SUPPLEMENTAL INFORMATION

Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers

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SUPPLEMENTAL METHODS

Oxylipin nomenclature

The International Union of Pure and Applied Chemistry (IUPAC) has adopted abbreviations for oxidized fatty acids following the recommendations of Smith *et al.*[1, 2]. Briefly, compounds are named using position, number, and standardized abbreviations of functional groups, carbon chain length, and degree of unsaturation. Plural chemical moieties are listed as Di (two), Tr (three), T (four), P (five), He (six). Abbreviations of chemical moieties are: Ep – Epoxide; H – hydroxy; Hp – hydroperoxide; K – keto. Carbon numbers appearing in this report

are abbreviated O (octadeca *i.e.* 18), E (eicosa *i.e.* twenty) and Do (docosa *i.e.* 22). Therefore, 14(15)-epoxyeicostri-(5*Z*,8*Z*,11*Z*)-enoic acid is reduced to 14(15)-EpETrE while 9(10)-epoxyoctadec-(12Z)-enoic acid becomes 9(10)-EpOME. Dihydroxy lipids are named similarly, such that 14,15-dihydroxyeicostri-(5*Z*,8*Z*,11*Z*)-enoic acid becomes 14,15-DiHETrE, while 20-hydroxyeicosatetra-(5*Z*,8*Z*,11*Z*,14*Z*)-enoic acid is 20-HETE.

1. Smith DL, Willis AL. A suggested shorthand nomenclature for the eicosanoids. *Lipids.* 1987;22:983-986.
2. Smith WL, Borgeat P, Hamberg M, Roberts LJ, 2nd, Willis A, Yamamoto S, Ramwell PW, Rokach J, Samuelsson B, Corey EJ, et al. Nomenclature. *Methods Enzymol.* 1990;187:1-9.

Table S1: Oxylipin Internal Standards

Compound	CID Mass Transition (Da)	Internal Standard
Internal Standards		
CUDA	340.3 > 214.1	
PHAU	249.2 > 130.1	
Surrogate Standards		
d4 6-keto-PGF1α	373.3 > 167.1	PHAU
d4-TXB2	373.3 > 173.15	PHAU
d4-PGE2	355.3 > 275.2	PHAU
d4-PGD2	355.3 > 275.2	PHAU
d3-LTE4	441.4 > 336.3	CUDA
d4-LTB4	339.3 > 163.15	CUDA
d6-20-HETE	325.3 > 281.15	CUDA
d4-9(S)-HODE	299.3 > 172.1	CUDA
d8-12(S)-HETE	327.2 > 184.15	CUDA
d8-5(S)-HETE	327.2 > 116.1	CUDA
d8-11(12)-EpETrE	327.2 > 171.15	CUDA

Table S2: Eighteen Carbon Oxylipins

Compound	CID Mass Transition (Da)	Surrogate Standard
Linoleic Acid Metabolites		
9,12,13-TriHOME	329.2 > 211.2	d4 6-keto-PGF1α
9,10,13-TriHOME	329.2 > 171.1	d4 6-keto-PGF1α
12,13-DHOME	313.2 > 183.1	d4-9(S)-HODE
9,10-DHOME	313.2 > 201.1	d4-9(S)-HODE
13-HODE	295.2 > 195.2	d4-9(S)-HODE
9-HODE	295.2 > 171.1	d4-9(S)-HODE
13-KODE	293.2 > 195.2	d4-9(S)-HODE
9-KODE	293.2 > 185.1	d4-9(S)-HODE
12(13)-EpOME	295.2 > 195.1	d8-11(12)-EpETrE
9(10)-EpOME	295.2 > 171.1	d8-11(12)-EpETrE
alpha Linolenic Acid Metabolites		
15,16-DiHODE	311.2 > 235.15	d4-9(S)-HODE
12,13-DiHODE	311.2 > 183.1	d4-9(S)-HODE
9,10-DiHODE	311.2 > 201.15	d4-9(S)-HODE
9-HOTE	293.35 > 171.15	d4-9(S)-HODE
13-HOTE	293.35 > 195.15	d4-9(S)-HODE
15(16)-EpODE	293.2 > 275.15	d8-11(12)-EpETrE
9(10)-EpODE	293.2 > 275.15	d8-11(12)-EpETrE
12(13)-EpODE	293.2 > 183.1	d8-11(12)-EpETrE

