

Supplementary Materials

Targeted Lipidomics for Characterization of PUFAs and Eicosanoids in Extracellular Vesicles

Madlen Reinicke [†], Saikal Shamkeeva [†], Max Hell, Berend Isermann, Uta Ceglarek and Mitja L. Heinemann ^{*}

Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig University Hospital, Paul-List-Str. 13-15, 04103 Leipzig, Germany; madlen.reinicke@medizin.uni-leipzig.de (M.R.); saikal.shamkeeva@medizin.uni-leipzig.de (S.S.); mhell97@gmx.de (M.H.); berend.isermann@medizin.uni-leipzig.de (B.I.); uta.ceglarek@medizin.uni-leipzig.de (U.C.)
^{*} Correspondence: mitja.heinemann@medizin.uni-leipzig.de (M.L.H.)
[†] These authors contributed equally to this work.

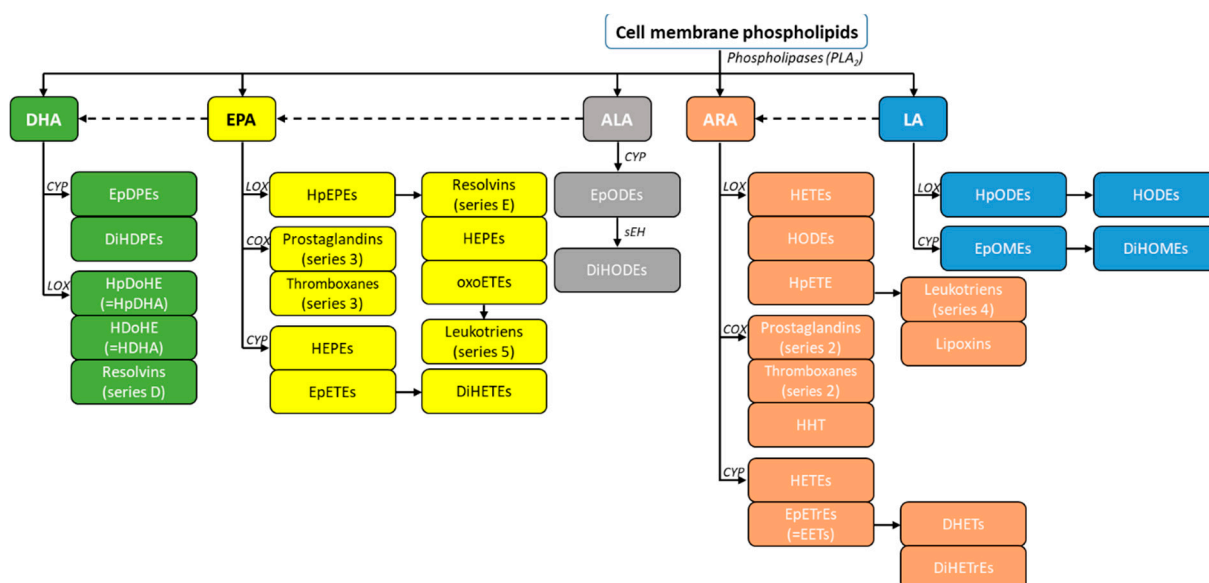


Figure S1. Simplified PUFA metabolism with the most relevant enzyme classes. ALA - alpha linolenic acid; ARA - arachidonic acid; COX - cyclooxygenase; CYP - cytochrome P450; DHA - docosahexaenoic acid; DHET - dihydroxyeicosatrienoic acid; DiHETe - dihydroxyeicosatrienoic acid; DiHDPE - dihydroxydocosapentaenoic acid; DiHETE - dihydroxyeicosatetraenoic acid; DiHODE - dihydroxyoctadecadienoic acid; DiHOME - dihydroxyoctadecamonoenoic acid; EPA - eicosapentaenoic acid; EpDPE - epoxydocosapentaenoic acid; EpETE - epoxyeicosatetraenoic acid; EpETe (=EET) - epoxyeicosatrienoic acid; EpODE - epoxyoctadecadienoic acid; EpOME - epoxyoctadecamonoenoic acid; HDoHE (=HDHA) - hydroxydocosahexaenoic acid; HEPE - hydroxyeicosapentaenoic acid; HETE - hydroxyeicosatetraenoic acid; HHT - hydroxyheptadecatrienoic acid; HODE - hydroxyoctadecadienoic acid; HpDoHE (=HpDHA) - hydroperoxydocosahexaenoic acid; HpEPE - hydroperoxyeicosapentaenoic acid; HpETE - hydroperoxyeicosatetraenoic acid; HpODE - hydroperoxyoctadecadienoic acid; LA - linoleic acid; LOX - lipoxygenase; oxoETE - oxoeicosatetraenoic acid; PLA₂ - phospholipase A₂; PUFA - polyunsaturated fatty acid; sEH - soluble epoxide hydrolase.

