Supplementary material

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Contents:

SPE-LC-MS/MS method validation

The mixture of standards of deuterated and native oxylipins at 100 ng/mL in methanol were prepared for MS parameters optimization. Stock solution of native oxylipins was 1 µg/mL in methanol for all analytes except 15-HETrE, which was at 250 ng/mL; PGD3 and 18-HEPE, which were at 500 ng/mL. Working solutions for calibration curve and recovery experiments were prepared by serial dilution. ISs mixture stock solution was prepared in methanol at 1 µg/mL. All mixtures were stored at -80°C.

The analytical method was characterized a validated in terms of linearity, intraday and interday precision and accuracy, method detection limit (MDL), lower method quantification limit (MQL) and SPE recoveries. During method validation each LC-MS run was repeated thrice. Seven-point calibration curve was prepared by spiking serum samples prior to SPE with 0.01, 0.025, 0.1, 0.5, 1, 5, 10 and 25 ng of native oxylipins mixture (for 15-HETre: 0.0025, 0.00625, 0.025, 0.125, 0.25, 1.25, 2.5 and 6.25 ng; for PGD3 and 18-HEPE: 0.005, 0.0125, 0.05, 0.25, 0.5, 2.5, 5, 12.5 ng). Each calibration point was prepared in triplicate. Standard curves were constructed by plotting the ratio of the peak area of each analyte to assigned IS (see Supplementary Table S1), corrected for blank samples (without the addition of analytes) peak ratios. Linearity was expressed by the coefficient of determination (R^2) value. Goodness of fit was evaluated by calculating relative standard deviation of the curve (RSD). Lower MDL was calculated from standard curve equation as (3.3x standard error of the curve)/slope); lower MQL was defined as the lowest calibration level at which signal-to-noise ratio (S/N) was > 10.

Accuracy was defined as the ratio of measured and known standard concentration and 80-120 was considered satisfactory. Precision was defined as coefficient of variation (CV) for three independent replicates and working range acceptance criterion for precision was < 20%. Intraday and interday accuracy and precision were determined at two spike levels: I - 0.5 ng of analytes standards (for 15-HETrE: 0.125 ng; for PGD3 and 18-HEPE: 0.25 ng) and II – 5 ng of analytes standard mixture (for 15-HETrE: 1.25 ng; for PGD3 and 18-HEPE: 2.5 ng). Intraday precision and accuracy was determined for three runs performed on the same day, interday precision was determined for triplicate sample runs on three consecutive days.

Extraction efficiency (EE, recovery) was also tested at level I and level II in three independent experiments. EE was calculated as peak area of analyte in sample spiked before SPE extraction to peak area of analyte spiked after SPE, adjusted for blank sample peak area and expressed as a percentage.

SPE-LC-MS/MS method characterization

The chromatographic method development was preceded by direct infusion experiment wherein optimization of spectrometer working parameters for each oxylipin was performed. This encompassed parent and daughter ions m/z adjustment, quadrupoles voltage, collision energy and dwell time. Two specific MRM transitions were chosen for each compound in order to ensure sensitive detection for quantification and specific identification

of isobaric compounds. For most of the oxylipins the MRM transition yielding highest peak intensity was chosen. The exceptions were made in cases of isobaric compounds for which we could not achieve a chromatographic separation e.g. 9-HODE and 13-HODE (see Supplementary Figure S2). The most abundant fragment ion for both of these oxylipins was $[M\text{-}H\text{-}H_2O]^-$ at m/z 277.40 and could not be used to distinguish between them. Fragment ions resulting from cleavage of C-C bond adjacent to the -OH group [1] were used instead for 9-HODE and 13-HODE quantification: m/z 171.30 and m/z 195.45 respectively. During the method optimization stage the ESI ionization efficiency depending on organic phase content was tested between less polar acetonitrile and more polar methanol (see Supplementary Figure S5) and methanol was chosen for better relative peak intensities. The influence of injection solvent on ionization was considered and analyses were performed for oxylipins dissolved in different ratios of polar and organic phase of which three parts of phase B (methanol) and one part of phase A (0.1% formic acid) was the most suitable for majority of analytes (see Supplementary Figure S5). Chromatographic separation conditions allowed for analysis of 28 oxylipins and 7 IS. To minimize the influence of matrix effects, each calibration point was prepared by spiking pooled serum samples (from > 100 bariatric patients) and performing the SPE sample preparation. Since we did not have access to oxylipin-free serum, blank pooled serum sample was prepared along each calibration curve and used to correct peak areas of spiked samples. The LC-MS/MS method R^2 was above 0.99 for each analyte, indicating good linearity, and the RSD of the calibration curves was between 0.75 – 6.62%. The MDLs ranged from 2 pg (18.5 pM, PDX) to 394 pg (4.01 nmol/L, 2,3-dinor-8-iso-PGF2α).

Recovery of IS was evaluated at one concentration of 10 ng spike into 300 µL of serum and was between 90 (4)% (RvE1-d4) and 97 (4)% (14(15)-DiHET-d11) (n = 3, Supplementary Figure S6). For all analytes the accuracy, precision and EE of the SPE-LC-MS/MS method was evaluated during one analytical sequence (interday) and between three consecutive days (intraday) at two concentration levels and the results are presented in Supplementary Table S6. On average the accuracy of 100 (16.2)% at level I and 101 (8.5)% at level II. The analytes outside of the desirable accuracy range were 15d-PGJ2, 7S-Mar1, 8-epi-PGF2α, 2,3-dinor-PGF2α and RvD1. The precision of the method averaged 12.5 (7.3)% at concentrations level I, and 12.1 (6.9)% at level II, except prostaglandins PGD2, PGD3 and PGF2α. The SPE procedure was characterized by excellent extraction efficiency for 27 analytes, mean recovery at level I was 97.2 (12.6)% and 96.8 (11.5)% at level II. In case of 2,3-dinor-8-iso-PGF2α the EE was

well below 80% and this analyte was removed from further analysis. The validation parameters we were able to achieve indicate that the applied method is a useful tool for the analysis of free oxylipins in serum samples.

1 Yuan Z-X, Rapoport SI, Soldin SJ, et al. Identification and profiling of targeted oxidized linoleic acid metabolites in rat plasma by quadrupole time-of-flight mass spectrometry (Q-TOFMS). Biomedical Chromatography. 2013; 27: 422–32.

Supplementary Table S1. Oxylipin nomenclature and fatty acid of origin.

