Supplemental Material

Bioactive products formed in humans from fish oils.

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Supplemental Figures

Supplemental Figure 1

Human study "High doses of fish oils". **(a)** CONSORT diagram of study participants; **(b)** spontaneous systolic and diastolic blood pressure and **(b)** spontaneous heart rate assessed during outpatient study visits to the Clinical Translational Research Center (CTRC). Boxplots indicate median, 25% and 75% quartiles and whiskers drawn to the furthest point within 1.5 x interquartile range (IQR) from the box.

Supplemental Figure 2

Human study "Evoked Endotoxemia". **(a)** Spontaneous systolic and diastolic blood pressure; **(b)** spontaneous heart rate; and **(c)** sublingual temperature during out- and inpatient study visits to the Clinical Translational Research Center (CTRC). Boxplots indicate median, 25% and 75% quantiles and whiskers drawn to the furthest point within 1.5 x interquartile range (IQR) from the box. The blue line connects the means. Note that time point zero (0) denotes the condition pre-LPS which coincides with day 50±6.8 of supplementing the healthy volunteers with 2 capsules Lovaza^{TM} BID.

Supplemental Figure 3

(a) This representative chromatogram shows how the limit of detection was calculated for RvD1. Peak integration of the labeled internal standard, 200 pg d_2-RvD1, generated an area of 1910.35 at retention time (RT) 13.65 minutes (red, lower panel). The integrated peaks with a signal-to-noise ratio of three or greater that eluted close or at the expected

retention time for RvD1 generated an area of 35 (orange peak #1 at RT 13.08 min), 15 (orange peak #2 at RT 13.37 min), and 23 (orange peak #3 at RT 13.65 min, all in upper panel). These areas translate to amounts of 3.7 pg, 1.6 pg, and 2.4 pg, respectively.

(b) Representative chromatograms from plasma samples of a single study participant before and after supplementation with fish oil show signals for d_2-protectin D1 in red and authentic protectin DX in blue; transitions monitored m/z 359→m/z 153 (PDX), m/z 361→m/z 153 (d_2-PD1), CE 15 eV. By using the unlabeled PDX sourced from Cayman Chemicals, Inc., we were able to ascertain the retention time of the PDX in relation to the d_2-PD1 and integrate the endogenous PDX peak. The two compounds, PD1 and PDX, differ only in the orientations of an –OH group and of a double bond, so that the MS characteristics are essentially the same, with an obvious difference in retention time.

Supplemental Figure 4

LPS-induced changes in AA-derived lipids are well established, less so for EPA/DHA-derived lipids. The prostaglandin metabolite, PGIM, and the thromboxane metabolite, 11-dehydro-TxB, in particular, respond dose-dependently to intravenous LPS in healthy participants [McAdam et al. J Clin Invest. 2000 May;105(10):1473-82; PMID 10811855]. In the present study, both interventions, supplementation with 4 g/d Lovaza fish oil and treatment with LPS 0.6 ng/kg body weight, modulated PGIM and 11-dehydro-TxB excretion as expected. Lovaza fish oil decreased PGIM by 45.1±12.5% from baseline and 11-dehydro-TxB by 30.5±11.8%. LPS completely reversed this suppression at 4-6 hours post administration when excretion of PGIM and 11-dehydro-TxB exceeded the concentrations observed for the pre-fish oil condition by 9.7±16.2%

and 20.3±19.2%, respectively. Effects of LPS on PGIM and 11-dehydro-TxB excretion ceased 12-18 hours post administration.

Supplemental Figure 5

Calibration curves from the mass spectrometry analyses of 11(12)-EpETE, 14(15)-EpETE, 16(17)-EpDPE, 17(18)-EpETE, 19(20)-EpDPE, and 8(9)-EpETE (dots indicate epoxides measured [ng] versus epoxides added [ng] with line of fit in blue).

Supplemental Figure 6

(a) Scans of the authentic unlabeled SPM and the corresponding labeled internal standard.

(b) To address the potential generation of non-enzymatically formed lipids, donor plasma (n=3) was treated *ex vivo* with DHA/EPA at sample processing conditions similar to our clinical studies. For each SPM the peak areas is integrated for the labeled internal standard (red) and if present for the unlabeled authentic compound (blue). Notably, only PD1 was found above the limit of detection (upper two panels) while no signal was detected for maresin (lower two panels). Compared to the acetonitrile (ACN) control, sample processing of plasma treated with DHA/EPA showed a 3.3-4.7-fold increase in PD1, translating to an increase from a mean 1.1 pg/ml to 4.7 pg/ml. Since plasma is devoid of any cellular components of blood, these results support a non-enzymatic degradation of DHA into products which elute close to the retention time of d_2-PD1.

(c) To address whether the labeled internal standards lead to ion suppression of the analyte of interest. Donor plasma (n=3) was treated ex vivo with authentic unlabeled Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg) then analyzed with and without the deuterated internal standards. On average, peak areas for RvE1 were 2.1 smaller for the unspiked samples (averaging the difference in peak area between spiked and unspiked

sample), 6.7 smaller for PD1, and 86.1 smaller for maresin. RvD1 was below the limit of detection (BLD) in all samples. We find that the internal standards we used for our studies (d_4-RvE1, d_2-RvD1, d_2-NPD1, and d_2-Maresin) might only lead marginally, if at all, to ion suppression of the corresponding authentic endogenous metabolite.

(d) MS scans of the deuterated internal standards to illustrate the deuterium distribution. Quantification was based on the main isotope.

(e) Treatment of urine samples in storage from the *Human study "High doses of fish oils"* with beta-glucuronidase increases Maresin concentrations up to 20-30 pg/mg creatinine, however, a consistent pattern that these concentrations are modulated by the high dose fish oil supplementation is not evident (upper two panels). No effect of beta-glucuronidase is seen for PD1 (lower two panels). Conditions are pre-fish oil (left column), fish oil supplementation (center column) and post-fish oil (right column).

(f) Treatment of urine samples with beta-glucuronidase increased RvD1 concentrations in 6 out of 9 samples to the low pictogram range (ranging from 0.3 to 6.5 pg/mg creatinine). These are concentrations close to the limit of detection. A modulation, however, by the high dose fish oil supplementation is not evident.

Supplemental Tables

Supplemental Table 1. Demographics of study participants.

Subject ID#	Age	Gender	Ethnicity	Race	BMI	Treatment Condition
28	32	F	Non-Hispanic	African-American	26.1	Lovaza 21 g/d
29	24	M	Non-Hispanic	Caucasian	26.8	Lovaza 21 g/d
30	24	F	Non-Hispanic	Caucasian	21.5	Lovaza 21 g/d
31	28	F	Non-Hispanic	African-American	21.6	Lovaza 21 g/d
32	23	F	Non-Hispanic	Asian	30.5	Lovaza 21 g/d
33.1	55	M	Non-Hispanic	Caucasian	27.4	Lovaza 21 g/d
34	24	F	Non-Hispanic	Caucasian	18.7	Lovaza 21 g/d
35	55	M	Non-Hispanic	Caucasian	30	Lovaza 21 g/d
36.2	27	F	Non-Hispanic	Asian	29.8	Lovaza 21 g/d
37.1	26	F	Non-Hispanic	Caucasian	20.5	Lovaza 21 g/d
38.2	26	M	Non-Hispanic	Caucasian	19.5	Lovaza 21 g/d
39	25	M	Non-Hispanic	Caucasian	34.1	Lovaza 21 g/d
001	20	M	Non-Hispanic	Caucasian	22	Lovaza 4 g/d & LPS 0.6 ng/kg body weight
013	28	F	Non-Hispanic	Caucasian	25.9	Lovaza 4 g/d & LPS 0.6 ng/kg body weight
041	29	F	Non-Hispanic	Asian	24.6	Lovaza 4 g/d & LPS 0.6 ng/kg body weight
071	19	M	Non-Hispanic	Asian	27.1	Lovaza 4 g/d & LPS 0.6 ng/kg body weight

087	23	F	Non-Hispanic	Asian	25.5	Lovaza 4 g/d & LPS 0.6 ng/kg body weight
107	27	M	Non-Hispanic	Caucasian	20	Lovaza 4 g/d & LPS 0.6 ng/kg body weight

LPS: lipopolysaccharide

Supplemental Table 2. Screening and exit clinical safety laboratory tests for the human study "*High doses of fish oils*".

