	0.128±0.047	0.304±0.506	0.081±0.042	0.192±0.110	0.103±0.027
6-keto-$PGF_{1\alpha}$	AA	COX	0.057±0.055	0.060±0.092	0.047±0.064	0.059±0.055	0.058±0.062	0.055±0.06	0.100±0.083	0.043±0.030
PGJ_2	AA	COX	0.068±0.077	0.092±0.114	0.165±0.105	0.084±0.067	0.043±0.059	0.086±0.098	0.062±0.053	0.124±0.154
15-deoxy-$\Delta^{12,14}$-PGJ_2	AA	COX	0.126±0.087	0.309±0.654	0.132±0.048	0.104±0.051	0.106±0.051	0.138±0.067	0.124±0.046	0.121±0.065
TxB_2	AA	COX	13.1±14.0	14.8±14.8	16.4±16.6	32.7±30.5	16.7±15.8	11.0±9.6	20.8±27.1	6.64±2.30
12-HHTrE	AA	COX	20.4±20.3	18.8±17.8	21.6±23.1	48.7±45.0	23.5±22.3	12.8±14.6	30.0±37.9	10.4±3.75
PGB_2	AA	NA	0.060±0.038	0.187±0.548	0.045±0.023	0.049±0.003	0.052±0.019	0.063±0.040	0.135±0.147	0.080±0.056
LTB_4	AA	5-LOX	0.195±0.183	0.287±0.430	0.231±0.339	0.354±0.489	0.263±0.342	0.133±0.109	0.181±0.236	0.231±0.253
6-*trans*-LTB_4	AA	5-LOX	0.605±1.13	1.64±1.67	0.350±0.842	0.073±0.089	0.233±0.342	1.04±1.05	0.035±0.017	LOD
20-carboxy-LTB_4	AA	5-LOX	0.176±0.089	0.492±1.00	0.175±0.082	0.231±0.186	0.188±0.071	0.140±0.101	0.135±0.036	0.236±0.022
LXA_4	AA	5-LOX	0.554±1.15	1.02±1.15	0.380±0.883	0.132±0.075	0.205±0.260	0.847±0.909	0.095±0.101	0.092±0.016
5-HETE	AA	5-LOX	43.9±104	102±119	31.3±83.1	2.71±1.94	18.0±41.6	105±128	3.74±1.44	3.77±0.863
5-KETE	AA	5-LOX	1.35±2.48	4.63±4.76	1.55±4.02	0.152±0.101	0.728±0.915	4.24±5.15	0.305±0.245	0.319±0.293
5,15-DiHETE	AA	5-LOX	1.87±3.19	2.80±3.20	1.53±2.59	LOD	0.729±0.798	1.88±1.84	0.066±0.036	0.081±0.020
8,15-DiHETE	AA	5-LOX	0.213±0.320	0.449±0.481	0.214±0.426	0.137±0.085	0.142±0.096	0.252±0.269	0.114±0.079	0.189±0.204
8-HETE	AA	ROS	1.72±3.34	6.71±9.91	1.52±3.51	0.513±0.406	0.726±1.00	3.94±4.72	0.516±0.345	0.519±0.189
9-HETE	AA	ROS	37.8±36.3	30.6±22.4	43.6±34.6	116±181	43.9±31.7	29.0±24.3	72.3±108	16.4±7.92
11-HETE	AA	ROS	2.28±3.27	5.49±5.39	2.25±3.74	1.78±2.06	1.37±1.06	3.76±3.96	1.31±1.11	0.636±0.144
12-HETE	AA	12-LOX	38.4±37.3	31.4±23.1	43.9±34.7	119±186	43.7±32.7	29.5±24.4	73.7±111	17.0±7.56
12-KETE	AA	12-LOX	5.65±6.26	11.8±12.0	6.17±6.31	6.05±6.09	4.31±2.86	8.71±8.05	4.94±4.75	3.65±1.79
15-HETE	AA	15-LOX	3.27±3.87	6.58±5.83	3.24±3.97	3.24±4.08	2.54±1.35	5.07±4.61	2.90±1.67	1.36±0.555
15-KETE	AA	15-LOX	1.57±2.03	3.26±3.06	1.64±2.35	0.770±0.263	1.02±0.650	2.99±2.88	0.743±0.317	0.951±0.256
20-HETE	AA	CYP	0.863±0.493	1.19±1.56	1.06±0.425	1.04±0.163	0.846±0.417	0.845±0.540	1.32±1.11	0.934±0.431
5(6)-EpETrE	AA	CYP	0.674±0.323	0.749±0.637	0.828±0.398	0.916±0.239	0.938±0.655	0.580±0.194	0.985±0.560	1.31±0.658
8(9)-EpETrE	AA	CYP	0.460±0.569	0.781±0.755	0.504±0.808	0.400±0.436	0.278±0.211	0.577±0.540	0.342±0.224	0.546±0.330
11(12)-EpETrE	AA	CYP	0.233±0.109	0.289±0.318	0.248±0.114	0.250±0.108	0.274±0.167	0.202±0.084	0.275±0.146	0.301±0.223