Abbreviation	Cat. no.	Origin	Common name	IUPAC name
12-HETE-d8	*334570*		*(±)15-HETE-d_8*	*(5Z,8Z,10E,12S,14Z)-12-hydroxy-5,8,10,14-icosatetraenoic-5,6,8,9,11,12,14,15-d_8 acid*
12-HETE	sc-200942	ARA	12(S)-HETE	(5Z,8Z,10E,12S,14Z)-12-hydroxy-5,8,10,14-icosatetraenoic acid
20-HETE	sc-205102	ARA	20-HETE	(5Z,8Z,11Z,14Z)-20-hydroxy-5,8,11,14-icosatetraenoic acid
14(15)-DiHET-d11	*10008040*		*(±)14(15)-DiHET-d_{11}*	*(5Z,8Z,11Z)-14,15-dihydroxy-5,8,11-icosatrienoic-16,16,17,17,18,18,19,19,20,20,20-d_{11} acid*
PDX	10008128	DHA	protectin DX / 10(S),17(S)-DiHDHA	(4Z,7Z,10S,11E,13Z,15E,17S,19Z)-10,17-dihydroxy-4,7,11,13,15,19-docosahexaenoic acid
RvD1	sc-204877	DHA	resolvin D_1	(4Z,7S,8R,9E,11E,13Z,15E,17S,19Z)-7,8,17-trihydroxy-4,9,11,13,15,19-docosahexaenoic acid
RvD2	sc-351847	DHA	resolvin D_2	(4Z,7S,8E,10Z,12E,14E,16R,17S,19Z)-7,16,17-trihydroxy-4,8,10,12,14,19-docosahexaenoic acid
5-HETE-d8	*334230*		*5(S)-HETE-d_8*	*(6Z,8Z,11Z,14Z)-5S-hydroxy-6,8,11,14-icosatetraenoic-5,6,8,9,11,12,14,15-d_8 acid*
15-HETE	sc-205023	ARA	(±)15-HETE	(5Z,8Z,11Z,13E)-15-hydroxy-5,8,11,13-icosatetraenoic acid
5-HETE	sc-205136	ARA	(±)5-HETE	(6E,8Z,11Z,14Z)-5-hydroxy-6,8,11,14-icosatetraenoic acid
LXA4	sc-201060	ARA	5(S),6(R)-lipoxin A_4	(5S,6R,7E,9E,11Z,13E,15S)-5,6,15-trihydroxy-7,9,11,13-icosatetraenoic acid
LXB4	sc-205151	ARA	lipoxin B_4	(5S,6E,8Z,10E,12E,14R,15S)-5,14,15-trihydroxy-6,8,10,12-icosatetraenoic acid
8-iso-PGF2α-d4	*316350*		*8-epi $PGF_{2\alpha}$-d_4*	*(5Z,8β,9α,11α,13E,15S)-9,11,15-trihydroxyprosta-5,13-dien-1-oic-3,3,4,4-d_4 acid*
15d-PGJ2	sc-201262	ARA	15-deoxy-$\Delta^{12,14}$-PGJ_2	(5Z,12E,14E)-11-oxoprosta-5,9,12,14-tetraen-1-oic acid
2,3-dinor-8-iso-PGF2α	sc-205096	ARA	2,3-dinor-8-*iso*-$PGF_{2\alpha}$	(3Z)-5-{(1S,2R,3R,5S)-3,5-dihydroxy-2-[(1E,3R)-3-hydroxy-1-octen-1-yl]cyclopentyl}-3-pentenoic acid
8-epi-PGF2α	sc-201261	ARA	8-*iso* prostaglandin $F_{2\alpha}$	(5Z,8β,9α,11α,13E,15S)-9,11,15-trihydroxyprosta-5,13-dien-1-oic acid
PGD2	sc-201221	ARA	prostaglandin D_2	(5Z,9α,13E,15S)-9,15-dihydroxy-11-oxoprosta-5,13-dien-1-oic acid
PGD3	sc-205449	EPA	prostaglandin D_3	(5Z,9α,13E,15S,17Z)-9,15-dihydroxy-11-oxoprosta-5,13,17-trien-1-oic acid
PGE2	sc-201225	ARA	prostaglandin E_2	(5Z,11α,13E,15S)-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid

Supplementary Table S1. Cont. Oxylipin nomenclature and fatty acid of origin.

Abbreviation	Cat. no.	Origin	Common name	IUPAC name
8-iso-PGF2α-d4:				
PGF2α	sc-201227	ARA	prostaglandin $F_{2\alpha}$	(5Z,8β,9β,11β,12α,13E,15S)-9,11,15-trihydroxyprosta-5,13-dien-1-oic acid
TXB2	sc-201452	ARA	thromboxane B_2	(5Z,9β,13E,15S)-9,11,15-trihydroxythromboxa-5,13-dien-1-oic acid
9-HODE-d4	*338410*		*9(S)-HODE-d_4*	*(10E,12Z)-9S-hydroxy-10,12-octadecadienoic-9,10,12,13-d_4 acid*
12-HHT	sc-200969	ARA	12(S)-HHT / 12(S)-HHTrE	(5Z,8E,10E,12S)-12-hydroxy-5,8,10-heptadecatrienoic acid
13-HODE	sc-204991	LA	(±)13-HODE	(9E,11E)-13-hydroxy-9,11-octadecadienoic acid
15-HETrE	sc-205043	DGLA	15(S)-HETrE	(8Z,11Z,13E,15S)-15-hydroxy-8,11,13-icosatrienoic acid
9(10)-EpOME	sc-221169	LA	(±)9(10)EpOME	8-{3-[(2Z)-2-octen-1-yl]-2-oxiranyl}octanoic acid
9-HODE	sc-205184	LA	(±)9-HODE	(10E,12Z)-9-hydroxy-10,12-octadecadienoic acid
LTB4-d4	*320110*		*leukotriene B_4-d_4*	*(5S,6Z,8E,10E,12R,14Z)-5,12-dihydroxy-6,8,10,14-icosatetraenoic-6,7,14,15-d_4 acid*
LTB4	sc-201043	ARA	leukotriene B_4	(5S,6Z,8E,10E,12R,14Z)-5,12-dihydroxy-6,8,10,14-icosatetraenoic acid
RvE1-d4	*10009854*		*resolvin E_1-d_4*	*(5S,6Z,8E,10E,12R,14Z,16E,18R)-5,12,18-trihydroxy-6,8,10,14,16-icosapentaenoic-6,7,14,15-d_4 acid*
18-HEPE	sc-205067	EPA	(±)18-HEPE	(5Z,8Z,11Z,14Z,16E)-18-hydroxy-5,8,11,14,16-icosapentaenoic acid
7S-Mar1	sc-358748	DHA	7-epi maresin 1	(4Z,7S,8E,10E,12Z,14S,16Z,19Z)-7,14-dihydroxy-4,8,10,12,16,19-docosahexaenoic acid
Mar1	10878	DHA	maresin 1	(4Z,7R,8E,10E,12Z,14S,16Z,19Z)-7,14-dihydroxy-4,8,10,12,16,19-docosahexaenoic acid
Mar2	16369	DHA	maresin 2	(4Z,7Z,9E,11E,13R,14S,16Z,19Z)-13,14-dihydroxy-4,7,9,11,16,19-docosahexaenoic acid
RvE1	10007848	EPA	resolvin E_1	(5S,6Z,8E,10E,12R,14Z,16E,18R)-5,12,18-trihydroxy-6,8,10,14,16-icosapentaenoic acid

Oxylipins are sorted according to deuterated internal standard (*in* italics) used for their quantification. Abbreviations: ARA, arachidonic acid; DGLA, dihomo-γ-linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.

Supplementary Table S2 Metabolic characteristics of study patients.

	Pre-OAGB	FU1	FU2	Pre-OAGB vs FU1	Pre-OAGB vs FU2	FU1 vs FU2
hsCRP, mg/L †	0.50 (0.44-0.60)	0.58 (0.39-0.97)	0.38 (0.33-0.41)	power < 0.800		
LDL-C, mg/dL	94.2 (15.2)	106 (20.6)	90.1 (19.4)	power < 0.800		
HDL-C, mg/dL	53.6 (13.9)	55.3 (10.8)	66.1 (14.9)	0.006	0.003	0.64
Total cholesterol, mg/dL	207 (35.7)	215 (31.3)	223 (38.6)	power < 0.800		
Triglicerides, mg/dL	111 (42.1)	118 (33.4)	105 (50.5)	power < 0.800		
Glucose, mg/dL †	127 (114-135)	108 (101-117)	95.1 (81.2-102)	0.51	< 0.001	0.03
Albumin, g/dL	4.29 (0.26)	5.02 (0.53)	4.87 (0.37)	< 0.001	< 0.001	0.30
Total protein, g/dL	7.03 (0.51)	8.03 (0.95)	7.92 (0.68)	0.001	0.007	0.53

Values are mean (SD) or median (1^{st} quartile – 3^{rd} quartile) for not normally distributed values as indicated with †; p-value from RM ANOVA followed by *post-hoc* test with Holm-Sidak method (or Friedman test followed by *post hoc* Holm-Sidak test, for not normally distributed values), numerical value given if test power is > 0.800, power < 0.800 indicates that the *post hoc* test was not performed. Abbreviations: FU1, patients at 2 weeks follow-up, FU2, patients at 6-9 months follow-up; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; pre-OAGB, patients before bariatric surgery; NS, not significant.