Human Study "High doses of fish oils"	Unit	Screen	Exit	Normal Range	n
Hct	**%**	**39.6±3.2**	**38.4±3.1**	**36-46**	**12**
Hgb	**g/dL**	**13.7±1.1**	**13.3±1.2**	**12-16**	**12**
MCH	**pg**	**31.0±1.4**	**30.8±1.0**	**27-33**	**12**
MCHC	**g/dL**	**34.8±0.8**	**34.8±0.6**	**31-36**	**12**
MCV	**fL**	**89.3±3.5**	**89.2±4.2**	**80-100**	**12**
Platelets	**THO/uL**	**225.5±55.0**	**227.5±51.3**	**150-400**	**12**
RBC	**MIL/uL**	**4.4±0.4**	**4.3±0.4**	**3.8-5.3**	**12**
RDW	**%**	**12.7±0.7**	**12.6±0.6**	**11.5-14.5**	**12**
WBC	**THO/uL**	**6.3±1.3**	**5.6±0.3**	**4-11**	**12**
ALT	**U/L**	**20.7±11.1**	**19.8±11.9**	**14-54**	**12**
AST	**U/L**	**23.7±5.6**	**21.0±5.7**	**15-41**	**12**
Albumin	**g/dL**	**4.0±0.4**	**3.8±0.3**	**3.5-4.8**	**12**
BUN	**mg/dL**	**9.8±3.1**	**10.0±3.3**	**8-20**	**12**
Total bilirubin	**mg/dL**	**0.9±0.4**	**0.9±0.4**	**0.3-1.2**	**12**
Creatinine	**mg/dL**	**0.8±0.2**	**0.8±0.2**	**0.44-1.03**	**12**
Glucose	**mg/dL**	**76.4±10.1**	**84.7±6.0**	**74-99**	**12**
Total protein	**g/dL**	**6.7±0.4**	**6.7±0.4**	**6.1-7.9**	**12**
GGT	**U/L**	**20.3±11.1**	**18.7±3.5**	**7-50**	**12**
LDH	**U/L**	**143.8±14.6**	**131.6±16.7**	**98-192**	**12**

Chol	**mg/dL**	**181.5±27.6**	**179.3±26.4**	**100-200**	**12**
HDL-C	**mg/dL**	**58.2±14.3**	**57.1±14.6**	**40-59**	**12**
LDL-C	**mg/dL**	**109.1±24.6**	**106.9±26.0**	**0-129**	**12**
Triglycerides	**mg/dL**	**70.7±44.6**	**76.4±36.0**	**25-150**	**12**
INR	**-**	**0.98±0.08**	**1.0±0.1**	**-**	**12**
PT	**second(s)**	**12.0±0.8**	**12.2±0.9**	**10.8-13.3**	**12**
PTT	**second(s)**	**27.3±2.1**	**28.3±2.8**	**21-32.5**	**12**

Supplemental Table 3 Specialized pro-resolving lipid mediators present in plasma samples from subjects treated with high doses of fish oil or bacterial lipopolysaccharide (LPS) on a dietary background conditioned with clinical doses of fish oil.

Human Study "High doses of fish oils"	**Unit**	**Baseline**		**Supplementation with fish oil supplements**		**Discontinuation of fish oil supplements**	
		Day -14	**Day -7**	**Day 14**	**Day 25**	**Day 7**	**Day 14**
PD1	pg/mL	0.96±0.82	1.19±0.78	4.09±2.79	2.72±1.35	1.7±1.27	1.40±0.88

Human Study "Evoked Endotoxemia"	**Unit**	**Pre-fish oil**	**0 hrs pre-LPS**	**2 hrs post-LPS**	**6 hrs post-LPS**	**12 hrs post-LPS**	**24 hrs post-LPS**	**48-72 hrs post-LPS**
			After ≈8 weeks of 4 g/d fish oil					
Maresin	pg/mL	4.2±1.4	4.3±1.8	6.3±2.8	4.2±3.5	5.0±1.6	4.5±3.0	5.8±3.5
PD1	pg/mL	3.0±1.5	3.1±1.2	2.9±1.0	2.5±1.8	2.8±2.8	2.7±1.5	2.8±0.4
PDX	pg/mL	2.5±1.3	2.9±1.0	2.9±0.5	1.9±0.6	2.5±1.4	2.9±1.5	2.3±0.5

Supplemental Table 4. ω-3 and ω-6-PUFA-derived epoxides at baseline and supplementation with fish oil as absolute concentrations and percent-changes from baseline.

Human Study "High doses of fish oils"	**Unit**	**Baseline**		**Supplementation with fish oil supplements**		**Discontinuation of fish oil supplements**	
		Day -14	Day -7	Day 14	Day 25	Day 7	Day 14
8(9)-EpETE	ng/mL	0.03±0.07	0.3±0.25	0.49±0.36	0.16±0.19	0.08±0.07	0.01±0.02
	%	-	-	3425±6577	2221±4870	714±1701	207±568
11(12)-EpETE	ng/mL	0.013±0.008	0.02±0.04	0.36±0.12	0.40±0.32	0.09±0.06	0.06±0.03
	%	-	-	2586±1639	2722±3520	601±540	374±390
14(15)-EpETE	ng/mL	0.02±0.01	0.03±0.04	0.46±0.23	0.49±0.38	0.11±0.08	0.07±0.04
	%	-	-	2401±1320	2173±1613	517±386	301±177
17(18)-EpETE	ng/mL	0.14±0.09	0.18±0.14	2.0±0.8	1.74±1.37	0.60±0.39	0.42±0.29
	%	-	-	1820±1319	1175±792	424±434	237±184
16(17)-EpDPE	ng/mL	0.05±0.03	0.06±0.03	0.28±0.12	0.29±0.20	0.14±0.10	0.11±0.07
	%	-	-	490.2±256.7	415.1±180.5	173.7±140.0	105.4±63.2
19(20)-EpDPE	ng/mL	0.32±0.11	0.34±0.13	1.54±0.69	1.60±1.23	0.72±0.36	0.57±0.27
	%	-	-	411.2±256.0	355.4±226.8	125.8±84.7	78.2±42.7
5(6)-EET	ng/mL	0.26±0.14	0.29±0.2	0.34±0.16	0.28±0.18	0.21±0.1	0.18±0.13
	%	-	-	56.1±108.5	9.7±28.0	-5.3±56.6	-27.0±23.4
8(9)-EET	ng/mL	0.26±0.15	0.28±0.2	0.29±0.14	0.25±0.17	0.21±0.12	0.16±0.1
	%	-	-	52.4±135.7	3.2±34.3	-2.6±70.5	-29.7±24.1
11(12)-EET	ng/mL	0.13±0.07	0.13±0.07	0.17±0.08	0.15±0.11	0.1±0.05	0.09±0.06
	%	-	-	65.2±115.5	25.2±40.0	4.3±66.6	-22.6±22.8
14(15)-EET	ng/mL	0.26±0.13	0.23±0.11	0.3±0.14	0.25±0.14	0.21±0.1	0.17±0.05
	%	-	-	43.3±71.8	5.3±31.5	-6.1±47.1	-21.4±25.9

Supplemental Table 5 ω-3 and ω-6-PUFA-derived epoxide concentrations in plasma at baseline, after supplementation with fish oil and after administration of LPS.

Human Study "Evoked Endotoxemia"	Unit	**Pre-fish oil**	**After 8 weeks of fish oil & pre-LPS**	**2 hours post-LPS**	**6 hours post-LPS**	**12 hours post-LPS**	**24 hours post-LPS**	**48-72 hours post-LPS**
8(9)-EpETE	pg/mL	16.9±12.7	64.2±19.6	32.9±16.1	49.8±36.9	46.9±22.3	43.8±18.1	43.2±25.3
11(12)-EpETE	pg/mL	10.8±18.3	50.2±17.9	24.6±13.7	41.1±36.2	35.8±16.2	33.6±16.0	27.7±15.7
14(15)-EpETE	pg/mL	34.7±35.5	116.7±33.5	63.5±33.7	103.7±78.5	81.5±28.6	84.0±31.1	79.3±38.0
17(18)-EpETE	pg/mL	98.0±76.1	296.7±80.7	128.6±56.2	210.4±130.0	183.3±81.7	206.1±76.7	243.6±117.6
16(17)-EpDPE	pg/mL	5.3±3.5	13.7±3.2	6.7±4.4	10.7±8.8	10.3±5.8	12.1±4.9	10.8±5.0
19(20)-EpDPE	pg/mL	116.6±153.2	309.9±156.7	64.1±33.2	228.1±316.2	178.2±154.5	214.4±174.2	158.5±117.8
5(6)-EET	pg/mL	263.5±241.2	204.9±95.7	93.1±30.0	166.7±130.4	163.9±87.0	214.9±98.5	149.4±58.9
8(9)-EET	pg/mL	120.7±54.2	96.4±30.6	43.6±19.2	91.6±57.1	92.7±54.5	117.3±46.0	84.1±32.9
11(12)-EET	pg/mL	94.2±75.3	70.0±26.3	29.3±8.7	57.6±47.0	54.5±30.8	70.3±20.3	55.6±21.7
14(15)-EET	pg/mL	164.6±107.6	123.3±41.0	66.0±18.0	109.2±69.7	97.9±39.6	127.2±31.3	111.0±44.4

Supplemental Table 6 Adverse event profile study "*High doses of fish oils*"

Subject #	Adverse Event	Severity*	Relation Lovaza™&	Action Taken$
28	Tooth disorder	Mild	0	0
28	Flatulence	Mild	3	0
28	Diarrhea	Mild	3	0
28	Dry skin	Mild	1	0
28	Stomach pain	Mild	2	0
28	Diarrhea	Mild	2	0
28	Toothache	Mild	1	0
29	Steatorrhea	Mild	3	0
29	Steatorrhea	Mild	3	0
29	Steatorrhea	Moderate	3	0
30	Fecal incontinence	Mild	4	0
31	Diarrhea	Mild	1	0
31	Diarrhea	Mild	3	0
31	Rash acneiform	Mild	3	0
31	Constipation	Mild	2	0
31	Diarrhea	Mild	2	0
31	Emesis	Mild	2	0
31	Diarrhea	Mild	2	0
32	Headache	Moderate	0	0
32	Diarrhea	Mild	3	0
32	Menorrhagia	Mild	2	0
32	Headache	Mild	2	0
32	Stomach pain	Mild	1	0
33.1	Dyspepsia	Mild	3	0

