SUPPLEMENTARY

Oxylipin profiles in plasma of patients with Wilson's disease

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Compound nome	CAS Number	Retention	Target ion	(m/z)		Matching internal standard
TXB2	54397-85-2	9,465	369.2	(m/z) 195.1	+/- (-)	TXB2-d4
PGF2a	551-11-1	10.27	353.2	193,1	(-)	PGF2a-d4
PGE2	363-24-6	10,7	351,2	271,2	(-)	PGE2-d4
PGD2	41598-07-6	11,1	351,2	271,2	(-)	PGD2-d4
LTE4	75715-89-8	12,72	440,3	189,1	(+)	LTC4-d5
PGA2	13345-50-1	13,02	333,2	271,2	(-)	PGD2-d4
17,18-DiHETE	116477-53-3	13,94	335,2	247,2	(-)	LTB4-d4
LTB4	71160-24-2	14,12	335,2	195,1	(-)	LTB4-d4
12,13-DiHOME	263399-35-5	14,46	313,2	183,1	(-)	LTB4-d4
9,10-DiHOME	263399-34-4	14,63	313,2	201,2	(-)	LTB4-d4
12-keto-LTB4	136696-10-1	14,74	333,2	179,1	(-)	LTB4-d4
14,15-DHET	77667-09-5	15,04	337,2	207,2	(-)	LTB4-d4

 Table S1. UPLC-MS/MS parameters of the identified lipids.

12-HHT	54397-84-1	15,155	279,2	179,1 (-)	LTB4-d4
11,12-DHET	192461-95-3	15,36	337,2	167,1 (—)	LTB4-d4
8,9-DHET	192461-96-4	15,54	337,2	127,1 (-)	LTB4-d4
9-HOTrE	89886-42-0	15,66	293,2	171,1 (—)	LTB4-d4
20-carboxy-AA	79551-84-1	15,67	333,2	297,2 (—)	LTB4-d4
19-HETE	115461-40-0	15,95	319,2	275,2 (—)	LTB4-d4
12-HEPE	81187-21-5	16,385	317,2	179,1 (—)	15-HETE-d8
5-HEPE	83952-40-3	16,45	317,2	115,1 (—)	15-HETE-d8
Lyso-PAF	52691-62-0	16,49	482,3	104,2 (+)	PAF-d4
13-HODE	73804-64-5	16,6	295,2	195,1 (—)	15-HETE-d8
9-HODE	98524-19-7	16,6	295,2	171,1 (—)	15-HETE-d8
20-HDoHE	90906-41-5	16,77	343,2	241,2 (—)	15-HETE-d8
15-HETE	73836-87-0	16,93	319,2	219,2 (—)	15-HETE-d8
13-KODE	54739-30-9	17	293,2	113,1 (—)	15-HETE-d8
16-HDoHE	90780-51-1	17,05	343,2	233,2 (—)	12-HETE-d8
9-KODE	54232-59-6	17,12	293,2	185,1 (—)	12-HETE-d8
11-HETE	73804-65-6	17,125	319,2	167,1 (—)	12-HETE-d8
13-HDoHE	90780-53-3	17,15	343,2	193,1 (—)	12-HETE-d8
10-HDoHE	90780-50-0	17,2	343,2	153,1 (—)	12-HETE-d8
8-HETE	79495-84-4	17,2	319,2	155,1 (-)	12-HETE-d8
14-HDoHE	87042-40-8	17,24	343,2	205,2 (—)	12-HETE-d8
12-HETE	54397-83-0	17,28	319,2	179,1 (—)	12-HETE-d8
8-HDoHE	90780-54-4	17,37	343,2	109,1 (—)	5-HETE-d8
5-HETE	70608-72-9	17,38	319,2	115,1 (—)	5-HETE-d8
15-HETrE	92693-02-2	17,44	321,2	221,2 (—)	5-HETE-d8
4-HDoHE	90906-40-4	17,67	343,2	101,1 (—)	5-HETE-d8
AEA	94421-68-8	18,21	348,2	62,1 (+)	OEA-d4
OEA	111-58-0	19,14	326,2	62,1 (+)	OEA-d4
EPA	10417-94-4	19,415	301,2	257,2 (—)	EPA-d5
DHA	6217-54-5	20,16	327,2	283,2 (-)	DHA-d5
AA	506-32-1	20,32	303,2	303,2 (-)	AA-d8