Supplementary Table S3. MS working parameters in MRM mode.

Oxylipin	MRM transition, [a] m/z	Dt, ms	Q1, V	CE, eV	Q3, V
12-HETE-d8	*327.10 > 184.50*	*22*	*15*	*15*	*12*
	327.10 > 264.50	22	28	18	20
12-HETE	*319.20 > 179.50*	*22*	*20*	*14*	*11*
	319.20 > 257.50	22	16	15	11
20-HETE	*319.20 > 289.45*	*22*	*15*	*16*	*20*
	319.20 > 301.45	22	16	18	13
14(15)-DiHET-d11	*348.40 > 207.45*	*22*	*22*	*20*	*20*
	348.40 > 140.45	22	23	21	14
PDX	*359.40 > 153.45*	*22*	*23*	*17*	*14*
	359.40 > 206.45	22	24	17	13
RvD1	*375.30 > 141.35*	*42*	*24*	*16*	*13*
	375.30 > 215.50	42	24	19	13
RvD2	*375.20 > 175.40*	*42*	*18*	*24*	*11*
	375.20 > 141.40	42	18	17	28
5-HETE-d8	*327.20 > 309.50*	*22*	*15*	*13*	*14*
	327.20 > 116.35	22	19	16	18
15-HETE	*319.20 > 301.50*	*22*	*30*	*13*	*18*
	319.20 > 219.45	22	16	13	21
5-HETE	*319.20 > 301.50*	*22*	*12*	*15*	*10*
	319.20 > 115.25	22	12	16	14
LXA4	*351.30 > 115.30*	*42*	*23*	*20*	*22*
	351.30 > 217.50	42	23	22	14
LXB4	*351.30 > 221.40*	*27*	*22*	*17*	*14*
	351.30 > 233.45	27	22	15	15
8-iso-PGF2α-d4	*357.20 > 313.40*	*42*	*17*	*21*	*14*
	357.20 > 197.35	42	17	26	12
15d-PGJ2	*315.30 > 271.50*	*22*	*20*	*14*	*12*
	315.30 > 203.45	22	20	21	12
2,3-dinor-8-iso-PGF2α	*325.30 > 237.50*	*122*	*22*	*14*	*24*
	325.30 > 137.40	122	21	19	29
8-epi-PGF2α	*353.20 > 193.40*	*42*	*17*	*26*	*12*
	353.20 > 309.45	42	17	21	10
PGD2	*351.30 > 271.45*	*27*	*23*	*18*	*25*
	351.30 > 189.40	27	23	21	12
PGD3	*349.30 > 269.50*	*34*	*23*	*16*	*12*
	349.30 > 233.40	34	23	13	10
PGE2	*351.30 > 271.50*	*27*	*23*	*18*	*18*
	351.30 > 315.40	27	22	13	10
PGF2α	*353.30 > 309.45*	*42*	*23*	*19*	*14*
	353.30 > 193.40	42	17	26	12
TXB2	*369.30 > 169.45*	*42*	*24*	*18*	*17*
	369.30 > 195.40	42	24	15	12

Supplementary Table S3 Cont. MS working parameters in MRM mode.

Oxylipin	MRM transition, [a] m/z	Dt, ms	Q1, V	CE, eV	Q3, V
9-HODE-d4	*299.20 > 281.50*	*22*	*19*	*19*	*12*
	299.20 > 172.40	22	19	20	11
12-HHT	*279.40 > 179.40*	*22*	*18*	*14*	*12*
	279.40 > 217.50	22	19	15	21
13-HODE	*295.20 > 195.45*	*22*	*18*	*18*	*18*
	295.20 > 277.40	22	18	19	12
15-HETrE	*321.50 > 221.30*	*22*	*12*	*15*	*10*
	321.50 > 303.40	22	12	16	14
9(10)-EpOME	*295.20 > 171.40*	*22*	*15*	*14*	*12*
	295.20 > 277.40	22	16	16	16
9-HODE	*295.20 > 171.30*	*22*	*18*	*20*	*10*
	295.20 > 277.40	22	29	18	26
LTB4-d4	*339.30 > 197.45*	*22*	*22*	*17*	*12*
	339.30 > 321.50	22	12	14	15
LTB4	*335.20 > 195.40*	*22*	*21*	*16*	*12*
	335.20 > 317.50	22	21	15	21
RvE1-d4	*353.20 > 197.30*	*122*	*11*	*17*	*12*
	353.20 > 109.30	122	17	22	10
18-HEPE	*317.20 > 299.35*	*22*	*15*	*12*	*14*
	317.20 > 215.40	22	15	13	13
7S-Mar1	*359.10 > 177.45*	*13*	*10*	*17*	*11*
	359.10 > 246.35	13	30	16	20
Mar1	*359.10 > 177.45*	*22*	*10*	*17*	*11*
	359.10 > 250.30	22	10	15	17
Mar2	*359.40 > 221.40*	*22*	*12*	*12*	*10*
	359.40 > 232.30	22	14	15	23
RvE1	*349.20 > 195.30*	*97*	*10*	*17*	*12*
	349.20 > 107.25	97	17	22	15

[a] Q1>Q3 parent>daughter transitions, *in italics* – transition used for quantification.
Abbreviations: CE, collision energy; Dt, dwell time; MRM, multiple reaction monitoring; Rt, retention time; Q1, first quadrupole voltage; Q3, second quadrupole voltage. For names of the analytes please refer to Supplementary Table S1.

Supplementary Table S4. LC-MS/MS instrument parameters.

Parameter	**Value**
Interface temperature	300°C
Desolvation temperature	526°C
Desolvation line temperature	250°C
Heat block temperature	400°C
Drying gas flow	10 L/min
Nebulizing gas flow	3 L/min
Heating gas flow	10 L/min
Interface voltage	4 kV

Supplementary Table S5. Characteristics of LC-MS/MS method for oxylipin quantification.