33.1	Flatulence	Mild	3	0
33.1	Steatorrhea	Mild	3	0
33.1	Steatorrhea	Mild	3	0
33.1	Steatorrhea	Mild	3	0
33.1	Diarrhea	Mild	2	0
34	Diarrhea	Mild	3	0
34	Steatorrhea	Mild	4	0
34	Steatorrhea	Mild	3	0
34	Steatorrhea	Mild	3	0
34	Bruising	Mild	2	0
34	Pharyngitis	Mild	1	0
34	Fecal incontinence	Moderate	3	3
35	Headache	Mild	0	0
35	Constipation	Mild	2	0
35	Diarrhea	Mild	2	0
35	Dry mouth	Mild	2	0
35	Headache	Mild	1	0
35	Taste alteration	Mild	2	0
35	Taste alteration	Mild	2	0
36.2	Dyspepsia	Mild	2	0
36.2	Flu-like symptoms	Mild	1	0
37.1	Allergic rhinitis	Mild	1	0
37.1	Dyspepsia	Mild	2	0
37.1	Steatorrhea	Moderate	3	3
37.1	Mucositis oral	Mild	1	0
37.1	Hypersensitivity	Mild	2	0
37.1	Flu-like symptoms	Mild	1	0
37.1	Irregular menstruation	Mild	2	0

38.2	Laceration of foot	Mild	1	0
38.2	Dyspepsia	Mild	3	0
38.2	Fecal incontinence	Mild	3	0
38.2	Steatorrhea	Moderate	3	0
38.2	Steatorrhea	Mild	3	0
38.2	Flatulence	Mild	2	0
38.2	Steatorrhea	Mild	3	0
38.2	Fecal incontinence	Moderate	3	0
39	Flatulence	Mild	2	0
39	Headache	Mild	2	0
39	Steatorrhea	Mild	2	0
39	Joint disorder	Mild	2	0
39	Headache	Mild	1	0
39	Bruising	Mild	2	0

Criteria:

*Severity normal, mild, moderate, severe, life-threatening;

[&] Relation Lovaza™: 0= not related, 1= unlikely, 2= possibly, 3= probably, 4= definitely;

[$] Action Taken: 0= none, 1= study agent temporarily stopped, 2= study agent discontinued, 3=dosing regimen of study agent changed.

Supplemental Table 7 Adverse event profile study "*Evoked Endotoxemia*"

Subject #	Adverse Event	Severity *	Relation LPS[&]	Relation Lovaza™[&]	Action Taken[$]
1	Seasonal allergies	1	0	0	0
13	Common cold	1	0	0	0
41	Dizziness	1	3	2	0
71	Strep throat infection	3	0	1	0
71	Bloated	1	1	2	0
71	Anxiety attack	1	2	1	0
87	Common cold	1	0	0	0
107	Common cold	1	0	0	0
107	Drop of >2g Hemoglobin	3	4	0	0
107	Emesis	1	0	1	0

Criteria:

*Severity 0= normal, 1=mild, 2=moderate, 3=severe, 4=life-threatening;

[&] Relation LPS / Lovaza™: 0= not related, 1= unlikely, 2= possibly, 3= probably, 4= definitely;

[$] Action Taken: 0= none, 1= study agent temporarily stopped, 2= study agent discontinued.

Supplemental Methods & Results

Exploring potential non-enzymatic formation of SPMs

Some of the peaks integrated for PD1 (high dose fish oil, Figure 1b) and maresin (lipopolysaccharide on a low dose fish oil background, Figure 2b) show wide signals or elute alongside another peak, respectively. To address the possibility that this might be caused non-enzymatically during sample processing, we incubated plasma from untreated healthy donors (1 mL each, n=3) with i) ACN alone or ii) ACN with 50 ng DHA/EPA each for 1 hr at RT, followed by extraction and analysis as described in the method section.

Supplemental Table 8 below and Supplemental Figure 6b show for each SPM the peak areas integrated for the labeled internal standard ("IS", i.e. 1 ng d_4-RvE1 and 0.2 ng each of d_2-RvD1, d_2-PD1, d_2-Maresin) and, if present, for the unlabeled authentic compound. Notably, only PD1 was formed above the limit of detection (BLD). Compared to the acetonitrile (ACN) control, sample processing of plasma treated with DHA/EPA showed a 3.3-4.7-fold increase in PD1, corresponding to an increase from a mean 1.1 pg/ml to 4.7 pg/ml. Since plasma is devoid of any cellular components of blood, these results support non-enzymatic degradation of DHA into products which elute close to the retention time of d_2-PD1.

Supplemental Table 8 Exploring potential non-enzymatic formation of SPMs

		RvE1		RvD1	
Subject ID#	**Treatment (ex vivo)**	Area	IS Area	Area	IS Area
Donor_1	ACN	BLD	1854.0	BLD	490.2
Donor_2	ACN	BLD	2464.2	BLD	397.1

Donor_3	ACN	BLD	2399.7	BLD	594.0
Donor_1	50 ng DHA/EPA each in ACN	BLD	1991.6	BLD	539.9
Donor_2	50 ng DHA/EPA each in ACN	BLD	2512.7	BLD	436.5
Donor_3	50 ng DHA/EPA each in ACN	BLD	2468.5	BLD	634.5

		PD1			**Maresin**	
Subject ID#	**Treatment (ex vivo)**	Area	IS Area	pg/mL	Area	IS Area
Donor_1	ACN	331.2	7694.8	1.4	BLD	2613.6
Donor_2	ACN	397.5	9143.9	1.4	BLD	3070.3
Donor_3	ACN	213.4	10180.1	0.4	BLD	3611.7
Donor_1	50 ng DHA/EPA each in ACN	1225.5	8490.1	5.4	BLD	3023.1
Donor_2	50 ng DHA/EPA each in ACN	1323.7	9393.1	5.2	BLD	3814.9
Donor_3	50 ng DHA/EPA each in ACN	1009.3	10384.0	3.4	BLD	4039.0

Exploring potential ion suppression of the co-eluting endogenous SPMs

Plasma from 3 healthy untreated donors, 1 mL each, was supplemented *ex vivo* with labeled authentic and unlabeled SPMs, i.e. Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), PD1 (5 pg), incubated at RT for 1 hour, taken through extraction the process and analyzed as described in the method section. Likewise, the control plasma sample, 1 mL each, was treated but this time only the unlabeled authentic SPMs were added in identical amounts.

We find that the internal standards we use for our studies (1 ng d_4-RvE1 and 0.2 ng each of d_2-RvD1, d_2-NPD1, d_2-Maresin) might only lead marginally, if at all, to ion suppression of the corresponding authentic endogenous metabolite. On average, peak areas for RvE1 were 2.1 smaller for the unspiked samples (averaging the difference in peak area between spiked and unspiked sample), 6.7 smaller for PD1, and 86.1 smaller for maresin. RvD1 was below the limit

of detection (BLD) in all samples. Traces for PD1 and maresin are shown in Supplementary Figure 6c.

Supplemental Table 9 Exploring potential ion suppression of the co-eluting endogenous SPMs

			RvE1		PD1		Maresin	
Subject ID#	**Sample Treatment (ex vivo)**	**Spike**	Area	IS Area	Area	IS Area	Area	IS Area
Donor_1	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	N	BLD	-	278.3	-	193.9	-
Donor_2	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	N	67.3	-	372.5	-	237.5	-
Donor_3	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	N	74.9	-	130.6	-	241.0	-
Donor_1	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	Y	BLD	2650.1	216.5	12189.3	BLD	3998.2
Donor_2	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	Y	68.5	3048.4	366.7	10478.1	485.4	3824.3
Donor_3	Maresin (5 pg), RvD1 (5 pg), RvE1 (10 pg), NPD1 (5 pg)	Y	80.0	3023.3	218.1	10196.0	165.2	4439.9

Exploring potential contamination of deuterated internal standards with authentic unlabeled lipids

Deuterated internal standards, i.e. d_{11}-8(9)-EET, d_{11}-11(12)-EET, d_{11}-14(15)-EET, d_8-AA, d_5-EPA, and d_5-DHA, and the unlabeled authentic compounds were injected, each at 1 ng/uL, into the Waters Xevo TQ-S and scanned from 5 mass units below D0 to 20 units above. As shown in Supplementary Figure 6a, we have no evidence that the deuterated internal standards are contaminated with the authentic unlabeled lipid.

Exploring the effects of beta-glucuronidase treatment of urine samples on detecting SPM

Urine samples (1 mL) in storage at -80°C from the Human study "High doses of fish oils" (n=3 subjects for the conditions "pre-fish oil", "fish oil supplementation" and "post-fish oil") were mixed with 1 mL sodium acetate (0.2M, pH=5.0) and 100 µL of β-Glucuronidase from Helix pomatia (Sigma, St. Louis, MO, USA). After hydrolysis at 37°C overnight, samples were spiked with internal standards (1 ng d_4-RvE1, and 0.2 ng each d_2-RvD1, d_2-PD1, d_2-Maresin, and 5 ng of d_4-8,12-iso-iP$F_{2\alpha}$ -VI) before SPE.

We find that:

- Treatment of urine samples with beta-glucuronidase increases Maresin concentrations up to 20-30 pg/mg creatinine (see Supplemental Table 10 below), however, a consistent pattern that these concentrations are modulated by the high dose fish oil supplementation is not evident. Traces are shown in Supplemental Figure 6e.

Supplemental Table 10 Exploring the effects of beta-glucuronidase treatment of urine samples on detecting maresin

			Maresin		
Subject ID#	**Visit**	**beta-glucuronidase**	Area	IS Area	pg/mg creatinine
32	Pre-fish oil	N		987.7	
32	Pre-fish oil	Y		1864.9	
32	High dose fish oil supplementation	N		2275.2	
32	High dose fish oil supplementation	Y		2121.0	
32	Post-fish oil	N	39.8	1686.1	0.6
32	Post-fish oil	Y		1440.8	
35	Pre-fish oil	N	22.3	1245.6	0.4
35	Pre-fish oil	Y	1355.4	2906.9	18.2
35	High dose fish oil supplementation	N		2475.5	

35	High dose fish oil supplementation	Y	1285.7	2143.8	23.6
35	Post-fish oil	N		2440.5	
35	Post-fish oil	Y	1429.5	1889.1	29.8
39	Pre-fish oil	N		2568.9	
39	Pre-fish oil	Y	1517.6	2288.6	26.2
39	High dose fish oil supplementation	N		3014.4	
39	High dose fish oil supplementation	Y	1446.7	2859.4	19.8
39	Post-fish oil	N		2205.1	
39	Post-fish oil	Y	1410.7	2409.6	23

- Treatment of urine samples with beta-glucuronidase has no effect on PD1 concentrations (see Supplemental Table 11 below). Traces are shown in Supplemental Figure 6e.

