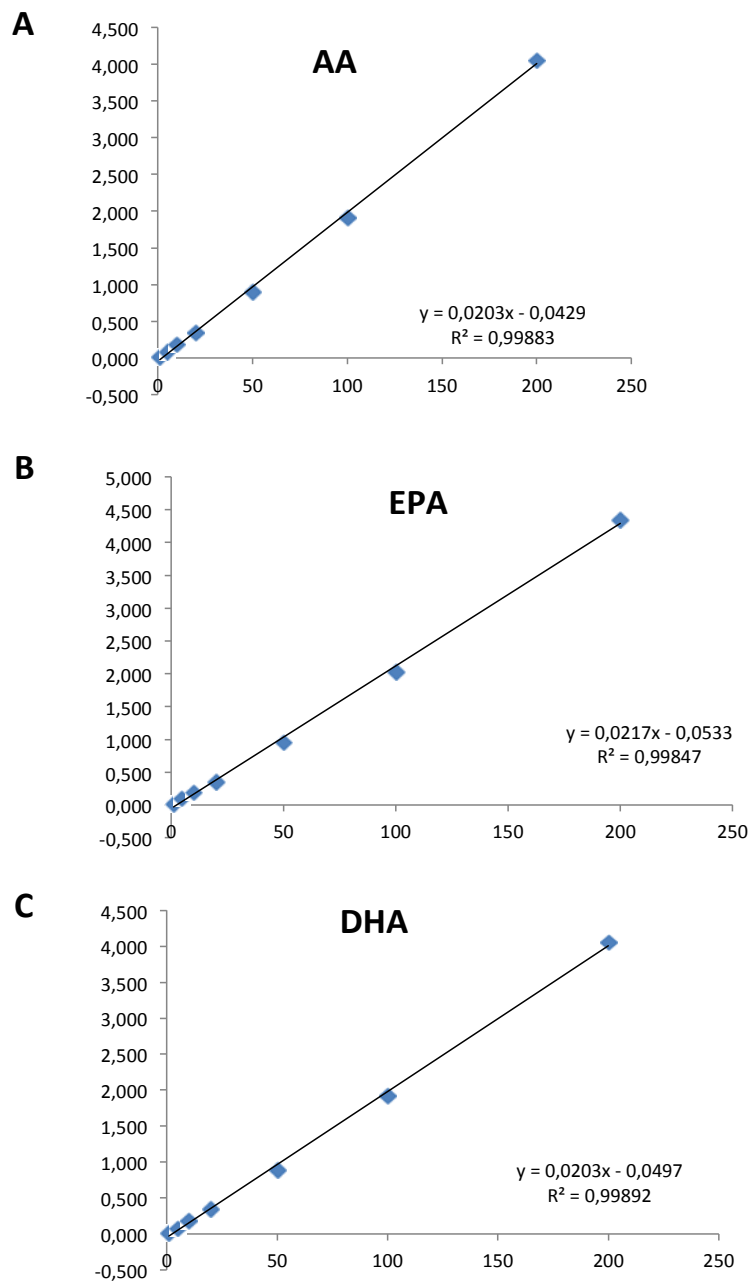
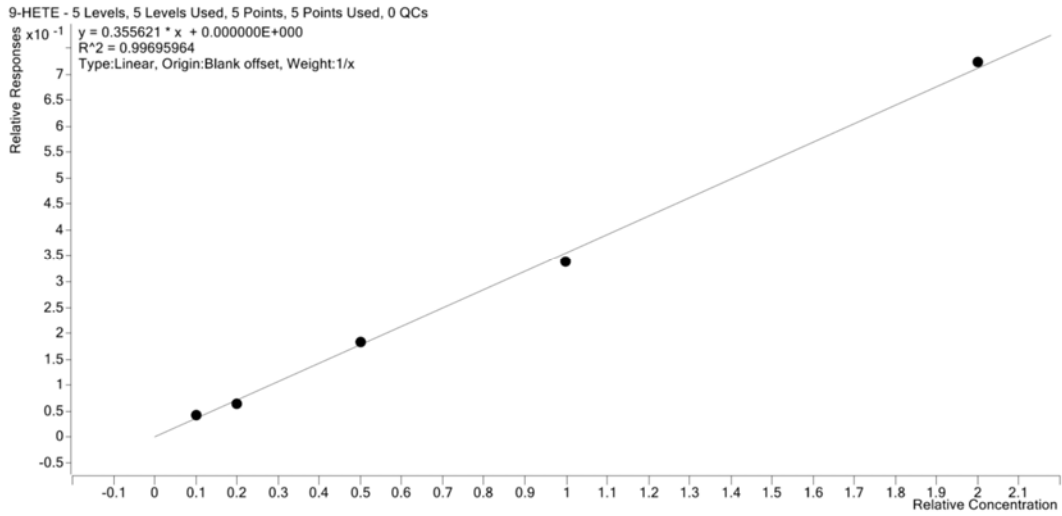
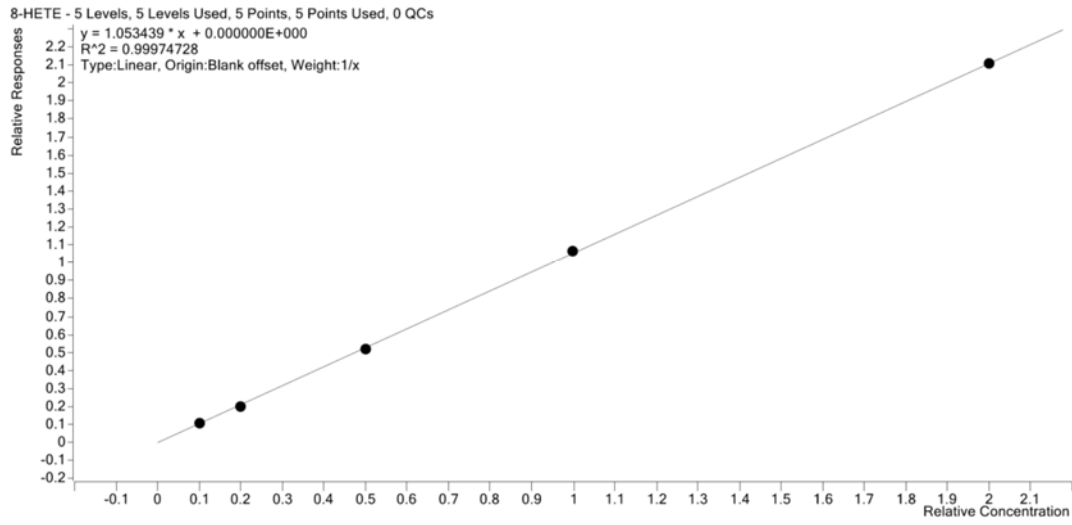
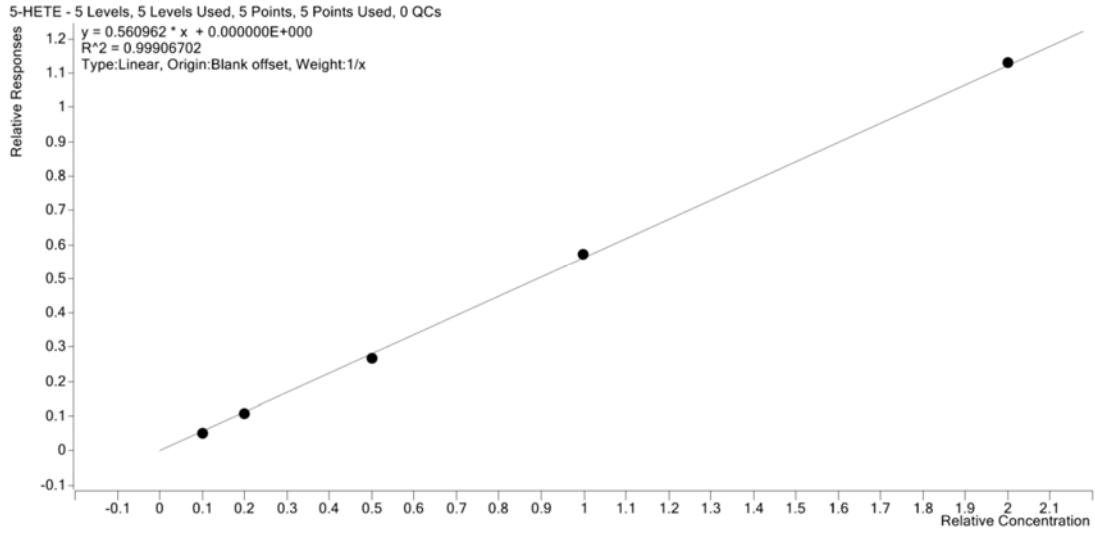