Lipid mediator (nM)	PUFA	Pathway	Male				Female			
			Healthy	Smokers	COPD	COPD ExS	Healthy	Smokers	COPD	COPD ExS
5,6-DiHETrE	AA	EH	0.340±0.154	0.809±0.548	0.475±0.331	0.334±0.107	0.356±0.232	0.713±0.459	0.295±0.117	0.324±0.123
8,9-DiHETrE	AA	EH	0.197±0.071	0.266±0.110	0.295±0.135	0.326±0.295	0.181±0.076	0.215±0.064	0.223±0.130	0.192±0.084
11,12-DiHETrE	AA	EH	0.464±0.105	0.630±0.174	0.642±0.152	0.899±0.944	0.396±0.059	0.462±0.112	0.595±0.243	0.482±0.132
14,15-DiHETrE	AA	EH	0.632±0.154	0.911±0.232	0.947±0.249	0.856±0.543	0.594±0.106	0.680±0.194	0.875±0.35	0.664±0.233
13-HODE	LA	15-LOX	16.1±13.6	19.7±11.2	14.0±17.1	10.1±3.06	13.3±7.68	17.3±12.9	12.8±7.78	21.0±11.8
13-KODE	LA	15-LOX	14.0±10.3	16.9±10.9	14.7±12.0	10.6±3.11	11.9±4.51	16.3±10.6	10.8±6.19	16.5±6.06
9-HODE	LA	NA	16.9±17.9	22.6±16.4	13.8±20.5	8.01±3.18	12.3±7.85	20.1±16.5	11.6±7.16	18.8±9.69
9-KODE	LA	NA	2.65±1.50	3.13±1.65	2.59±2.15	1.64±0.449	3.46±3.26	2.57±1.63	3.28±2.57	5.03±2.59
9,10,13-TriHOME	LA	NA	0.868±1.26	1.84±4.37	1.14±1.26	0.920±0.669	1.54±1.68	0.717±1.03	1.25±0.985	2.02±2.39
9,12,13-TriHOME	LA	NA	0.684±0.954	1.26±2.79	0.755±1.03	0.757±0.294	0.841±0.973	0.563±0.641	0.800±0.557	1.37±1.45
EKODE	LA	NA	8.21±3.70	7.64±4.81	7.18±4.13	12.3±13.2	14.8±15.8	6.18±3.55	8.45±6.17	11.0±6.04
9(10)-EpOME	LA	CYP	3.30±1.34	2.75±1.43	2.82±0.946	2.96±0.853	3.78±2.04	2.83±1.71	3.45±2.20	5.24±1.86
12(13)-EpOME	LA	CYP	5.45±1.70	5.11±2.62	5.27±2.46	3.96±1.35	5.64±2.24	5.42±3.57	5.60±2.64	7.40±2.42
9,10-DiHOME	LA	EH	3.28±3.68	2.40±1.70	2.61±2.26	2.39±1.78	2.23±0.892	3.67±5.74	2.25±1.3	2.35±0.661
12,13-DiHOME	LA	EH	4.58±2.09	3.96±1.94	3.72±2.05	3.65±1.81	4.32±1.72	4.17±2.39	4.86±2.74	4.88±2.20
PGD_1	DGLA	COX	0.039±0.023	0.312±1.11	0.048±0.052	0.022±0.005	0.038±0.025	0.052±0.040	0.027±0.003	0.031±0.002
5-HETrE	DGLA	5-LOX	2.22±5.17	5.65±8.04	2.25±5.75	0.450±0.309	1.47±3.73	4.84±5.97	0.641±0.519	0.481±0.033
8-HETrE	DGLA	ROS	1.17±2.26	2.29±2.31	1.23±2.82	0.297±0.296	0.623±0.630	1.78±1.87	0.466±0.243	0.334±0.196
15-HETrE	DGLA	15-LOX	0.677±1.34	1.28±1.41	0.837±1.84	0.244±0.292	0.352±0.324	1.03±1.16	0.307±0.157	0.141±0.080
9-HOTrE	ALA	NA	0.064±0.056	0.094±0.075	0.081±0.133	0.029±0.019	0.063±0.093	0.083±0.105	0.066±0.068	0.104±0.071
9-KOTrE	ALA	NA	0.299±0.212	0.281±0.176	0.443±0.469	0.149±0.084	0.444±0.611	0.239±0.142	0.355±0.388	0.418±0.260
13-HOTrE	ALA	15-LOX	0.959±0.797	1.33±0.801	1.02±1.47	0.508±0.373	0.785±0.511	0.963±0.677	0.901±0.629	1.64±1.08
12(13)-EpODE	ALA	CYP	0.282±0.133	0.246±0.167	0.347±0.413	0.237±0.102	0.288±0.200	0.207±0.117	0.299±0.176	0.453±0.178
LXA_5	EPA	5-LOX	2.31±1.36	1.82±1.800	2.27±1.40	2.07±0.908	2.19±1.03	1.58±1.16	2.15±1.09	2.19±1.59
5-HEPE	EPA	5-LOX	10.6±25.	17.9±19.4	3.29±7.74	0.858±0.764	3.82±7.09	21./±27.7	1.14±1.77	1.04±0.411
8-HEPE	EPA	ROS	0.850±1.54	1.38±1.60	0.396±0.840	0.235±0.178	0.341±0.378	1.32±1.50	0.310±0.496	0.142±0.088
9-HEPE	EPA	ROS	1.28±2.15	1.66±2.03	0.523±0.965	0.078±0.017	0.519±0.614	1.56±1.93	0.348±0.633	0.132±0.109
11-HEPE	EPA	ROS	0.724±1.36	1.46±1.80	0.352±0.742	0.298±0.307	0.356±0.458	1.26±1.52	0.262±0.399	0.098±0.064
12-HEPE	EPA	12-LOX	5.07±4.79	3.68±3.29	2.68±1.18	16.1±25.7	5.44±3.74	4.28±3.98	5.86±7.92	1.59±1.31
15-HEPE	EPA	15-LOX	0.891±1.66	1.60±2.05	0.372±0.531	0.356±0.481	0.395±0.512	1.35±1.76	0.416±0.601	0.222±0.057
18-HEPE	EPA	15-LOX	1.37±2.27	2.32±2.49	0.732±0.920	0.884±0.877	0.986±0.734	2.17±2.34	1.05±1.75	0.520±0.426
14(15)-EpETE	EPA	CYP	0.151±0.085	0.197±0.092	0.110±0.038	0.111±0.016	0.132±0.105	0.167±0.077	0.229±0.336	0.253±0.007
17(18)-EpETE	EPA	CYP	0.208±0.171	0.361±0.242	0.147±0.058	0.140±0.015	0.288±0.208	0.219±0.203	0.298±0.369	0.294±0.124