Supplemental Table 11 Exploring the effects of beta-glucuronidase treatment of urine samples on detecting PD1

			PD1		
Subject ID#	**Visit**	**beta-glucuronidase**	Area	IS Area	pg/mg creatinine
32	Pre-fish oil	N	83.6	4568.5	0.2
32	Pre-fish oil	Y		6634.6	
32	High dose fish oil supplementation	N	121.2	6539.3	0.3
32	High dose fish oil supplementation	Y		5682.0	
32	Post-fish oil	N	130.1	4814.7	0.5
32	Post-fish oil	Y		3373.3	
35	Pre-fish oil	N		3834.9	0.0
35	Pre-fish oil	Y	133.6	6994.3	0.6
35	High dose fish oil supplementation	N	141.1	8537.2	0.0
35	High dose fish oil supplementation	Y		7560.8	
35	Post-fish oil	N	127.1	7871.3	0.0
35	Post-fish oil	Y		5654.5	
39	Pre-fish oil	N	149.0	8013.5	0.1

39	Pre-fish oil	Y	7833.8	
39	High dose fish oil supplementation	N	6713.7	0.0
39	High dose fish oil supplementation	Y	7033.5	
39	Post-fish oil	N	7068.3	0.0
39	Post-fish oil	Y	8825.4	

- Treatment of urine samples with beta-glucuronidase increased RvD1 concentrations in 6 out of 9 samples to the low pictogram range, concentrations close to the limit of detection (see Supplemental Table 12 below). A modulation, however, by the high dose fish oil supplementation is not evident. Traces are shown in Supplemental Figure 6f.
- RvE1 concentrations were not affected by beta-glucuronidase treatment (see Supplemental Table 12 below). In two samples RvE1 reached concentrations a magnitude higher, in the ng/mg creatinine range. This is not consistent with our findings of our initial urine analysis. Therefore, we interpret these inconsistent results as artifacts. Traces not shown.

Supplemental Table 12 Exploring the effects of beta-glucuronidase treatment of urine samples on detecting RvD1 and RvE1

			RvD1	**RvE1**
Subject ID#	**Visit**	**beta-glucuronidase**	pg/mg creatinine	pg/mg creatinine
32	Pre-fish oil	N	0.0	0.0
32	Pre-fish oil	Y	6.5	0.0
32	High dose fish oil supplementation	N	0.0	22871.8
32	High dose fish oil supplementation	Y	1.0	23956.4
32	Post-fish oil	N	0.0	0.0
32	Post-fish oil	Y	0.7	0.0
35	Pre-fish oil	N	0.0	0.0
35	Pre-fish oil	Y	5.2	0.0

35	High dose fish oil supplementation	N	0.0	0.0
35	High dose fish oil supplementation	Y	0.0	0.0
35	Post-fish oil	N	0.0	0.0
35	Post-fish oil	Y	0.0	0.0
39	Pre-fish oil	N	0.0	0.0
39	Pre-fish oil	Y	3.5	0.0
39	High dose fish oil supplementation	N	0.0	1060.5
39	High dose fish oil supplementation	Y	0.4	0.0
39	Post-fish oil	N	0.0	0.0
39	Post-fish oil	Y	0.3	0.0

- We used the abundant isoprostane, 8,12-*iso*-iPF$_{2a}$-VI, as positive control. Beta-glucuronidase treatment of urine samples from study subjects treated with high doses of Lovaza fish oil showed a consistent increase in urine 8,12-*iso*-iPF$_{2a}$-VI concentrations averaged (±SD) as a 1.7±0.5-fold change across the different conditions pre-fish oil, fish oil supplementation, and post-fish oil (note that fish oil supplementation only marginally modulated 8,12-iso-iPF2a-VI concentrations in urine). Effects of long-term storage of these human urine samples are evident in the 5.6±1.0-fold increase in 8,12-*iso*-iPF$_{2a}$-VI concentrations between our initial and current analyses, underscoring the non-enzymatic formation of isoprostanes during thaw-freeze cycles (Supplemental Table 13).

Supplemental Table 13 Exploring the effects of beta-glucuronidase treatment of urine samples on detecting the isoprostane, 8,12-*iso*-iPF$_{2\alpha}$-VI

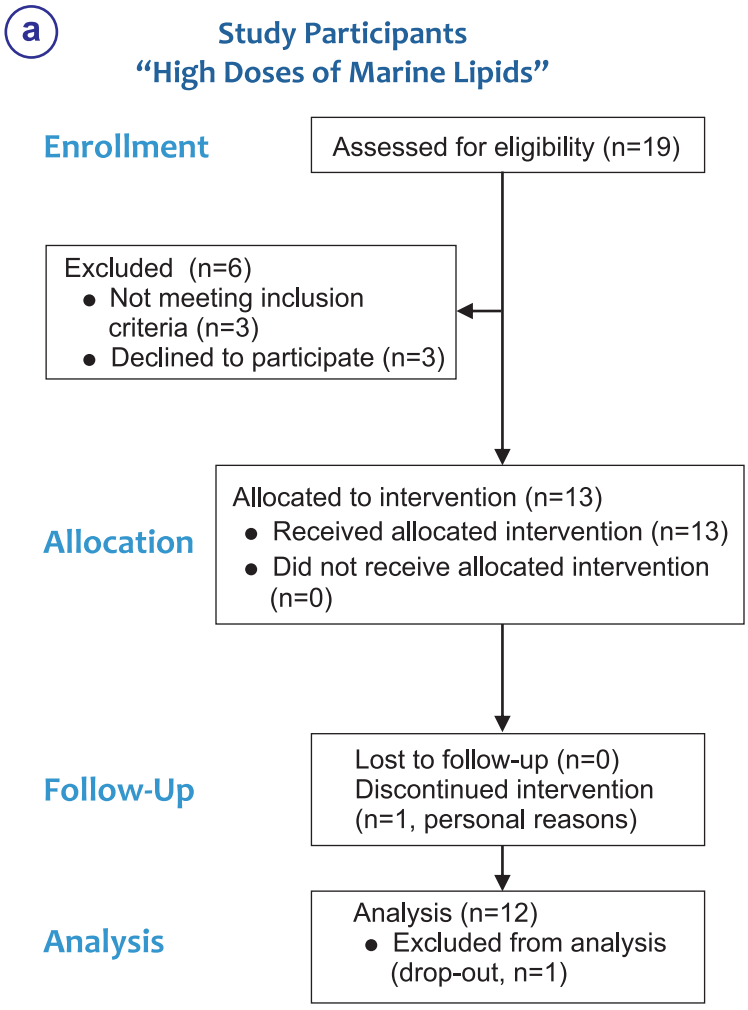
					8,12-*iso*-iPF$_{2\alpha}$-VI	
Subject ID#	Visit	beta-glucuroni-dase	ng/mg creatinine *June 2015*	Fold-change -/+ β-glu *June 2015*	ng/mg creatinine *June 2012*	Fold-change *June 2015/2012*
32	Pre-fish oil	N	26.6		4.6	5.8

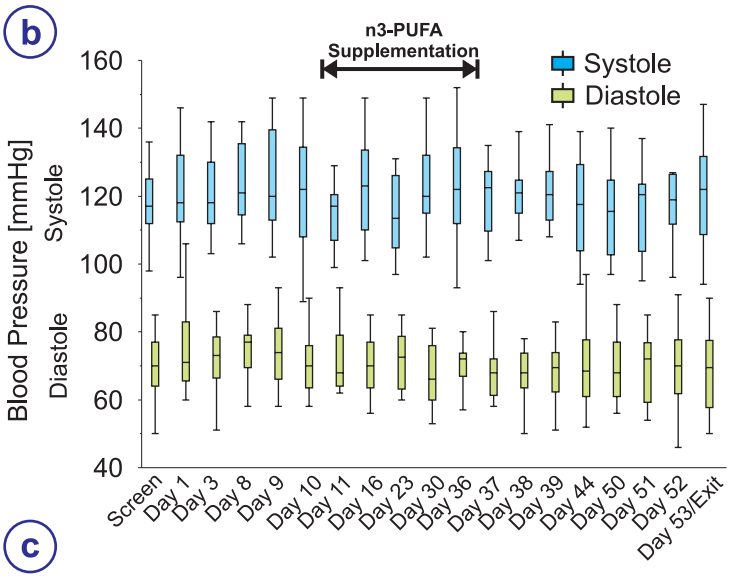
32	Pre-fish oil	Y	35.4	1.3	*not analyzed*	
32	High dose fish oil supplementation	N	24.8		4.9	5.0
32	High dose fish oil supplementation	Y	34.3	1.4	*not analyzed*	
32	Post-fish oil	N	18.0		2.5	7.2
32	Post-fish oil	Y	35.0	1.9	*not analyzed*	
35	Pre-fish oil	N	19.2		2.7	7.2
35	Pre-fish oil	Y	53.2	2.8	*not analyzed*	
35	High dose fish oil supplementation	N	14.3		3.1	4.6
35	High dose fish oil supplementation	Y	28.9	2.0	*not analyzed*	
35	Post-fish oil	N	7.0		1.3	5.4
35	Post-fish oil	Y	12.9	1.8	*not analyzed*	
39	Pre-fish oil	N	15.8		3.6	4.4
39	Pre-fish oil	Y	22.6	1.4	*not analyzed*	
39	High dose fish oil supplementation	N	17.5		3.5	5.0
39	High dose fish oil supplementation	Y	23.2	1.3	*not analyzed*	
39	Post-fish oil	N	14.1		2.6	5.4
39	Post-fish oil	Y	20.2	1.4	*not analyzed*	
			Mean	*1.7*		*5.6*
			Standard Deviation	*0.5*		*1.0*