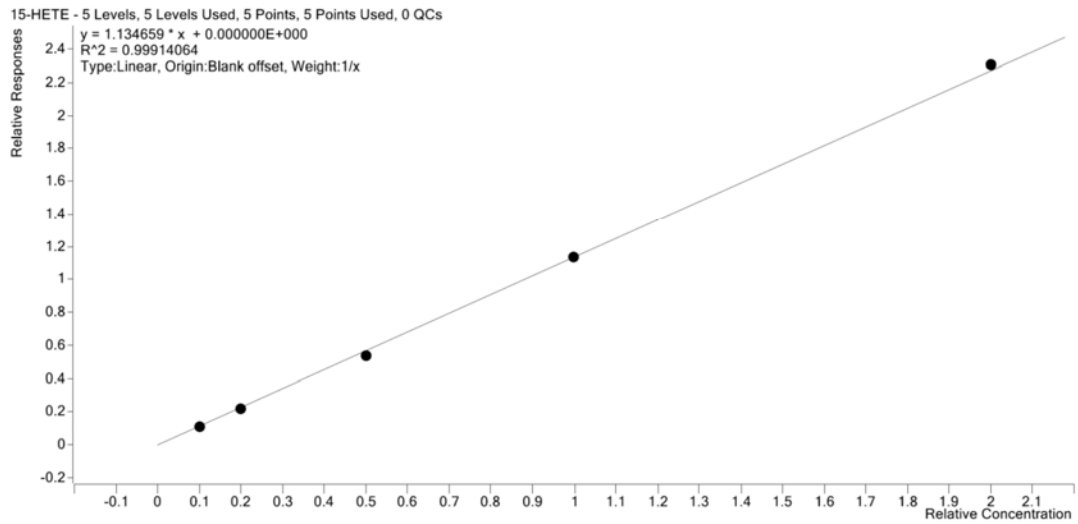
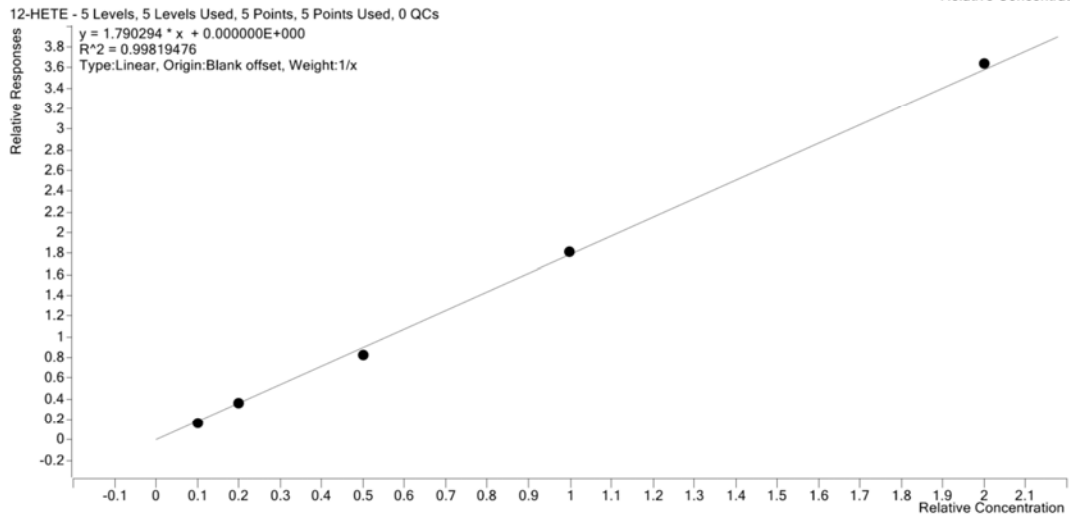
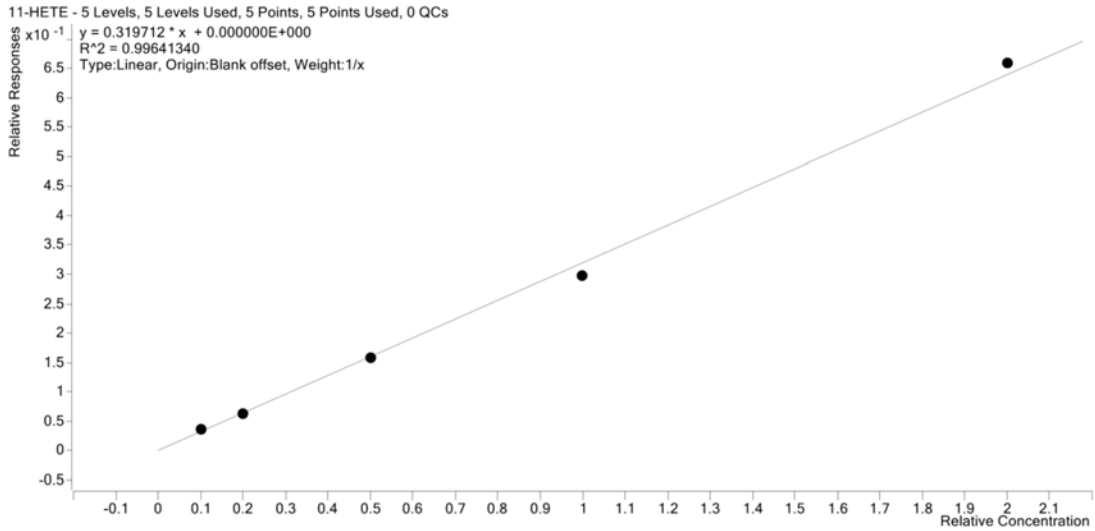
# Supplementary Materials: Quantitative Profiling of Hydroxy Lipid Metabolites in Mouse Organs Reveals Distinct Lipidomic Profiles and Modifications Due to Elevated n-3 Fatty Acid Levels

Cheng-Ying Chiu, Christopher Smyl, Inci Dogan, Michael Rothe and Karsten-H Weylandt