Lipid mediator (nM)	PUFA	Pathway	Male				Female			
			Healthy	Smokers	COPD	COPD ExS	Healthy	Smokers	COPD	COPD ExS
14,15-DiHETE	EPA	EH	0.907±0.562	1.04±0.679	0.887±0.552	1.08±1.30	0.901±0.617	0.942±0.355	1.10±0.781	0.852±0.388
17,18-DiHETE	EPA	EH	7.38±3.32	8.95±5.74	8.03±3.81	10.4±10.2	7.77±3.97	7.45±2.61	10.6±7.14	6.23±1.18
8-HDoHE	DHA	ROS	3.35±6.37	5.45±5.75	2.60±6.14	0.789±0.218	1.70±1.98	6.16±7.61	1.02±1.88	0.956±0.807
11-HDoHE	DHA	ROS	2.33±3.51	3.49±3.06	1.52±2.78	1.88±2.48	1.62±1.10	3.38±3.85	1.22±1.59	0.589±0.475
14-HDoHE	DHA	ROS	17.7±15.8	15.5±11.6	11.5±7.89	37.5±68.5	19.8±13.6	16.2±14.1	17.1±22.4	4.98±3.02
17-HDoHE	DHA	15-LOX	3.82±6.41	9.00±5.92	3.08±5.00	1.70±1.67	2.03±1.95	7.24±7.68	1.72±2.16	1.08±0.736
19(20)-EpDPE	DHA	CYP	0.601±0.412	1.19±0.552	0.539±0.275	0.588±0.684	0.937±1.36	1.07±0.303	0.742±0.971	1.07±0.569
19,20-DiHDPA	DHA	EH	1.90±0.627	2.02±1.07	1.75±0.469	1.61±0.624	2.78±2.8	2.78±2.29	1.73±0.625	1.62±0.231

Table E6. Univariate statistics for 9-lipid panel identified in Figure 2A. Statistical significance for the comparisons of all groups from the COSMIC study. Groups are described by gender in the first letter (M for males and F for females) and smoking/disease status by the following letters: HNS=healthy never smoker, HS=healthy smoker, CS=COPD smoker, CXS=COPD ex-smoker. Values shown in red are considered significant at the <0.05 level.

Lipid	Sig	Males						Females					
		MHNS vs MHS	MHNS vs MCS	MHNS vs MCXS	MHS vs MCS	MHS vs MCXS	MCS vs MCXS	FHNS vs FHS	FHNS vs FCS	FHNS vs FCXS	FHS vs FCS	FHS vs FCXS	FCS vs FCXS
9-KODE	p	2.96E-01	7.34E-01	4.56E-01	6.50E-01	8.11E-02	2.96E-01	9.48E-02	5.25E-01	3.95E-01	4.40E-02	6.28E-02	7.91E-01
	q	6.41E-01	9.16E-01	7.61E-01	8.64E-01	3.48E-01	6.41E-01	3.84E-01	8.05E-01	7.17E-01	2.59E-01	3.17E-01	9.41E-01
TXB2	p	5.66E-09	6.64E-07	5.44E-02	7.17E-01	3.09E-01	1.27E-01	5.48E-04	2.45E-05	2.79E-01	4.40E-02	9.19E-02	5.91E-03
	q	5.75E-07	4.50E-05	2.93E-01	9.08E-01	6.46E-01	4.32E-01	1.05E-02	1.06E-03	6.26E-01	2.59E-01	3.78E-01	6.95E-02
5-KETE	p	9.65E-06	4.15E-05	1.88E-01	7.17E-01	1.35E-01	1.01E-01	7.33E-03	3.54E-04	8.93E-01	2.85E-02	8.13E-02	5.91E-03
	q	5.09E-04	1.60E-03	5.05E-01	9.08E-01	4.36E-01	3.90E-01	8.15E-02	7.81E-03	9.85E-01	2.08E-01	3.48E-01	6.95E-02
12-HHTrE	p	1.56E-04	2.59E-04	2.50E-02	8.77E-01	7.63E-01	6.76E-01	1.04E-01	5.06E-04	4.24E-01	1.08E-02	3.03E-01	7.11E-03
	q	4.37E-03	6.35E-03	1.91E-01	9.85E-01	9.36E-01	8.82E-01	3.90E-01	9.99E-03	7.47E-01	1.11E-01	6.44E-01	8.03E-02
12,13-DiHOME	p	6.53E-02	7.10E-02	9.73E-02	7.43E-01	4.77E-01	6.45E-01	7.79E-01	1.08E-02	4.64E-01	2.02E-02	7.25E-01	1.54E-02
	q	3.20E-01	3.29E-01	3.84E-01	9.20E-01	7.78E-01	8.63E-01	9.39E-01	1.11E-01	7.64E-01	1.69E-01	9.10E-01	1.42E-01
9,10-DiHOME	p	2.08E-02	2.88E-02	1.38E-01	7.69E-01	6.82E-01	1.00E+00	9.25E-01	7.20E-03	2.88E-01	1.23E-02	4.00E-01	1.19E-03
	q	1.71E-01	2.08E-01	4.41E-01	9.36E-01	8.87E-01	9.97E-01	9.97E-01	8.07E-02	6.34E-01	1.21E-01	7.21E-01	1.97E-02
9(10)-EpOME	p	6.96E-02	5.77E-01	3.66E-01	1.69E-01	3.65E-02	2.33E-01	2.92E-04	1.97E-01	9.35E-01	4.65E-05	1.64E-03	2.85E-01
	q	3.29E-01	8.31E-01	6.98E-01	4.76E-01	2.36E-01	5.68E-01	6.92E-03	5.10E-01	9.97E-01	1.66E-03	2.48E-02	6.34E-01
12(13)-EpOME	p	4.28E-01	7.88E-01	3.66E-01	2.74E-01	9.69E-02	3.82E-01	2.39E-03	1.04E-01	8.08E-01	7.61E-05	7.68E-03	1.26E-01
	q	7.50E-01	9.41E-01	6.98E-01	6.21E-01	3.84E-01	7.12E-01	3.31E-02	3.90E-01	9.46E-01	2.52E-03	8.41E-02	4.32E-01
9,10,13-TriHOME	p	2.62E-02	9.00E-01	2.73E-02	5.57E-02	1.32E-03	6.14E-02	3.36E-05	8.68E-02	4.64E-01	1.58E-06	1.64E-03	5.96E-01
	q	1.98E-01	9.91E-01	2.04E-01	2.96E-01	2.13E-02	3.13E-01	1.33E-03	3.66E-01	7.64E-01	1.00E-04	2.48E-02	8.44E-01