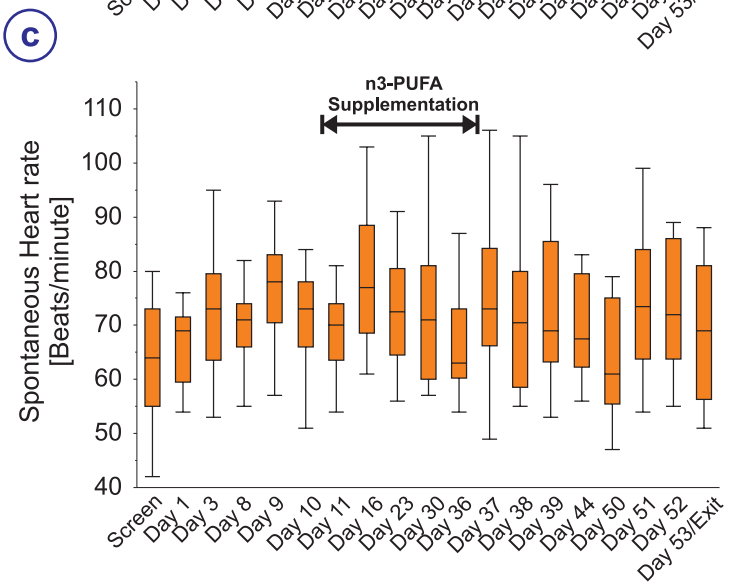
Summary

We summarize, (i) that non-enzymatic formation of low concentrations of PD1 from DHA can be observed, (ii) that the internal standards we use for SPMs in our studies might only lead marginally, if at all, to ion suppression of the corresponding authentic endogenous lipid, (iii) that the deuterated internal standards for EETs, DHA and EPA are not contaminated by authentic unlabeled lipids, and (iv) that treatment of urine samples with beta-glucuronidase shows the presence of some maresin and RvD1 without, however, a consistent pattern that these concentrations are modulated by the high doses of fish oil supplementation.