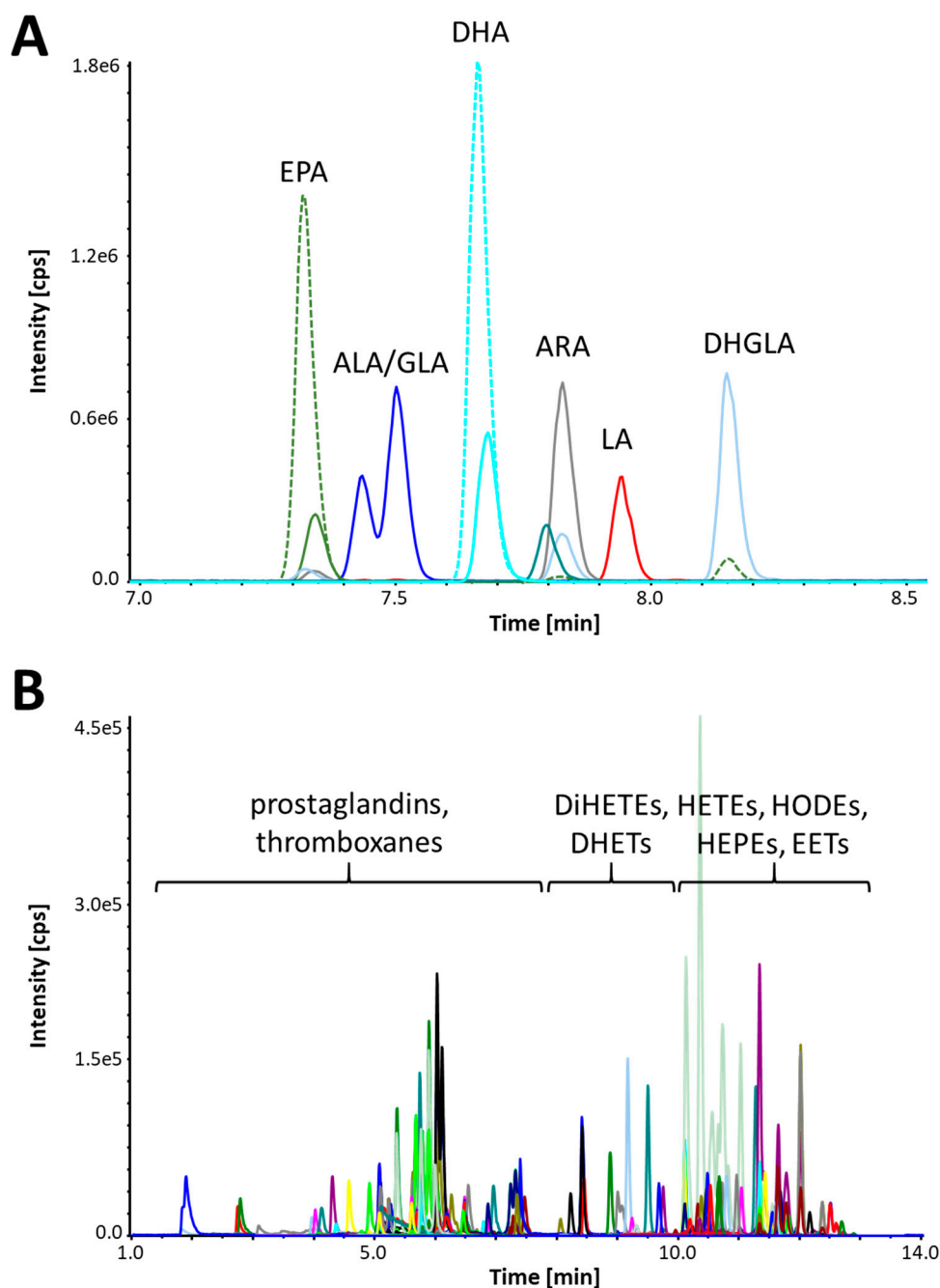


Figure S2. LC-MS/MS chromatogram of a standard mixture of (A) PUFAs, 1 µg/mL, (B) eicosanoids, 1 ng/mL. ALA - alpha linolenic acid; ARA - arachidonic acid; DHA - docosahexaenoic acid; DHET - dihydroxyeicosatrienoic acid; DHGLA - dihomogamma-linolenic acid; DiHETE - dihydroxyeicosatetraenoic acid; EET - epoxyeicosatrienoic acid; EPA - eicosapentaenoic acid; GLA - gamma linolenic acid; HEPE - hydroxyeicosapentaenoic acid; HETE - hydroxyeicosatetraenoic acid; HODE - hydroxyoctadecadienoic acid; LA - linoleic acid; LC-MS/MS - liquid chromatography - tandem mass spectrometry; PUFA - polyunsaturated fatty acid.

Table S1. Selection of mass spectrometric methods for lipid analysis of EVs.

Author	EV Isolation method	Lipid extraction	Method for lipid analysis	Lipid analytes
Subra <i>et al.</i> 2010 (18)	ultracentrifugation	Bligh and Dyer (25) and ethyl acetate	GC-MS	differently saturated FA (C ₁₄ -C ₂₄), PG series 2
Esser <i>et al.</i> 2010 (15)	ultracentrifugation / filtration	chloroform/MeOH (2:1) followed by hydrolysis	LC-MS/MS	LT serie 4, 5-HETE, 5-KETE, 15-HETE, 12-HETE, 15-KETE (samples were incubated with ARA, LTA ₄)
Llorente <i>et al.</i> 2013 (26)	ultracentrifugation	chloroform/MeOH (2:1)	LC-MS/MS	HexCer, LacCer and SM lipid classes; PC lipid class