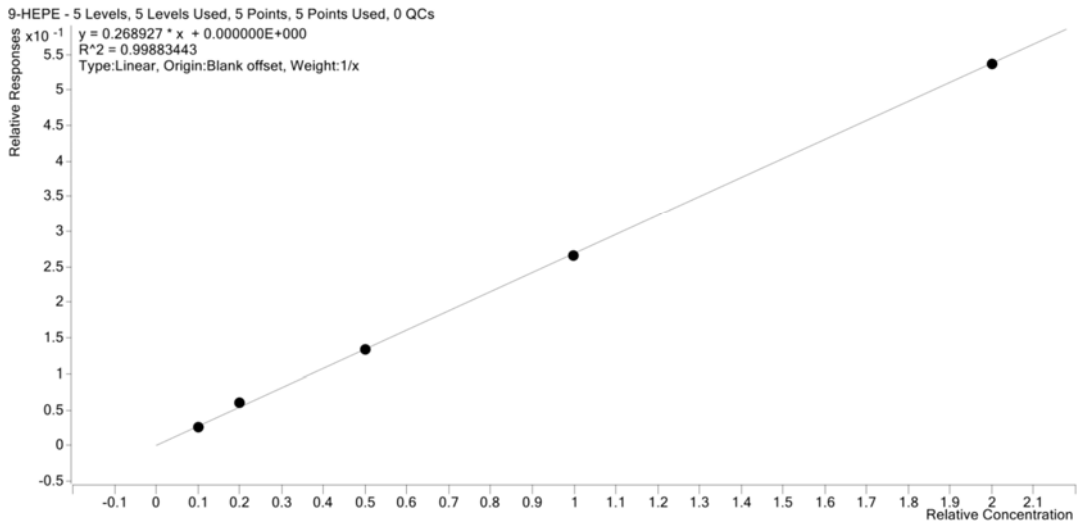
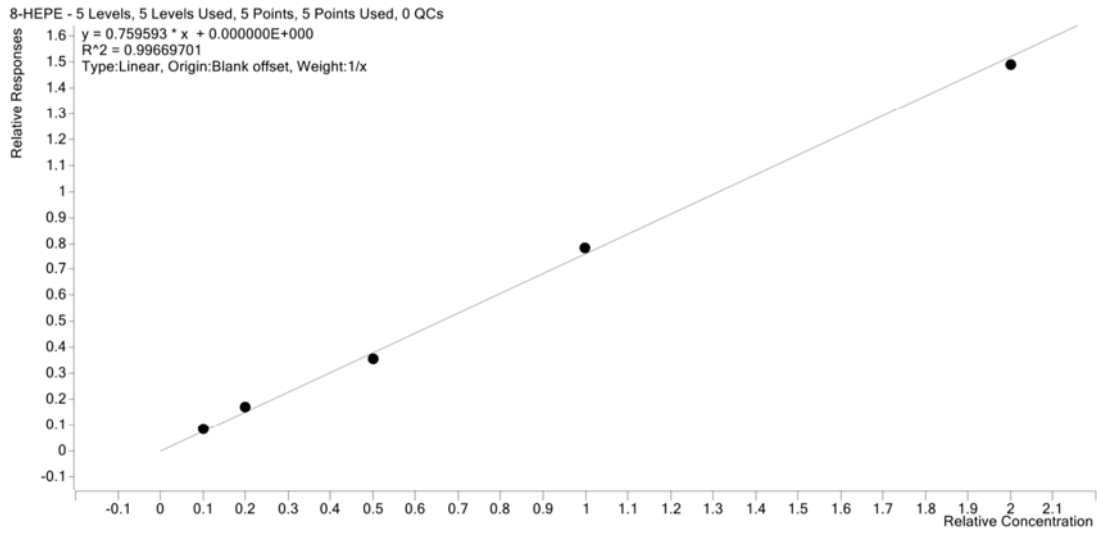
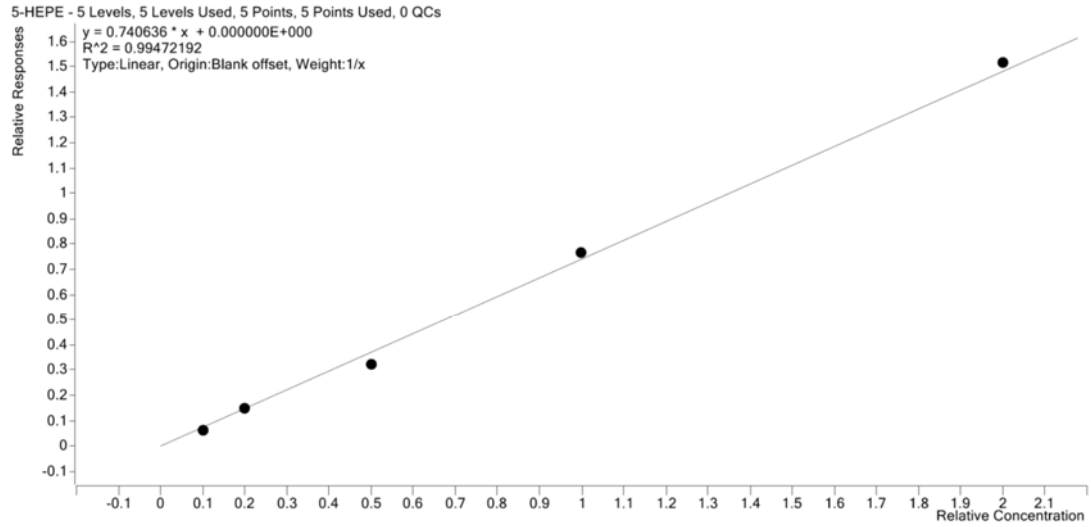


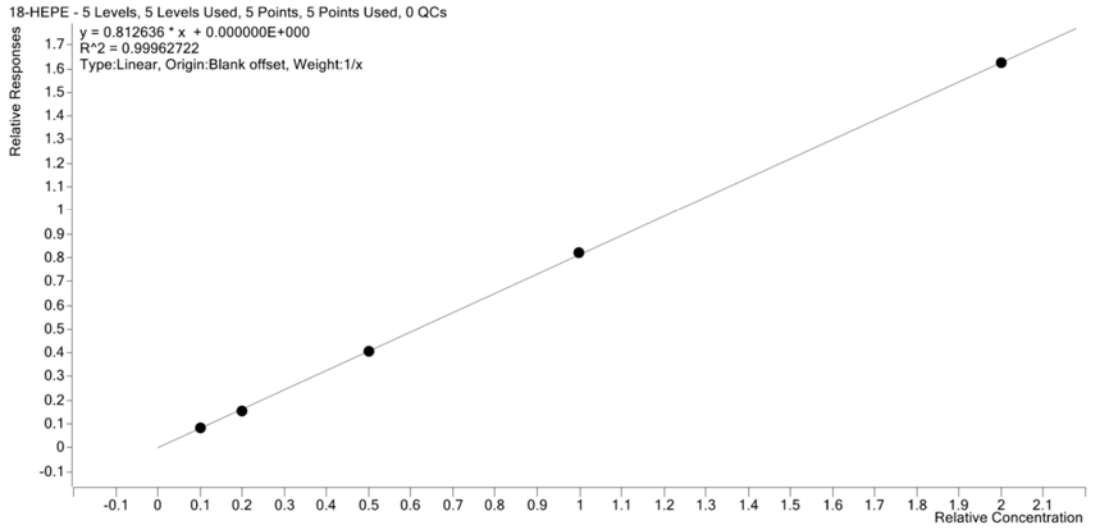
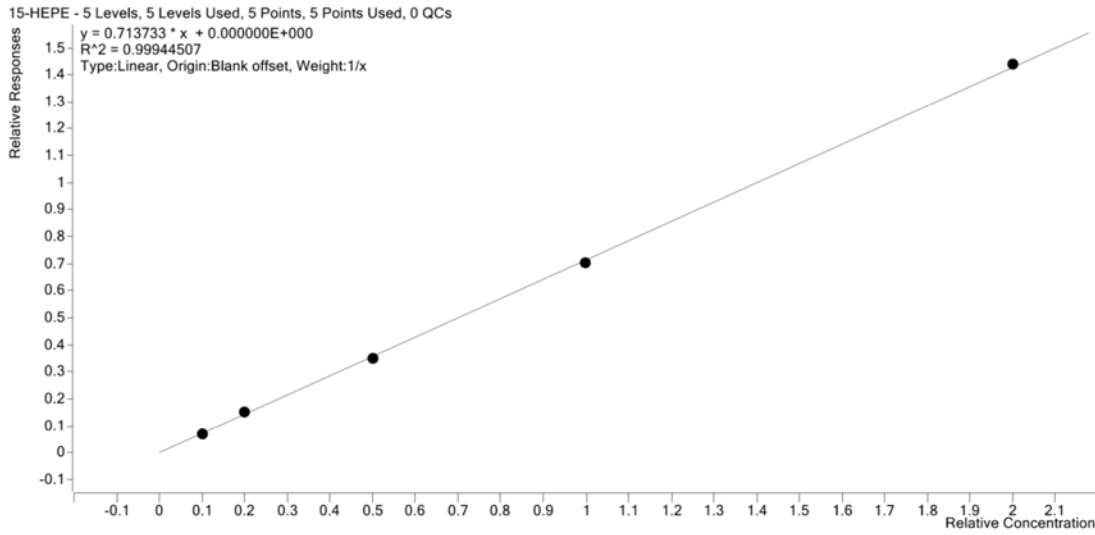
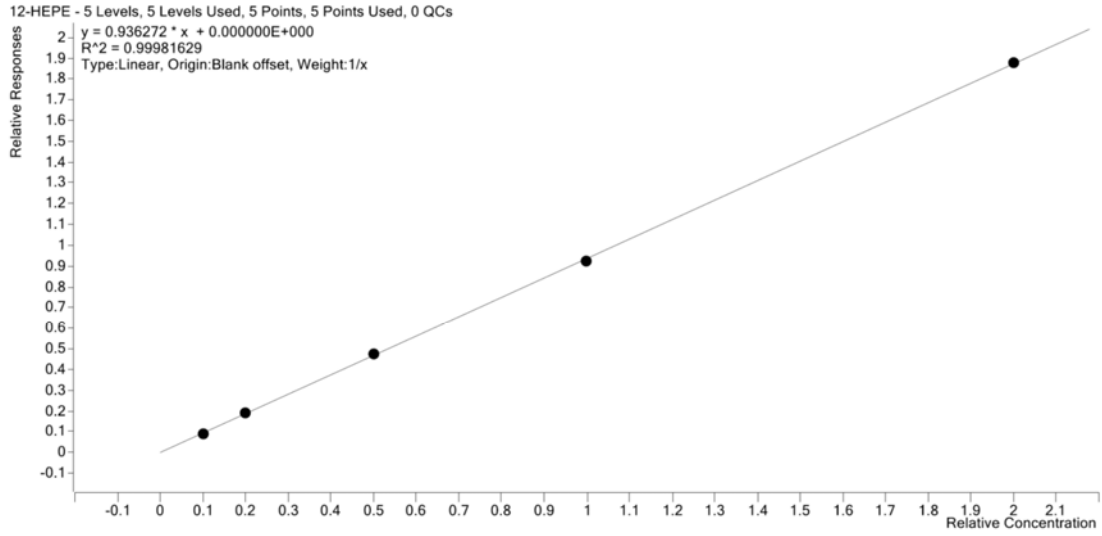
**Figure S1.** Standard curves for the assayed fatty acids, (A) arachidonic acid (AA); (B) eicosapentaenoic acid (EPA); and (C) docosahexaenoic acid (DHA). Calibrators used were 1, 5, 10, 20, 50, 100 and 200 µg of fatty acid.