 Table S2. Intraday reproducibility

	Slope	Intercept	Error (σ) slope	Error (σ) intercept	Relative error slope	Relative error intercept	R^2	Standard error of regression (SER)	Relative standard deviation (RSD. %)
6-keto- PGF1a-d4	2444.071	-38.804	82.861	85.872	0.034	-2.213	0.973	311.855	6.191
TXB2-d4	7593.666	178.751	297.693	308.511	0.039	1.726	0.962	1120.394	7.015

PGF2a-d4	1948.939	42.945	126.526	131.124	0.065	3.053	0.903	476.192	11.638
PGE2-d4	6875.689	246.933	319.630	331.246	0.046	1.341	0.947	1202.958	8.259
PGD2-d4	5306.940	8.195	176.488	182.902	0.033	22.319	0.973	664.229	6.026
LTC4-d5	2997.273	67.945	127.417	132.047	0.043	1.943	0.956	479.546	7.612
PGA2-d4	8550.508	312.019	304.138	315.190	0.036	1.010	0.968	1144.651	6.304
LTB4-d4	3066.559	-6.019	140.428	145.531	0.046	-24.177	0.951	528.512	8.312
15-HETE- d8	3344.472	63.796	201.106	208.414	0.060	3.267	0.916	756.880	10.796
12-HETE- d8	1661.877	-52.008	126.950	131.563	0.076	-2.530	0.877	477.786	14.061
5-HETE-d8	2753.773	44.770	148.207	153.593	0.054	3.431	0.932	557.792	9.677
OEA-d4	123070.914	21076.465	7207.485	7469.403	0.059	0.354	0.907	27126.025	9.396
EPA-d5	968.423	4.449	45.248	31.218	0.047	7.016	0.952	174.788	8.485
DHA-d5	583.910	11.467	41.140	42.635	0.070	3.718	0.888	154.835	12.647
15- deoxyPGJ2- d4	7493.587	685.609	525.081	544.162	0.070	0.794	0.882	1976.190	12.056

 Table S3. Interday reproducibility

	Slope	Intercept	Error (σ) slope	Error (σ) intercept	Relative error slope	Relative error intercept	R^2	Standard error of regression (SER)	Relative standard deviation (RSD. %)
6-keto- PGF1a-d4	3306.820	-760.694	350.301	476.203	0.106	-0.626	0.898	526.787	11.147
TXB2-d4	10491.141	-1193.387	1569.479	2133.569	0.150	-1.788	0.832	2216.397	13.322
PGF2a-d4	2570.495	421.726	687.243	934.247	0.267	2.215	0.663	960.557	19.150
PGE2-d4	6875.689	246.933	319.630	331.246	0.046	1.341	0.947	1202.958	8.259
PGD2-d4	5253.891	452.015	420.706	469.987	0.080	1.040	0.910	649.210	8.810
LTC4-d5	3082.120	90.765	244.881	273.566	0.079	3.014	0.910	410.043	9.217

PGA2-d4	12064.274	295.065	2673.015	3633.727	0.222	12.315	0.720	3570.758	16.196
LTB4-d4	4370.303	-466.032	781.735	1062.699	0.179	-2.280	0.778	1072.299	14.599
15-HETE- d8	3299.651	483.735	417.004	465.851	0.126	0.963	0.805	699.740	13.149
12-HETE- d8	2157.892	-137.694	402.466	547.117	0.187	-3.973	0.770	565.802	16.700
5-HETE-d8	2668.431	487.706	360.060	402.237	0.135	0.825	0.785	553.064	13.001
OEA-d4	102653.248	54762.382	9706.689	10843.723	0.095	0.198	0.890	20271.788	9.002
EPA-d5	4453.004	45.328	229.126	202.359	0.051	4.464	0.940	451.668	10.911
DHA-d5	677.110	26.284	87.825	110.185	0.130	4.192	0.743	178.435	13.099
15- deoxyPGJ2- d4	4669.416	5804.435	1430.199	1944.229	0.306	0.335	0.733	2579.111	16.831

Table S4. Accuracy, LOD, LOQ.