Table E7. Classification of independent female COPD cohort based upon multivariate modeling of BALF lipid mediator levels

	Members	Correct	COPD	Smoker
COPD	6	83.3%	5	1
Smoker	7	100%	0	7
Total	13	92.3%	5	8

The OPLS model from Figure 2A was used to classify an independent cohort of female Smokers and COPD (from the validation cohort). Fisher's exact test p=0.005.

The validation cohort consisted of 7 current female smokers (49–61 years; BMI 20.5–32.6) with normal lung function and 6 current female smokers with COPD (48–73 years; BMI 20.0–27.7; GOLD stage II-III; FEV_1=44-68%; $FEV_1/FVC<70$) with arterial oxygen saturation (SaO_2) >90%. Further information on the validation cohort can be found in the original publication by Blidberg *et al.,* 2013 (8).

Table E8. Classification of independent male COPD cohort based upon multivariate modeling of BALF lipid mediator levels

	Members	Correct	COPD	Smoker
COPD	5	60.0%	3	2
Smoker	9	55.6%	4	5
Total	14	57.1%	7	7

The OPLS model from Figure 2A was used to classify an independent cohort of male Smokers and COPD (from the validation cohort). Fisher's exact test p=0.5.

The validation cohort consisted of 9 current male smokers (49–61 years; BMI 20.5–32.6) with normal lung function and 5 current male smokers with COPD (48–73 years; BMI 20.0–27.7; GOLD stage II-III; FEV_1=44-68%; $FEV_1/FVC<70$) with arterial oxygen saturation (SaO_2) >90%. Further information on the validation cohort can be found in the original publication by Blidberg *et al.,* 2013 (8).

Figure E1. Overview of synthetic pathways for the lipid mediators quantified in BALF and serum. The synthetic route of each lipid species as well as the polyunsaturated fatty acid (PUFA) substrate is provided. The compounds LTA_4, PGH_x and PGI_2 are unstable intermediates that were not measured. Lipid nomenclature is provided in Table S4. The primary enzyme responsible for each conversion, when known, is indicated in the ovals. Lipid synthetic routes are not unambiguous and many compounds can be produced via multiple routes (*e.g.,* 11(*R*)-HETE can be produced by acetylated COX). An "x" (*e.g.,* PGE_x) indicates that there are multiple PUFA substrates possible for a compound, with x=2 for arachidonic acid-derived compounds and x=1 for dihomo-gamma-linolenic acid-derived compounds. LA=linoleic acid, ALA=alpha-linolenic acid, AA=arachidonic acid, DHGLA=dihomo-gamma-linolenic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid. LOX=lipoxygenase, COX=cyclooxygenase, CYP=cytochrome P450, EH=epoxide hydrolase, ROS=reactive oxygen species, NA=not assigned.