Table S3: Twenty Carbon Oxylipins

Compound	CID Mass Transition (Da)	Internal Standard
dihomo gamma Linoleic Acid Metabolites		
15(S)-HETrE	321.2 > 221.15	d8-11(12)-EpETrE
Arachidonic Acid Metabolites		
6-keto-PGF1α	369.2 > 163.1	d4 6-keto-PGF1α
TXB2	369.3 > 195.2	d4 6-keto-PGF1α
PGF2α / F2-isoprostanes	353.2 > 193.1	d4 6-keto-PGF1α
20-carboxy-LTB4	365.2 > 347.2	d4 6-keto-PGF1α
20-hydroxy-LTB4	351.2 > 195.15	d4 6-keto-PGF1α
11,12,15 THET	353.2 > 167.15	d4 6-keto-PGF1α
Lipoxin A4	351.3 > 217.15	d4 6-keto-PGF1α
8,15-DiHETE	335.3 > 235.15	d4-9(S)-HODE
5,15-DiHETE	335.3 > 173.15	d4-9(S)-HODE
LTB4	335.2 > 195.15	d4-LTB4
14,15-DHET	337.2 > 207.1	d4-9(S)-HODE
11,12-DHET	337.2 > 167.1	d4-9(S)-HODE
8,9-DHET	337.2 > 127.1	d4-9(S)-HODE
5,6-DHET	337.2 > 145.1	d4-9(S)-HODE
20-HETE	319.2 > 275.2	d4-9(S)-HODE
19-HETE	319.2 > 275.2	d4-9(S)-HODE
15-HETE	319.2 > 219.1	d4-9(S)-HODE
11-HETE	319.2 > 167.1	d4-9(S)-HODE
12-HETE	319.2 > 179.1	d4-9(S)-HODE
9-HETE	319.2 > 123.1	d4-9(S)-HODE
8-HETE	319.2 > 155.1	d4-9(S)-HODE
5-HETE	319.2 > 115.1	d8-5(S)-HETE
15-KETE	317.3 > 273.2	d8-11(12)-EpETrE
5-KETE	317.2 > 203.15	d8-11(12)-EpETrE
14(15)-EET	319.2 > 219.1	d8-11(12)-EpETrE
11(12)-EET	319.2 > 208.1	d8-11(12)-EpETrE
8(9)-EET	319.2 > 155.1	d8-11(12)-EpETrE
5(6)-EET	319.2 > 191.1	d8-11(12)-EpETrE
Eicosapentaenoic Acid Metabolites		
Resolvin E1	349.3 > 195	d4 6-keto-PGF1α
17,18-DiHETE	335.3 > 247.2	d4-9(S)-HODE
14,15-DiHETE	335.3 > 207.15	d4-9(S)-HODE
15(S)-HEPE	317.2 > 219.15	d4-9(S)-HODE
12(S)-HEPE	317.3 > 179.2	d4-9(S)-HODE
5(S)-HEPE	317.3 > 115.2	d4-9(S)-HODE
17(18)-EpETE	317.2 > 259.5	d8-11(12)-EpETrE
14(15)-EpETE	317.2 > 247.5	d8-11(12)-EpETrE

Table S4: Twenty-two Carbon Oxylipins

Compound	CID Mass Transition (Da)	Internal Standard
Docosapentaenoic Acid Metabolites		
Resolvin D1	375.3 >121.0	d4 6-keto-PGF1α
19,20-DiHDPE	361.5 > 273.5	d4-9(S)-HODE
17(R)-HDoHE	343.2 > 281.2	d8-11(12)-EpETrE
19(20)-EpDPE	343.5 > 281.2	d8-11(12)-EpETrE
16(17)-EpDPE	343.5 > 273.5	d8-11(12)-EpETrE

Table S5: Oxylipin assay UPLC solvent gradient

Time (min)	Solvent A (%)
0.0	75
1.0	60
2.5	58
4.5	50
10.5	35
12.5	25
14.0	15
14.5	5
15.0	75
16.0	75