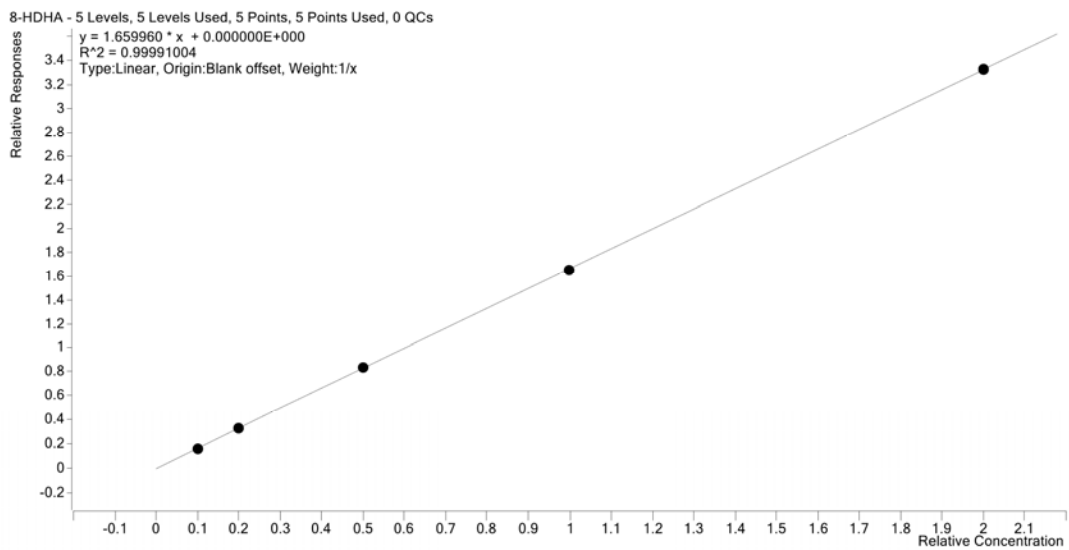
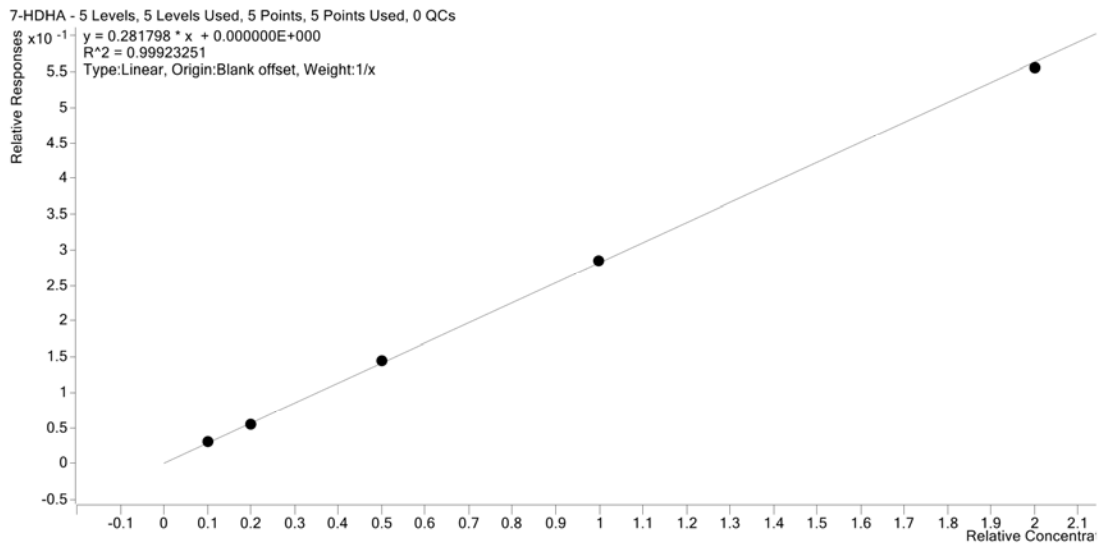
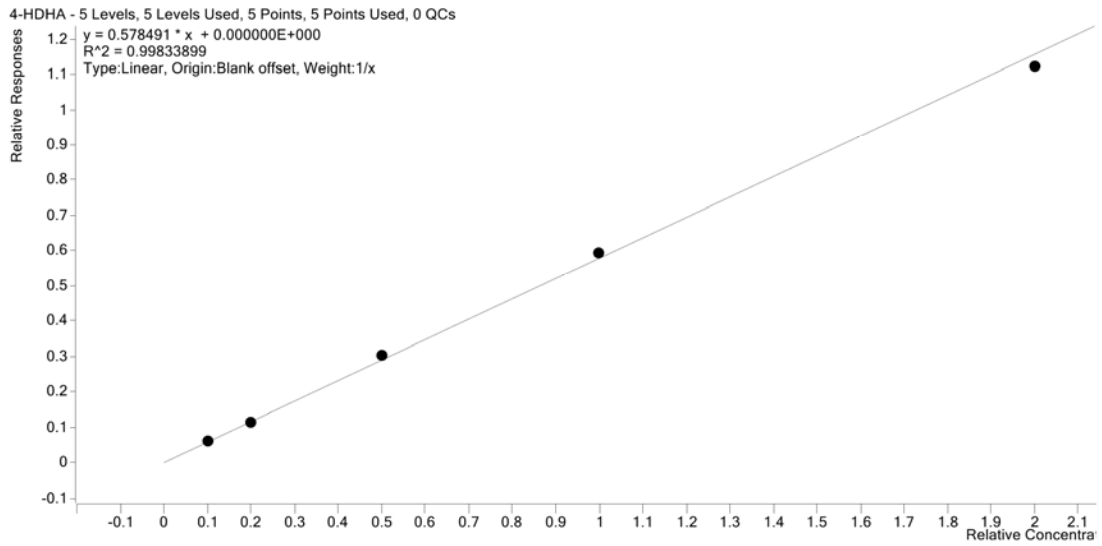