Oksylipin	MRM for quantification, m/z	Rt,[a] min	Working range,[b] ng of spike	Calibration curve equation[c]	RSD,[d] %	R^2	MDL,[e] ng
12-HETE-d8	327.10 > 184.50	17.82					
12-HETE	319.20 > 179.50	17.89	0.1 – 25	0.1404x + 0.0063	2.01	0.9997	0.005
20-HETE	319.20 > 289.45	17.39	0.01 – 1	0.0297x – 0.0010	3.94	0.9998	0.004
14(15)-DiHET-d11	348.40 > 207.45	16.22					
PDX	359.40 > 153.45	15.17	0.01 – 0.5	0.0888x + 0.0002	1.68	0.9999	0.002
RvD1	375.30 > 141.35	11.31	0.025 – 10	0.0615x + 0.0039	3.44	0.9992	0.015
RvD2	375.20 > 175.40	10.36	0.1 – 5	0.0295x + 0.0015	2.84	0.9997	0.032
5-HETE-d8	327.20 > 309.50	18.26					
15-HETE	319.20 > 301.50	17.61	0.025 – 10	0.4121x – 0.0058	2.71	0.9999	0.014
5-HETE	319.20 > 301.50	18.31	0.025 – 25	0.2906x + 0.0004	1.18	0.9997	0.016
LXA4	351.30 > 115.30	11.39	0.01 – 1	0.6100x + 0.0015	4.25	0.9998	0.004
LXB4	351.30 > 221.40	10.08	0.025 – 10	0.2999x + 0.0039	1.56	0.9999	0.011
8-iso-PGF2α-d4	357.20 > 313.40	8.59					
15d-PGJ2	315.30 > 271.50	16.08	0.5 – 10	0.8459x – 0.0743	0.75	0.9980	0.013
2.3-dinor-8-iso-PGF2α	325.30 > 237.50	5.09	0.5 – 25	0.6476x + 0.1877	6.62	0.9969	0.394
8-epi-PGF2α	353.20 > 193.40	8.61	0.1 – 25	0.2805x + 0.0120	1.56	0.9999	0.045
PGD2	351.30 > 271.45	9.88	0.01 – 5	2.6990x + 0.0015	2.31	0.9996	0.008
PGD3	349.30 > 269.50	7.49	0.05 – 12.5	1.3457x + 0.0129	6.12	0.9984	0.036
PGE2	351.30 > 271.50	9.65	0.01 – 10	5.0227x – 0.2700	1.67	0.9998	0.003
PGF2α	353.30 > 309.45	10.54	0.1 – 10	0.7936x – 0.0131	3.50	0.9995	0.013
TXB2	369.30 > 169.45	9.59	0.1 – 10	2.9828x – 0.0819	2.39	0.9997	0.070
9-HODE-d4	299.20 > 281.50	17.40					
12-HHT	279.40 > 179.40	16.13	0.1 – 25	0.0249x + 0.0001	1.68	0.9995	0.045
13-HODE	295.20 > 195.45	17.41	0.025 – 25	0.0934x – 0.0080	1.14	0.9999	0.016
15-HETrE	321.50 > 221.30	18.14	0.00625 – 1.25	0.2085x – 0.0018	0.87	0.9999	0.009
9(10)-EpOME	295.20 > 171.40	18.12	0.025 – 10	0.0218x – 0.0002	2.96	0.9997	0.013
9-HODE	295.20 > 171.30	17.43	0.1 – 10	0.0600x + 0.0006	2.13	0.9998	0.036
LTB4-d4	339.30 > 197.45	15.55					
LTB4	335.20 > 195.40	15.58	0.025 – 25	0.0734x -0.0017	0.99	0.9999	0.009
RvE1-d4	353.20 > 197.30	5.63					
18-HEPE	317.20 > 299.35	16.92	0.05 – 2.5	0.2958x – 0.0091	3.77	0.9995	0.020
7S-Mar1	359.10 > 177.45	14.78	0.1 – 10	0.1090x – 0.0159	4.53	0.9964	0.058
Mar1	359.10 > 177.45	15.44	0.025 – 5	0.1272x – 0.0029	0.88	0.9999	0.014
Mar2	359.40 > 221.40	15.99	0.01 – 5	0.3075x – 0.0097	1.75	0.9999	0.017
RvE1	349.20 > 195.30	5.70	0.025 – 5	0.2530x + 0.0012	1.03	0.9998	0.012

[a] Retention time drift was < 0.05 min.

[b] Working range from lower MQL (S/N > 10) to highest concentration for which CV was < 15%.

[c] Linear regression equation from three independently prepared concentrations of seven-point calibration curve that were analyzed in triplicate. [d] Relative standard deviation of the curve.

[e] Method detection limit calculated from calibration curve.

Abbreviations: MDL, method detection limit; MRM, multiple reaction monitoring; RSD, relative standard deviation; Rt, retention time; For oxylipin names please refer to Supplementary Table S1.

Supplementary Table S6. Intraday and interday accuracy, precision and extraction efficiency

Oxylipin	Spike concentration level I [a]						Spike concentration level II [b]					
	Interday [c]			Intraday [d]			Interday [c]			Intraday [d]		
	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]
12-HETE	106.7	14.4	85.8 (3.7)	101.3	0.2	98.5 (0.4)	101.1	10.0	100.9 (12.2)	100.6	5.9	101.3 (4.9)
20-HETE	106.4	13.5	95.9 (12.2)	98.7	5.8	97.6 (1.4)	outside of the calibration range					
PDX	97.8	12.3	102.1 (9.5)	104.4	7.9	96.0 (8.8)	outside of the calibration range					
RvD1	96.6	8.8	93.6 (7.3)	89.4	9.2	101.0 (3.6)	113.2	10.6	92.0 (6.9)	107.4	3.9	97.9 (6.8)
RvD2	117.9	14.4	81.3 (0.1)	97.2	2.8	101.8 (6.9)	120.8	11.8	82.7 (6.3)	100.4	2.5	100.4 (8.8)
15-HETE	101.1	5.6	98.8 (12.4)	107.8	2.8	88.7 (2.7)	100.2	7.1	99.0 (7.2)	108.0	1.7	95.0 (4.0)
5-HETE	101.4	9.8	93.8 (3.0)	106.0	5.8	97.6 (7.6)	104.2	5.2	97.9 (5.2)	98.8	2.7	105.1 (5.2)
LXA4	90.0	13.3	109.2 (13.1)	98.7	4.5	101.2 (9.3)	outside of the calibration range					
LXB4	93.2	12.7	113.3 (16.6)	104.0	4.9	98.1 (7.7)	97.6	8.9	100.7 (13.2)	104.9	2.0	94.7 (4.8)
15d-PGJ2	138.7	8.9	119.9 (11.0)	169.3	5.3	109.5 (9.9)	118.0	13.1	79.1 (27.3)	143.0	3.8	94.4 (12.9)
2,3-dinor-8-iso-PGF2α	42.6	14.9	60.6 (8.8)	63.8	4.7	72.9 (1.4)	96.2	11.2	61.5 (10.1)	93.7	4.2	65.8 (1.7)
8-epi-PGF2α	104.0	10.6	98.8 (2.9)	124.6	6.9	93.5 (5.0)	102.5	15.5	99.9 (10.8)	104.2	2.1	89.7 (5.1)
PGD2	89.5	16.3	106.2 (7.5)	96.3	8.2	96.6 (9.0)	84.4	22.9	118.2 (9.4)	97.2	6.9	114.6 (2.2)
PGD3	104.3	42.3	94.5 (9.2)	102.9	1.0	81.5 (1.8)	111.2	33.6	82.4 (12.0)	106.6	5.4	94.9 (1.4)
PGE2	92.8	11.7	100.7 (5.1)	105.7	6.2	95.9 (6.9)	86.2	11.2	114.2 (4.6)	86.2	6.7	112.0 (6.6)
PGF2α	95.3	17.5	92.1 (2.0)	99.3	0.2	106.2 (6.3)	94.0	26.5	102.7 (8.3)	86.8	1.3	114.1 (3.6)
TXB2	90.0	4.2	110.7 (0.2)	106.5	5.3	110.4 (2.5)	91.1	9.0	107.2 (13.6)	96.2	3.7	94.8 (2.0)

Supplementary Table S6 Cont. Intraday and interday accuracy, precision and extraction efficiency