Solvent A = 0.1% acetic acid
Solvent B = 90:10 v/v acetonitrile/isopropanol

Table S6: Oxylipin Surrogate Recoveries

Analytical Surrogates	Recoveries (Mean ± SD)
d4 6-keto PGF1a	58% ± 15%
d4-TXB2 [a]	1% ± 1%
d4-PGE2 [a]	0% ± 0%
d4-PGD2 [a]	2% ± 2%
d3-LTE4 [a]	0% ± 0%
d4-LTB4	75% ± 13%
10,11-DHHep	87% ± 10%
d11-14,15-DiHETrE	90% ± 7%
d6-20-HETE	47% ± 6%
d4-9(S)-HODE	71% ± 9%
d8-12(S)-HETE	64% ± 8%
d8-5(S)-HETE	59% ± 15%
d8-11(12)-EpETrE	60% ± 15%

a – These surrogates and their associated analytical targets are not alkali stable.

SUPPLEMENTAL RESULTS

Table S7: Fatty acid mol% composition in platelet, RBC, and plasma samples pre- and post- P-OM3 treatment [a]

	Platelet			RBC			Plasma		
	Week 0	Week 4	*p*	Week 0	Week 4	*p*	Week 0	Week 4	*p*
Saturated Fatty Acids									
14:0	0.39±0.03	0.34±0.02		0.3 ± 0.02	0.3 ± 0.02		0.7±0.04	0.7±0.05	
16:0	18±0.40	19±0.50		21±0.30	21±0.30		22±0.40	21±0.30	
18:0	21±0.30	20±0.40		18±0.20	18±0.20		9.4±0.20	9.3±0.30	
Mono Unsaturated Fatty Acids									
16:1n7	2.7±0.50	2.7±0.50		0.23±0.02	0.17±0.02	**	1.2±0.09	0.94±0.06	***
16:1n7t	0.5±0.09	0.33±0.07		0.12±0.01	0.11±0.01		0.24±0.01	0.22±0.01	
18:1n9	14±0.20	14±0.40		13±0.10	13±0.10	**	16±0.30	15±0.30	***
18:1t	1.2±0.07	1.1±0.07		2.3±0.20	2.2±0.20		1±0.06	1±0.08	
20:1n9	0.57±0.03	0.66±0.10		0.27±0.01	0.29±0.01		0.24±0.01	0.24±0.01	
Poly Unsaturated Fatty Acids									
18:2n6	7.8±0.40	8.2±0.50		13±0.20	12±0.20	***	30±0.60	29±0.60	
18:3n6	0.07±0.01	0.058±0.01		0.1±0.01	0.09±0.01	**	0.43±0.03	0.28±0.03	***
18:3n3	0.12±0.01	0.15±0.02		0.14±0.01	0.13±0.01	*	0.67±0.05	0.66±0.07	
20:2n6	0.42±0.02	0.46±0.08		0.32±0.01	0.31±0.01		0.39±0.02	0.33±0.03	
20:3n6	1.5±0.08	1.3±0.06	*	1.8±0.08	1.4±0.06	*	2.4±0.10	1.6±0.07	***
Highly Unsaturated Fatty Acids									
20:4n6	23±0.40	21±0.60	*	18±0.20	16±0.20	***	10±0.30	8.1±0.20	***
22:4n6	3.0±0.10	1.9±0.10	**	4.2±0.10	3.8±0.10	***	0.45±0.02	0.22±0.01	***
22:5n6	0.41±0.02	0.2±0.01	***	0.86±0.03	0.71±0.02	***	0.35±0.02	0.14±0.01	***
20:5n3	0.32±0.08	1.8±0.10	***	0.37±0.05	1.8±0.08	***	0.53±0.08	3.5±0.20	***
22:5n3	1.7±0.08	2.3±0.08	***	2.3±0.10	2.8±0.08	***	0.73±0.06	1.1±0.05	***
22:6n3	1.8±0.10	3.1±0.20	***	3.6±0.20	5.1±0.20	***	2.1±0.10	5±0.20	***

a - Results are means ± SEMs. Significance of means tested by 2-tailed t-test after false discovery rate corrections (q=0.2) for multiple comparisons are indicated at p<0.05 (*), p<0.01 (**), and p<0.001 (***).

