Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Material

Intra-individual variance of human plasma oxylipin pattern: Low inter-day variability in fasting blood samples versus high variability during the day

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Study B

Details on the study population

Participants were recruited from the general population in Hannover, Germany by advertisements. Subjects were pre-selected via screening questionnaires according to the following inclusion criteria: Male sex, age between 20 and 40 years, body mass index (BMI) between 20 and 27 kg/m², mixed diet with low meat and fish consumption. Exclusion criteria were defined as followed: Smoking, serum triglyceride (TG) levels ≥150 mg/dl (≥1.7 mmol/l); serum total cholesterol levels ≥200 mg/dl (≥5.2 mmol/l); a relative amount of ΣEPA+DHA in red blood cells ≤3 and ≥6%, intake of fish (>2 times per week) as well as addiction to alcohol, drugs and/or medications and diseases: chronic diseases (e.g. malignant tumors, manifest cardiovascular disease, insulin-dependent type 1 and 2 diabetes, severe renal or liver diseases); chronic gastrointestinal disorders (especially small intestine, pancreas, liver) as well as prior gastrointestinal surgical procedures (e.g. gastrectomy); hormonal disorders (e.g. Cushing´s syndrome and untreated hyperthyroidism); uncontrolled hypertension; blood coagulation disorders and intake of coagulation-inhibiting drugs; periodic intake of laxatives; intake of anti-inflammatory drugs (incl. acetylsalicylic acid); intake of lipid lowering drugs or supplements during the last 3 months before baseline examination. Inclusion and exclusion criteria were assessed via questionnaires. The pre-selected subjects were invited to a screening examination to collect fasting blood for the analysis of serum lipid levels, liver enzymes and fatty acid patterns in blood cells.

Pre-screening

Subjects evaluated eligible based on a screening questionnaire (including inclusion and exclusion criteria) were invited to the screening examination 3 weeks before the start of the run-in period. Fasting blood was drawn from the subjects to determine the fatty acid composition in red blood cells, serum triglyceride and serum total cholesterol levels.

Serum triglyceride and total cholesterol were analyzed in the LADR laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V.), Hannover, Germany. For analysis of fatty acids in blood cells, the cell sediment after centrifugation and removal of plasma was reconstituted in PBS to the initial blood volume, transferred into 1.5 mL Eppendorf tubes and immediately frozen and stored at -80 °C until extraction and analysis. Lipids were extracted from 50 µL diluted blood cells using MTBE/MeOH and concentrations of fatty acids were determined by by means of gas chromatography with flame ionization detection (GC-FID) following (trans-)esterification to fatty acid methy esters (FAMEs) as described (1) using methyl pentacosanoate (C25:0 methyl ester) as internal standard (IS) for quantification.

(1) Ostermann, A.I., Müller, M., Willenberg, I., and Schebb, N.H. (2014). Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters using gas chromatography – a comparison of different derivatization and extraction procedures. Prostaglandins Leukot. Essent. Fat. Acids PLEFA 91, 235–241.

Table S1: Daily energy, macronutrient and fatty acid intake of study participants from the study on inter-day and intra-day variation of free oxylipins in plasma on a standardized diet (Study B) during the whole period of the standardized nutrition **(A)** and energy, macronutrient and fatty acid intake of meals from t0 to t24 (Day 1) of the standardized nutrition **(B)**.

A)	**Day 1**		**Day 2**		**Day 3**	
Portion size	**small**	**large**	**small**	**large**	**small**	**large**
Energy intake (kcal)[a]	2924	3152	2687	2946	2907	3179
Carbohydrates (g)[a]	337	378	321	375	335	375
Protein (g)[a]	122	128	103	110	125	136
Total fat intake (g)[a]	82.0	82.3	106	108	85.33	85.32
SFA (g)[a]	37.06	35.31	40.88	38.55	38.50	37.34
MUFA (g)[a]	17.49	16.64	20.87	20.06	18.05	17.67
PUFA (g)[a]	3.98	4.00	10.20	10.16	4.86	5.32
LA (g)[b]	3.25	3.27	9.49	9.45	4.00	4.36
ALA (g)[b]	0.53	0.52	0.51	0.49	0.67	0.74
ARA (g)[b]	0.10	0.11	0.12	0.12	0.11	0.11
EPA (g)[b]	0.03	0.03	0.03	0.03	0.03	0.03
DPAn3 (g)[b]	< 0.01	0.02	< 0.01	0.02	0.01	0.02
DHA (g)[b]	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Levels are shown at day 1, 2 and 3 of standardized nutrition for small and large portion size.

B)	**Breakfast**	**Lunch**	**Snack**		**Dinner**	
Portion size	**small / large**	**small / large**	**small**	**large**	**small**	**large**
Energy intake (kcal)[a]	900	919	223	319	882	1014
Carbohydrates (g)[a]	95.9	125	33.3	48.1	83.3	109
Protein (g)[a]	34.8	37.7	8.00	10.0	41.2	45.3
Total fat intake (g)[a]	36.6	6.80	3.78	4.10	34.9	35.1
SFA (g)[a]	17.82	2.71	2.16		14.37	12.62
MUFA (g)[a]	8.68	1.24	1.07		6.49	5.64
PUFA (g)[a]	1.86	0.33	0.12		1.67	1.69
LA (g)[b]	1.54	0.24	0.09		1.38	1.40
ALA (g)[b]	0.22	0.07	0.02		0.21	0.20
ARA (g)[b]	0.05	0.01	< 0.01		0.04	0.04
EPA (g)[b]	0.02	< 0.01	< 0.01		0.01	0.01
DPAn3 (g)[b]	< 0.01	< 0.01	< 0.01		< 0.01	0.01
DHA (g)[b]	< 0.01	< 0.01	< 0.01		< 0.01	< 0.01

Levels are shown for breakfast, lunch, snack and dinner of standardized nutrition for small and large portion size.

ARA: arachidonic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPAn3: docosapentaenoic acid; LA: linoleic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

[a] Energy, carbohydrate and protein intake were calculated with PRODI®

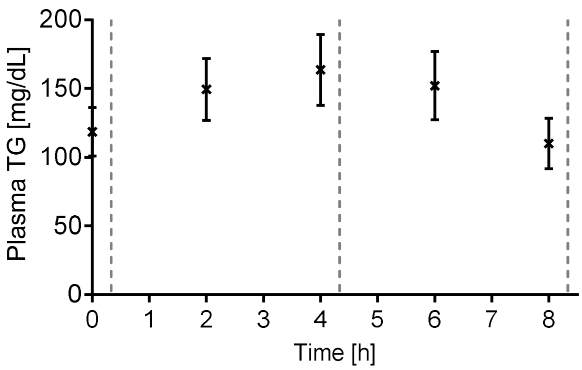
[b] Total fat, SFA, MUFA and PUFA LA, ALA, AA, EPA, DPAn3 and DHA intake were calculated from own analyses of meals that were provided by the Institute of Food Science and Human Nutrition

Table S2: Baseline clinical, biochemical and anthropometric parameters of study participants from the study on inter-day and intra-day variation of free oxylipins in plasma on a standardized diet (Study B). Shown are mean ± SD (n=13).

	mean	±	SD
Age (years)	24.6	±	2.47
Weight (kg)	83.6	±	9.10
BMI (kg/m^2)	24.6	±	2.02
Sys BP (mmHg)	132	±	19.9
Dias BP (mmHg)	81.5	±	9.13
AST (U/l)	26.7	±	7.35
ALT (U/l)	23.7	±	15.3
GGT (U/l)	21.5	±	8.52
TC (mg/dl)	177	±	43.1
HDL (mg/dl)	50.2	±	8.69
LDL (mg/dl)	111	±	33.5
TG (mg/dl)	136	±	67.5

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: body mass index; dias BP: diastolic blood pressure; GGT: Gamma-glutamyl transpeptidase; HDL: high density lipoprotein; LDL: low density lipoprotein; SD: standard deviation; sys BP: systolic blood pressure; TC: total cholesterol; TG: triglycerides.

A)



B)

Individual	Plasma TG [mg/dL] t0	t2	t4	t6	t8
1	74	124	116	103	91
2	48	62	78	67	52
3	91	109	125	147	89
4	68	68	78	92	69
5	198	308	368	240	185
6	85	99	122	86	74
7	151	163	194	159	131
8	66	80	107	94	62
9	131	151	199	136	90
10	219	277	229	296	215
11	86	128	117	120	53
12	83	111	79	83	65
13	240	261	317	353	256

Figure S1: Plasma triglyceride levels of study participants from the study on inter-day and intra-day variation of free oxylipins in plasma on a standardized diet (Study B). Shown are mean ± SEM (n=13) **(A)** and individual values **(B)**. Dotted lines in the diagram indicate food intake (20 min post sample collection at t0, t4 and t8).

Table S3: List of analytes included in LC-MS analysis and data evaluation in both studies. Shown are the analyte name and the LLOQ in plasma (2).