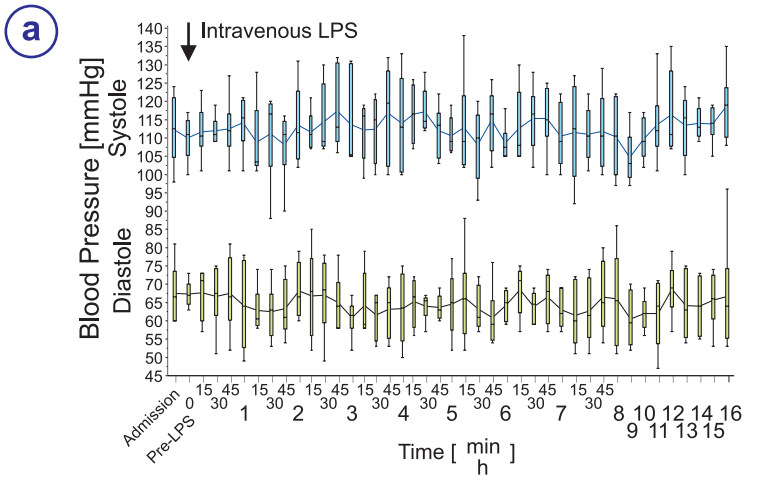
Supplemental Figure 1

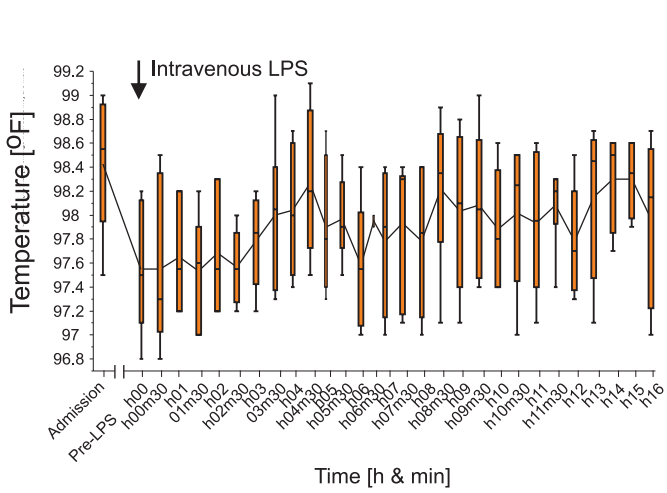


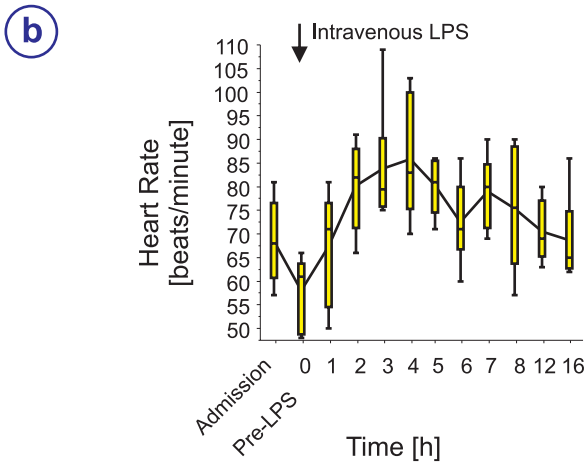




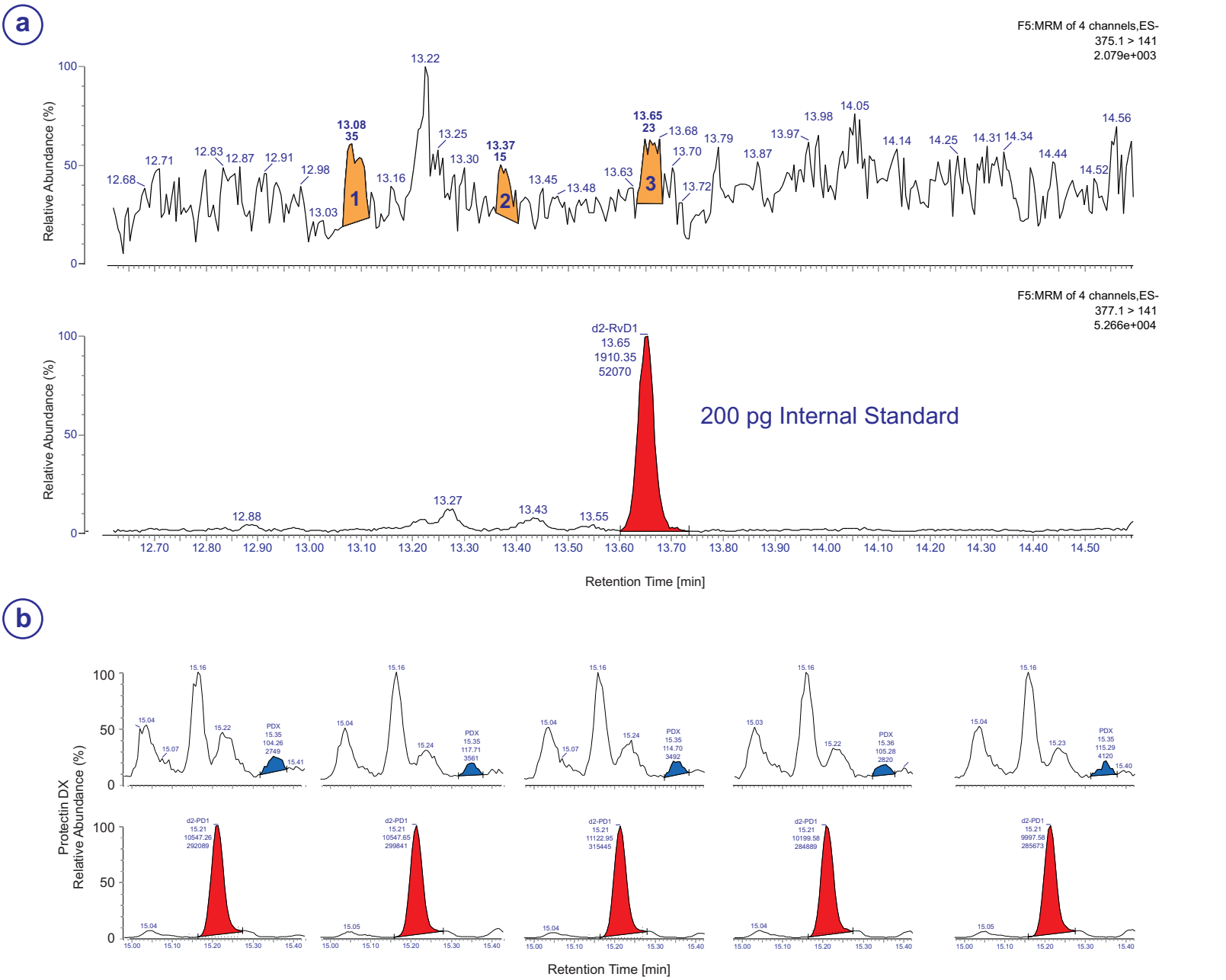
Supplemental Figure 2



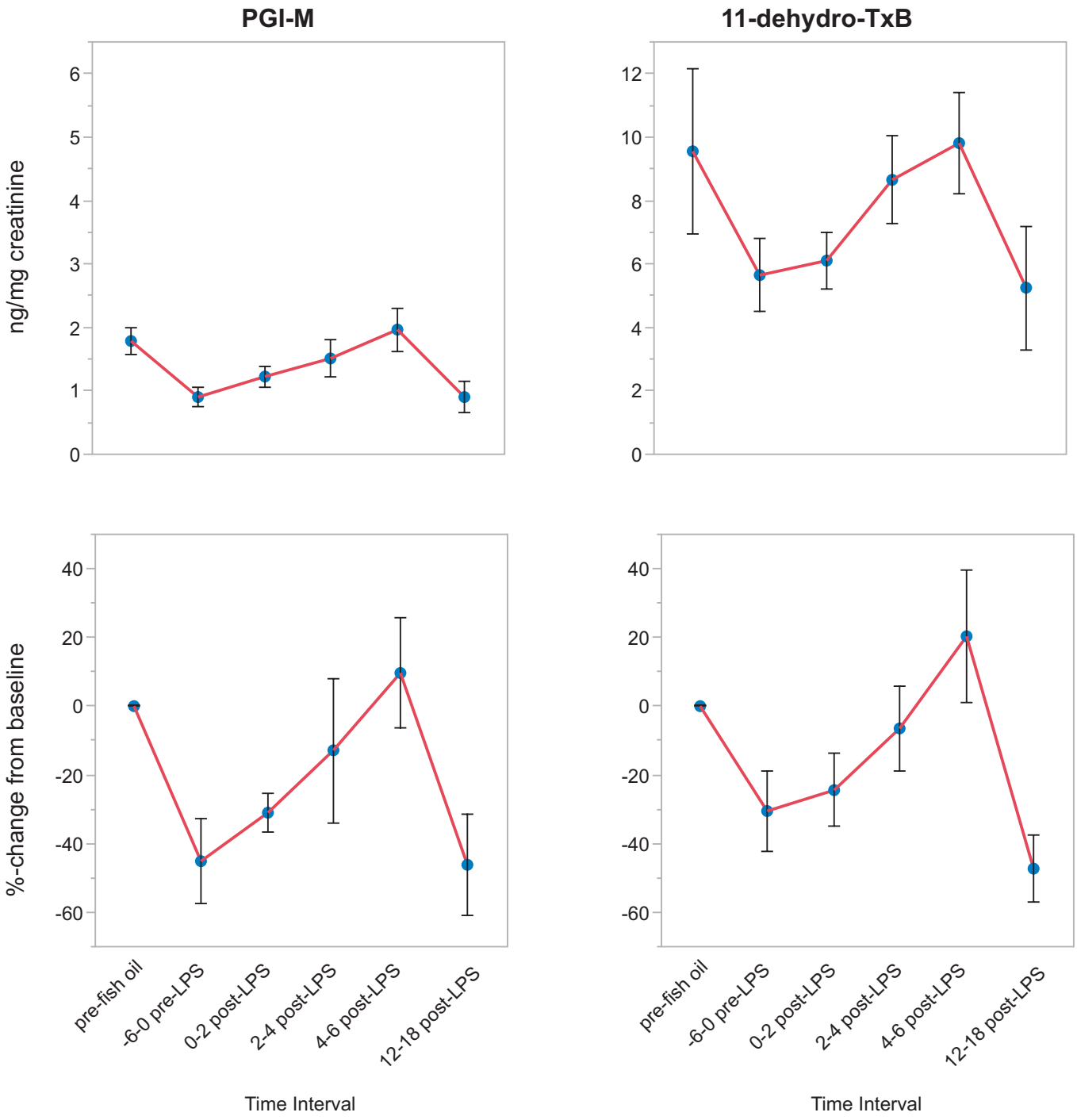




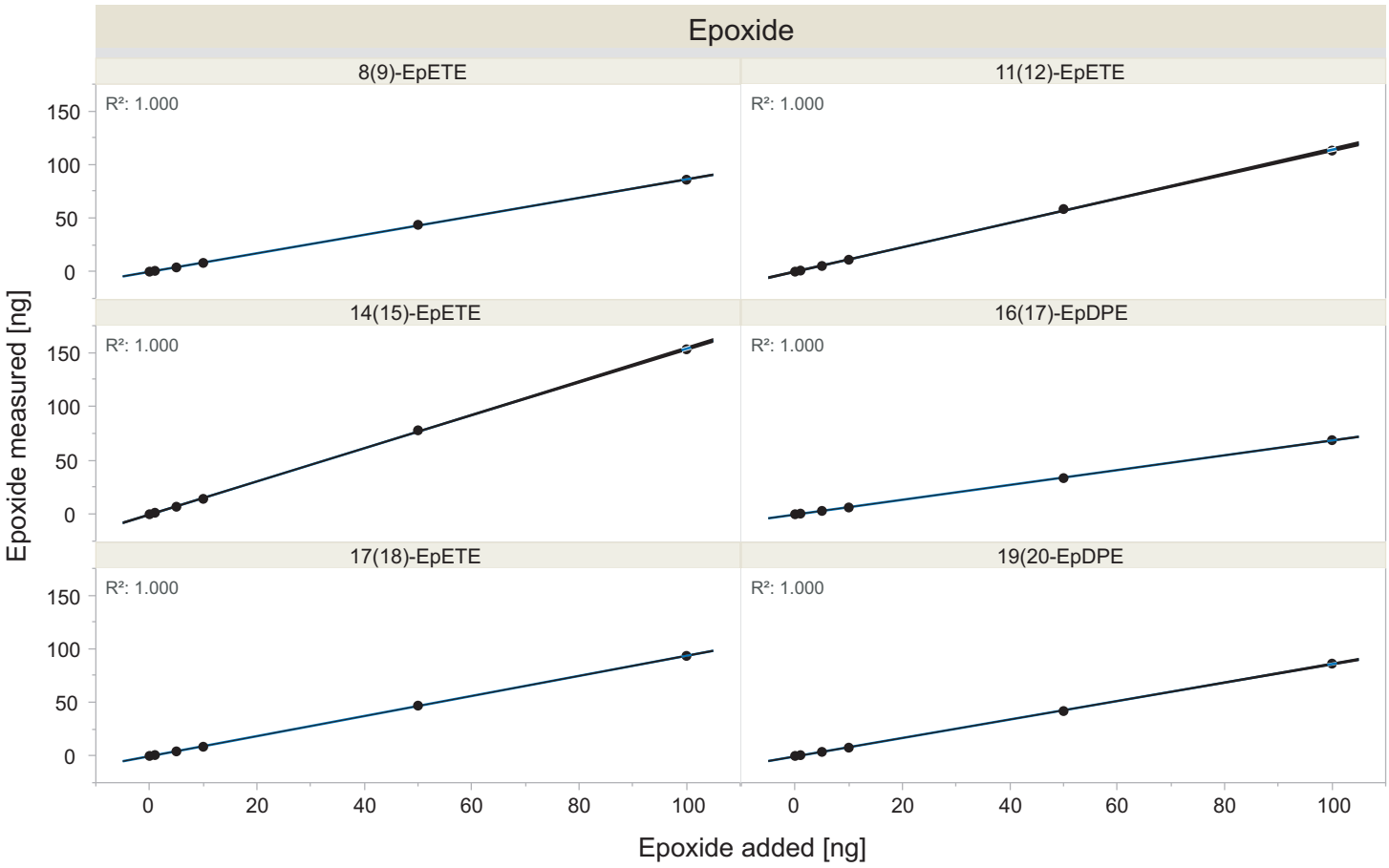