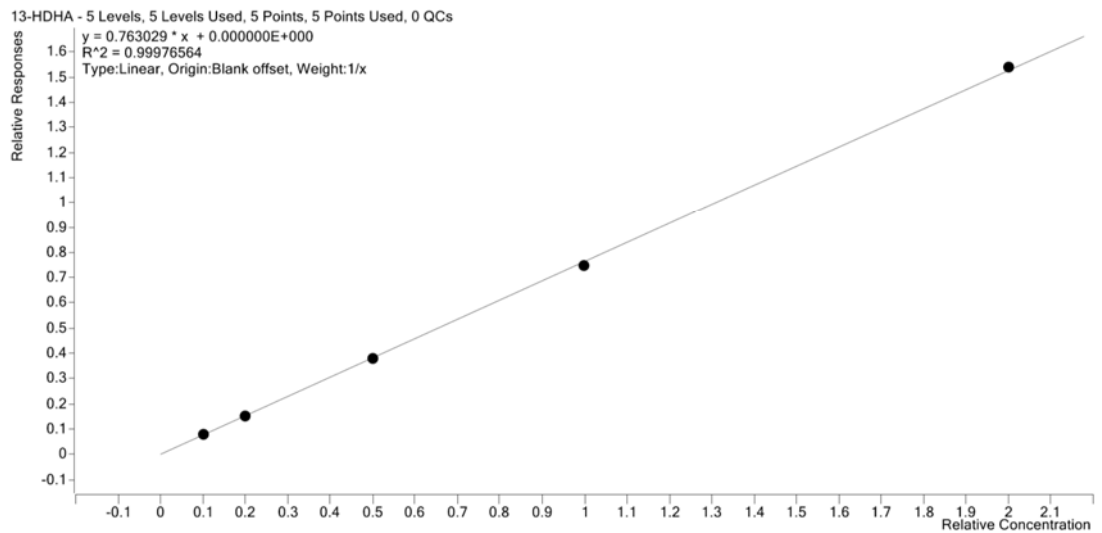
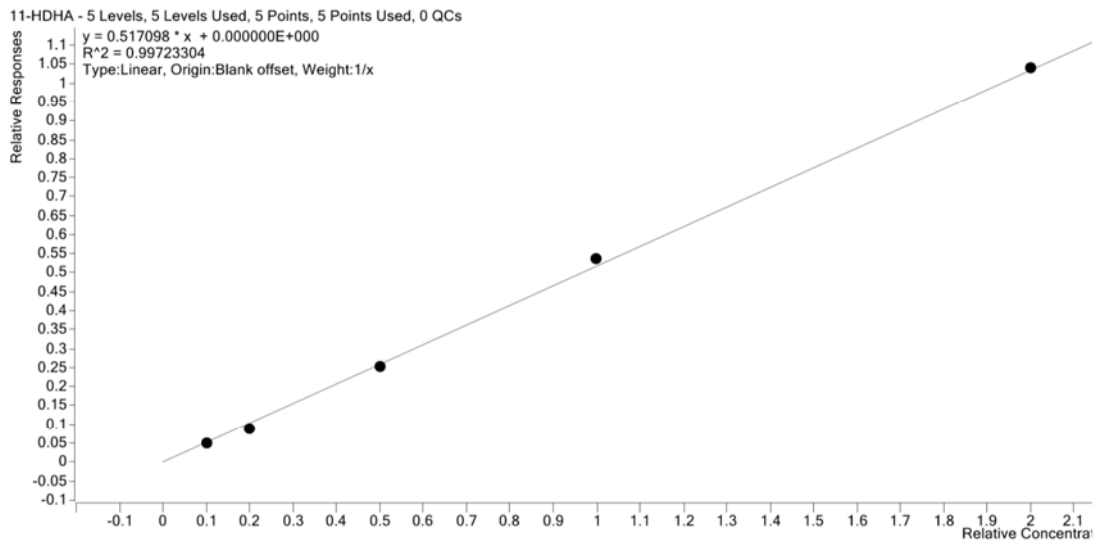
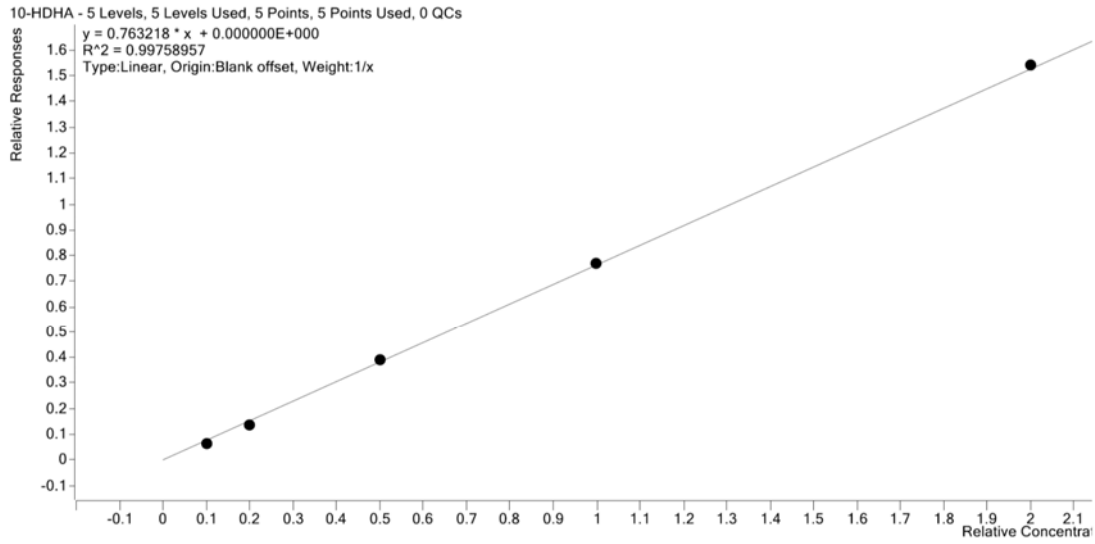
(A) HETE

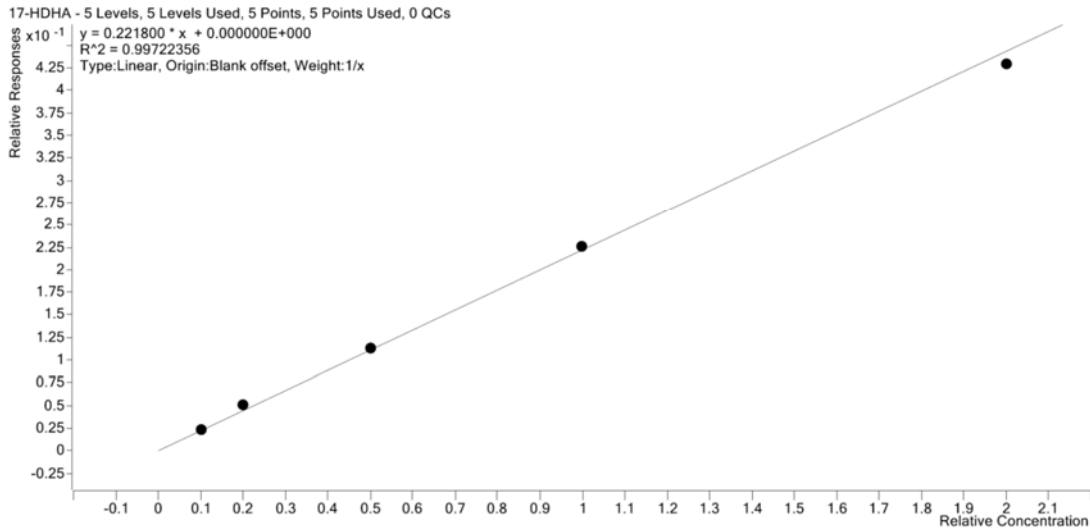
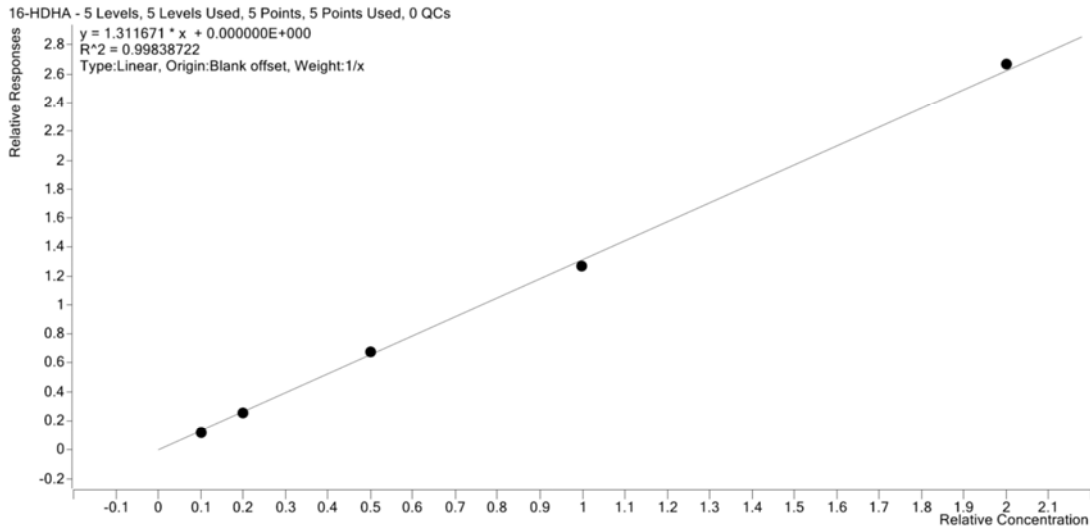
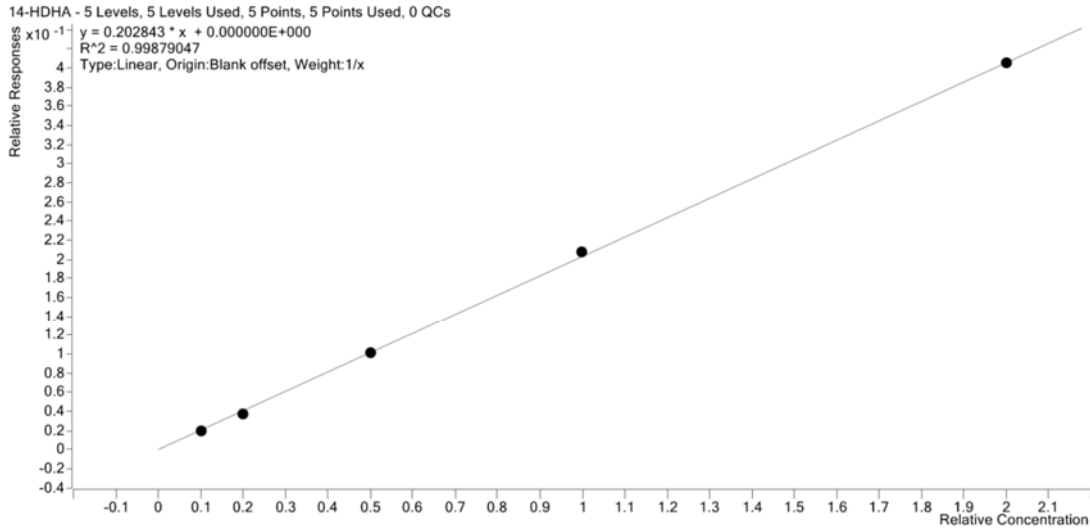