Table S8: Total plasma eicosanoid and docosanoid oxylipins concentrations (nM)

Oxylipin	Class	Pre (Mean ± SEM)	Post (Mean ± SEM)	*p* [a]
		20:3n6 Metabolite		
15-HETrE	Alcohol	52 ± 5.2	32 ± 2.5	<0.001
		20:4n6 Metabolites		
F2-isoprostanes [b]	Triol	2.84 ± 0.37	2.31 ± 0.24	-
11,12,15 THET	Triol	5.25 ± 0.67	4.44 ± 0.66	-
8,15-DiHETE	Diol	1.08 ± 0.23	0.733 ± 0.096	-
5,15-DiHETE	Diol	0.175 ± 0.016	0.151 ± 0.013	-
14,15-DiHETrE	Diol	2.13 ± 0.32	2.02 ± 0.3	-
11,12-DiHETrE	Diol	1.61 ± 0.13	1.38 ± 0.11	-
8,9-DiHETrE	Diol	4.25 ± 0.24	3.4 ± 0.15	<0.001
5,6-DiHETrE	Diol	15.8 ± 1.4	13.2 ± 1	0.092
14(15)-EpETrE	Epoxide	17 ± 3.7	13.8 ± 2.5	-
11(12)-EpETrE	Epoxide	24.3 ± 5.5	19.1 ± 3.7	-
8(9)-EpETrE	Epoxide	9.25 ± 2.1	7.72 ± 1.6	-
15-HETE	Alcohol	168 ± 14	135 ± 9	<0.05
12-HETE	Alcohol	99 ± 14	74.6 ± 5.2	-
11-HETE	Alcohol	113 ± 12	93.3 ± 9.3	0.062
9-HETE	Alcohol	85.5 ± 12	61.8 ± 3.9	<0.05
8-HETE	Alcohol	118 ± 21	81.1 ± 8.2	<0.05
5-HETE	Alcohol	148 ± 15	116 ± 7.5	0.087
15-KETE	Ketone	416 ± 47	349 ± 28	-
12-KETE	Ketone	616 ± 43	538 ± 28	-
5-KETE	Ketone	101 ± 12	74.6 ± 6.9	-
		20:5n3 Metabolites		
Resolvin E1	Triol	< 0.1	< 0.1	
17,18-DiHETE	Diol	6.96 ± 1.1	14 ± 2.5	<0.05
14,15-DiHETE	Diol	31 ± 1.8	33.3 ± 2.1	-
17(18)-EpETE	Epoxide	0.715 ± 0.18	5.08 ± 1.1	<0.001
14(15)-EpETE	Epoxide	0.411 ± 0.18	4.04 ± 0.94	<0.001
15-HEPE	Alcohol	2.75 ± 0.46	16.8 ± 1.3	<0.001
12-HEPE	Alcohol	6.45 ± 1.1	36.5 ± 3.3	<0.001
5-HEPE	Alcohol	8.51 ± 1.6	47.3 ± 4	<0.001
		22:6n3 Metabolites		
Resolvin D1	Triol	0.524 ± 0.04	0.658 ± 0.064	0.097
19,20-DiHDPA	Diol	0.479 ± 0.051	1.17 ± 0.17	<0.001
19(20)-EpDPE	Epoxide	3.68 ± 1.2	8.64 ± 2	<0.05
16(17)-EpDPE	Epoxide	3.45 ± 0.85	6.79 ± 1.2	<0.05
17-HDoHE	Alcohol	39.3 ± 3.6	94 ± 7	<0.001

a – Mean differences were assessed by paired 2-tailed t-tests after normality transformation, p > 0.1 not shown (-).
b – The F2 isoprostanes were quantified as an unresolved mixture of peaks sharing the PGF2α mass transition (m/z 353.2 > 193.1) as shown in Figure S2.