Analyte	LLOQ+	Study A	Study B
20-OH-PGE_2	0.025	Y	Y
ent-16(*R*,*S*)-13-*epi*-ST-Δ^{14}-9-PhytoF 1	0.024	N	Y
ent-16(*R*,*S*)-13-*epi*-ST-Δ^{14}-9-PhytoF 2	0.026	N	Y
ent-16-*epi*-16-F_{1t}-PhytoP	0.050	N	Y
ent-16-F_{1t}-PhytoP	0.10	N	Y
ent-9-F_{1t}-PhytoP	0.025	N	Y
ent-9-*epi*-9-F_{1t}-PhytoP	0.050	N	Y
Δ^{17}-6-keto-$PGF_{1\alpha}$	0.10	Y	Y
2,3-dinor-TxB_1	0.50	Y	Y
15(*R*,*S*)-2,3-dinor-15-F_{2t}-IsoP	0.050	N	Y*
2,3-dinor-TxB_2	0.10	Y	Y
6-keto-$PGF_{1\alpha}$	0.18	Y	Y
8-F_{3t}-IsoP	0.10	N	Y
8-*epi*-8-F_{3t}-IsoP	0.10	N	Y
RvE1	0.12	Y	Y
20-COOH-LTB_4	0.10	Y	Y
TxB_3	0.025	Y	Y
20-OH-LTB_4	0.025	Y	Y
5(*R*,*S*)-5-F_{3t}-IsoP	0.20	N	Y
13,14-dihydro-15-keto-tetranor-PGE_2	0.025	Y	Y
TxB_1	0.050	Y	Y
15-F_{2t}-IsoP (8-*iso*-$PGF_{2\alpha}$)	0.050	Y	Y
TxB_2	0.13	Y	Y
11-dehydro-TxB_3	0.10	Y	Y
PGE_3	0.030	Y	Y
11β-$PGF_{2\alpha}$	0.050	Y	Y
10-F_{4t}-NeuroP	0.050	N	Y
10-*epi*-10-F_{4t}-NeuroP	0.10	N	Y
5(*R,S*)-5-F_{2t}-IsoP (5-$iPF_{2\alpha}$-VI)	0.050	Y	Y
PGD_3	0.10	Y	Y
16-B_1-PhytoP	0.025	N	Y
9-L_1-PhytoP	0.025	N	Y
$PGF_{2\alpha}$	0.070	Y*	Y
14(*R*,*S*)-14-F_{4t}-NeuroP	2.0	N	Y
$PGF_{1\alpha}$	0.025	Y	Y
PGE_2	0.025	Y	Y
11-dehydro-TxB_2	0.050	Y	Y
PGE_1	0.033	Y	Y*
4(*R*,*S*)-4-F_{4t}-NeuroP	0.10	N	Y
PGD_1	0.05	Y	Y
PGD_2	0.10	Y	Y
15-keto-$PGF_{1\alpha}$	0.025	Y	Y
4(*R*,*S*)-ST-Δ^5-8-NeuroF	4.0	N	Y
17(*R*,*S*)-17-F_{2t}-dihomo-IsoP 1	0.13	N	Y
17(*R*,*S*)-17-F_{2t}-dihomo-IsoP 2	0.075	N	Y
7(*R*,*S*)-ST-Δ^8-11-dihomo-IsoF	0.20	N	Y
ent-7(*R*,*S*)-7-F_{2t}-dihomo-IsoP	0.050	N	Y
11,12,15-TriHETrE	0.050	Y	Y
LXA_4	0.018	Y	Y
RvD1	0.025	Y	Y
13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.050	Y	Y
13,14-dihydro-15-keto-PGE_1	0.050	Y	Y

Analyte	LLOQ+	Study A	Study B
dihomo-$PGF_{2\alpha}$	0.010	Y	Y
4(*R,S*)-4-F_{3t}-$NeuroP_{n6}$	0.10	N	Y
17(*R*,*S*)-10-*epi*-SC-Δ^{15}-11-dihomo-IsoF 1	0.10	N	Y
17(*R*,*S*)-10-*epi*-SC-Δ^{15}-11-dihomo-IsoF 2	0.10	N	Y
RvE2	0.20	Y	Y
PGJ_2	0.16	Y	Y
Δ^{12}-PGJ_2	0.10	N	Y
LTB_5	0.010	Y	Y
PGB_2	0.040	Y	Y
THF diol	0.025	Y	Y
18(*S*)-RvE3	0.1	Y	Y
12-OH-17(18)-EpETE	0.05	Y	Y
15,16-DiHODE	0.1	Y	Y
9,10-DiHODE	0.02	Y	Y
12,13-DiHODE	0.1	Y	Y
8,15-DiHETE	0.08	Y	Y
10(*S*),17(*S*)-diH n3 DPA	0.1	N	Y
18(*R*)-RvE3	0.05	Y	Y
NPD1	0.05	N	Y
6-*trans*-LTB_4	0.05	Y	Y
5,15-DiHETE	0.025	Y	Y
17,18-DiHETE	0.025	Y	Y
LTB_4	0.025	Y	Y
7(*S*),17(*S*)-diH n3 DPA	0.075	N	Y
14,15-DiHETE	0.025	Y	Y
11,12-DiHETE	0.025	Y	Y
12,13-DiHOME	0.05	Y	Y
8,9-DiHETE	0.05	Y	Y
10(*S*),17(*S*)-diH n6 DPA	0.025	N	Y
9,10-DiHOME	0.05	Y	Y
10(*S*),17(*S*)-diH AdA	0.1	N	Y
12(*S*)-HHTrE	0.05	N	Y
14,15-DiHETrE	0.01	Y	Y
19,20-DiHDPE	0.05	Y	Y
LTB_3	0.05	Y	Y
9,10-diH-stearic acid	0.2	Y	Y
16,17-DiHDPE	0.05	Y	Y
11,12-DiHETrE	0.025	Y	Y
19-HEPE	0.071	Y	Y
13,14-DiHDPE	0.025	Y	Y
20-HEPE	0.1	Y	Y
9-HOTrE	0.05	Y	Y
10,11-DiHDPE	0.025	Y	Y
8,9-DiHETrE	0.05	Y	Y
13-HOTrE	0.06	Y	Y
18-HEPE	0.1	Y	Y
15-deoxy-PGJ_2	0.05	Y	Y
19-HETE	1.0	N	Y
7,8-DiHDPE	0.10	Y	Y
20-HETE	0.10	Y	Y
15-HEPE	0.13	Y	Y
5,6-DiHETrE	0.050	Y	Y

Analyte	LLOQ+	Study A	Study B
11-HEPE	0.050	Y	Y
8-HEPE	0.063	Y	Y
12-HEPE	0.063	Y*	Y
9-HEPE	0.050	Y	Y
21-HDHA	0.17	Y	Y
5-HEPE	0.050	Y	Y
22-HDHA	0.28	Y	Y
4,5-DiHDPE	0.20	Y	Y
13-HODE	0.50	Y	Y
9-HODE	0.50	Y	Y
20-HDHA	0.05	Y	Y
15(16)-EpODE	0.05	Y	Y
15-HETE	0.13	Y	Y
9(10)-EpODE	0.04	Y	Y
17(18)-EpETE	0.10	Y	Y
16-HDHA	0.03	Y	Y
17-HDHA	0.20	Y	Y
13-HDHA	0.05	Y	Y
12(13)-EpODE	0.05	Y	Y
13-oxo-ODE	0.10	Y*	Y
11-HETE	0.05	Y	Y
10-HDHA	0.05	Y	Y
14-HDHA	0.10	Y*	Y
9-oxo-ODE	0.10	Y*	Y
15-oxo-ETE	0.05	Y*	Y
14(15)-EpETE	0.05	Y	Y
8-HETE	0.13	Y	Y
12-HETE	0.05	Y*	Y
11(12)-EpETE	0.05	Y	Y*
11-HDHA	0.03	Y*	Y
7-HDHA	0.10	Y	Y
8(9)-EpETE	0.10	Y	Y
9-HETE	0.25	Y	Y
15-HETrE	0.05	Y	Y
8-HDHA	0.05	Y	Y
5-HETE	0.05	Y	Y
4-HDHA	0.03	Y	Y
19(20)-EpDPE	0.05	Y	Y
12(13)-EpOME	0.03	Y	Y
14(15)-EpETrE	0.05	Y	Y
9(10)-EpOME	0.03	Y	Y
16(17)-EpDPE	0.05	Y	Y
13(14)-EpDPE	0.05	Y	Y
5-oxo-ETE	0.20	Y	Y
10(11)-EpDPE	0.03	Y	Y
11(12)-EpETrE	0.05	Y	Y
8(9)-EpETrE	0.10	Y	Y
5(6)-EpETrE	0.20	Y	Y*
5-HETrE	0.02	Y	Y
9(10)-ep-stearic acid	0.20	Y	Y

N - analyte not included in LC-MS method

Y - Analyte included in LC-MS method and evaluated

Y* - Analyte included in LC-MS method, but no evaluation

+ Lower limit of quantification (LLOQ) in plasma [nM]

(2) Rund, K.M., Ostermann, A.I., Kutzner, L., Galano, J.-M., Oger, C., Vigor, C., Wecklein, S., Seiwert, N., Durand, T., and Schebb, N.H. (2017). Development of an LC-ESI(-)-MS/MS method for the simultaneous quantification of 35 isoprostanes and isofurans derived from the major n3- and n6-PUFAs. Anal. Chim. Acta. 2018; DOI: *doi.org/10.1016/j.aca.2017.11.002.*