Supplemental Figure 3



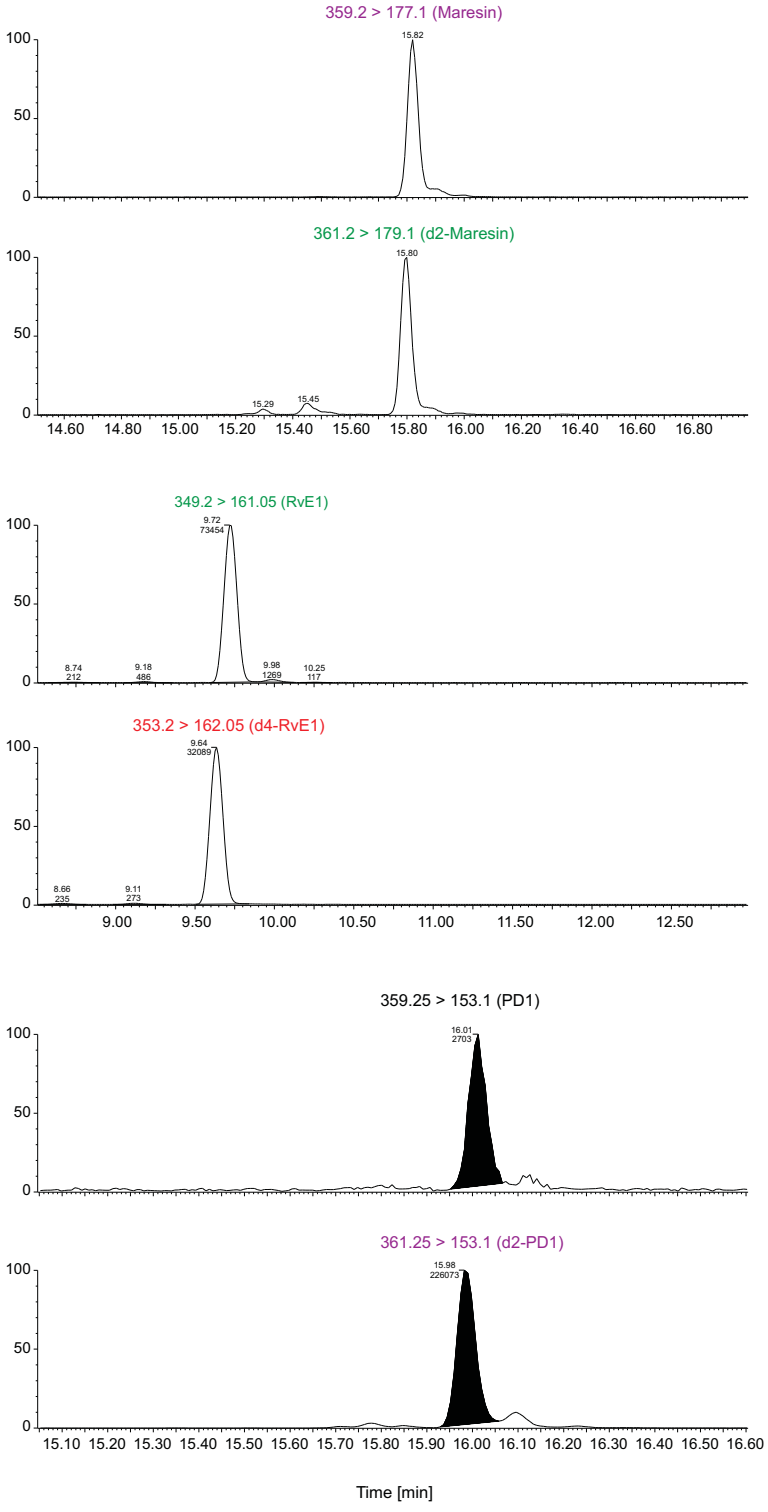
Supplemental Figure 4



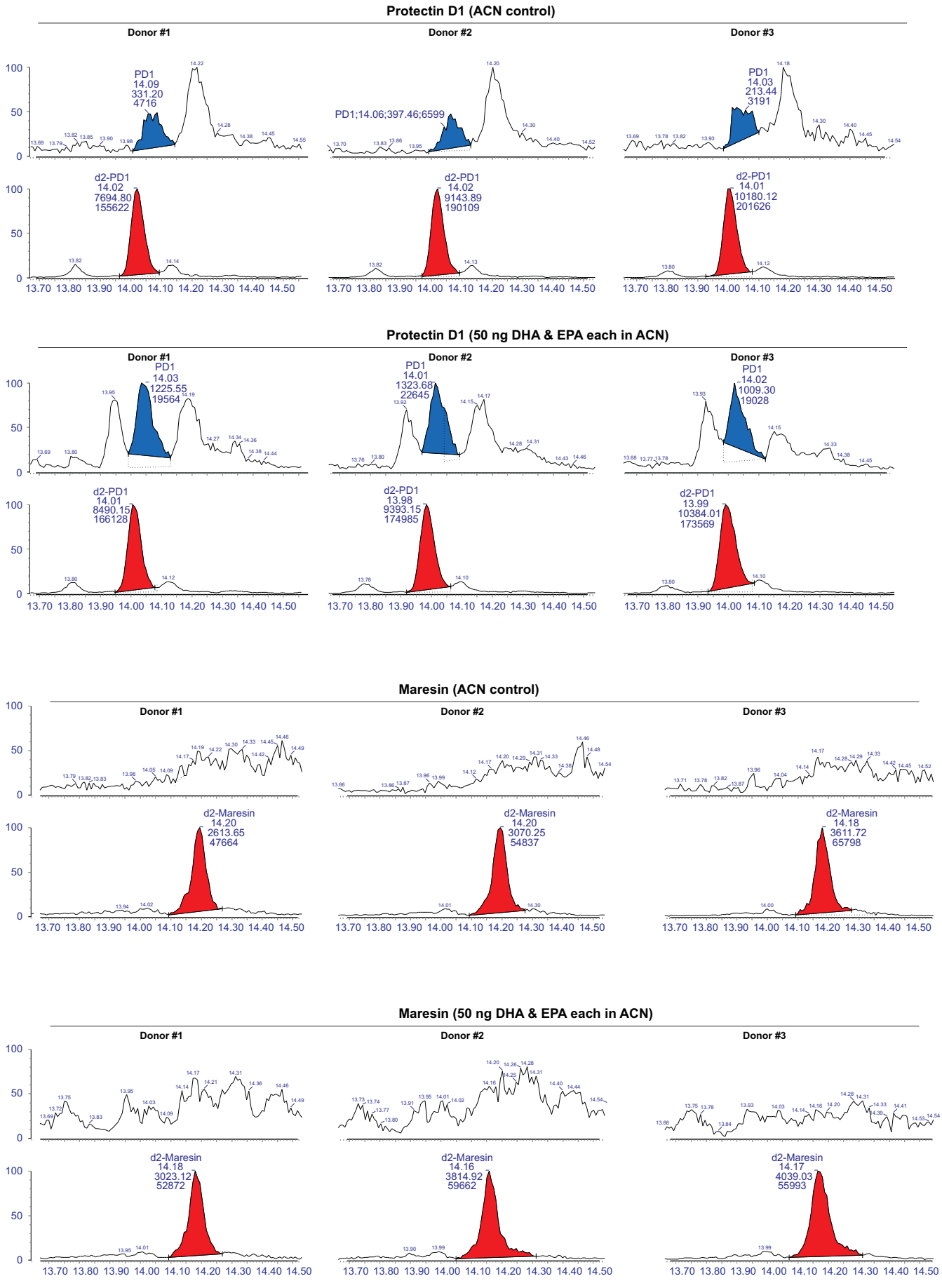
Supplemental Figure 5



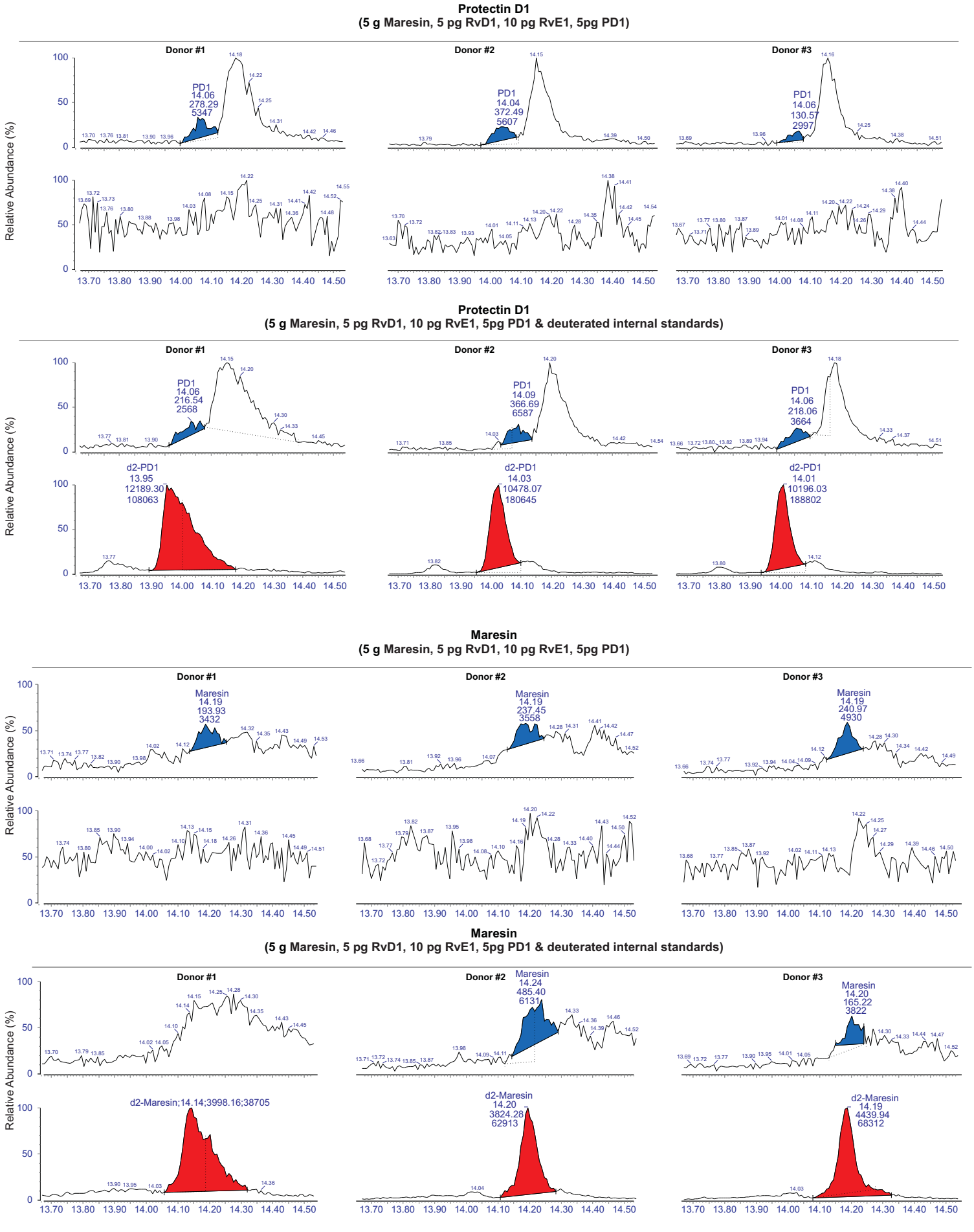
Supplemental Figure 6a



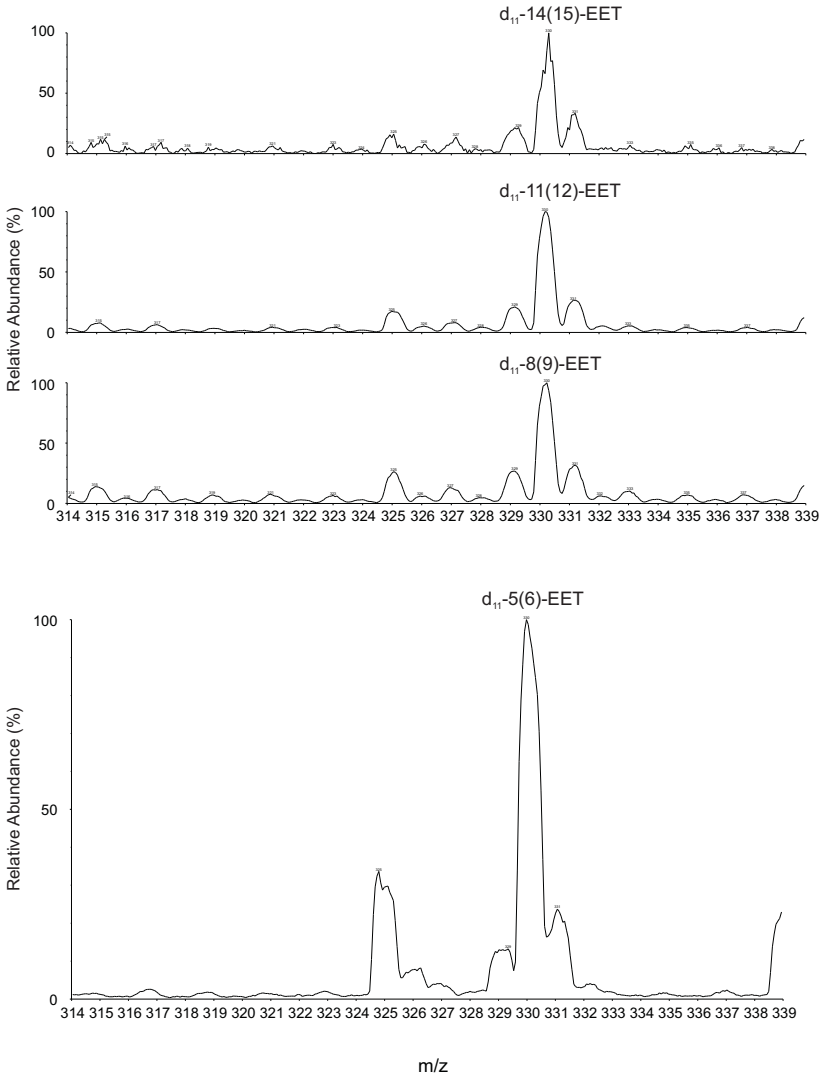