Oxylipin	Spike concentration level I [a]						Spike concentration level II [b]					
	Interday [c]			Intraday [d]			Interday [c]			Intraday [d]		
	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]	Accuracy, % [e]	Precision, % [f]	EE, % [g]
12-HHT	108.7	11.6	100.6 (3.6)	115.9	4.2	100.8 (11.9)	102.0	15.9	100.2 (6.0)	109.3	10.5	106.8 (3.9)
13-HODE	105.1	19.7	97.0 (27.6)	104.8	9.7	105.1 (6.2)	98.4	4.4	102.2 (8.3)	106.4	3.6	103.3 (4.4)
15-HETrE	102.7	6.2	93.4 (3.8)	95.1	1.3	97.9 (6.5)	99.2	15.6	100.9 (2.7)	102.4	4.6	98.2 (9.5)
9(10)-EpOME	99.6	16.2	77.2 (11.9)	104.4	14.4	88.5 (5.3)	103.9	11.6	88.6 (11.3)	104.7	1.3	97.3 (7.4)
9-HODE	79.8	17.4	124.7 (35.8)	111.4	1.0	78.8 (1.8)	93.3	6.8	107.1 (8.5)	97.0	7.9	103.3 (8.4)
LTB4	97.4	10.4	101.2 (11.0)	98.8	6.1	90.7 (3.1)	100.9	7.4	97.1 (4.4)	105.3	6.9	92.5 (2.6)
18-HEPE	118.7	4.2	91.2 (8.3)	99.7	12.9	88.4 (12.5)	103.5	11.6	96.8 (6.7)	97.3	8.6	85.2 (15.6)
7S-Mar1	121.5	9.7	95.1 (6.1)	111.3	6.5	105.2 (13.4)	96.9	6.9	94.2 (6.4)	107.8	6.6	100.2 (2.4)
Mar1	105.7	15.4	91.6 (8.5)	96.7	8.8	101.6 (7.7)	103.3	13.2	96.9 (1.5)	104.5	9.3	96.0 (10.1)
Mar2	102.0	6.5	97.1 (6.1)	106.0	5.2	92.2 (4.0)	102.5	10.0	97.7 (6.9)	102.6	2.1	97.9 (2.9)
RvE1	99.0	2.9	96.2 (4.6)	98.9	3.7	93.4 (4.1)	98.6	3.4	99.9 (6.4)	98.7	0.2	98.2 (2.0)

[a] Spike of 0.5 ng of analytes standards (for 15-HETrE: 0.125 ng; for PGD3 and 18-HEPE: 0.25 ng) into 300 µL of serum.
[b] Spike of 5 ng of analytes standard mixture (for 15-HETrE: 1.25 ng; for PGD3 and 18-HEPE: 2.5 ng).
[c] Calculated form triplicate sample runs on three consecutive days.
[d] Calculated from three runs performed on the same day.
[e] Expressed as a ratio of calculated concentration to known concentration.
[f] Expressed as coefficient of variance for three independent samples.
[g] Calculated as ratio of relative peak area of analyte added to serum before SPE to the relative peak area of analytes added to serum after SPE, including correction for blank serum, mean (SD).
Abbreviations: EE, extraction efficiency; for oxylipin names please refer to Supplementary Table S1.

Supplementary Table S7. Results of quantification of oxylipins in serum of bariatric patients.

	Origin	Pre-OAGB (n = 15)			FU1 (n = 15)			FU2 (n = 15)			p-value [a]		
		> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	Pre-OAGB vs FU1	Pre-OAGB vs FU2	FU1 vs FU2
12-HETE	ARA	10	10	32.4 (11.0)	14	14	27.5 (3.90)	12	12	20.0 (3.92)	power < 0.800		
20-HETE	ARA	9	9	2.71 (0.300)	12	12	3.79 (0.367)	11	11	2.71 (0.213)	0.02	0.38	0.003
PDX†	DHA	1	0	< LOQ	1	0	< LOQ	1	0	< LOQ	not calculated		
RvD1	DHA	0	0	ND	0	0	ND	0	0	ND	-	-	-
RvD2	DHA	0	0	ND	0	0	ND	0	0	ND	-	-	-
15-HETE	ARA	12	11	1.46 (0.187)	14	14	1.76 (0.214)	13	13	1.25 (0.128)	power < 0.800		
5-HETE	ARA	15	11	1.32 (0.313)	14	14	2.49 (0.318)	14	13	1.45 (0.243)	0.002	0.27	0.03
LXA4†	ARA	1	0	< LOQ	0	0	ND	0	0	ND	power < 0.800		
LXB4†	ARA	0	0	ND	0	0	ND	0	0	ND	-	-	-
15d-PGJ2	ARA	13	2	6.12 (0.239)	0	0	ND	7	0	< LOQ	< 0.001	< 0.001	0.53
2,3-dinor-8-iso-PGF2α	ARA	0	0	ND	0	0	ND	0	0	ND	-	-	-
8-epi-PGF2α	ARA	0	0	ND	0	0	ND	0	0	ND	-	-	-
PGD2†	ARA	0	0	ND	7	1	< LOQ	10	1	< LOQ	0.02	0.13	1.00
PGD3	EPA	0	0	ND	0	0	ND	0	0	ND	-	-	-
PGE2	ARA	3	0	< LOQ	8	0	< LOQ	15	1	< LOQ	not calculated		
PGF2α†	ARA	0	0	ND	0	0	ND	3	0	< LOQ	1.00	0.69	0.69
TXB2	ARA	5	3	< LOQ	14	13	2.55 (0.488)	13	13	2.44 (0.398)	< 0.001	0.001	0.82
12-HHT	ARA	4	4	4.07 (0.107)	12	12	10.9 (1.49)	14	14	14.6 (2.90)	0.02	< 0.001	0.14
15-HETrE	DGLA	13	13	0.513 (0.045)	13	13	0.383 (0.024)	14	14	0.329 (0.028)	0.096	0.004	0.13
13-HODE	LA	13	13	18.6 (1.79)	14	14	13.0 (1.14)	15	15	18.4 (2.12)	power < 0.800		
9(10)-EpOME	LA	11	11	1.54 (0.332)	11	9	0.969 (0.121)	10	9	1.26 (0.270)	power < 0.800		
9-HODE	LA	13	13	12.2 (0.831)	14	14	10.2 (0.703)	14	14	11.4 (1.18)	power < 0.800		
LTB4	ARA	9	4	0.822 (0.151)	10	10	1.11 (0.254)	12	11	0.637 (0.071)	power < 0.800		

Supplementary Table S7 Cont. Results of quantification of oxylipins in serum of bariatric patients

	Origin	pre-OAGB (n = 15)			FU1 (n = 15)			FU2 (n = 15)			p-value [A]		
		> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	> MDL [b]	> LMQL [c]	Concentration, [d] nmol/L	pre-OAGB vs FU1	pre-OAGB vs FU2	FU1 vs FU2
18-HEPE	EPA	8	6	1.55 (0.106)	9	8	1.87 (0.289)	10	10	1.25 (0.175)	power < 0.800		
7S-Mar1	DHA	0	0	ND	0	0	ND	0	0	ND	-	-	-
Mar1	DHA	0	0	ND	0	0	ND	0	0	ND	-	-	-
Mar2	DHA	0	0	ND	0	0	ND	0	0	ND	-	-	-
RvE1	EPA	0	0	ND	0	0	ND	0	0	ND	-	-	-

[a] p-value from RM ANOVA followed by *post-hoc* test with Holm-Sidak method or Friedman test on ranks followed by *post-hoc* Holm-Sidak test for not normally distributed variables (indicated with †) or with unequal variance. Numerical value given if test power is > 0.800. Power < 0.800 indicated that the statistical test lacked sufficient power to calculate the p-value. Not calculated means that the differences in median or mean values among groups are not great enough to ensure that the difference is not to the random sampling variability, therefore the *post hoc* test was not performed. Dashes indicated that the comparisons were not performed due to not detecting the analyte in the sample.

[b] Number of samples above limit of detection calculated from calibration curve.

[c] Number of samples in which quantification was possible.

