Targeted lipidomics of low-level lipids

Sample preparation

Targeted profiling of lipids was conducted at Emory University using previously published methods[60,61]. Powdered tissue samples (10 mg) were homogenized in 100 µl PBS with Bead Ruptor (Omni International, Kennesaw, GA). Homogenized samples were diluted with 300 µL 20% methanol and spiked with 1% BHT solution to a final BHT concentration of 0.1% and pH of 3.0 by acetic acid addition. Samples were then centrifuged (10 minutes, 14,000 rpm), and the supernatants were transferred to 96-well plates for further extraction. The supernatants were loaded to Isolute C18 SPE columns (conditioned with 1000 µL ethyl acetate and 1000 µL 5% methanol). The SPE columns were then washed with 800 µL water and 800 µL hexane. The oxylipins were eluted with 400 µl methyl formate. Automated solid phase extraction (SPE) was conducted with a Biotage Extrahera (Uppsala, Sweden). The eluate was dried with nitrogen and then reconstituted with 200 µL methanol prior to LC-MS analysis. Sample blanks, pooled extract samples used as quality controls (QC), and consortium reference samples, were prepared for analysis using the same methods. The external standards consisted of prostaglandin E2 ethanolamide, oleoyl ethanolamide, palmitoyl ethanolamide, arachidonoyl ethanolamide, docosahexaenoyl ethanolamide, linoleoyl ethanolamide, stearoyl ethanolamide, oxy-arachidonoyl ethanolamide, 2-arachidonyl glycerol, docosatetraenoyl ethanolamide, α-linolenoyl ethanolamide, oleamide, dihomo-γ-linolenoyl ethanolamide, decosanoyl ethanolamide, 9,10 DiHOME, prostaglandin E2-1-glyceryl ester, 20-HETE, 9-HETE, 14,15 DiHET, 5(S)-HETE, 12(R)-HETE, 11(12)-DiHET, 5,6-DiHET, thromboxane B2, 12(13)-EpOME, 13 HODE, prostaglandin F2α, 14(15)-EET, 8(9)-EET, 11(12)-EET, leukotriene B4, 8(9)-DiHET, 13-OxoODE, 13(S)-HpODE, 9(S)-HpODE, 9(S)-HODE, resolvin D3, resolvin E1, resolvin D1, resolvin D2, 9(S)HOTrE, 13(S)HOTrE, 8-iso Progstaglandin F2α. All external standards were purchased from Cayman Chemical (Ann Arbor, Michigan) at a final concentration in the range 0.01-20 µg/ml.

Data collection

Targeted oxylipin and endocannabinoid data were acquired using an ExionLC/Qtrap5500 (SCIEX, Waltham, MA) LC-MS system. For chromatography, a ThermoFisher Scientific AccucoreTM C18 column (100 mm × 4.6, 2.6 µm particle size) was used. The mass spectrometry data acquisition was conducted in both positive and negative ion modes. The mobile phases were water with 10 mM ammonium acetate (mobile phase A), and acetonitrile with 10 mM ammonium acetate (mobile phase B). The chromatographic method used the following gradient program shown in Table 14. The column temperature was set to 50°C, and the injection volume was 10 µL with negative ion mode and 2 µL with positive ion mode. For mass spectrometry analysis, the heated electrospray ionization source was operated at a vaporizer temperature of 650°C, a spray voltage of 5.5 kV for positive ion mode and 4.5 kV for negative ion mode, curtain gas, ion source gas 1, ion source gas 2 were 20, 60 and 50, respectively. The declustering potential, entrance potential, collision energy, and collision cell exit potential were 200, 10, 40, and 10 for the negative ion mode, respectively and 90, 10, 47

and 18, respectively, for positive ion mode. MRM transitions are shown in Table 15 (negative mode) and Table 16 (positive mode).

Table 14. Targeted lipidomics LC-MS gradient program

Time	%A	%B	Flow (mL/min)
0	90	10	0.5
0.5	90	10	0.5
1	50	50	0.5
2	50	50	0.5
2.1	25	75	0.5
5	25	75	0.5
7	15	85	0.5
12	15	85	0.5
12.1	90	10	0.5
18	90	10	0.5

Table 15. Targeted lipidomics negative mode MRM transitions

analyte	parent m/z	product m/z
9,10 DIHOME	313.2	201.0
PGE E2	351.2	315.1
20- HETE	319.2	289.2
9-HETE	319.2	167.2
14,15 DHET	337.2	207.0
5 HETE	319.2	115.1
12 R-HETE	319.2	179.1
11,12-DHET	337.2	167.1
8,9-DHET	337.2	127.2
5,6 EET	319.2	191.1
5,6-DHET	337.2	71.0
TXB2	369.2	169.1
PGF2A	353.2	193.3
14(15)-EET / 15-HETE	319.2	219.0
LTB4	335.2	195.1
8(9)-EET	319.2	69.2
11(12)-EET	319.2	167.1
Resolvin D1	375.3	215.1
Resolvin D2	375.3	215.2
Resolvin D3	375.3	147.1
Resolvin E1	349	109
Resolvin E1	349	161
12(13)-EpOME	295.2	195.1
13 HODE	295.2	195.1

13-OxoODE (13-KODE)	293.2	113.1
13(S)-HpODE	311.2	113.1
13(S)HOTrE	293.2	195.1
9(S)-HODE	295.2	277.6
9(S)-HODE	295.2	171.3
9(S)-HpODE	311.2	201.0
9(S)-HpODE	311.2	185.1
9(S)HOTrE	293.2	185.1
9(S)HOTrE	293.2	171.1
8-iso Progstaglandin F2α	353.2	255
8-iso Progstaglandin F2α	353.2	219.2
8-iso Progstaglandin F2α	353.2	193.1

Table 16. Targeted lipidomics positive mode MRM transitions

analyte	**parent m/z**	**product m/z**
PGE2 Ethanolamide (PGE2-EA)_271.3	396.5	271.3
PGE2 Ethanolamide (PGE2-EA)_358.3	396.5	358.3
PGE2 Ethanolamide (PGE2-EA)_376.3	396.5	376.3
Oleoyl Ethanolamide (OEA)	326.4	62.1
Palmitoyl Ethanolamide	300.4	62.1
ARA-Ethanolamide (AEA)	348.4	62.1
Docosahexaenoyl Ethanolamide (DHEA)	372.4	62.1
Linoleoyl Ethanolamide (LEA)	324.4	62.1
Stearoyl Ethanolamide (ceramid)	328.4	62.1
oxy-Arachidonoyl Ethanolamide (oxy-AEA)	364	76
2-Arachidonoyl Glycerol (2AG) trans1	379.4	135
2-Arachidonoyl Glycerol (2AG) trans2	379.4	161
Docosatetraenoyl Ethanolamide (DEA)	376.59	62.1
Alpha-Linolenoyl Ethanolamide (ALEA)	322.4	62.1
Oleamide	282.5	247.4
Dihomo-Gamma-Linolenoyl Ethanolamide	350.4	62.1
Docosanoyl Ethanolamide	384.5	62.1

Data processing and quality control

Sciex OS (AB SCIEX, Version 1.6.1) was used to process the raw LC-MS data. Standard curves were built for each oxylipin/endocannabinoid and calibrated against external standards, i.e, all concentration points should be in the linear portion of the curve with an R-squared value no less than 0.9. Additionally, features with a high coefficient of variation (CV), defined as CV > 150%, in pooled QCs were removed from the dataset. Pearson correlation among the QCs for each tissue type were calculated with the *Hmisc* R library, and figures documented in QC report were plotted with the *corrplot* R library[62,63].