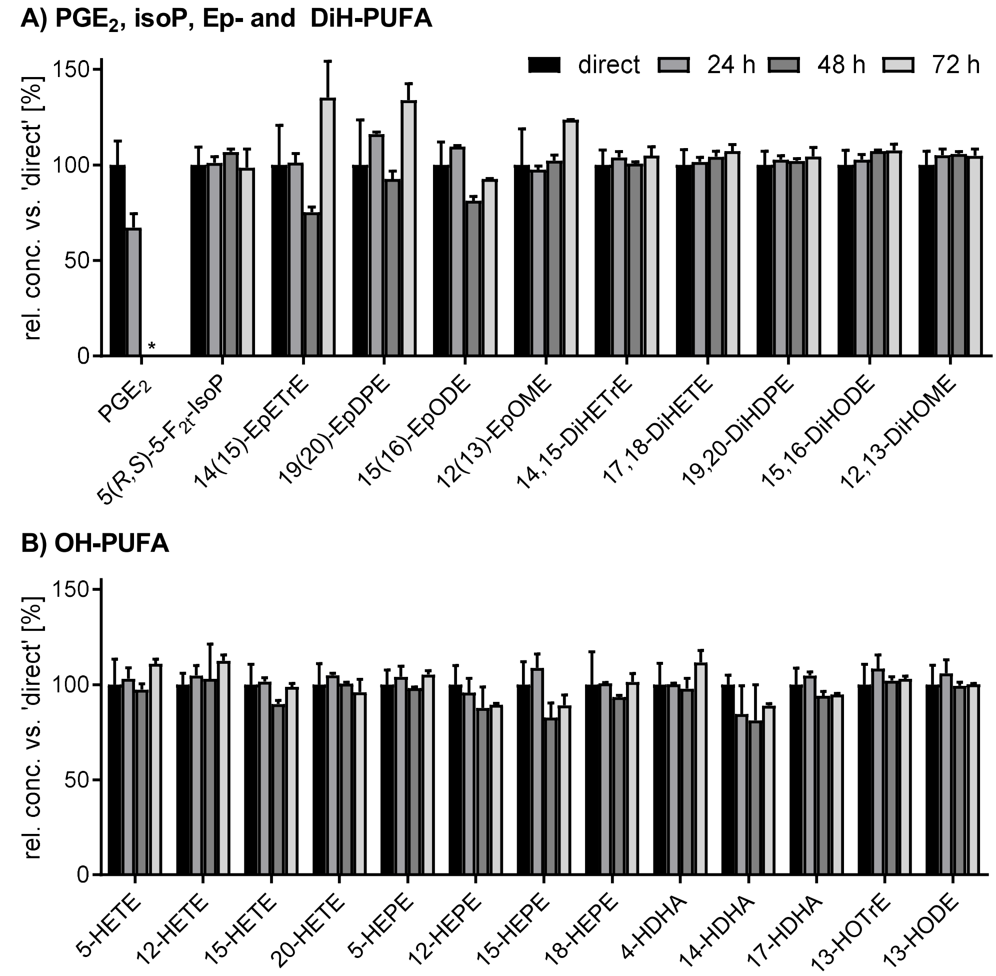


Figure S2: Changes in selected oxylipins when freezing samples for up to 72 h following protein precipitation, i.e. before solid phase extraction. Relative concentrations of PGE_2 as well as selected isoP, ep-, and diH-PUFA **(A)** and OH-PUFA **(B)** in samples stored for 24-72 h versus direct extraction (30 min) are shown (n=3 for direct analysis; n=2 for 24 h, 48 h and 72 h). For sample preparation, internal standards, antiox-mix and methanol were added to plasma samples. Samples were stored for 30 min, 24 h, 48 h and 72 h at -80°C before extraction of free oxylipins by solid phase extraction (Bond Elut Certify II, Agilent). * PGE_2 was found at very low concentrations in plasma (< 2-fold LLOQ) and was <LLOQ after storage for 48 and 72 h.

Table S4: Concentration of oxylipins found in human quality control plasma. Shown are mean ± 95% interval of the standard deviation (95%SD, n=15) as well as the relative 95%SD. A color code was assigned to each analyte based on the relative 95%SD as a measure for the quality of the analytes‘ analytical variance.

Analytes			LLOQ [nM]*	Mean±95%SD [nM]	rel 95%SD [%]	color code
LA	epoxy-PUFA	9(10)-EpOME	0.03	1.8 ± 0.25	14	
		12(13)-EpOME	0.03	4.6 ± 0.36	7.7	
	vic dihydroxy-PUFA	9,10-DiHOME	0.05	4.2 ± 0.27	6.3	
		12,13-DiHOME	0.05	4.8 ± 0.32	6.6	
	hydroxy-PUFA	9-HODE	0.50	10 ± 0.62	6.2	
		13-HODE	0.50	10.0 ± 0.57	5.7	
	others	13-oxo-ODE	0.10	0.23 ± 0.053	23	
		9-oxo-ODE	0.10	0.98 ± 0.14	14	
DGLA	hydroxy-PUFA	5-HETrE	0.02	0.20 ± 0.016	7.9	
		15-HETrE	0.05	0.53 ± 0.033	6.2	
ARA	prostanoids	PGE_2	0.03	0.081 ± 0.015	18	
		$PGF_{2\alpha}$	0.07	8.0 ± 3.7	46	
		13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.05	0.097 ± 0.035	36	
		TxB_2	0.13	0.73 ± 0.11	15	
	isoprostanes	5(*R,S*)-5-F_{2t}-IsoP	0.05	0.12 ± 0.025	22	
	epoxy-PUFA	11(12)-EpETrE	0.02	0.079 ± 0.023	29	
		14(15)-EpETrE	0.05	0.20 ± 0.030	15	
	vic dihydroxy-PUFA	5,6-DiHETrE	0.05	0.33 ± 0.10	31	
		8,9-DiHETrE	0.05	0.26 ± 0.018	6.9	
		11,12-DiHETrE	0.03	0.60 ± 0.034	5.7	
		14,15-DiHETrE	0.01	0.66 ± 0.032	4.8	
	hydroxy-PUFA	5-HETE	0.05	0.86 ± 0.067	7.7	
		8-HETE	0.13	0.34 ± 0.052	15	
		11-HETE	0.05	0.34 ± 0.031	9.1	
		12-HETE	0.05	1.7 ± 0.27	15	
		15-HETE	0.13	1.2 ± 0.12	10	
		19-HETE	1.00	1.1 ± 0.22	20	
		20-HETE	0.10	0.98 ± 0.075	7.7	
		12-HHTrE	0.05	1.1 ± 0.084	7.6	
	others	15-oxo-ETE	0.05	0.067 ± 0.019	28	
ALA	epoxy-PUFA	9(10)-EpODE	0.04	0.20 ± 0.077	38	
		12(13)-EpODE	0.05	0.23 ± 0.040	17	
		15(16)-EpODE	0.05	3.2 ± 0.26	8.2	
	vic dihydroxy-PUFA	9,10-DiHODE	0.02	0.25 ± 0.013	5.1	
		12,13-DiHODE	0.10	0.27 ± 0.034	12	
		15,16-DiHODE	0.10	11 ± 0.66	5.8	
	hydroxy-PUFA	9-HOTrE	0.05	0.53 ± 0.035	6.6	
		13-HOTrE	0.06	0.62 ± 0.053	8.5	

Table S4: Continued.

Analytes			LLOQ [nM]*	Mean±95%SD [nM]	rel 95%SD [%]	color code
EPA	vic dihydroxy-FA	8,9-DiHETE	0.05	0.085 ± 0.016	19	
		11,12-DiHETE	0.03	0.050 ± 0.005	11	
		14,15-DiHETE	0.03	0.083 ± 0.010	12	
		17,18-DiHETE	0.03	0.66 ± 0.043	6.5	
	hydroxy-PUFA	5-HEPE	0.05	0.23 ± 0.031	13	
		8-HEPE	0.06	0.16 ± 0.016	10	
		9-HEPE	0.05	0.10 ± 0.019	19	
		11-HEPE	0.05	0.062 ± 0.012	19	
		12-HEPE	0.06	0.21 ± 0.036	17	
		15-HEPE	0.13	0.18 ± 0.035	19	
		18-HEPE	0.10	0.34 ± 0.033	9.8	
		19-HEPE	0.07	0.83 ± 0.14	17	
		20-HEPE	0.10	0.62 ± 0.100	16	
DHA	epoxy-PUFA	10(11)-EpDPE	0.03	0.14 ± 0.020	14	
		13(14)-EpDPE	0.05	0.067 ± 0.025	37	
		16(17)-EpDPE	0.05	0.083 ± 0.023	27	
		19(20)-EpDPE	0.05	0.23 ± 0.026	12	
	vic dihydroxy-PUFA	4,5-DiHDPE	0.20	0.55 ± 0.068	12	
		10,11-DiHDPE	0.03	0.20 ± 0.016	8.1	
		13,14-DiHDPE	0.03	0.21 ± 0.017	8.3	
		16,17-DiHDPE	0.05	0.29 ± 0.021	7.2	
		19,20-DiHDPE	0.05	3.1 ± 0.19	6.2	
	hydroxy-PUFA	4-HDHA	0.03	0.17 ± 0.015	9.2	
		8-HDHA	0.05	0.44 ± 0.051	12	
		10-HDHA	0.05	0.086 ± 0.012	14	
		11-HDHA	0.03	0.16 ± 0.034	21	
		13-HDHA	0.05	0.12 ± 0.020	17	
		14-HDHA	0.10	0.94 ± 0.21	22	
		16-HDHA	0.03	0.15 ± 0.015	10	
		17-HDHA	0.20	0.66 ± 0.11	17	
		20-HDHA	0.05	0.34 ± 0.038	11	
		21-HDHA	0.17	2.8 ± 0.25	9.0	
		22-HDHA	0.28	2.2 ± 0.18	8.2	
oleic acid	epoxy-PUFA	9(10)-Ep-stearic acid	0.20	11 ± 1.3	12	
	vic dihydroxy-PUFA	9,10-DiH-stearic acid	0.20	7.5 ± 0.64	8.5	