Other parameters					Accuracy		
	LOD (ng)	(3SER/Slope)	LOQ (ng)	(10SER/Slope)	0.2-0.8 ng/probe	0.9-1.3 nh/probe	1.4-2 ng/probe
6-keto-PGF1a- d4	0.478		1.593		121.792	74.277	60.848
TXB2-d4	0.634		2.113		126.785	84.188	64.595
PGF2a-d4	1.121		3.737		121.831	86.374	56.817
PGE2-d4	0.525		1.750		130.322	75.973	62.709
PGD2-d4	0.371		1.236		126.294	79.850	66.416
LTC4-d5	0.399		1.330		128.523	86.228	82.958
PGA2-d4	0.888		2.960		125.301	76.412	68.110
LTB4-d4	0.736		2.454		111.328	75.837	68.053
15-HETE-d8	0.636		2.121		64.111	71.416	59.792
12-HETE-d8	0.787		2.622		134.304	61.427	76.821

5-HETE-d8	0.622	2.073	90.726	80.260	81.628
OEA-d4	0.592	1.975	28.132	58.189	68.068
EPA-d5	0.304	1.014	33.678	69.117	46.610
DHA-d5	0.791	2.635	13.867	73.099	75.697
15-deoxyPGJ2- d4	1.657	5.523	145.978	73.897	60.536



Figure S1. The Balanced Error Rate (BER) and overall error rate estimated via cross-validation for different component numbers.

Table S5. AUC (Area Under	Curve) values and	d p-value estimated	1 for different number of	Ê
components.				

ncomp	AUC	p-value
ncomp1	0.7917000	0.00086591
ncomp2	0.8958	6.172e-06
ncomp3	0.9097	2.883e-06
ncomp4	0.8785	1.545e-05
ncomp5	0.8611	3.725e-05



Figure S2. ROC (receiver operating characteristic) curve for chosen number of components (3).

Table S6. Mean +/- standard deviation of relative concentrations for healthy controls (HC) and Wilson disease patients (WD).

	Relative concentration			
illness	НС	WD		
10-HDoHE	0.0121±0.01	0.0271±0.0274		
11,12-DHET	0.1055±0.068	0.1135±0.0692		
11-HETE	0.0573±0.045	0.0918±0.0754		
12,13-DiHOME	1.1567±0.717	1.6706±1.2151		
12-HEPE	0.0131±0.0097	0.0336±0.0422		
12-HETE	0.8281±0.7086	1.2521±1.221		
12-ННТ	0.0118±0.0143	0.0464±0.0372		
12-keto-LTB4	0.0433±0.0578	0.0208±0.0139		

13-HDoHE	0.0093±0.0112	0.0145±0.0142
13-HODE	1.2115±1.0144	1.3636±0.9827
13-KODE	0.1574±0.1113	0.1131±0.1163
14,15-DHET	0.1221±0.0739	0.1912±0.0864
14-HDoHE	0.0438±0.0267	0.103±0.16
15-HETE	0.0378±0.0253	0.0553±0.0402
15-HETrE	0.0151±0.0081	0.0356±0.0333
16-HDoHE	0.0177±0.0128	0.0317±0.0424
17,18-DiHETE	0.0647±0.0536	0.0763±0.0403
19-HETE	0.907±0.3395	0.7024±0.2777
20-HDoHE	0.0132±0.0136	0.0145±0.0156
20-carboxy-AA	0.1172±0.0994	0.0388±0.0495
4-HDoHE	0.0248±0.0181	0.0207±0.0239
5-HEPE	0.0069±0.0069	0.016±0.0165
5-HETE	0.084±0.0591	0.1237±0.1374
6-trans-LTB4+LTB4	0.0064±0.0039	0.0333±0.0671
8,9-DHET	0.0056 ± 0.0002	0.0111±0.0068
8-HDoHE	0.0197±0.0123	0.0505±0.0827
8-HETE	0.0232±0.0174	0.0272±0.0193
9,10-DiHOME	1.5115±1.067	1.5454±1.4252
9-HODE	0.4867±0.1988	0.8474±0.5325
9-HOTrE	0.0049±0.0049	0.007±0.0027
9-KODE	0.0837±0.0541	0.0542±0.0339
АА	9.5848±7.1545	8.6858±5.5443
AEA	1.8321±1.5384	2.3297±1.1781
DHA	46.2452±36.2903	41.8502±23.264