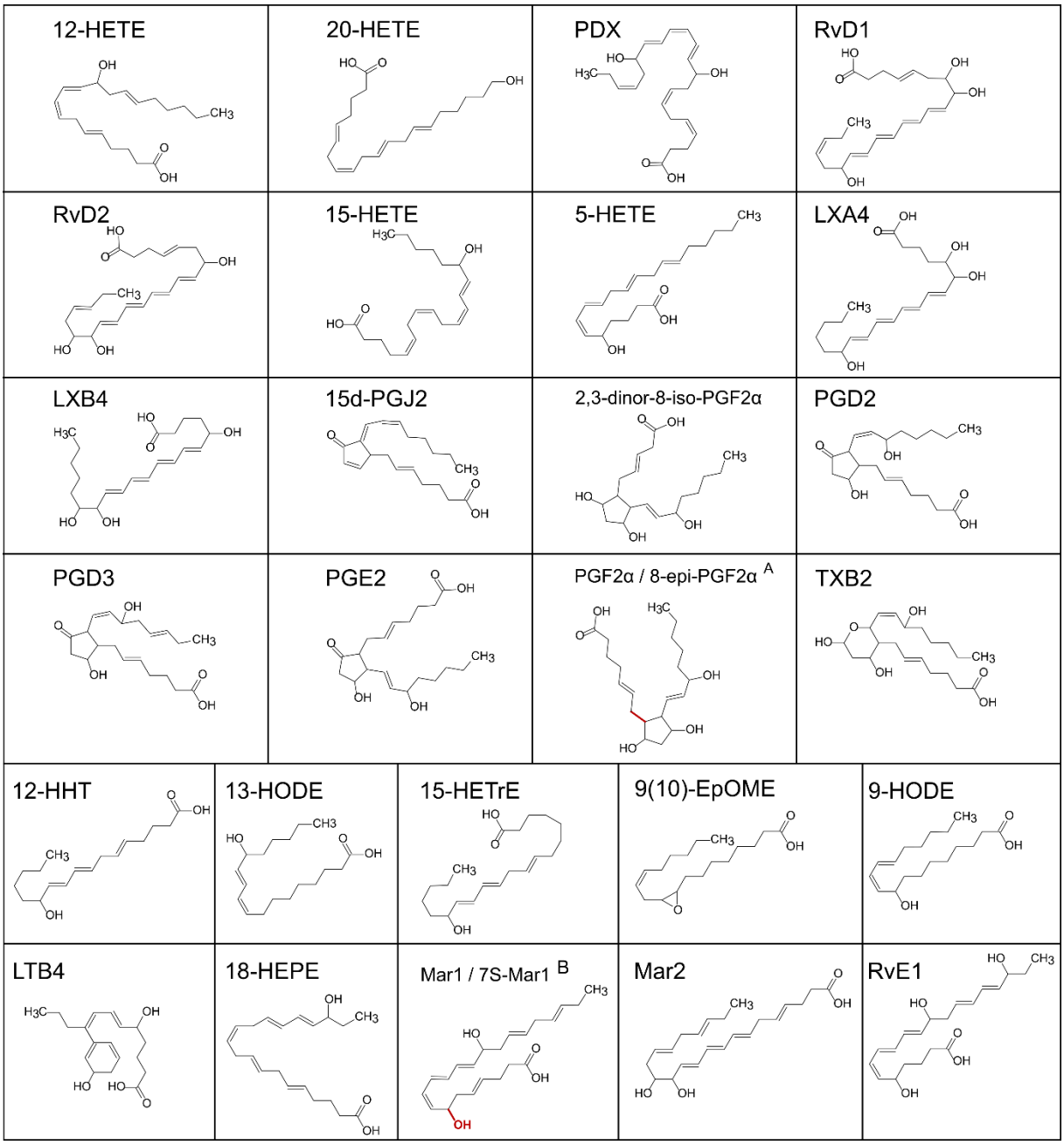
[d] Mean (SD) for samples above lower MQL, median (interquartile range) for variables without normal distribution, when appropriate (indicated with †).

Abbreviations: LMQL, lower limit of method quantification; MLD, method's limit of detection; ND, not detected. For names of the analytes please refer to Supplementary Table S1.

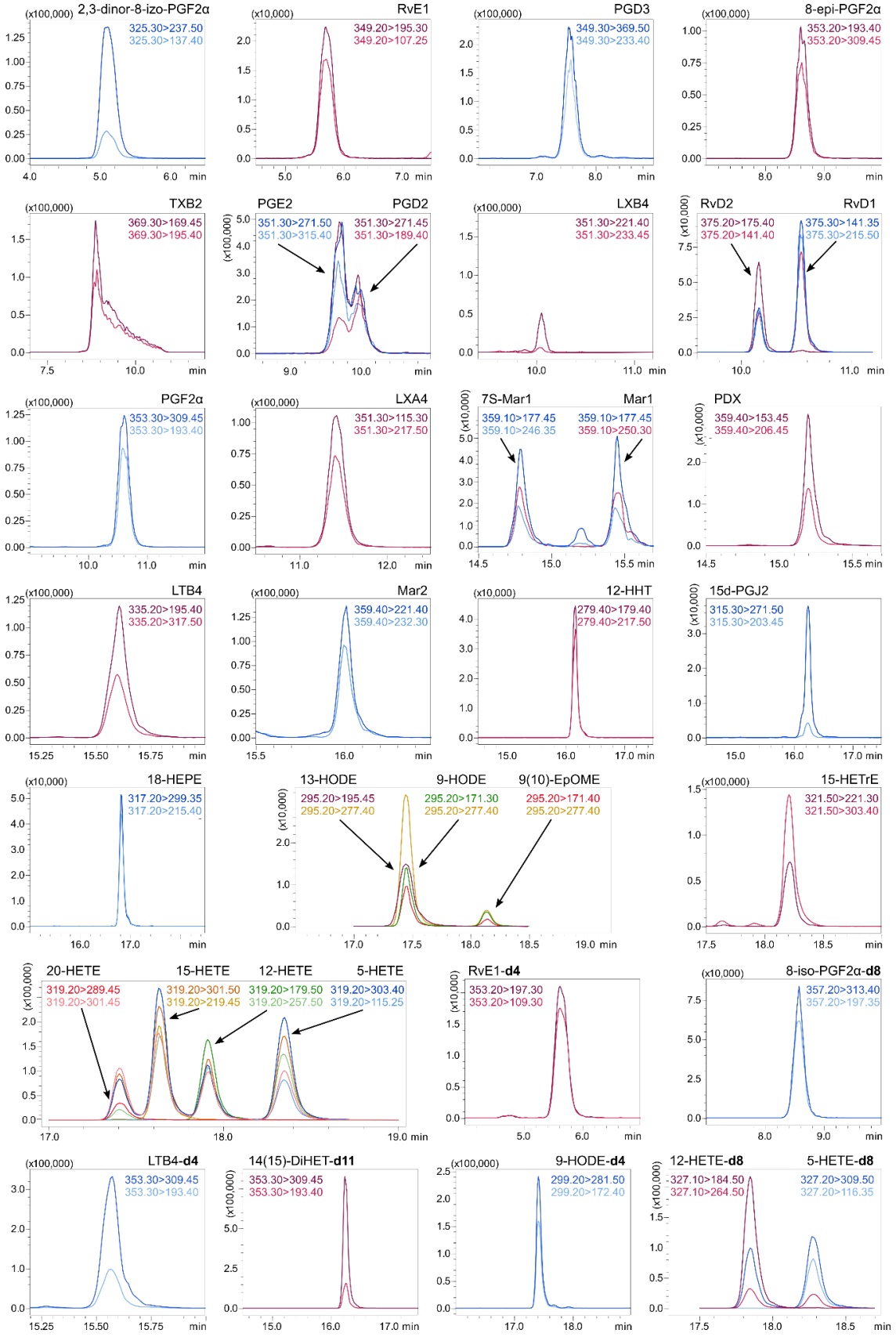
Supplementary Table S8. Correlations between oxylipin concentration and CRP and weight loss parameters.