Legend: Variance (x)
- x < 10%
- 10% < x < 20%
- x > 20%

* Lower limit of quantification [nM] in plasma

Table S5: Concentration of oxylipins found in plasma of study participants from the study on inter-day variation of free oxylipins in plasma on a non-standardized diet (Study A). Shown are mean ± SEM (n=18). The LLOQ is shown in case the analyte's concentration was <LLOQ in more than 50% of the samples. All analytes are shown which were quantified at least at one time point. The color code refers to the analytes' variation in quality control plasma (relative 95%SD <10%: green, 10-20%: orange, >20% red; see Table S4). Statistics were done with paired t-tests and Wilcoxon-tests. No statistical differences between t0 and t48 were found ($p \leq 0.05$; p-values not shown).

Analytes			color code (see Table S4)	t0	t48	factor high *vs.* low*	within analytical variance?⁺
LA	epoxy-PUFA	9(10)-EpOME	orange	1.7 ± 0.23	1.4 ± 0.16	1.26	N
		12(13)-EpOME	green	4.1 ± 0.61	3.3 ± 0.47	1.23	N
	vic dihydroxy-PUFA	9,10-DiHOME	green	5.3 ± 0.76	4.7 ± 0.66	1.12	N
		12,13-DiHOME	green	5.9 ± 0.65	5.9 ± 0.73	1.00	Y
	hydroxy-PUFA	9-HODE	green	14 ± 1.9	15 ± 2.6	1.12	N
		13-HODE	green	20 ± 2.9	23 ± 3.6	1.11	N
DGLA	prostanoids	13,14-dihydro-15-keto-PGE_1		0.11 ± 0.026	0.074 ± 0.015	1.56	-
	hydroxy-FA	15-HETrE	green	0.26 ± 0.017	0.27 ± 0.019	1.01	Y
ARA	prostanoids	PGE_2	orange	0.079 ± 0.015	0.059 ± 0.010	1.33	N
		13,14-dihydro-15-keto-$PGF_{2\alpha}$	red	0.14 ± 0.007	0.15 ± 0.009	1.08	Y
		TxB_2	orange	0.48 ± 0.057	0.53 ± 0.055	1.10	Y
	epoxy-PUFA	5(6)-EpETrE		0.37 ± 0.066	0.34 ± 0.046	1.09	-
		8(9)-EpETrE		0.37 ± 0.059	0.44 ± 0.075	1.21	-
		11(12)-EpETrE	red	0.062 ± 0.011	0.063 ± 0.007	1.02	Y
		14(15)-EpETrE	orange	0.093 ± 0.012	0.093 ± 0.011	1.00	Y
	vic dihydroxy-PUFA	5,6-DiHETrE	red	0.21 ± 0.022	0.22 ± 0.014	1.03	Y
		8,9-DiHETrE	green	0.23 ± 0.014	0.24 ± 0.013	1.06	Y
		11,12-DiHETrE	green	0.58 ± 0.046	0.59 ± 0.037	1.01	Y
		14,15-DiHETrE	green	0.69 ± 0.044	0.69 ± 0.033	1.00	Y
	hydroxy-PUFA	5-HETE	green	0.54 ± 0.069	0.58 ± 0.063	1.08	Y
		8-HETE	orange	0.31 ± 0.023	0.31 ± 0.019	1.01	Y
		11-HETE	green	0.26 ± 0.021	0.25 ± 0.018	1.01	Y
		15-HETE	orange	0.87 ± 0.073	0.89 ± 0.064	1.03	Y
		20-HETE	green	0.84 ± 0.15	0.83 ± 0.11	1.01	Y
ALA	epoxy-PUFA	9(10)-EpODE	red	0.18 ± 0.025	0.16 ± 0.022	1.12	Y
		12(13)-EpODE	orange	0.13 ± 0.019	0.11 ± 0.013	1.17	Y
		15(16)-EpODE	green	3.7 ± 0.73	3.1 ± 0.43	1.21	N
	vic dihydroxy-PUFA	9,10-DiHODE	green	0.41 ± 0.14	0.28 ± 0.045	1.45	N
		12,13-DiHODE	orange	0.28 ± 0.034	0.28 ± 0.032	1.01	Y
		15,16-DiHODE	green	18 ± 3.0	16 ± 2.1	1.13	N
	hydroxy-PUFA	9-HOTrE	green	0.70 ± 0.071	0.78 ± 0.13	1.11	N
		13-HOTrE	green	1.0 ± 0.11	1.3 ± 0.30	1.26	N
EPA	prostanoids	TxB_3		< 0.025	0.042 ± 0.007	-	
	vic dihydroxy-PUFA	8,9-DiHETE	orange	< 0.05	< 0.05	-	
		11,12-DiHETE	orange	0.041 ± 0.006	0.033 ± 0.004	1.24	N
		14,15-DiHETE	orange	0.083 ± 0.008	0.076 ± 0.006	1.10	Y
		17,18-DiHETE	green	0.50 ± 0.054	0.47 ± 0.037	1.06	Y
	hydroxy-PUFA	5-HEPE	orange	0.16 ± 0.022	0.16 ± 0.017	1.02	Y
		15-HEPE	orange	0.13 ± 0.016	< 0.13	-	
		18-HEPE	green	0.20 ± 0.022	0.18 ± 0.016	1.07	Y
		19-HEPE	orange	0.68 ± 0.092	0.68 ± 0.067	1.01	Y
		20-HEPE	orange	0.41 ± 0.068	0.43 ± 0.048	1.05	Y
DHA	epoxy-PUFA	13(14)-EpDPE	red	0.13 ± 0.018	0.13 ± 0.015	1.00	Y
		19(20)-EpDPE	orange	0.22 ± 0.032	0.23 ± 0.029	1.06	Y
	vic dihydroxy-PUFA	4,5-DiHDPE	orange	0.66 ± 0.070	0.72 ± 0.078	1.09	Y
		10,11-DiHDPE	green	0.17 ± 0.021	0.17 ± 0.018	1.01	Y
		13,14-DiHDPE	green	0.22 ± 0.019	0.22 ± 0.018	1.02	Y
		16,17-DiHDPE	green	0.30 ± 0.025	0.29 ± 0.022	1.04	Y
		19,20-DiHDPE	green	2.7 ± 0.24	2.7 ± 0.22	1.01	Y
	hydroxy-PUFA	4-HDHA	green	0.28 ± 0.042	0.28 ± 0.039	1.00	Y
		7-HDHA		0.14 ± 0.016	0.15 ± 0.020	1.10	-
		8-HDHA	orange	0.44 ± 0.055	0.44 ± 0.055	1.01	Y
		10-HDHA	orange	0.27 ± 0.030	0.29 ± 0.031	1.06	Y
		13-HDHA	orange	0.17 ± 0.020	0.16 ± 0.018	1.00	Y
		16-HDHA	orange	0.17 ± 0.013	0.17 ± 0.013	1.01	Y
		17-HDHA	orange	0.78 ± 0.083	0.86 ± 0.12	1.11	Y
		20-HDHA	orange	0.41 ± 0.037	0.42 ± 0.040	1.03	Y
		21-HDHA	green	2.0 ± 0.17	2.1 ± 0.24	1.06	Y
		22-HDHA	green	2.1 ± 0.27	2.2 ± 0.29	1.03	Y
Oleic Acid	epoxy-PUFA	9(10)-Ep-stearic acid	orange	11 ± 1.2	8.8 ± 0.81	1.23	N
	vic dihydroxy-PUFA	9,10-DiH-stearic acid	green	6.0 ± 0.78	5.9 ± 0.79	1.01	Y

* Higher mean concentration of both time points (t0 and t48) was divided by the lower mean concentration

⁺ The factor 'high *vs.* low' was compared against the analytical variance; Y – variation between time points within analytical variance; N – variation between time point not within analytical variance

Table S6: Concentration of oxylipins found in plasma of study participants from the study on inter-day and intra-day variation o (Study B). Shown are mean ± SEM (n=13) at t24, t48 and t72. The LLOQ is shown in case the analyte's concentration was analytes are shown which were quantified at least at one time point. The color code refers to the analytes' variation in quality 10-20%: orange, >20% red; see Table S4). Statistics for normally distributed variables were done with ANOVA with repeated samples with Bonferroni-correction and for not-normally distributed variables with Friedman Tests and post-hoc Dunn-Bonferro