Duchez <i>et al.</i> 2015 (16)	ultracentrifugation	chloroform/MeOH (2:1)	LC-MS/MS	12-LOX (Enzyme), 12-HETE
Haraszti <i>et al.</i> 2016 (27)	ultracentrifugation	chloroform/MeOH (1:1)	MS/MS	untargeted lipidomics, including differently saturated FA (C ₁₂ -C ₂₂),
Dang <i>et al.</i> 2017 (72)	ultracentrifugation / filtration	MeOH/DCM (2:0,9)	MS/MS	untargeted lipidomics, including differently saturated FA (C ₁₄ -C ₂₂)
Brzozowski <i>et al.</i> 2018 (29)	ultrafiltration	chloroform/MeOH (1:1)	LC-MS/MS	glycerolipids, glycerophospholipids, sphingolipids, fatty acids and sterol lipids
Hough <i>et al.</i> 2018 (17)	ultracentrifugation	chloroform/MeOH (1:2)	MS/MS	untargeted lipidomics, including sphingomyelins, glycerophospholipids and ceramides
Chen <i>et al.</i> 2019 (40)	ultracentrifugation	MeOH/DCM (1:1) with 5 mM ammonium acetate	MS/MS	untargeted lipidomics, including saturated and unsaturated species (e.g. Cer, LPC, PC)
Lacy <i>et al.</i> 2019 (73)	ultracentrifugation	directly from precipitate	LC-MS/MS	PG series 2
Nishida-Aoki <i>et al.</i> 2020 (28)	ultracentrifugation / filtration	MeOH/chloroform/water (10:5:3)	MS/MS	untargeted lipidomics, including FA, cholesterol, CE and TG
Surmiak <i>et al.</i> 2020 (38)	ultracentrifugation / filtration	tert-butyl-ether/MeOH (80:20) after acidification	LC-MS/MS	LT series 4, 5-HETE, 12-HETE, 5-oxo-EETE, 13,14-dihydro-15-keto-PGE ₂