Supplemental Figure 6b

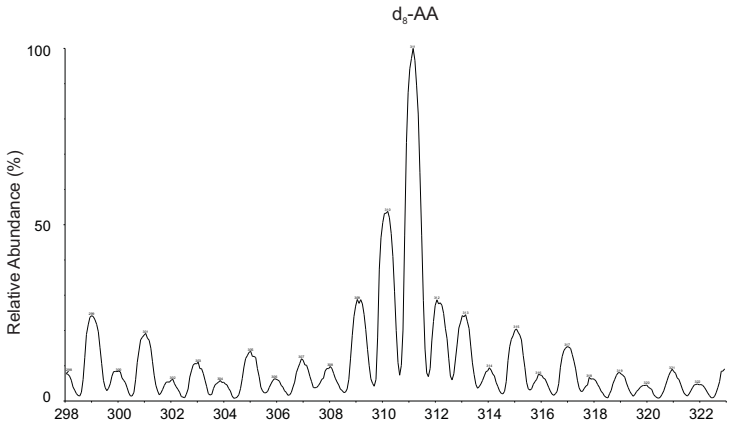


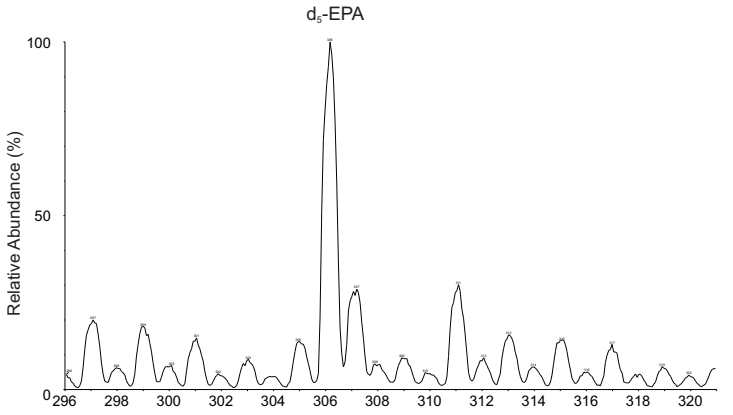
Supplemental Figure 6c

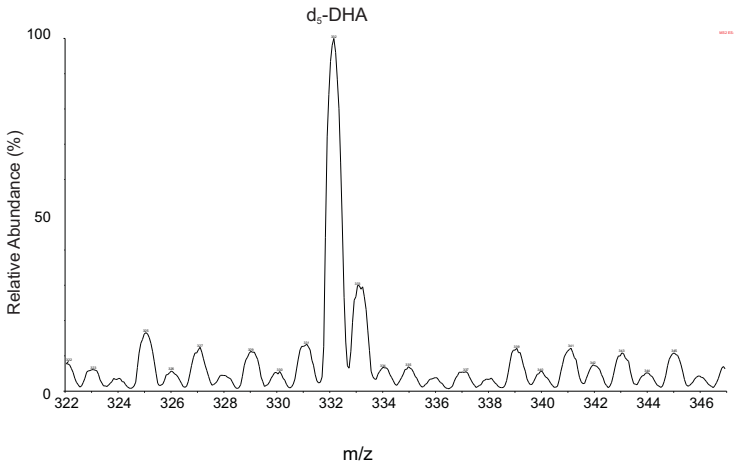


Supplemental Figure 6d

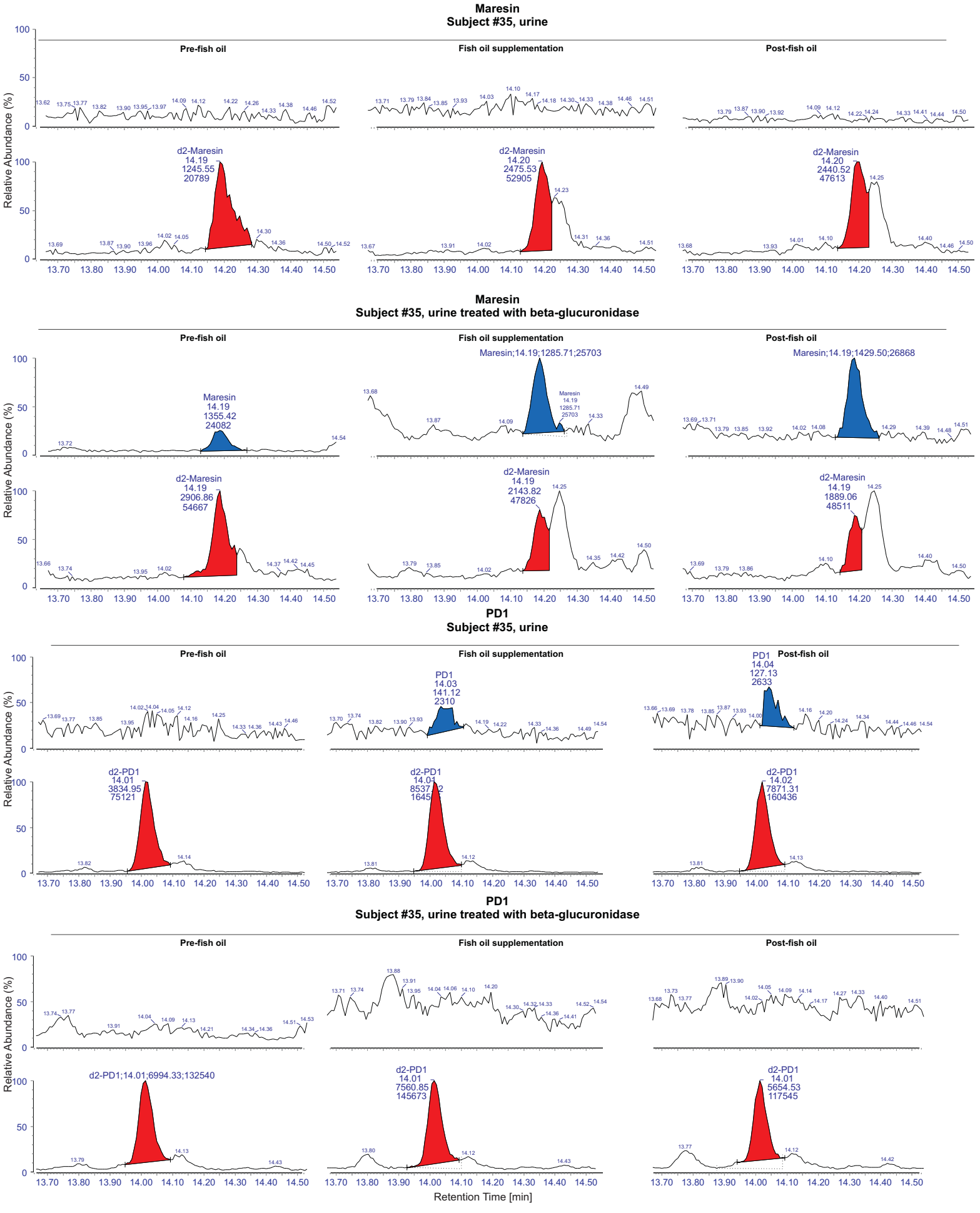








Supplemental Figure 6e



Supplemental Figure 6f