Analytes			color code (see Table S4)	t24	t48	P t24-48	t72
LA	epoxy-PUFA	9(10)-EpOME	orange	1.7 ± 0.36	1.4 ± 0.25	-	1.4 ± 0.26
		12(13)-EpOME	green	5.0 ± 1.2	4.8 ± 1.2	-	4.7 ± 1.1
	vic dihydroxy-PUFA	9,10-DiHOME	green	4.3 ± 0.55	4.3 ± 0.62	-	4.2 ± 0.72
		12,13-DiHOME	green	6.6 ± 0.54	6.6 ± 0.97	-	6.6 ± 1.1
	hydroxy-PUFA	9-HODE	green	11 ± 1.1	10 ± 0.93	-	9.8 ± 0.89
		13-HODE	green	14 ± 1.2	13 ± 1.2	-	13 ± 1.3
	others	13-oxo-ODE	red	0.20 ± 0.019	0.19 ± 0.036	-	0.19 ± 0.030
		9-oxo-ODE	orange	1.2 ± 0.13	1.0 ± 0.064	-	0.95 ± 0.046
DGLA	prostanoids	13,14-dihydro-15-keto-PGE_1		0.11 ± 0.031	< 0.05	-	0.15 ± 0.059
	hydroxy-PUFA	5-HETrE	green	0.13 ± 0.011	0.10 ± 0.009	-	0.11 ± 0.010
		15-HETrE	green	0.44 ± 0.021	0.43 ± 0.019	-	0.41 ± 0.020
ARA	prostanoids	PGD_2		0.20 ± 0.018	0.19 ± 0.028	-	0.20 ± 0.024
		PGE_2	orange	0.24 ± 0.030	0.27 ± 0.043	-	0.27 ± 0.037
		$PGF_{2\alpha}$	red	4.7 ± 1.1	4.6 ± 1.1	-	4.6 ± 1.1
		13,14-dihydro-15-keto-PGF_{2a}	red	0.16 ± 0.015	0.17 ± 0.020	-	0.16 ± 0.016
		TxB_2	orange	0.46 ± 0.049	0.45 ± 0.068	-	0.40 ± 0.055
	isoprostanes	5(*R,S*)-5-F_{2t}-IsoP	red	0.11 ± 0.006	0.096 ± 0.005	n.s.	0.095 ± 0.007
	epoxy-PUFA	11(12)-EpETrE	red	0.087 ± 0.007	0.089 ± 0.007	-	0.077 ± 0.006
		14(15)-EpETrE	orange	0.20 ± 0.017	0.20 ± 0.015	-	0.18 ± 0.012
	vic dihydroxy-PUFA	5,6-DiHETrE	red	0.30 ± 0.020	0.28 ± 0.022	-	0.28 ± 0.020
		8,9-DiHETrE	green	0.22 ± 0.013	0.21 ± 0.013	n.s.	0.21 ± 0.014
		11,12-DiHETrE	green	0.56 ± 0.025	0.54 ± 0.024	-	0.53 ± 0.026
		14,15-DiHETrE	green	0.67 ± 0.031	0.67 ± 0.029	-	0.70 ± 0.030
	hydroxy-PUFA	5-HETE	green	0.96 ± 0.11	0.63 ± 0.042	0.032	0.59 ± 0.040
		8-HETE	orange	0.39 ± 0.027	0.32 ± 0.015	0.032	0.31 ± 0.018
		9-HETE		0.39 ± 0.032	0.30 ± 0.030	-	0.33 ± 0.029
		11-HETE	green	0.61 ± 0.035	0.57 ± 0.053	-	0.51 ± 0.037
		12-HETE	orange	1.5 ± 0.16	1.6 ± 0.12	-	1.4 ± 0.12
		15-HETE	orange	1.2 ± 0.075	1.1 ± 0.060	n.s.	1.0 ± 0.082
		20-HETE	green	0.87 ± 0.048	0.87 ± 0.074	-	0.77 ± 0.050
		12-HHTrE	green	0.74 ± 0.079	0.71 ± 0.098	-	0.65 ± 0.078
		15-oxo-ETE	red	0.070 ± 0.008	< 0.05	-	< 0.05

Table S6: Continued.

Analytes			color code (see Table S4)	t24	t48	P t24-48	t72	P t24-72	P Friedman/ ANreM	factor high *vs.* low*	within analytical variance?[+]
ALA	epoxy-PUFA	9(10)-EpODE		0.20 ± 0.032	0.18 ± 0.037	-	0.17 ± 0.035	-	n.s.	1.17	Y
		12(13)-EpODE		0.20 ± 0.019	0.20 ± 0.037	-	0.19 ± 0.034	-	n.s.	1.09	Y
		15(16)-EpODE		3.7 ± 0.51	3.2 ± 0.70	-	3.1 ± 0.61	-	n.s.	1.03	Y
	vic dihydroxy-PUFA	9,10-DiHODE		0.21 ± 0.019	0.22 ± 0.051	-	0.23 ± 0.057	-	n.s.	1.06	Y
		12,13-DiHODE		0.28 ± 0.030	0.30 ± 0.067	-	0.32 ± 0.077	-	n.s.	1.16	Y
		15,16-DiHODE		12 ± 1.0	12 ± 1.4	-	12 ± 1.5	-	n.s.	1.02	Y
	hydroxy-PUFA	9-HOTrE		0.60 ± 0.061	0.51 ± 0.065	-	0.54 ± 0.066	-	n.s.	1.19	N
		13-HOTrE		0.64 ± 0.064	0.57 ± 0.079	-	0.67 ± 0.085	-	n.s.	1.18	N
EPA	vic dihydroxy-PUFA	8,9-DiHETE		0.056 ± 0.006	< 0.05	-	< 0.05	-	-	-	
		11,12-DiHETE		0.037 ± 0.003	0.034 ± 0.002	-	0.034 ± 0.002	-	n.s.	1.11	Y
		14,15-DiHETE		0.068 ± 0.004	0.066 ± 0.004	-	0.070 ± 0.004	-	n.s.	1.05	Y
		17,18-DiHETE		0.46 ± 0.028	0.44 ± 0.031	-	0.45 ± 0.028	-	n.s.	1.05	Y
	hydroxy-PUFA	5-HEPE		0.15 ± 0.011	0.12 ± 0.009	n.s.	0.11 ± 0.009	0.010	0.005	1.27	N
		9-HEPE		0.067 ± 0.005	0.054 ± 0.006	-	0.055 ± 0.007	-	n.s.	1.23	N
		12-HEPE		0.14 ± 0.018	0.15 ± 0.012	-	0.13 ± 0.012	-	n.s.	1.16	Y
		18-HEPE		0.13 ± 0.007	0.12 ± 0.010	-	0.11 ± 0.011	-	n.s.	1.14	N
		19-HEPE		0.61 ± 0.046	0.60 ± 0.058	-	0.58 ± 0.047	-	n.s.	1.04	Y
		20-HEPE		0.54 ± 0.045	0.51 ± 0.041	-	0.48 ± 0.045	-	n.s.	1.11	Y
DHA	epoxy-PUFA	10(11)-EpDPE		0.082 ± 0.011	0.066 ± 0.006	-	0.064 ± 0.005	-	n.s.	1.24	N
		16(17)-EpDPE		0.059 ± 0.005	0.054 ± 0.006	-	< 0.05	-	-	-	
		19(20)-EpDPE		0.13 ± 0.012	0.12 ± 0.009	-	0.11 ± 0.009	-	n.s.	1.15	Y
	vic dihydroxy-PUFA	4,5-DiHDPE		0.39 ± 0.046	0.35 ± 0.034	-	0.34 ± 0.039	-	n.s.	1.13	Y
		10,11-DiHDPE		0.13 ± 0.014	0.11 ± 0.009	-	0.12 ± 0.011	-	n.s.	1.12	N
		13,14-DiHDPE		0.16 ± 0.015	0.16 ± 0.012	-	0.15 ± 0.013	-	n.s.	1.06	Y
		16,17-DiHDPE		0.20 ± 0.015	0.20 ± 0.012	-	0.21 ± 0.013	-	n.s.	1.04	Y
		19,20-DiHDPE		1.9 ± 0.15	1.8 ± 0.14	-	1.8 ± 0.12	-	n.s.	1.03	Y
	hydroxy-PUFA	4-HDHA		0.18 ± 0.021	0.12 ± 0.008	0.034	0.11 ± 0.006	0.009	<0.001	1.60	N
		8-HDHA		0.37 ± 0.032	0.29 ± 0.019	n.s.	0.27 ± 0.016	0.007	0.004	1.38	N
		10-HDHA		0.083 ± 0.011	0.065 ± 0.005	n.s.	0.060 ± 0.005	0.034	0.009	1.38	N
		11-HDHA		0.13 ± 0.013	0.12 ± 0.007	-	0.11 ± 0.007	-	n.s.	1.15	Y
		13-HDHA		0.16 ± 0.017	0.14 ± 0.015	-	0.12 ± 0.011	-	n.s.	1.35	N
		14-HDHA		0.86 ± 0.14	0.91 ± 0.11	-	0.78 ± 0.078	-	n.s.	1.17	Y
		16-HDHA		0.26 ± 0.019	0.26 ± 0.024	-	0.25 ± 0.029	-	n.s.	1.05	Y
		17-HDHA		0.47 ± 0.033	0.42 ± 0.031	n.s.	0.36 ± 0.031	0.010	0.005	1.31	N
		20-HDHA		0.28 ± 0.018	0.26 ± 0.013	n.s.	0.24 ± 0.013	0.026	0.015	1.15	Y
		21-HDHA		1.6 ± 0.17	1.5 ± 0.12	-	1.5 ± 0.13	-	n.s.	1.08	Y
		22-HDHA		1.3 ± 0.14	1.3 ± 0.11	-	1.2 ± 0.12	-	n.s.	1.13	N
Oleic Acid	epoxy-PUFA	9(10)-Ep-stearic acid		12 ± 2.1	10 ± 1.5	-	9.8 ± 1.5	-	n.s.	1.25	N
	vic dihydroxy-PUFA	9,10-DiH-stearic acid		7.3 ± 0.91	7.7 ± 1.5	-	7.4 ± 1.6	-	n.s.	1.06	Y