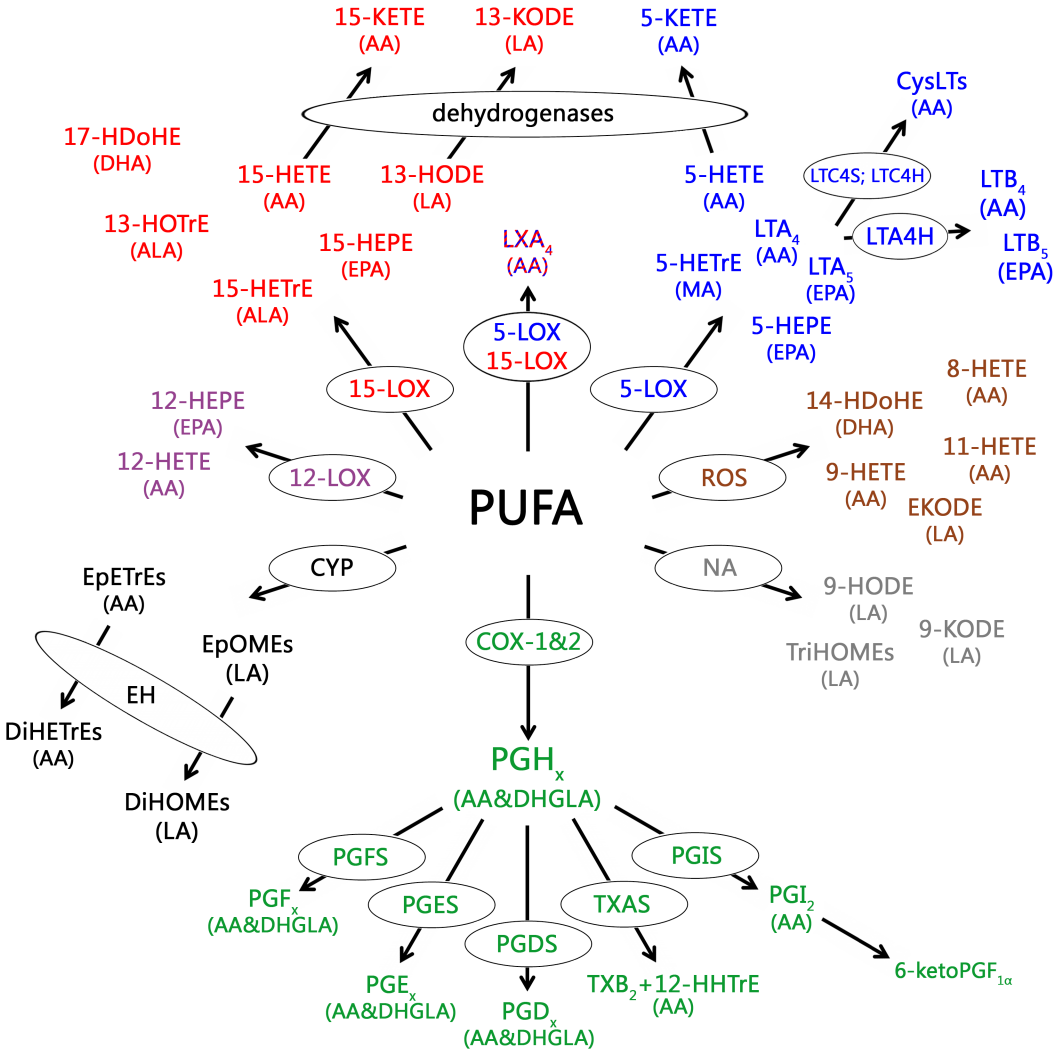


Figure E2. Concentration of cells in BALF and serum. A) BALF macrophages, **B)** BALF neutrophils, **C)** BALF eosinophils, **D)** blood platelets, **E)** blood leukocytes, and **F)** goblet cells (AB-PAS). Subjects are divided into smokers with normal lung function (Smokers, circles) and smokers with COPD (COPD, squares). Open symbols indicate males and closed symbols females. Significance is indicated by the non-parametric Mann-Whitney test. Data are expressed as millions of cells per L of BALF.

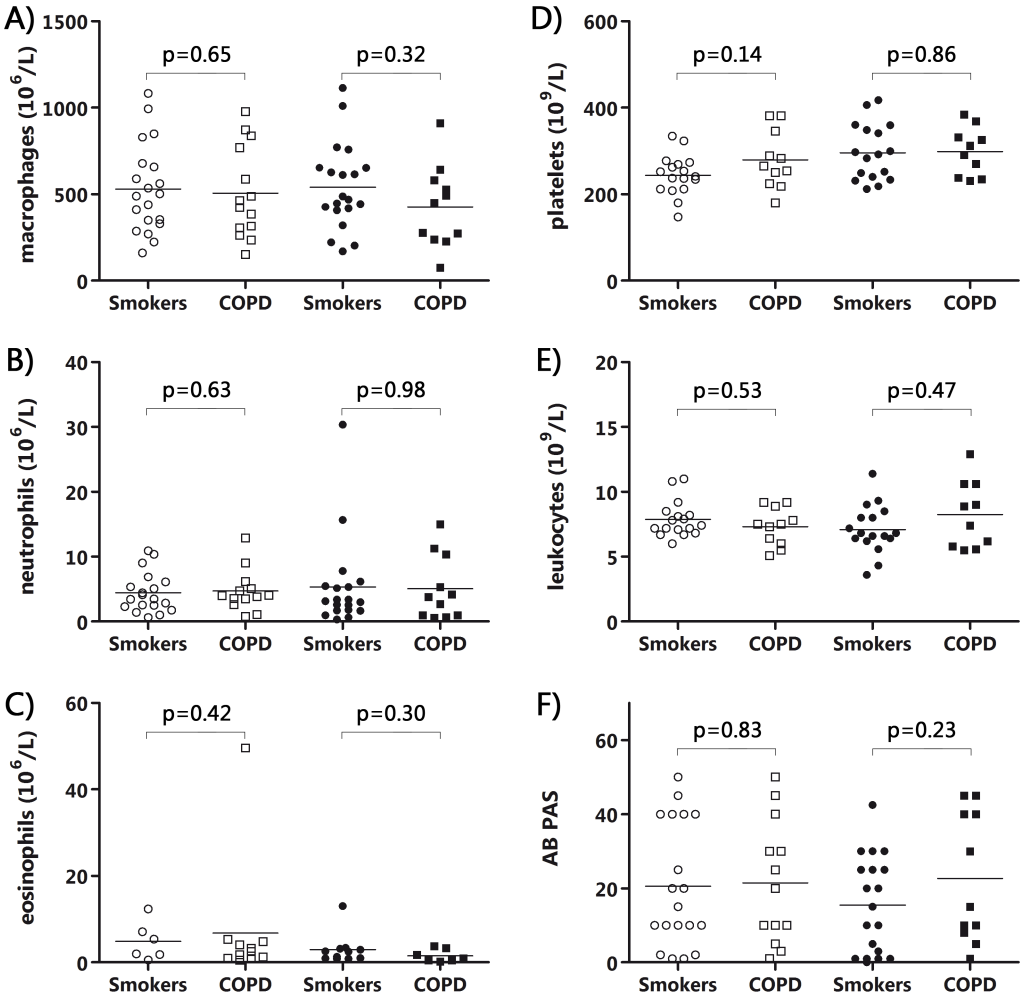


Figure E3. Variable selection to identify the lipid mediators driving the separation of female COPD patients and Smokers with normal lung function. Variables selected from the OPLS model based on all lipid mediators (1+1 components, R^2Y=0.75, Q^2=0.47, CV-ANOVA p=6.0x10^{-4}) was performed using a cutoff of $|p(corr)| \geq 0.4$ as well as variable importance in the projection (VIP)≥1.0. A total of 9 lipids fulfilled both selection criteria: 9,10,13-TriHOME, 12(13)-EpOME, 9(10)-EpOME, 9,10-DiHOME, 12,13-DiHOME, 12-HHTrE, 5-KETE, TXB_2 and 9-KODE (red dots). Green dots indicate those variables that did not fulfill the selection criteria.