Table S9: Total plasma octadecanoid oxylipin concentrations (nM)

Oxylipin	Class	Pre (Mean ± SD)	Post (Mean ± SD)	*p* [a]
18:2n6 Metabolites				
9,10-13-TriHOME	Triol	14.1 ± 2.1	14.4 ± 1.7	-
9,12,13-TriHOME	Triol	10.5 ± 1.5	10.3 ± 1.3	-
12,13-DiHOME	Diol	8.26 ± 0.78	8.93 ± 1.1	-
9,10-DiHOME	Diol	129 ± 11	132 ± 11	-
13-HODE	Alcohol	1160 ± 100	996 ± 71	-
9-HODE	Alcohol	833 ± 69	729 ± 51	-
13-KODE	Ketone	2110 ± 150	2010 ± 120	-
9-KODE	Ketone	434 ± 39	400 ± 30	-
12(13)Ep-9-KODE	Epoxy Ketone	124 ± 13	110 ± 10	-
12(13)-EpOME	Epoxide	45.6 ± 9.8	41.1 ± 8.4	-
9(10)-EpOME	Epoxide	49.4 ± 11	44.7 ± 9.2	-
18:3n3 Metabolites				
15,16-DiHODE	Diol	6.24 ± 0.71	4.81 ± 0.9	-
9,10-DiHODE	Diol	0.902 ± 0.1	0.945 ± 0.1	-
13-HOTE	Alcohol	8.42 ± 1.3	6.93 ± 0.61	-
9-HOTE	Alcohol	16 ± 1.6	14.6 ± 1.1	-
15(16)-EpODE	Epoxide	5.6 ± 1.6	4.46 ± 1.1	-
12(13)-EpODE	Epoxide	0.478 ± 0.11	0.41 ± 0.078	-
9(10)-EpODE	Epoxide	6.08 ± 1.4	4.87 ± 0.98	-

a – Mean differences were assessed by paired 2-tailed t-tests after normality transformation, p > 0.1 not shown (-).

Table S10: Treatment dependent change in subject plasma fatty acids.

Compound	Class	% Above Threshold	*p* (χ2)[a]	*p* (t-test)[b]
22:6n3	n3-HUFA	100%	<0.001	<0.001
20:5n3	n3-HUFA	97%	<0.001	<0.001
22:5n3	n3-HUFA	77%	0.04	0.005
24:1n9	MUFA	60%	-	-
24:0	SFA	57%	-	-
18:0	SFA	53%	-	-
14:0	SFA	50%	-	-
16:0	SFA	50%	-	-
16:1n7*t*	MUFA	50%	-	-
18:1n9*t*	MUFA	47%	-	-
18:1n9	MUFA	47%	-	-
20:1n9	MUFA	47%	-	-
16:1n7	MUFA	47%	-	-
20:2n6	n6-PUFA	43%	-	-
18:3n3	n3-PUFA	43%	-	-
18:2n6*tc*	n6-PUFA	43%	-	-
18:2n6*ct*	n6-PUFA	40%	-	-
18:2n6	n6-PUFA	40%	-	-
18:2n6*tt*	n6-PUFA	33%	0.2	-
18:3n6	n6-PUFA	33%	0.2	-
20:4n6	n6-HUFA	33%	0.2	-
20:3n6	n6-PUFA	30%	0.1	0.09
22:4n6	n6-HUFA	13%	0.005	<0.001
22:5n6	n6-HUFA	10%	0.002	<0.001

a - Observed population distribution above and below the change threshold was compared to the expected Ho of 50:50 using a χ2 test with 1 df.

b - Differences in analyte means before and after treatment were tested with paired 2-tailed t-tests.

Table S11: Treatment dependent changes in plasma fatty acid alcohol and ketones.