* Highest mean concentration from the three time points (t24, t48 and t72) was divided by the lowest mean concentration

[+] The factor 'high *vs.* low' was compared against the analytical variance; Y – variation between time point within analytical variance; N – variation between time point not within analytical variance

Figure S3: Comparison of inter-day variations of circulating oxylipins in subjects on a standardized (StA) and non-standardized (StB) diet. Shown are relative concentrations ± SEM of selected epoxy-PUFA (**A**), dihydroxy-PUFA (**B**), hydroxy-PUFA (**C**), prostanoids (**D**) (n=18 for StA; n=13 for StB). In StA, plasma was collected from study participants at baseline (t0) and after 48h without intervention; in StB plasma was collected after 24 and 72 h on a standardized diet. Relative concentrations of oxylipins for StA were calculated for t48 against baseline and for StB relative concentrations after 72 h on the standardized diet were calculated against 24 h (i.e. 48 h time interval between sample collections).

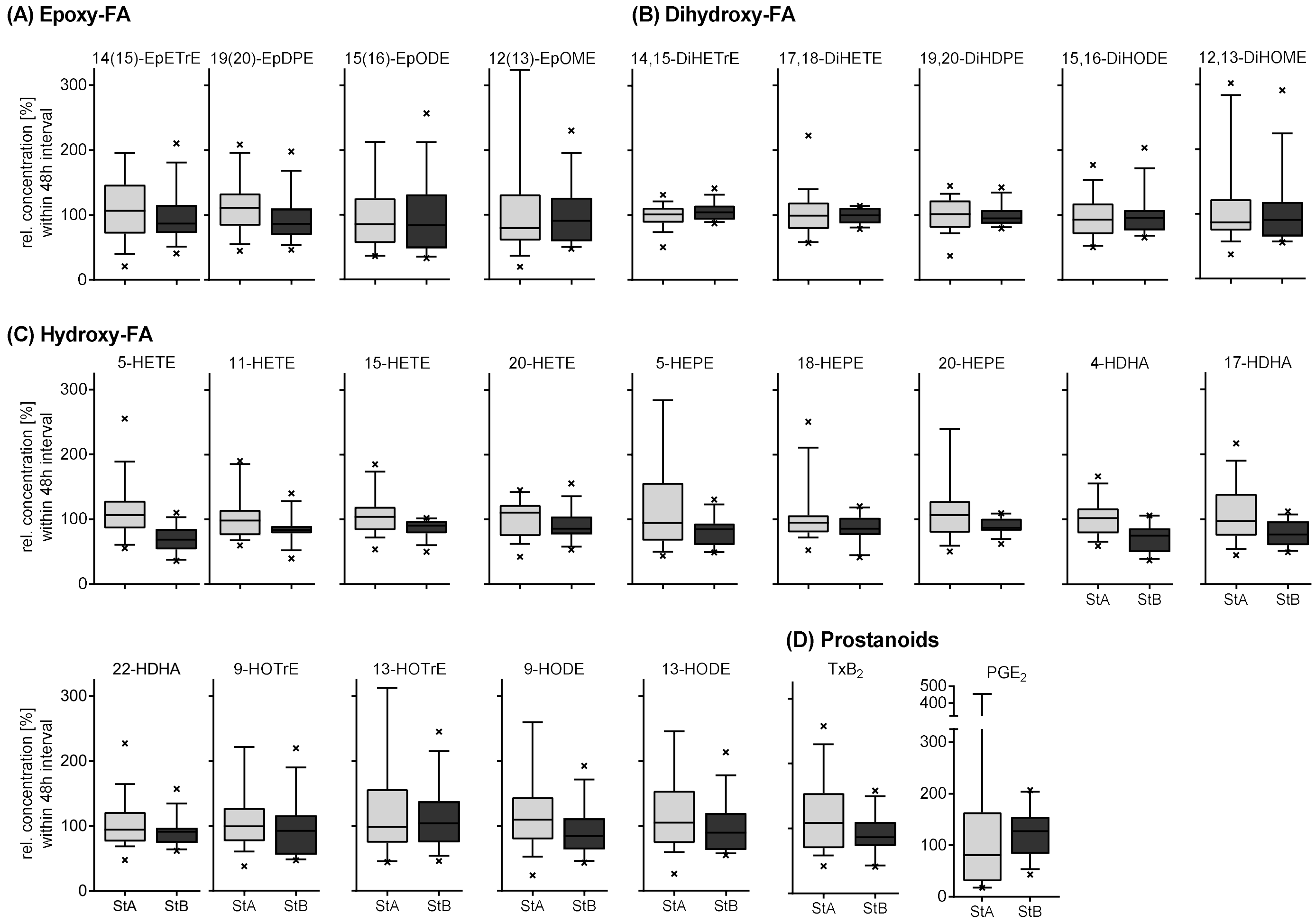


Table S7: Concentration of oxylipins found in plasma of study participants from the study on inter-day and intra-day variation of free oxylipins in plasma on a standardized diet (Study B). Shown are mean ± SEM (n=13) at t0, t2, t4, t6 and t8. The LLOQ is shown in case the analyte's concentration was <LLOQ in more than 50% of the samples. All analytes are shown which were quantified at least at one time point. Statistics for normally distributed variables were done with ANOVA with repeated measurements and post-hoc t-Tests for paired samples with Bonferroni-correction and for not-normally distributed variables with Friedman and post-hoc Dunn-Bonferroni tests; significance level p ≤ 0.05.