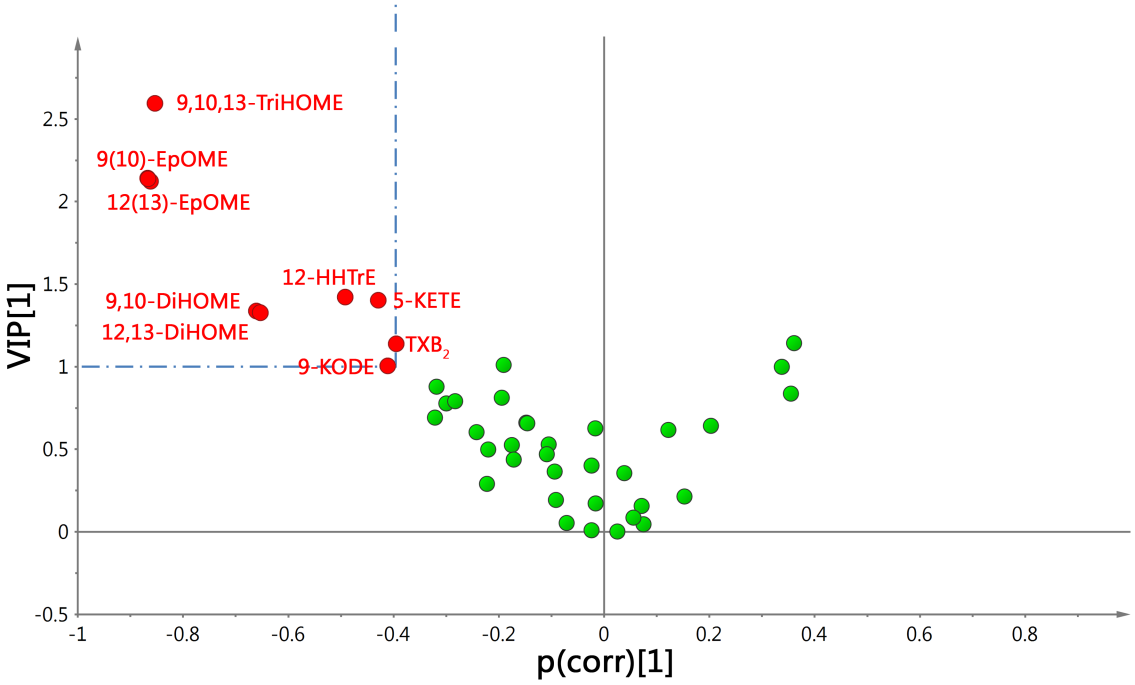


Figure E4. Subjecting the 9-lipid model to permutation tests confirmed that the observed differences did not occur by random, as confirmed by the low y-intercept of R^2, and the negative y-intercept of Q^2.