Oxylipin	hsCRP † (n = 45)		%TWL [a] (n=30)		%EWL [b] (n=30)	
	r	p		p		p
12-HETE	-0.212	0.23	-0.324	0.11	-0.204	0.32
12-HHT	-0.125	0.45	0.217	0.28	0.320	0.10
13-HODE	-0.303*	0.047	0.360	0.05	0.294	0.12
15-HETrE	0.072	0.67	-0.345	0.08	-0.428*	0.03
20-HETE	0.351	0,03	-0.517*	0.004	-0.549*	0.002
5-HETE	0.245	0.12	-0.513*	0.005	-0.427*	0.024
9(10)-EpOME	-0.130	0.41	0.269	0.15	0.281*	0.13
9-HODE	-0.116	0.48	0.094	0.64	0.141	0.47
TXB2	0.051	0.75	0.020	0,92	0.159	0.43
LTB4	-0.035	0.83	-0.056	0,78	0.030	0.88
18-HEPE	-0.161	0.32	-0.134	0.49	-0.171	0.38

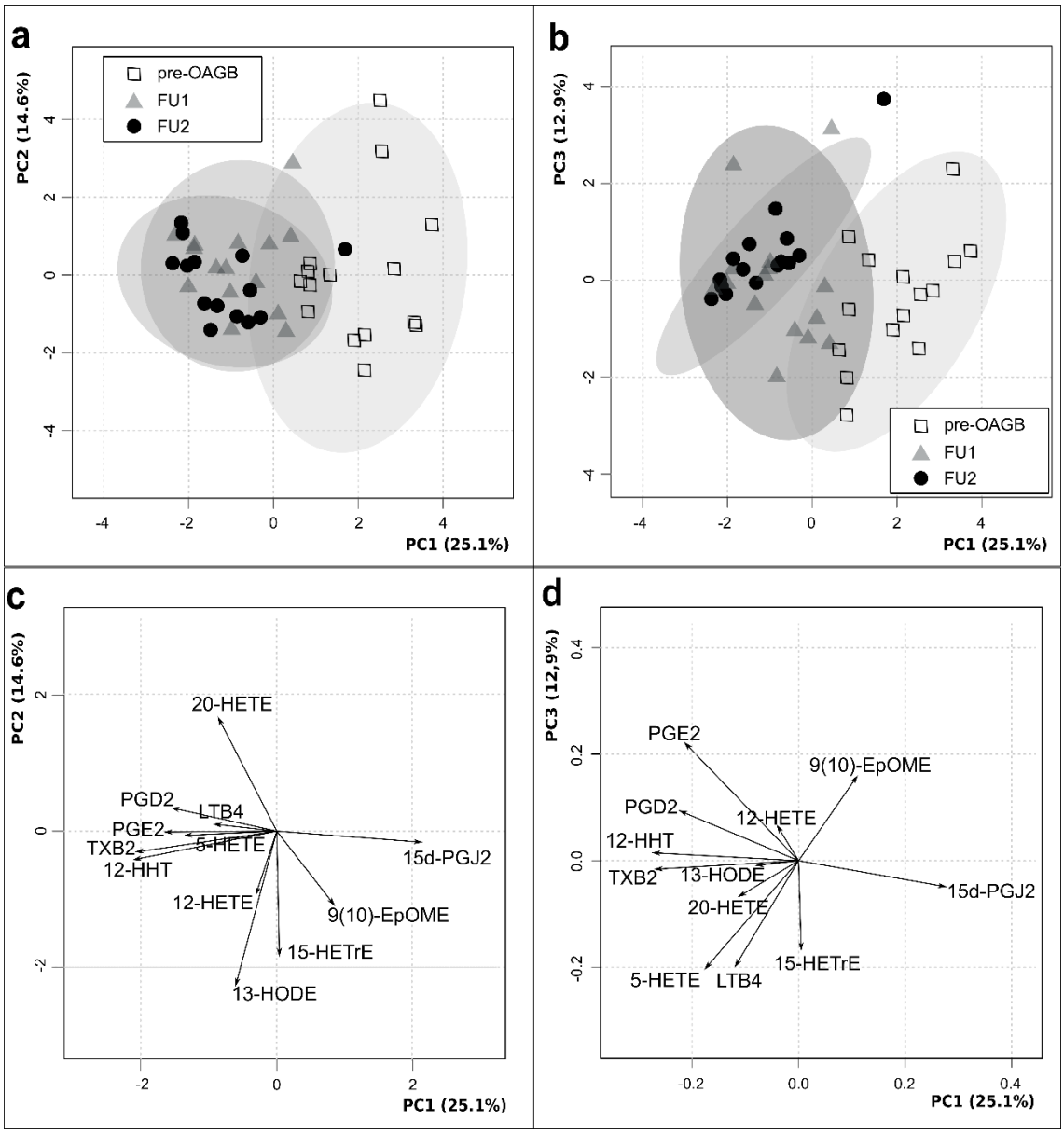
[a] Percentage total weight loss [(initial weight – current weight) / (initial weight)] * 100.
[b] Percentage excess weight loss [(initial weight – current weight) / (initial weight – ideal weight)] * 100, where ideal weight for each patient was weigh at which BMI was 25 kg/m^2.
Coefficient values from Pearson correlation or Spearman correlation for not normally distributed variables as indicated with †.
Oxylipins were included in the analysis only if > 50% of serum samples were > MQL, and values below MQL were substituted for extrapolated constants, for details on number of samples refer to Supplementary Table S7.



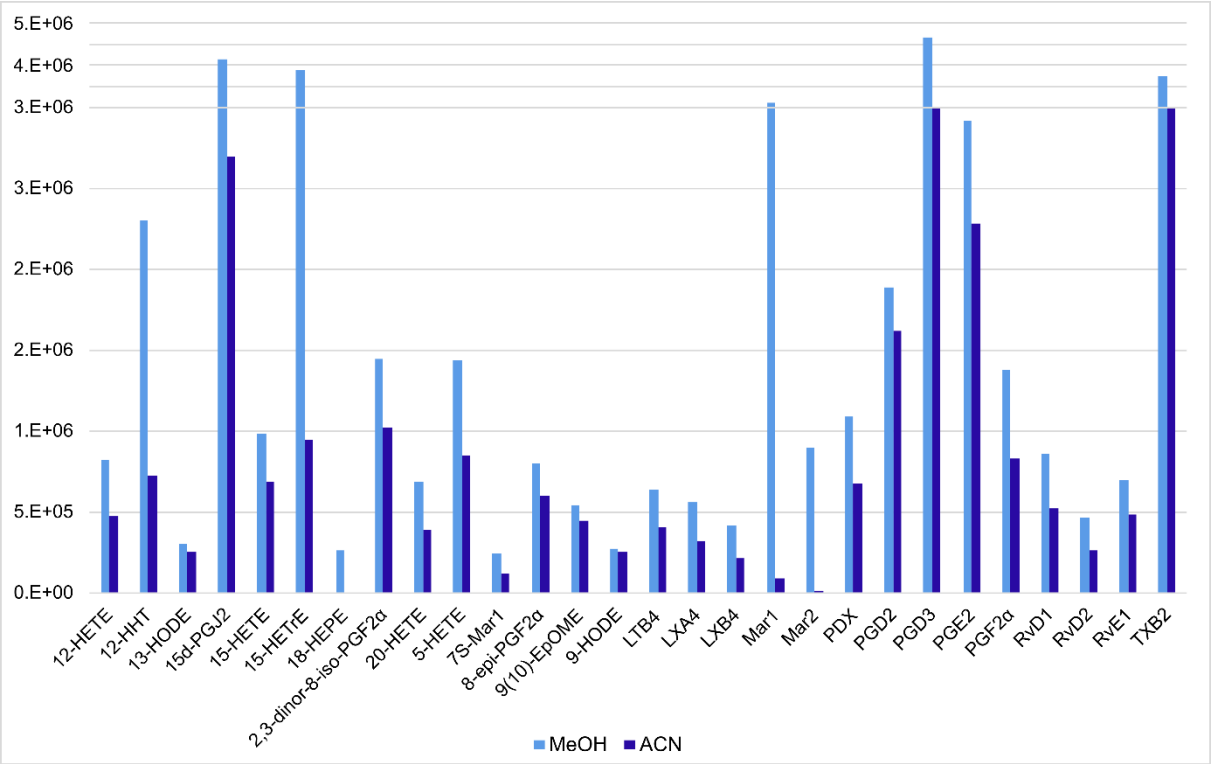
Supplementary Figure S1. Skeletal formulas of analytes.
A - 8-epi-PGF2α is a PGF2α isomer with inverted stereochemistry at the 8-position (in red);
B – Mar1 and 7S-Mar1 are epimers with differing stereochemistry at carbon with hydroxyl group. For names of oxylipins please refer to Table S1.