Compound	Parent FA	Chemical Class	% Above Threshold	*p* (χ2)[a]	*p* (t-test)[b]
5-HEPE	20:5n3	n3-HUFA Alcohol	100%	<0.001	<0.001
17-HDoHE	22:6n3	n3-HUFA Alcohol	100%	<0.001	<0.001
15-HEPE	20:5n3	n3-HUFA Alcohol	97%	<0.001	<0.001
12-HEPE	20:5n3	n3-HUFA Alcohol	97%	<0.001	<0.001
13-KODE	18:2n6	n6-PUFA Ketone	53%	-	-
9-HODE	18:2n6	n6-PUFA Alcohol	47%	-	0.2
9-KODE	18:2n6	n6-PUFA Ketone	47%	-	-
9-HOTE	18:3n3	n3-PUFA Alcohol	47%	-	-
15-KETE	20:4n6	n6-HUFA Ketone	47%	-	-
12-KETE	20:4n6	n6-HUFA Ketone	47%	-	0.1
13-HODE	18:2n6	n6-PUFA Alcohol	43%	-	0.2
13-HOTE	18:3n3	n3-PUFA Alcohol	40%	-	-
12-HETE	20:4n6	n6-HUFA Alcohol	37%	0.2	0.1
11-HETE	20:4n6	n6-HUFA Alcohol	37%	0.2	0.06
8-HETE	20:4n6	n6-HUFA Alcohol	37%	0.2	0.06
5-HETE	20:4n6	n6-HUFA Alcohol	37%	0.2	0.09
15-HETE	20:4n6	n6-HUFA Alcohol	33%	0.2	<0.05
9-HETE	20:4n6	n6-HUFA Alcohol	30%	0.1	<0.05
5-KETE	20:4n6	n6-HUFA Ketone	27%	0.07	0.11
15-HETrE	20:3n6	n6-PUFA Alcohol	23%	0.04	<0.001

a - Observed population distribution above and below the change threshold was compared to the expected Ho of 50:50 using a χ2 test with 1 df.

b - Differences in analyte means before and after treatment were tested with paired 2-tailed t-tests.

Table S12: Treatment dependent changes in plasma fatty acid epoxide, diol, and triols.

Compound	Parent FA	Chemical Class	% Above Threshold	*p* ($\chi 2$)[a]	*p* (t-test)[b]
17(18)-EpETE	20:5n3	n3-HUFA Epoxide	93%	<0.001	<0.001
14(15)-EpETE	20:5n3	n3-HUFA Epoxide	93%	<0.001	0.03
19,20-DiHDPA	22:6n3	n3-HUFA Diol	80%	0.02	<0.001
17,18-DiHETE	20:5n3	n3-HUFA Diol	77%	0.04	0.01
16(17)-EpDPE	22:6n3	n3-HUFA Epoxide	77%	0.04	<0.001
19(20)-EpDPE	22:6n3	n3-HUFA Epoxide	73%	0.07	0.1
9,10-13-TriHOME	18:2n6	n6-PUFA Triol	67%	0.2	-
12(13)-EpODE	18:3n3	n3-PUFA Epoxide	60%	-	-
14,15-DiHETE	20:5n3	n3-HUFA Diol	60%	-	-
9(10)-EpOME	18:2n6	n6-PUFA Epoxide	57%	-	-
9,12,13-TriHOME	18:2n6	n6-PUFA Triol	57%	-	-
14(15)-EpETrE	20:4n6	n6-HUFA Epoxide	57%	-	-
12(13)-EpOME	18:2n6	n6-PUFA Epoxide	53%	-	-
9,10-DiHOME	18:2n6	n6-PUFA Diol	53%	-	-
9(10)-EpODE	18:3n3	n3-PUFA Epoxide	53%	-	-
15(16)-EpODE	18:3n3	n3-PUFA Epoxide	53%	-	-
9,10-DiHODE	18:3n3	n3-PUFA Diol	53%	-	-
LTB4	20:4n6	n6-HUFA Diol	53%	-	-
12,13-DiHOME	18:2n6	n6-PUFA Diol	50%	-	-
11(12)-EpETrE	20:4n6	n6-PUFA Epoxide	50%	-	-
8(9)-EpETrE	20:4n6	n6-PUFA Epoxide	50%	-	-
F2 isoprostanes[c]	20:4n6	n6-HUFA Triol	50%	-	-
LTB5	20:5n3	n3-HUFA Diol	50%	-	-
Resolvin D1	22:6n3	n3-HUFA Triol	50%	-	0.1
14,15-DiHETrE	20:4n6	n6-HUFA Diol	47%	-	-
8,15-DiHETE	20:4n6	n6-HUFA Diol	47%	-	-
Lipoxin A4	20:4n6	n6-HUFA Diol	47%	-	-
12(13)-Ep-9-KODE	18:2n6	n6-PUFA Epoxide	43%	-	-
15,16-DiHODE	18:3n3	n3-PUFA Diol	40%	-	0.2
11,12-DiHETrE	20:4n6	n6-HUFA Diol	40%	-	0.1
5,15-DiHETE	20:4n6	n6-HUFA Diol	40%	-	-
11,12,15-THET	20:4n6	n6-HUFA Triol	40%	-	-
8,9-DiHETrE	20:4n6	n6-HUFA Diol	27%	0.07	0.006
5,6-DiHETrE	20:4n6	n6-HUFA Diol	27%	0.07	0.1

a - Observed population distribution above and below the change threshold was compared to the expected Ho of 50:50 using a $\chi 2$ test with 1 df.