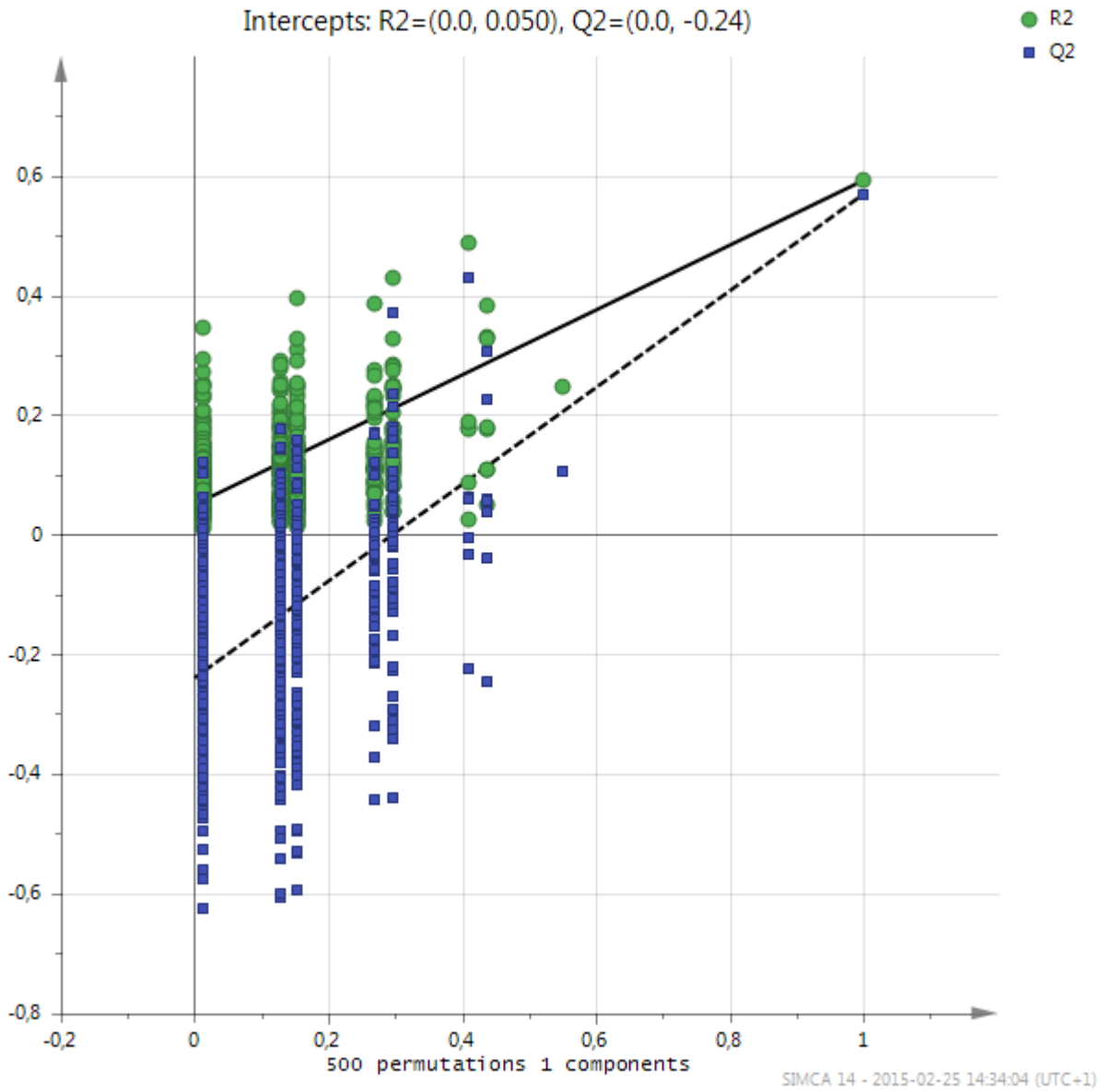


Figure E5. The effect of menopausal status and estrogen replacement therapy on the 9 lipid mediators from the multivariate (OPLS) model in Figure 2A among the female smokers with normal lung function. No separation with PCA was detected, and the displayed forced model indicates a homogeneous distribution of pre-menopausal (red), post-menopausal not taking estrogens (green), and post-menopausal subjects taking estrogen replacement therapy (blue). As such, it can be concluded that the observed differences with regards to the female smoking COPD group, where all subjects were post-menopausal without estrogen therapy, were not driven by these factors.

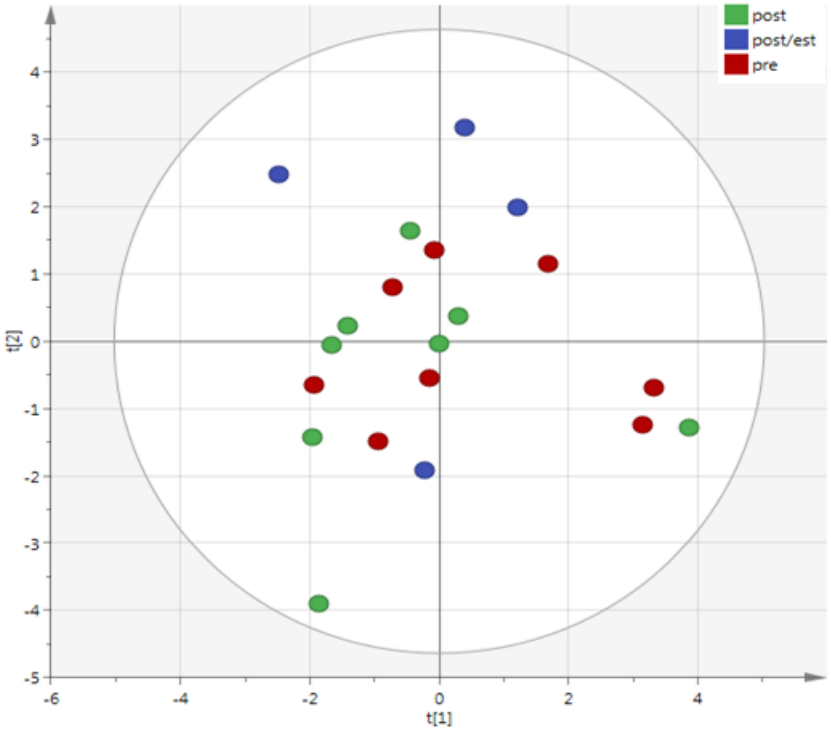


Figure E6. Shared and unique structures (SUS) plot for gender in lipid mediators in serum. Correlation analysis between the female and male OPLS models based on selected lipid mediators in serum from Smoker *vs.* COPD is displayed, with females displayed on the X-axis (1+1 components, $R^2Y=0.72$, $Q^2=0.67$, CV-ANOVA $p=5x10^{-6}$) and males on the Y-axis (1+1 components, $R^2Y=0.60$, $Q^2=0.45$, CV-ANOVA $p=0.03$). No lipid mediator falls in the areas selective for disease correlation in males or females.