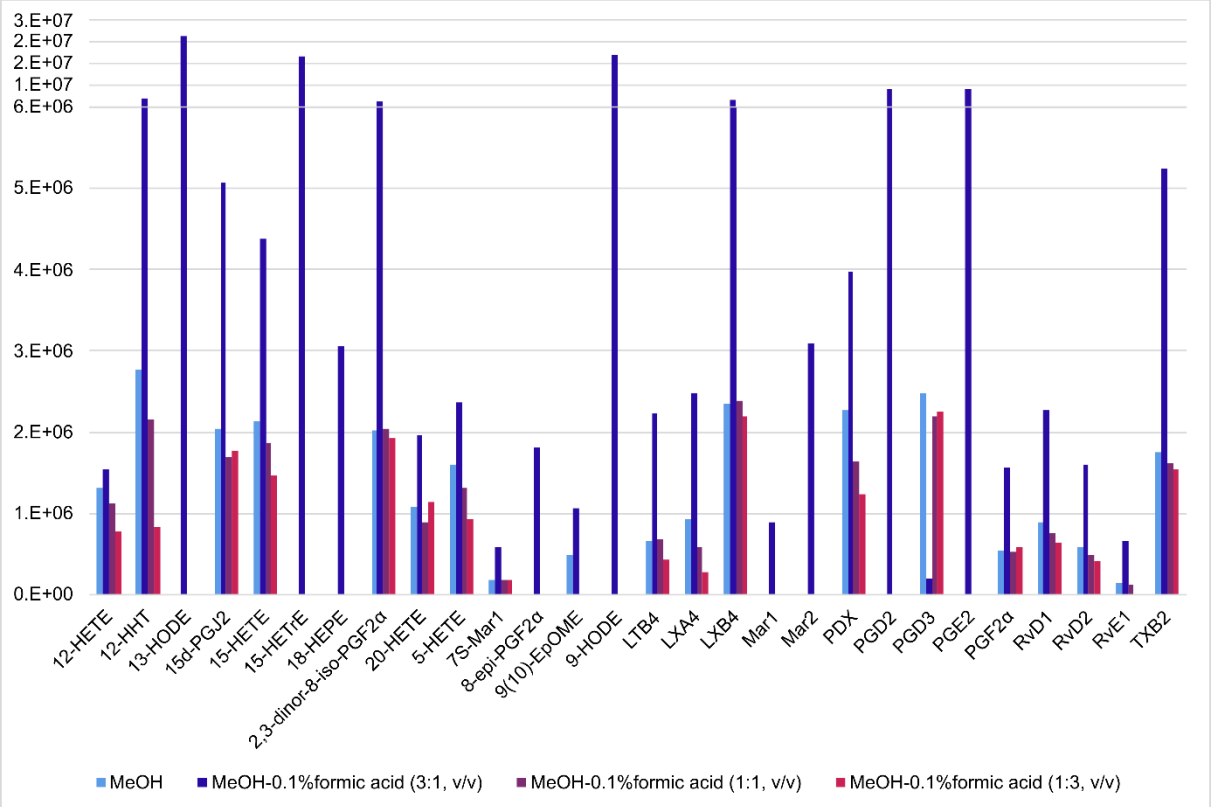
Supplementary Figure S2. Chromatograms of oxylipins obtained in MRM mode. For names of the analytes please refer to Supplementary Table S1. Axis X – retention time, axis Y – signal intensity.



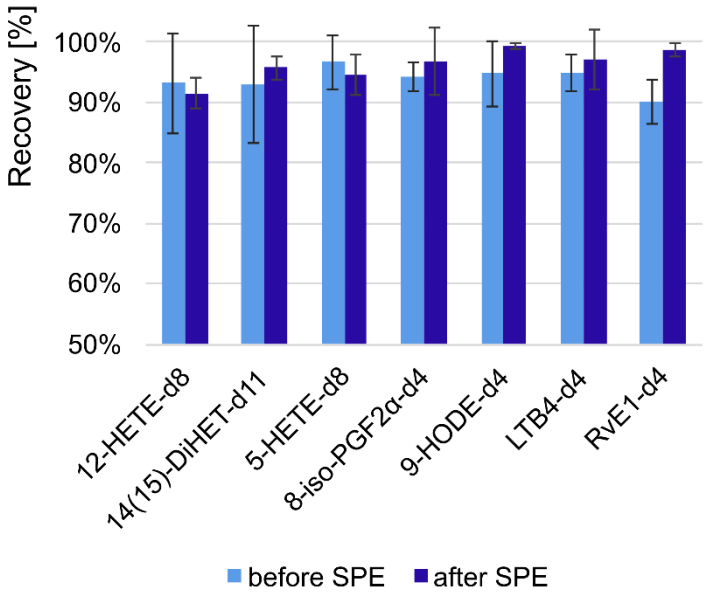
Supplementary Figure S3. The results of PCA bariatric patients before (pre-OAGB), 2 weeks after (FU1) and 6-9 months after (FU2) OAGB surgery, based on oxylipins concentrations Score plot of cases along axes of PC1 and PC2 (**a**) PC1 and PC3 (**b**), and corresponding variables plots (**c** and **d** respectively). Oxylipins were calculated in the analysis only if > 50% of serum samples were > MDL, and values below MQL were substituted for extrapolated constants, for details on number of samples refer to Supplementary Table S7.



Supplementary Figure S4. Relative peak intensities of oxylipin standards depending on phase B composition. For names of the analytes please refer to Supplementary Table S1.



Supplementary Figure S5. Relative peak intensities of oxylipin standards injected in different solvent compositions. For names of the analytes please refer to Supplementary Table S1.



Supplementary Figure S6. Recovery of deuterated internal standards (IS) used for quantification of oxylipins.

IS recovery was a ratio between peak intensity in samples where IS were added directly to serum (before SPE) to peak intensity in samples where IS were added to the SPE eluent. The recovery was determined at one level – addition of 10 ng of each IS to sample in three independent replicates. For names of the analytes please refer to Supplementary Table S1.