ARA - arachidonic acid; Cer - ceramides; COX - cyclooxygenase; CE - cholesteryl ester; DCM - dichloromethane; DHA - docosahexaenoic acid; EV - extracellular vesicle; ETE - eicosatetraenoic acid; FA - fatty acid; GC - gas chromatography; HETE - hydroxyeicosatetraenoic acid; HexCer - hexosylceramide; KETE - ketoico-

satetraenoic acid; LacCer - lactosylceramide; LOX - lipoxygenase; LT - leukotriene; LPC - lysophosphatidylcholine; LC-MS/MS - liquid chromatography - tandem mass spectrometry; LXA4 - Lipoxin A4, MeOH - methanol; PC - phosphatidylcholine; PG - prostaglandin; PGE2 - prostaglandin E2; PUFA - polyunsaturated fatty acid; SM - sphingomyelin; TG – triacylglycerol.

Table S2. Primer list for RT-qPCR targeting M1/M2 marker expression.

Number	Oligoname	Gene	Sequence	Target
Pr1	hACTB-F	ACTB	ATTGCCGACAGGATGCAGAA	THP-1 (M0)
Pr2	hACTB-R	ACTB	GCTGATCCACATCTGCTGGAA	THP-1 (M0)
Pr3	hTNF-a_F	TNF-alpha	CTTCTGCCTGCTGCACTTTG	THP-1 (M1)
Pr4	hTNF-a_R	TNF-alpha	GGCCAGAGGGCTGATTAGAGA	THP-1 (M1)
Pr5	hIL-1B_F	Interleukin-1-Beta	CAGTGGCAATGAGGATGACTTG	THP-1 (M1)
Pr6	hIL-1B_R	Interleukin-1-Beta	AGTGGTGGTCCGAGATTCGT	THP-1 (M1)
Pr7	hCXCL10_F	C-X-C motif chemokine 10	ATTCCTGCAAGCCAATTTTGTC	THP-1 (M1)
Pr8	hCXCL10_R	C-X-C motif chemokine 10	CATCTCTTCTCACCCCTTCTTTTCA	THP-1 (M1)
Pr9	hCD23_F	CD23	CCCGGAACGTCTCTCAAGTTT	THP-1 (M2)
Pr10	hCD23_R	CD23	TCAGCTCGAAGTTCCTCCAGTT	THP-1 (M2)
Pr11	hCD209_F	CD209 / DC-SIGN	TCAAGCAGTATTGGAACAGAGGA	THP-1 (M2)
Pr12	hCD209_R	CD209 / DC-SIGN	CAGGAGGCTGCGGACTTTTT	THP-1 (M2)

Table S3. Comparison of precipitation solutions. Statistics: one-way ANOVA with follow-up multiple comparison test (Dunnnett).

p value	one-way ANOVA	multiple comparison test (Dunnnett)		
		A vs. B	A vs. C	A vs. D
DHA	<0.0001	<0.0001	<0.0001	<0.0001
ARA	<0.0001	0.0124	0.001	0.0261
LA	<0.0001	0.0012	<0.0001	<0.0001
Tetranor-12-HETE	<0.0001	0.0016	<0.0001	0.0001
13-HODE	0.0003	0.0018	0.0001	0.0006
9-HODE	<0.0001	0.0007	<0.0001	0.0002
8-HETE/12-HETE	0.0006	0.0711	0.0003	0.0031

A: MeOH*BHT/H₂O*ZnSO₄ (80:20 v/v, 56 mg/L BHT, 17.8 g/L ZnSO₄), B: MeOH*BHT (56 mg/L BHT), C: n-hexane/iPrOH (60:40 v/v), D: ACN/H₂O (80:20 v/v)