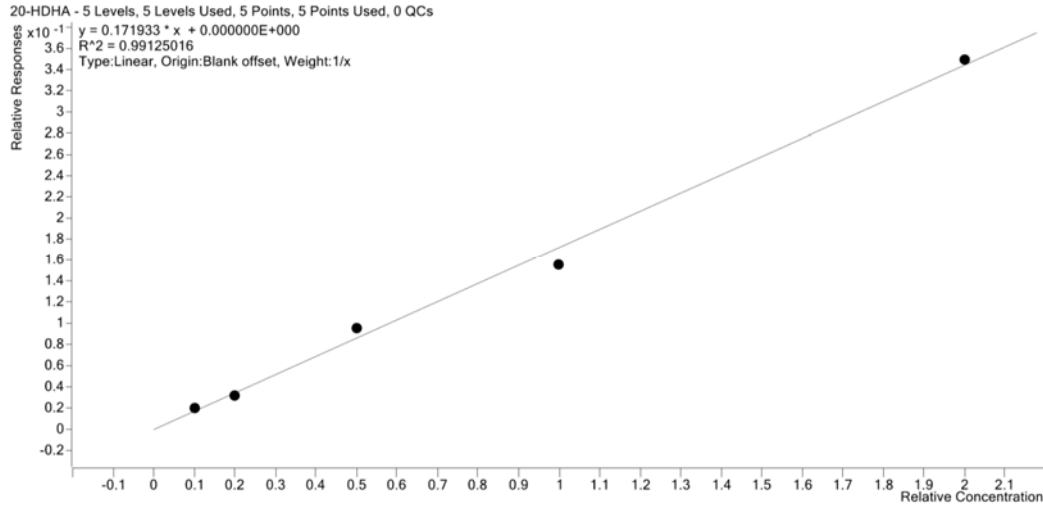
(B) HEPE



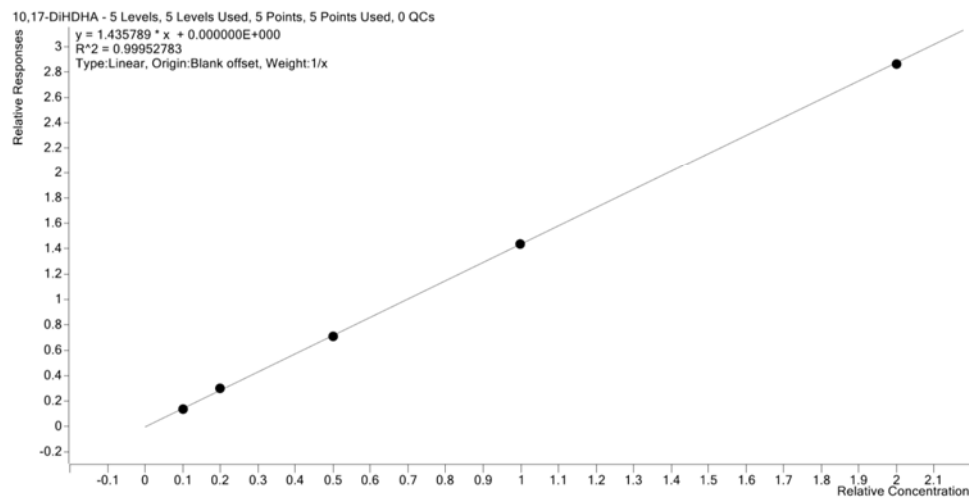
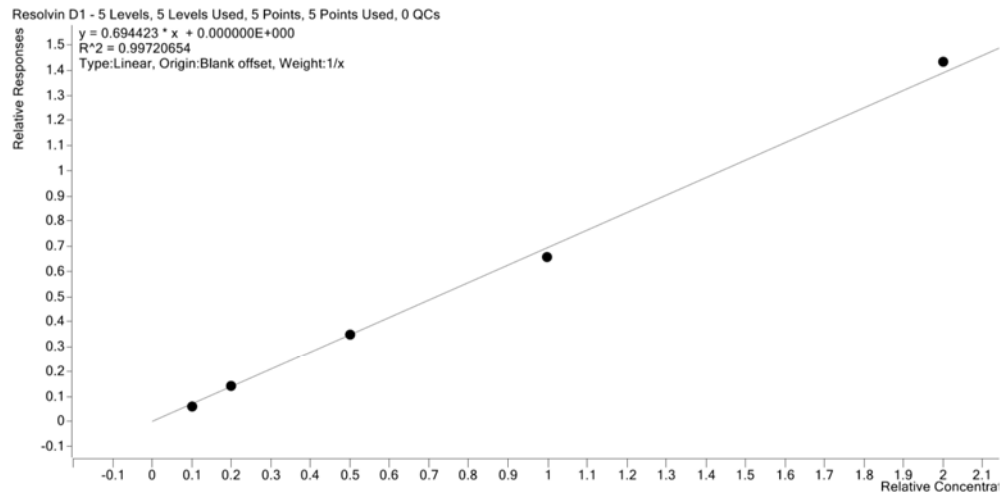








(C) HDHA



(D) Resolvin D1, 10,17-DiHDHA

**Figure S2.** Standard curves for the assayed lipid metabolites (A) hydroxyeicosatetraenoic acids (HETE); (B) hydroxyeicosapentaenoic acids (HEPE); (C) hydroxydocosahexaenoic (HDHA) acids; and (D) resolvin D1 and 10,17-DiHDHA. Calibrators used were 1, 2, 5, 10 and 20 ng of respective compounds.