EPA	8.5929±9.7091	24.6221±23.2701	
LTE4	0.0194±0.0204	0.0912±0.1524	
Lyso-PAF	326.5978±156.9061	431.2231±197.1076	
OEA	10.8608±6.6427	16.7657±8.5828	
PGA2	0.0438±0.0251	0.0319±0.0197	
PGD2	0.0027±0.0023	0.0095±0.0076	
PGE2	0.0102±0.0101	0.0231±0.0186	
PGF2a	0.0045±0.0029	0.0066±0.0036	
TXB2	0.1087±0.1195	0.4916±0.6527	

Table S7. Source acid and metabolic enzyme for the analyzed oxylipins.

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Chemical	Acid	Enzyme
10-HDoHE	DHA	LOX
11,12-DHET	AA	СҮР
11-HETE	AA	LOX
12,13-DiHOME	LA	СҮР
12-HEPE	EPA	LOX
12-HETE	AA	LOX
12-HHT	AA	COX
12-keto-LTB4	AA	LOX
13-HDoHE	DHA	LOX
13-HODE	LA	LOX
13-KODE	LA	LOX
14,15-DHET	AA	СҮР
14-HDoHE	DHA	LOX
15-HETE	AA	LOX

15-HETrE	DGLA	LOX
16-HDoHE	DHA	LOX
17,18-DiHETE	EPA	СҮР
19-HETE	AA	СҮР
20-HDoHE	DHA	СҮР
20-carboxy-AA	AA	СҮР
4-HDoHE	DHA	LOX
5-HEPE	EPA	ROS
5-HETE	AA	LOX
6-trans-LTB4+LTB4	AA	LOX
8,9-DHET	AA	СҮР
8-HDoHE	DHA	LOX
8-HETE	AA	LOX
9,10-DiHOME	LA	СҮР
9-HODE	LA	LOX
9-HOTrE	ALA	LOX
9-KODE	LA	LOX
AA	-	-
AEA	EA	-
DHA	-	-
EPA	-	-
LTE4	AA	LOX
Lyso-PAF	-	-
OEA	EA	-
PGA2	AA	COX
PGD2	AA	COX

PGE2	AA	COX
PGF2a	AA	COX
TXB2	AA	COX

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Figure S3 (A-D). A clustered image map was generated using euclidean distance and complete linkage clustering algorithm. On the figure each entry of that matrix is colored according to its value, rows represent subjects, columns - metabolites. Dendrograms are shown on the left side (for metabolites). Clustering was performed only for rows, columns are placed in ascending order according to studied characteristic (A) Cu, mkM in serum, (B) Shvab scale, (C) age, (D) debut age. Studied parameters values are labeled on the bottom side of CIM. Colorbar on the top side of the picture indicates whether the subject is WD (black) or HC (red).



Figure S4. Relative concentrations of separate metabolites which changed significantly in WD1 and/or WD2 in comparison with HC. Pairwise comparison of adjusted means was conducted taking into account age and sex of patients. * - p<0.05 (adjusted for multiple testing).



Figure S5. Relative concentrations of summed metabolites which changed significantly between group1 and group2. Concentrations of compounds were summed according to their acid precursors (AA, DHA, EPA, ALA, DGLA, EA, EPA) or via which metabolic pathways they were derived (COX, CYP, LOX or non-enzymatic ROS). Here only compounds which significantly differs between groups are shown. Pairwise comparison of adjusted means was conducted taking into account age and sex of patients. * - p<0.05 (adjusted for multiple testing).