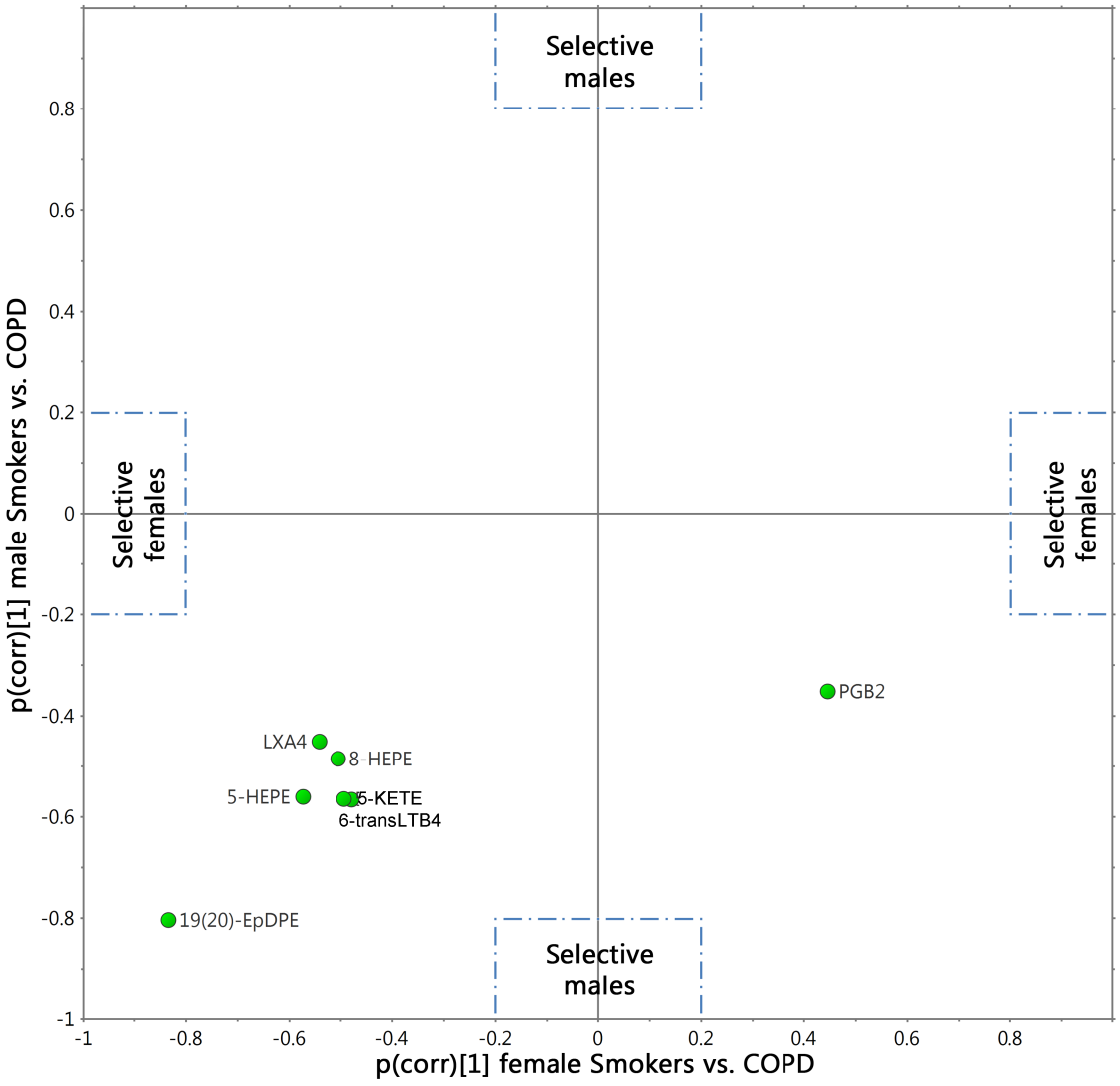


Figure E7. Cytochrome P450-derived arachidonate mediators in BALF in relation to gender, smoking status and disease. Lipid mediators from arachidonic acid: epoxy-eicosatrienoic acids, EpETrEs (A) and dihydroxy-eicosatrienoic acids, DiHETrEs (B). Subjects are divided into smokers with normal lung function (Smokers, circles) and smokers with COPD (COPD, squares). Open symbols indicate males and closed symbols females. Significance is indicated by the non-parametric Mann-Whitney test. Lipid mediator nomenclature is provided in Table E1

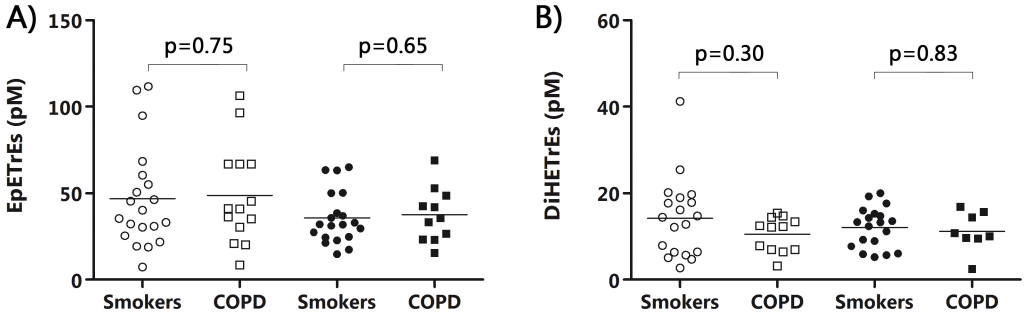
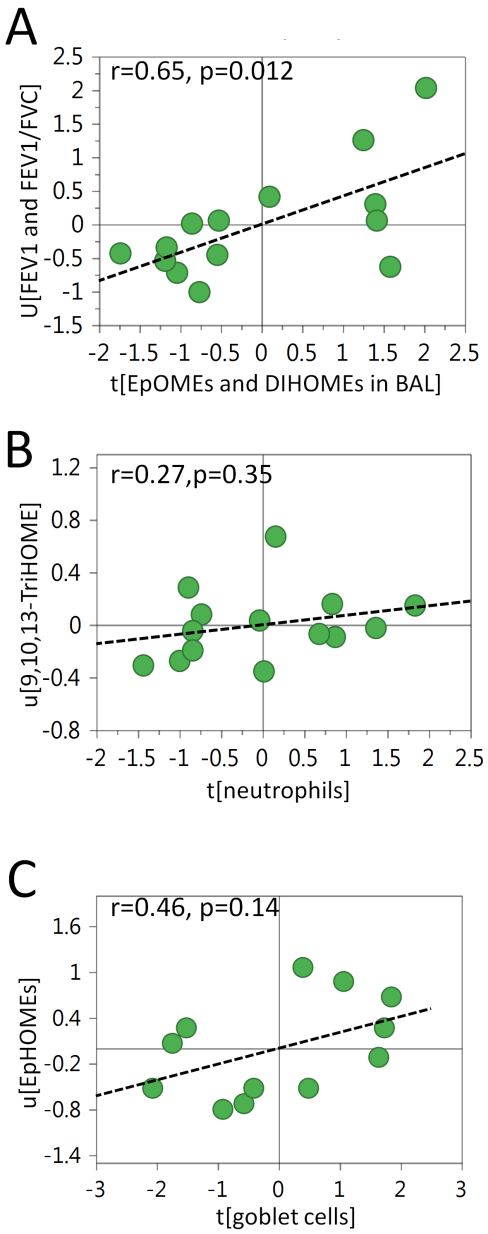


Figure E8. Correlation between lipid mediators in BALF with lung function, neutrophils and goblet cells. A) The correlation between lung function parameters (FEV_1 and FEV_1/FVC) *vs.* the concentrations of EpOMEs and DIHOMEs in male COPD smokers (PLS inner relation, r=0.65, p=0.012, n=14). **B)** The correlation between neutrophil abundance in BALF and 9,10,13-TriHOME (PLS inner relation, r=0.27, p=0.35, n=14). **C)** The correlation between goblet cell abundance *vs.* the 2 EpOME lipid mediators (leukotoxin and isoleukotoxin; PLS inner relation, r=0.46, p=0.14, n=12).



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