**Table S1.** Multiple reaction monitoring characterization of the measured compounds.

Compound	Precursor Ion	Product Ion	Fragmentor Voltage	Collision Energy [V]	Cell Accel. Voltage [V]	Ret Time (min)
12-HEPE	317.2	179.1	120	5	1	9.93
15-HEPE	317.2	219.1	120	5	1	9.58
18-HEPE	317.2	259.1	140	3	5	9.12
5-HEPE	317.2	115.1	110	6	1	10.12
8-HEPE	317.2	155.1	110	7	2	9.77
9-HEPE	317.2	167.1	110	5	1	9.99
11-HETE	319.2	149.1	130	17	1	11.30
12-HETE	319.2	179.1	110	5	1	11.62
15-HETE	319.2	219.1	140	4	8	10.88
5-HETE	319.2	115.1	110	10	1	12.00
8-HETE	319.2	155.1	120	8	1	11.53
9-HETE	319.2	151.1	130	5	2	11.83
15-HETE-d8	327.2	226.1	140	4	1	10.73
10-HDHA	343.2	153.1	110	9	1	11.43
11-HDHA	343.2	149.1	110	5	1	11.63
13-HDHA	343.2	193.1	110	7	1	11.23
14-HDHA	343.2	205.1	110	6	2	11.43
16-HDHA	343.2	233.1	130	6	5	11.00
17-HDHA	343.2	201.1	110	6	6	11.07
20-HDHA	343.2	241.1	130	5	3	10.55
4-HDHA	343.2	101.1	130	7	2	12.53
7-HDHA	343.2	141.1	110	4	1	11.75
8-HDHA	343.2	189.1	110	4	2	11.90
10,17-DIHDHA	359.2	153.1	90	10	8	7.42
Rv D1	375.2	141.1	110	9	1	6.27

**Table S2.** Monohydroxy lipid metabolite levels (ng/g) in colon of wildtype and fat-1 mice.

	Colon			
	Wild Type	Fat-1	<i>p</i> -Value	Change (-Fold)
5-HETE	447.43 ± 48.10	307.63 ± 36.66	0.078	0.69
8-HETE	128.43 ± 46.85	110.23 ± 56.76	0.815	0.86
9-HETE	120.30 ± 16.16	70.83 ± 5.67	0.053	0.59
11-HETE	359.21 ± 37.07	247.75 ± 51.45	0.108	0.69
12-HETE	800.59 ± 79.17	406.18 ± 25.73	0.006	0.51
15-HETE	2403.80 ± 180.52	1564.48 ± 170.00	0.013	0.65
5-HEPE	4.26 ± 1.15	91.73 ± 12.33	<0.001	21.55
8-HEPE	5.31 ± 1.42	56.675 ± 8.79	<0.001	10.66
9-HEPE	16.93 ± 7.10	580.03 ± 105.26	<0.001	34.26
12-HEPE	12.17 ± 2.13	107.28 ± 16.81	<0.001	8.81
15-HEPE	6.67 ± 1.20	99.25 ± 13.43	<0.001	14.88
18-HEPE	15.63 ± 3.12	405.03 ± 21.28	<0.001	25.92
4-HDHA	62.00 ± 5.72	95.10 ± 12.52	0.022	1.53
7-HDHA	21.79 ± 1.64	31.38 ± 2.77	0.011	1.44
8-HDHA	6.70 ± 1.56	7.90 ± 0.35	0.586	1.18
10-HDHA	17.61 ± 1.03	29.68 ± 3.52	0.002	1.68
11-HDHA	9.64 ± 2.43	20.23 ± 4.00	0.039	2.10
13-HDHA	62.30 ± 5.34	99.00 ± 11.56	0.009	1.59
14-HDHA	63.79 ± 7.77	113.15 ± 9.29	0.003	1.77
16-HDHA	122.93 ± 7.37	187.45 ± 23.00	0.009	1.52
17-HDHA	139.33 ± 12.38	217.88 ± 28.74	0.016	1.56
20-HDHA	240.03 ± 16.98	381.43 ± 30.75	0.002	1.59

**Table S3.** Monohydroxy lipid metabolite levels (ng/g) in liver of wildtype and fat-1 mice.

	Liver			
	Wild Type	Fat-1	<i>p</i> -Value	Change (-fold)
5-HETE	1176.06 ± 173.90	841.53 ± 95.23	0.206	0.72
8-HETE	625.86 ± 69.90	454.53 ± 54.20	0.129	0.73
9-HETE	442.17 ± 58.28	328.10 ± 49.93	0.221	0.74
11-HETE	236.37 ± 44.24	198.93 ± 39.01	0.586	0.84
12-HETE	861.40 ± 116.64	589.40 ± 61.98	0.132	0.68
15-HETE	2993.99 ± 259.15	2527.98 ± 209.38	0.254	0.84
5-HEPE	8.89 ± 1.59	88.75 ± 3.21	<0.001	9.99
8-HEPE	3.14 ± 1.63	32.75 ± 2.35	<0.001	10.42
9-HEPE	0.00	124.23 ± 63.99	0.025	
12-HEPE	4.63 ± 1.20	39.90 ± 7.13	<0.001	8.62
15-HEPE	5.24 ± 2.07	27.75 ± 5.12	<0.001	5.29
18-HEPE	14.13 ± 1.63	175.65 ± 24.10	<0.001	12.43
4-HDHA	232.84 ± 34.51	333.75 ± 12.00	0.063	1.43
7-HDHA	91.50 ± 11.49	131.25 ± 6.36	0.037	1.43
8-HDHA	33.81 ± 4.68	63.40 ± 6.09	0.004	1.87
10-HDHA	71.71 ± 8.71	123.35 ± 14.42	0.010	1.72
11-HDHA	70.37 ± 13.57	109.13 ± 12.13	0.090	1.55
13-HDHA	110.50 ± 15.27	181.65 ± 14.97	0.014	1.64
14-HDHA	179.87 ± 23.30	308.80 ± 25.87	0.007	1.72
16-HDHA	147.61 ± 16.87	244.40 ± 22.12	0.007	1.66
17-HDHA	230.36 ± 28.23	363.45 ± 34.77	0.017	1.58
20-HDHA	417.00 ± 46.47	684.30 ± 42.93	0.004	1.64

**Table S4.** Monohydroxy lipid metabolite levels (ng/g) in lung of wildtype and fat-1 mice.

	Lung			
	Wild Type	Fat-1	<i>p</i> -Value	Change (-Fold)
5-HETE	164.47 ± 11.17	144.63 ± 7.15	0.234	0.88
8-HETE	257.50 ± 70.89	171.43 ± 15.23	0.390	0.67
9-HETE	650.84 ± 65.00	691.08 ± 73.33	0.799	1.06
11-HETE	1154.61 ± 115.02	1062.95 ± 97.96	0.508	0.92
12-HETE	13,305.86 ± 1430.19	14,505.28 ± 1745.70	0.703	1.09
15-HETE	828.54 ± 43.68	714.38 ± 84.59	0.268	0.86
5-HEPE	0.57 ± 0.23	15.53 ± 3.71	<0.001	27.17
8-HEPE	0.63 ± 0.42	12.48 ± 1.93	<0.001	19.85
9-HEPE	10.41 ± 2.79	121.13 ± 16.73	<0.001	11.63
12-HEPE	142.71 ± 21.95	2869.40 ± 503.52	<0.001	20.11
15-HEPE	1.20 ± 0.58	22.90 ± 7.30	0.006	19.08
18-HEPE	0.61 ± 0.61	15.23 ± 3.89	0.002	24.78
4-HDHA	10.67 ± 2.54	22.93 ± 4.54	0.050	2.15
7-HDHA	4.06 ± 0.79	6.15 ± 0.43	0.146	1.52
8-HDHA	43.94 ± 7.15	105.90 ± 8.77	0.001	2.41
10-HDHA	15.66 ± 1.50	28.90 ± 3.14	0.004	1.85
11-HDHA	15.94 ± 2.93	41.35 ± 2.64	<0.001	2.59
13-HDHA	43.80 ± 8.45	84.45 ± 8.67	0.024	1.93
14-HDHA	491.19 ± 73.19	1132.30 ± 120.83	0.002	2.31
16-HDHA	22.39 ± 2.72	44.45 ± 3.59	0.002	1.99
17-HDHA	62.36 ± 9.48	98.95 ± 13.40	0.041	1.59
20-HDHA	30.59 ± 2.85	63.48 ± 9.63	0.006	2.08