Analyte			t0	t2	P t0-2	t4	P t0-4	t6	P t0-6	t8	P t0-8	P Friedman/ ANreM
LA	epoxy-PUFA	9(10)-EpOME	2.0 ± 0.32	1.5 ± 0.24	n.s.	2.7 ± 0.29	n.s.	0.97 ± 0.091	n.s.	0.59 ± 0.081	**< 0.001**	**< 0.001**
		12(13)-EpOME	6.1 ± 1.2	17 ± 4.4	**0.005**	23 ± 3.4	**< 0.001**	8.6 ± 1.1	n.s.	3.1 ± 0.40	n.s.	**< 0.001**
	vic dihydroxy-PUFA	9,10-DiHOME	5.7 ± 0.60	9.1 ± 0.72	n.s.	8.9 ± 0.69	n.s.	2.3 ± 0.20	n.s.	2.7 ± 0.57	n.s.	**< 0.001**
		12,13-DiHOME	8.6 ± 0.99	18 ± 1.6	**< 0.001**	16 ± 1.2	**< 0.001**	5.3 ± 0.57	**< 0.001**	4.0 ± 0.72	**0.011**	**< 0.001**
	hydroxy-PUFA	9-HODE	14 ± 1.2	18 ± 1.6	n.s.	28 ± 1.7	**0.006**	13 ± 1.2	n.s.	6.8 ± 0.67	n.s.	**< 0.001**
		13-HODE	16 ± 1.4	38 ± 3.4	**0.029**	42 ± 2.8	**0.005**	16 ± 1.5	n.s.	7.7 ± 0.93	n.s.	**< 0.001**
	others	13-oxo-ODE	0.22 ± 0.022	0.81 ± 0.13	-	0.96 ± 0.088	-	0.40 ± 0.033	-	< 0.1	-	-
		9-oxo-ODE	1.3 ± 0.061	1.0 ± 0.035	n.s.	1.3 ± 0.061	n.s.	1.0 ± 0.058	n.s.	0.82 ± 0.039	**< 0.001**	**< 0.001**
DGLA	prostanoids	13,14-dihydro-15-keto-PGE_1	< 0.05	< 0.05	-	0.12 ± 0.036	-	< 0.05	-	< 0.05	-	-
	hydroxy-PUFA	5(S) HETrE	0.12 ± 0.011	0.036 ± 0.006	**< 0.001**	0.072 ± 0.009	n.s.	0.041 ± 0.007	**< 0.001**	0.040 ± 0.008	**< 0.001**	**< 0.001**
		15(S)-HETrE	0.42 ± 0.022	0.22 ± 0.009	**< 0.001**	0.37 ± 0.026	n.s.	0.26 ± 0.016	**< 0.001**	0.32 ± 0.021	**0.020**	**< 0.001**
ARA	prostanoids	PGD_2	< 0.1	< 0.1	-	< 0.1	-	0.16 ± 0.016	-	0.19 ± 0.026	-	-
		PGE_2	0.088 ± 0.016	0.079 ± 0.014	n.s.	0.10 ± 0.018	n.s.	0.17 ± 0.028	**0.011**	0.24 ± 0.037	**0.019**	**< 0.001**
		$PGF_{2\alpha}$	5.3 ± 1.2	4.1 ± 0.97	n.s.	4.1 ± 0.97	n.s.	3.9 ± 0.98	n.s.	4.0 ± 0.95	n.s.	**0.011**
		13,14-dihydro-15-keto-$PGF_{2\alpha}$	0.14 ± 0.020	< 0.05	n.s.	0.088 ± 0.011	n.s.	0.066 ± 0.005	n.s.	0.074 ± 0.012	n.s.	**0.001**
		TxB_2	0.23 ± 0.031	0.19 ± 0.030	n.s.	0.29 ± 0.052	n.s.	0.32 ± 0.046	n.s.	0.48 ± 0.063	**0.029**	**< 0.001**
	isoprostanes	5(*R*,*S*)-5-F_{2t}-IsoP	0.10 ± 0.008	0.065 ± 0.006	**0.002**	0.096 ± 0.005	n.s.	0.073 ± 0.007	**0.024**	0.075 ± 0.006	n.s.	**< 0.001**
	epoxy-PUFA	8(9)-EpETrE	0.11 ± 0.015	< 0.1	-	< 0.1	-	< 0.1	-	< 0.1	-	-
		11(12)-EpETrE	0.086 ± 0.008	0.038 ± 0.004	**< 0.001**	0.058 ± 0.005	n.s.	0.043 ± 0.004	**< 0.001**	0.038 ± 0.004	**< 0.001**	**< 0.001**
		14(15)-EpETrE	0.20 ± 0.016	0.11 ± 0.009	**0.001**	0.15 ± 0.012	n.s.	0.10 ± 0.010	**< 0.001**	0.10 ± 0.007	**< 0.001**	**< 0.001**
	vic dihydroxy-PUFA	5,6-DiHETrE	0.29 ± 0.024	0.25 ± 0.020	n.s.	0.30 ± 0.025	n.s.	0.23 ± 0.019	**0.015**	0.23 ± 0.021	**0.026**	**< 0.001**
		8,9-DiHETrE	0.22 ± 0.014	0.19 ± 0.013	n.s.	0.23 ± 0.017	n.s.	0.19 ± 0.013	n.s.	0.18 ± 0.013	**0.008**	**< 0.001**
		11,12-DiHETrE	0.55 ± 0.027	0.40 ± 0.019	**< 0.001**	0.59 ± 0.037	n.s.	0.41 ± 0.028	**0.002**	0.40 ± 0.031	**0.002**	**< 0.001**
		14,15-DiHETrE	0.70 ± 0.036	0.51 ± 0.025	**< 0.001**	0.69 ± 0.036	n.s.	0.54 ± 0.029	**0.001**	0.55 ± 0.033	**0.001**	**< 0.001**
	hydroxy-PUFA	5-HETE	0.80 ± 0.050	0.45 ± 0.036	**< 0.001**	0.55 ± 0.034	**< 0.001**	0.40 ± 0.029	**< 0.001**	0.40 ± 0.029	**< 0.001**	**< 0.001**
		8-HETE	0.38 ± 0.024	0.20 ± 0.015	**< 0.001**	0.24 ± 0.016	**< 0.001**	0.19 ± 0.011	**< 0.001**	0.18 ± 0.017	**< 0.001**	**< 0.001**
		9-HETE	0.27 ± 0.019	< 0.25	-	< 0.25	-	< 0.25	-	0.25 ± 0.027	-	-
		11-HETE	0.38 ± 0.018	0.23 ± 0.014	**< 0.001**	0.33 ± 0.027	n.s.	0.32 ± 0.026	n.s.	0.45 ± 0.041	n.s.	**< 0.001**
		12-HETE	2.1 ± 0.24	0.99 ± 0.14	**< 0.001**	1.4 ± 0.12	n.s.	1.1 ± 0.11	**0.003**	0.96 ± 0.097	**< 0.001**	**< 0.001**
		15-HETE	1.0 ± 0.057	0.63 ± 0.048	**< 0.001**	0.88 ± 0.070	n.s.	0.79 ± 0.077	**0.024**	0.90 ± 0.093	n.s.	**< 0.001**
		19-HETE	< 1.0	< 1.0	-	< 1.0	-	< 1.0	-	< 1.0	-	-
		20-HETE	0.92 ± 0.057	0.49 ± 0.033	**< 0.001**	0.70 ± 0.051	**0.008**	0.45 ± 0.031	**< 0.001**	0.44 ± 0.040	**< 0.001**	**< 0.001**
		12-HHTrE	0.39 ± 0.045	0.31 ± 0.041	n.s.	0.44 ± 0.070	n.s.	0.49 ± 0.064	n.s.	0.71 ± 0.088	n.s.	**< 0.001**
		15-oxo-ETE	0.059 ± 0.009	< 0.05	-	0.053 ± 0.007	-	< 0.05	-	0.050 ± 0.007	-	-

Table S7: Continued.