b - Differences in analyte means before and after treatment were tested with paired 2-tailed t-tests after normality transformation. $p > 0.2$ not shown (-).

c – *b* – The F2 isoprostanes were quantified as an unresolved mixture of peaks sharing the PGF2α mass transition (m/z 353.2 > 193.1) as shown in Figure S2.

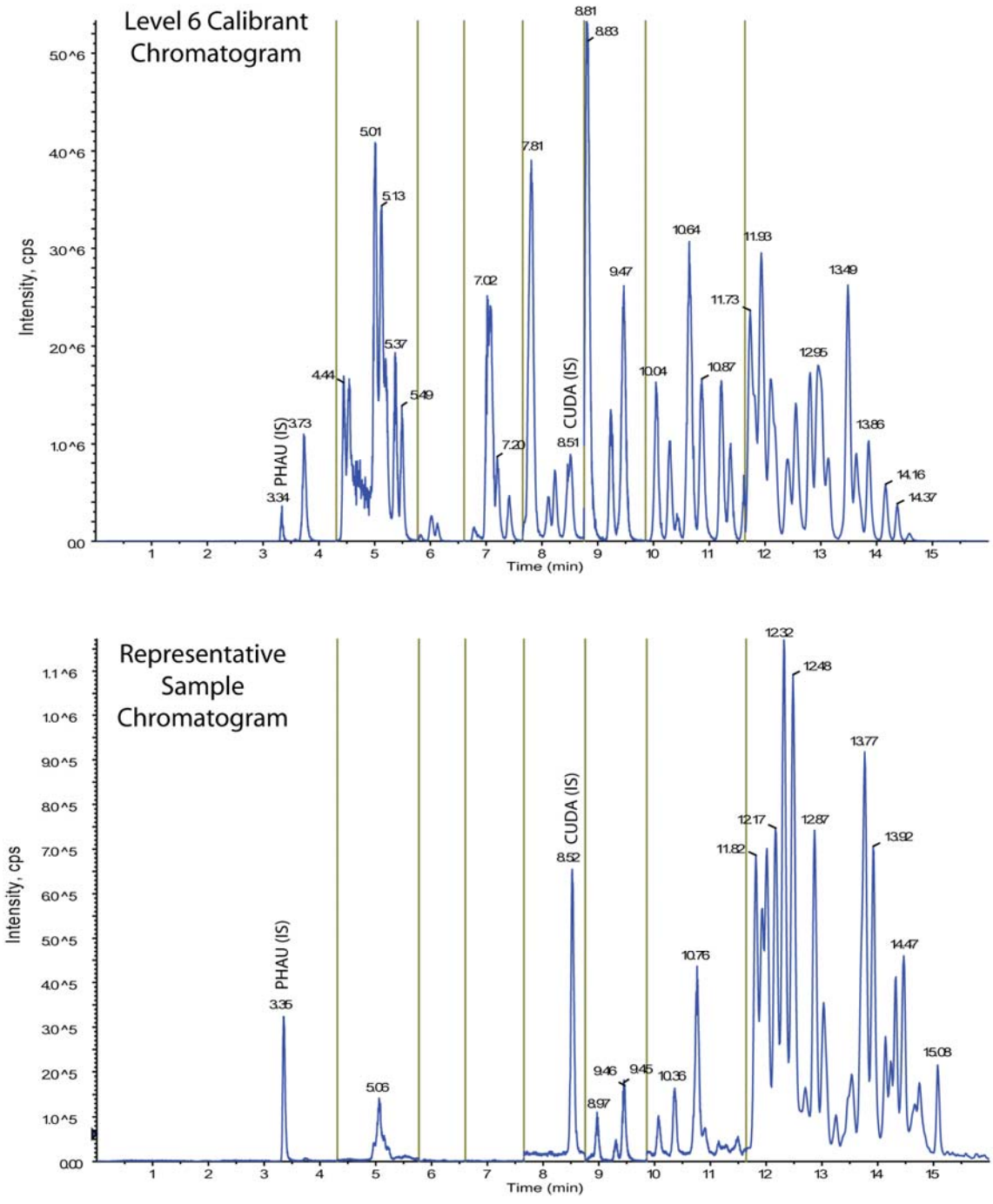

Figure S1: Representative total ion current chromatograms of a high level oxylipin calibration solution (top) and plasma sample alkali releasable/stable oxylipins (bottom). The internal standards (IS) 1-phenyl-3-hexanoic acid urea (PHAU) and 1-cyclohexyl-3dodecanoic acid urea (CUDA) are indicated in each trace. Vertical lines indicate multi-reaction monitoring window changes.

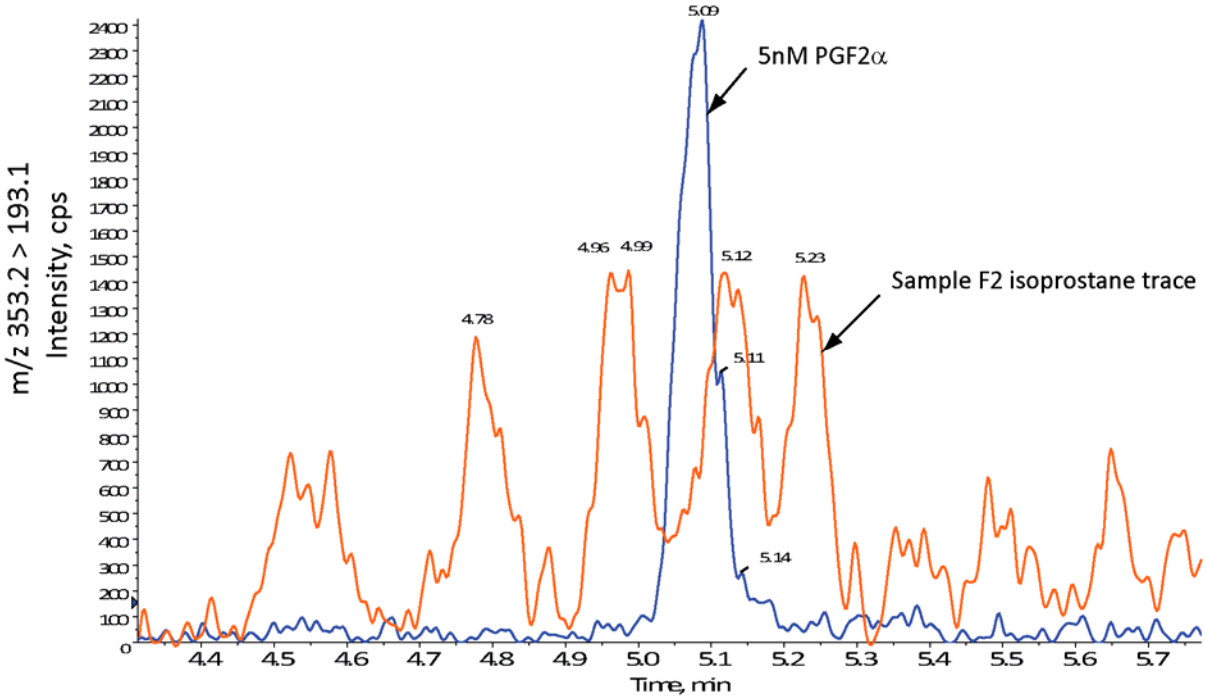

Figure S2: Representative total ion current chromatograms of prostaglanding F2α (PGF2α) mass transition for a low level oxylipin calibration solution (top) and an alkali digested plasma sample (bottom). The total area under the sample m/z 353.2 > 193.1 ion trace was quantified using the PGF2α calibration curve and used as an estimate of the total arachidonate derived F2 isoprostanes in the sample.