**Table S5.** Monohydroxy lipid metabolite levels (ng/g) in spleen of wildtype and fat-1 mice.

	Spleen			
	Wild Type	Fat-1	<i>p</i> -Value	Change (-Fold)
5-HETE	2093.74 ± 537.91	922.10 ± 236.90	0.152	0.44
8-HETE	1108.33 ± 113.16	631.28 ± 31.85	0.013	0.57
9-HETE	3138.36 ± 386.39	1820.30 ± 239.05	0.040	0.58
11-HETE	3859.66 ± 287.93	2548.80 ± 183.02	0.011	0.66
12-HETE	69,522.40 ± 4007.76	49,095.40 ± 1963.00	0.005	0.71
15-HETE	6788.66 ± 1062.22	3496.88 ± 355.96	0.051	0.52
5-HEPE	0.00	188.03 ± 31.06	<0.001	
8-HEPE	3.23 ± 1.46	111.88 ± 28.79	<0.001	34.65
9-HEPE	44.34 ± 12.30	660.60 ± 219.83	0.004	14.90
12-HEPE	322.46 ± 56.37	15,940.73 ± 1875.72	<0.001	49.44
15-HEPE	25.39 ± 6.67	374.35 ± 53.60	<0.001	14.75
18-HEPE	7.79 ± 3.14	435.68 ± 99.45	<0.001	55.96
4-HDHA	293.79 ± 68.04	333.63 ± 66.36	0.710	1.14
7-HDHA	75.10 ± 12.44	78.78 ± 18.84	0.869	1.05
8-HDHA	0.00	0.00		
10-HDHA	108.81 ± 22.44	165.48 ± 27.85	0.155	1.52
11-HDHA	164.00 ± 34.17	133.15 ± 24.44	0.549	0.81
13-HDHA	315.16 ± 56.65	333.98 ± 70.34	0.843	1.06
14-HDHA	3743.71 ± 486.24	4937.35 ± 305.42	0.120	1.32
16-HDHA	137.36 ± 32.51	210.53 ± 25.39	0.159	1.53
17-HDHA	867.60 ± 151.58	1002.48 ± 137.86	0.569	1.16
20-HDHA	289.57 ± 110.57	229.78 ± 83.72	0.720	0.79

**Table S6.** Monohydroxy lipid metabolite levels (ng/g) in heart of wildtype and fat-1 mice.

	Heart			
	Wild Type	Fat-1	<i>p</i> -Value	Change (-Fold)
5-HETE	1396.27 ± 278.09	2040.03 ± 772.40	0.364	1.46
8-HETE	532.61 ± 52.52	556.58 ± 167.84	0.869	1.04
9-HETE	545.84 ± 79.97	823.48 ± 314.83	0.300	1.51
11-HETE	257.60 ± 33.43	522.98 ± 223.20	0.148	2.03
12-HETE	1290.83 ± 140.35	1360.05 ± 354.28	0.833	1.05
15-HETE	4070.40 ± 385.24	4522.93 ± 1276.74	0.680	1.11
5-HEPE	5.64 ± 1.94	112.50 ± 28.89	<0.001	19.94
8-HEPE	3.37 ± 0.69	47.85 ± 9.32	<0.001	14.19
9-HEPE	0.00	94.18 ± 89.86	0.182	
12-HEPE	12.90 ± 5.15	137.85 ± 41.00	0.003	10.69
15-HEPE	3.20 ± 1.00	60.23 ± 14.65	<0.001	18.82
18-HEPE	11.51 ± 5.80	433.30 ± 104.04	<0.001	37.63
4-HDHA	1259.50 ± 456.93	3583.03 ± 1597.38	0.110	2.84
7-HDHA	238.16 ± 29.05	766.08 ± 293.73	0.037	3.22
8-HDHA	134.50 ± 13.86	589.85 ± 245.66	0.031	4.39
10-HDHA	217.59 ± 23.22	783.65 ± 316.13	0.037	3.60
11-HDHA	191.44 ± 22.41	645.40 ± 304.49	0.072	3.37
13-HDHA	368.99 ± 47.61	1394.90 ± 539.49	0.029	3.78
14-HDHA	729.54 ± 97.13	2398.75 ± 852.34	0.027	3.29
16-HDHA	695.97 ± 95.24	2623.25 ± 964.76	0.024	3.77
17-HDHA	976.11 ± 131.59	3866.85 ± 1415.13	0.021	3.96
20-HDHA	1830.47 ± 272.32	6202.93 ± 2246.36	0.028	3.39

**Table S7.** Monohydroxy lipid metabolite levels (ng/g) in skeletal muscle of wildtype and fat-1 mice.

	<b>Muscle</b>			
	<b>Wild Type</b>	<b>Fat-1</b>	<b>p-Value</b>	<b>Change (-Fold)</b>
5-HETE	250.34 ± 49.48	80.55 ± 12.01	0.033	0.32
8-HETE	108.33 ± 26.89	46.30 ± 5.13	0.124	0.43
9-HETE	111.09 ± 25.02	46.25 ± 4.28	0.089	0.42
11-HETE	109.29 ± 21.70	61.48 ± 18.13	0.172	0.56
12-HETE	538.19 ± 35.57	283.38 ± 46.99	0.002	0.53
15-HETE	1353.39 ± 140.96	539.33 ± 57.89	0.002	0.40
5-HEPE	0.00	8.65 ± 3.95	0.014	
8-HEPE	1.11 ± 0.42	5.05 ± 2.93	0.106	4.53
9-HEPE	0.00	15.15 ± 8.86	0.042	
12-HEPE	1.57 ± 0.55	39.90 ± 5.30	<0.001	25.39
15-HEPE	8.73 ± 2.59	22.03 ± 5.37	0.032	2.52
18-HEPE	8.01 ± 2.62	66.15 ± 5.79	<0.001	8.25
4-HDHA	352.77 ± 128.80	234.28 ± 13.47	0.515	0.66
7-HDHA	60.76 ± 11.70	86.25 ± 13.26	0.202	1.42
8-HDHA	0.00	0.00		
10-HDHA	83.56 ± 12.91	94.15 ± 22.17	0.666	1.13
11-HDHA	131.43 ± 8.25	166.28 ± 37.19	0.262	1.27
13-HDHA	216.47 ± 35.54	261.13 ± 29.88	0.419	1.21
14-HDHA	1062.19 ± 102.00	1262.18 ± 183.12	0.323	1.19
16-HDHA	116.83 ± 15.53	166.08 ± 27.24	0.122	1.42
17-HDHA	1305.61 ± 147.38	1320.80 ± 196.59	0.952	1.01
20-HDHA	429.26 ± 56.80	430.08 ± 73.70	0.993	1.00

**Table S8.** Monohydroxy lipid metabolite levels (ng/g) in kidney of wildtype and fat-1 mice.

	<b>Kidney</b>			
	<b>Wild Type</b>	<b>Fat-1</b>	<b>p-Value</b>	<b>Change (-Fold)</b>
5-HETE	924.41 ± 195.40	939.78 ± 366.66	0.865	1.02
8-HETE	305.83 ± 31.80	305.93 ± 86.71	0.894	1.00
9-HETE	443.16 ± 41.41	364.53 ± 75.13	0.459	0.82
11-HETE	470.84 ± 70.43	386.43 ± 148.42	0.639	0.82
12-HETE	1434.39 ± 170.77	1247.10 ± 177.96	0.662	0.87
15-HETE	1908.40 ± 270.32	2095.10 ± 754.03	0.697	1.10
5-HEPE	1.40 ± 0.92	149.63 ± 92.75	0.078	106.88
8-HEPE	1.64 ± 0.51	31.85 ± 13.85	0.025	19.39
9-HEPE	13.34 ± 4.03	206.90 ± 95.58	0.034	15.51
12-HEPE	12.79 ± 5.41	244.10 ± 87.57	0.009	19.09
15-HEPE	5.39 ± 2.41	56.98 ± 18.59	0.010	10.58
18-HEPE	5.73 ± 1.52	229.70 ± 120.68	0.046	40.10
4-HDHA	772.86 ± 155.78	1301.15 ± 627.17	0.310	1.68
7-HDHA	147.66 ± 21.42	221.98 ± 72.03	0.254	1.50
8-HDHA	0.00	0.00		
10-HDHA	240.07 ± 26.39	405.88 ± 133.80	0.150	1.69
11-HDHA	213.20 ± 41.47	186.80 ± 29.65	0.858	0.88
13-HDHA	472.06 ± 72.16	766.18 ± 236.41	0.147	1.62
14-HDHA	958.07 ± 212.12	1593.18 ± 438.81	0.075	1.66
16-HDHA	368.83 ± 43.70	477.85 ± 115.12	0.200	1.30
17-HDHA	1276.46 ± 173.59	1939.10 ± 464.98	0.095	1.52
20-HDHA	658.74 ± 163.23	1004.03 ± 430.90	0.305	1.52