Analyte			t0	t2	P t0-2	t4	P t0-4	t6	P t0-6	t8	P t0-8	P Friedman/ ANreM
ALA	epoxy-PUFA	9(10)-EpODE	0.23 ± 0.032	0.12 ± 0.020	n.s.	0.23 ± 0.024	n.s.	0.080 ± 0.009	0.002	0.056 ± 0.013	< 0.001	< 0.001
		12(13)-EpODE	0.20 ± 0.026	0.84 ± 0.14	0.005	0.99 ± 0.091	< 0.001	0.38 ± 0.031	n.s.	0.090 ± 0.016	n.s.	< 0.001
		15(16)-EpODE	4.4 ± 0.48	11 ± 1.3	< 0.001	16 ± 1.1	< 0.001	6.2 ± 0.56	n.s.	1.9 ± 0.30	0.005	< 0.001
	vic dihydroxy-PUFA	9,10-DiHODE	0.28 ± 0.046	0.46 ± 0.038	n.s.	0.49 ± 0.041	n.s.	0.16 ± 0.016	n.s.	0.14 ± 0.032	n.s.	< 0.001
		12,13-DiHODE	0.38 ± 0.066	0.56 ± 0.046	-	0.70 ± 0.054	-	0.20 ± 0.023	-	< 0.1	-	-
		15,16-DiHODE	15 ± 1.6	29 ± 1.7	< 0.001	27 ± 1.6	< 0.001	12 ± 0.52	n.s.	8.9 ± 1.3	0.024	< 0.001
	hydroxy-PUFA	9-HOTrE	0.75 ± 0.082	1.2 ± 0.11	0.031	1.7 ± 0.12	< 0.001	0.96 ± 0.092	n.s.	0.39 ± 0.043	0.016	< 0.001
		13-HOTrE	0.71 ± 0.10	2.3 ± 0.19	0.002	2.4 ± 0.16	0.001	1.5 ± 0.11	n.s.	0.56 ± 0.045	n.s.	< 0.001
EPA	vic dihydroxy-PUFA	8,9-DiHETE	< 0.05	< 0.05	-	< 0.05	-	< 0.05	-	< 0.05	-	-
		11,12-DiHETE	0.036 ± 0.003	< 0.025	-	0.038 ± 0.003	-	0.027 ± 0.002	-	< 0.025	-	-
		14,15-DiHETE	0.066 ± 0.005	0.055 ± 0.004	n.s.	0.070 ± 0.006	n.s.	0.061 ± 0.004	n.s.	0.058 ± 0.005	n.s.	< 0.001
		17,18-DiHETE	0.46 ± 0.027	0.42 ± 0.025	n.s.	0.53 ± 0.039	n.s.	0.46 ± 0.029	n.s.	0.45 ± 0.030	n.s.	< 0.001
	hydroxy-PUFA	5-HEPE	0.15 ± 0.011	0.060 ± 0.007	< 0.001	0.088 ± 0.009	n.s.	0.053 ± 0.007	< 0.001	0.056 ± 0.008	< 0.001	< 0.001
		9-HEPE	0.074 ± 0.006	< 0.05	-	< 0.05	-	< 0.05	-	< 0.05	-	-
		12-HEPE	0.18 ± 0.023	0.078 ± 0.014	< 0.001	0.11 ± 0.011	n.s.	0.092 ± 0.011	0.010	0.075 ± 0.009	< 0.001	< 0.001
		18-HEPE	0.14 ± 0.012	< 0.1	-	0.11 ± 0.012	-	< 0.1	-	0.097 ± 0.009	-	-
		19-HEPE	0.66 ± 0.054	0.51 ± 0.047	0.006	0.68 ± 0.065	n.s.	0.49 ± 0.038	0.005	0.45 ± 0.037	0.002	< 0.001
		20-HEPE	0.60 ± 0.050	0.44 ± 0.041	< 0.001	0.52 ± 0.042	0.046	0.43 ± 0.041	< 0.001	0.48 ± 0.054	0.007	< 0.001
DHA	epoxy-PUFA	10(11)-EpDPE	0.080 ± 0.010	0.036 ± 0.005	0.002	0.064 ± 0.007	n.s.	0.038 ± 0.005	0.002	0.026 ± 0.004	< 0.001	< 0.001
		16(17)-EpDPE	0.051 ± 0.008	< 0.05	-	< 0.05	-	< 0.05	-	< 0.05	-	
		19(20)-EpDPE	0.13 ± 0.012	0.061 ± 0.009	0.002	0.12 ± 0.015	n.s.	0.071 ± 0.010	0.003	0.059 ± 0.008	0.001	< 0.001
	vic dihydroxy-PUFA	4,5-DiHDPE	0.35 ± 0.044	0.21 ± 0.029	-	0.27 ± 0.036	-	< 0.2	-	< 0.2	-	-
		10,11-DiHDPE	0.12 ± 0.010	0.082 ± 0.007	< 0.001	0.12 ± 0.012	n.s.	0.092 ± 0.010	0.001	0.080 ± 0.008	0.001	< 0.001
		13,14-DiHDPE	0.16 ± 0.013	0.11 ± 0.008	< 0.001	0.16 ± 0.012	n.s.	0.12 ± 0.011	< 0.001	0.12 ± 0.010	0.006	< 0.001
		16,17-DiHDPE	0.20 ± 0.013	0.16 ± 0.010	< 0.001	0.22 ± 0.015	n.s.	0.19 ± 0.013	n.s.	0.18 ± 0.013	n.s.	< 0.001
		19,20-DiHDPE	1.9 ± 0.13	1.5 ± 0.11	< 0.001	2.1 ± 0.16	n.s.	1.8 ± 0.16	n.s.	1.7 ± 0.14	n.s.	< 0.001
	hydroxy-PUFA	4-HDHA	0.13 ± 0.012	0.078 ± 0.006	< 0.001	0.096 ± 0.008	0.015	0.070 ± 0.005	0.001	0.072 ± 0.003	0.006	< 0.001
		8-HDHA	0.39 ± 0.031	0.19 ± 0.018	0.001	0.25 ± 0.019	n.s.	0.16 ± 0.016	< 0.001	0.16 ± 0.015	< 0.001	< 0.001
		10-HDHA	0.078 ± 0.008	< 0.05	-	0.052 ± 0.005	-	< 0.05	-	< 0.05	-	-
		11-HDHA	0.14 ± 0.011	0.10 ± 0.007	0.002	0.12 ± 0.009	n.s.	0.093 ± 0.008	< 0.001	0.083 ± 0.005	< 0.001	< 0.001
		13-HDHA	0.12 ± 0.012	0.062 ± 0.007	< 0.001	0.092 ± 0.008	n.s.	0.079 ± 0.009	0.019	0.10 ± 0.009	n.s.	< 0.001
		14-HDHA	1.0 ± 0.15	0.52 ± 0.10	< 0.001	0.70 ± 0.11	n.s.	0.59 ± 0.082	0.029	0.45 ± 0.052	< 0.001	< 0.001
		16-HDHA	0.15 ± 0.008	0.10 ± 0.007	0.010	0.15 ± 0.014	n.s.	0.15 ± 0.015	n.s.	0.21 ± 0.021	n.s.	< 0.001
		17-HDHA	0.47 ± 0.048	0.24 ± 0.029	< 0.001	0.35 ± 0.036	n.s.	0.30 ± 0.047	0.024	0.28 ± 0.039	0.004	< 0.001
		20-HDHA	0.29 ± 0.020	0.18 ± 0.011	< 0.001	0.24 ± 0.013	n.s.	0.19 ± 0.014	< 0.001	0.19 ± 0.009	< 0.001	< 0.001
		21-HDHA	1.7 ± 0.15	0.73 ± 0.070	< 0.001	1.2 ± 0.12	< 0.001	0.70 ± 0.072	< 0.001	0.87 ± 0.12	< 0.001	< 0.001
		22-HDHA	1.4 ± 0.13	0.47 ± 0.043	< 0.001	0.96 ± 0.10	0.004	0.47 ± 0.059	< 0.001	0.55 ± 0.092	< 0.001	< 0.001
Oleic Acid	epoxy-PUFA	9(10)-Ep-stearic acid	14 ± 1.8	6.8 ± 0.37	< 0.001	9.1 ± 0.68	n.s.	7.2 ± 0.57	0.001	6.2 ± 0.47	< 0.001	< 0.001
	vic dihydroxy-PUFA	9,10-DiH-stearic acid	9.7 ± 1.6	4.2 ± 0.40	< 0.001	7.3 ± 1.1	n.s.	3.6 ± 0.35	< 0.001	5.2 ± 1.1	< 0.001	< 0.001

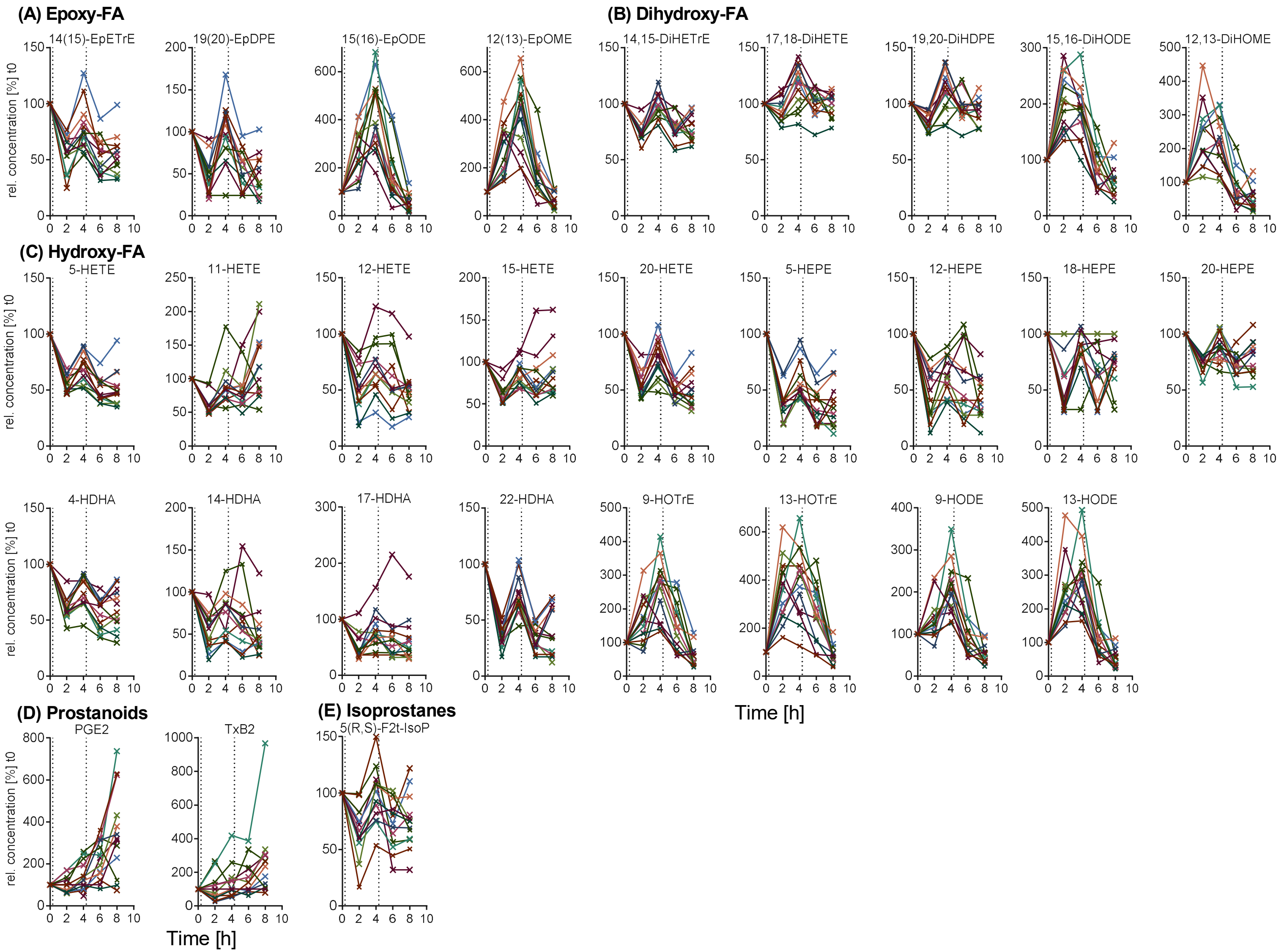


Figure S4: Individual values of intra-day variation of circulating oxylipins in plasma of subjects on a standardized diet. Shown are relative individual concentrations of selected epoxy-PUFA (**A**), dihydroxy-PUFA (**B**), hydroxy-PUFA (**C**), prostanoids (**D**) and isoprostanes (**E**) (n=13). Plasma was collected from study participants on a standardized diet at baseline and t2, t4, t6 and t8. Relative concentrations of oxylipins were calculated against baseline. Dotted lines in the diagrams indicate food intake (20 min post sample collection at t0 and t4).