

Open-flow Microperfusion Combined with Mass Spectrometry for *in vivo*

Liver Lipidomic Analysis

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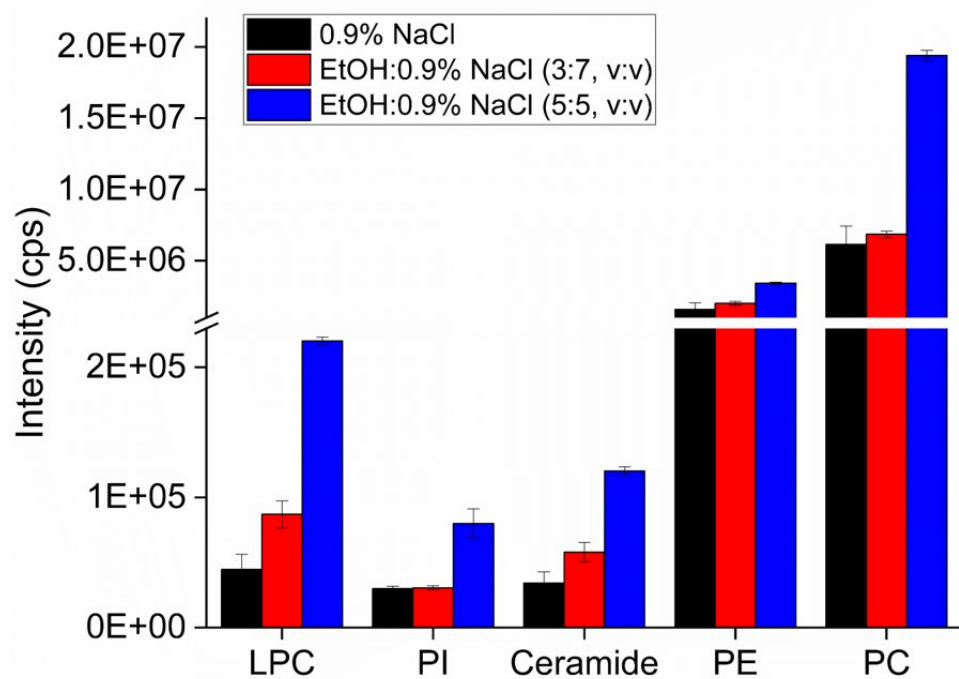


Fig. S1 Comparison the intensity of MS signals of lipid standards collected using different perfusates with the selected OFM probe (hole size: 50 μm , hole number: 60) at the perfusion rate (2 $\mu\text{L}/\text{min}$).

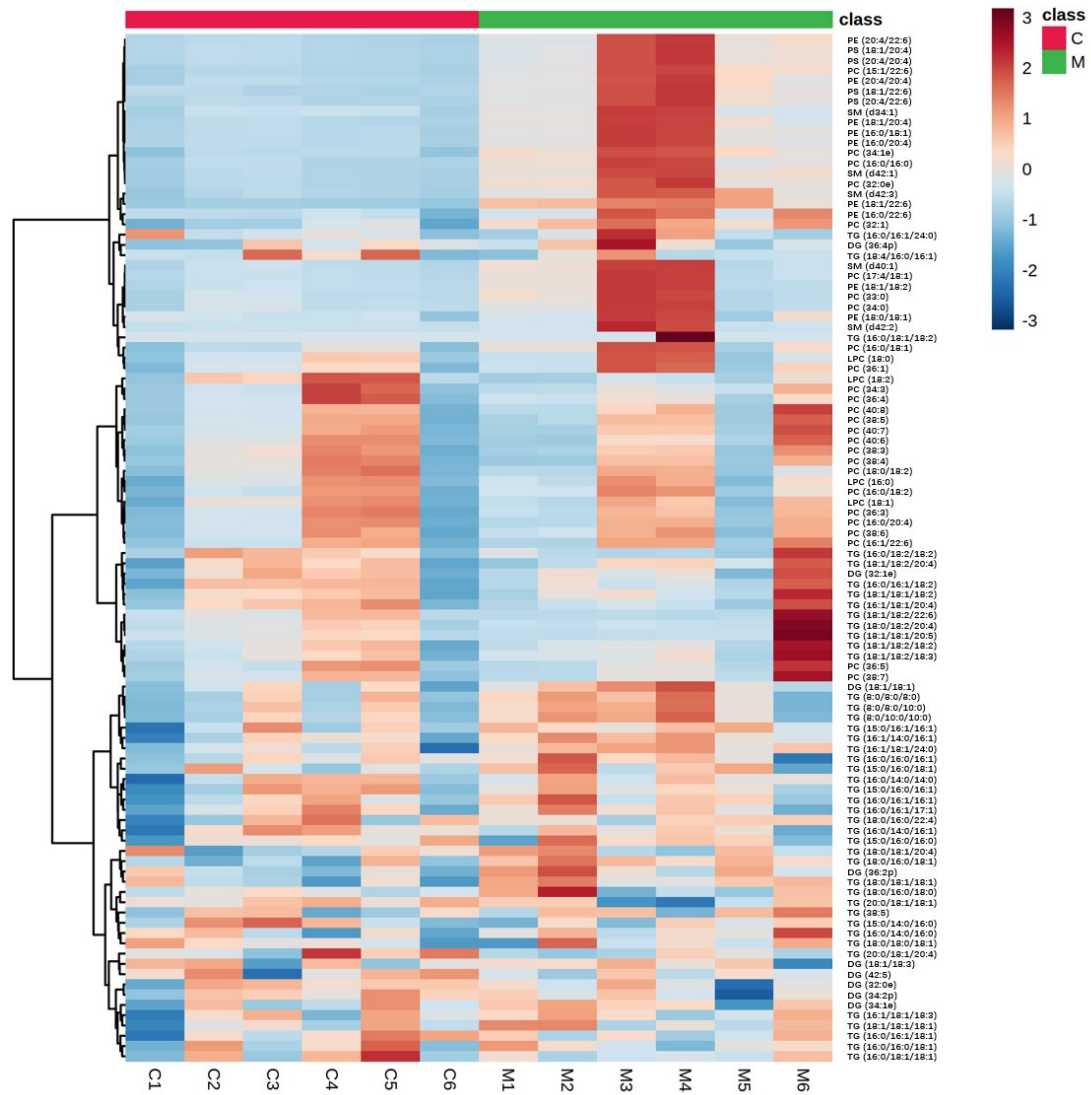


Fig. S2 Heat map denoting fold changes (over normalized means) of lipids between control group and model group.

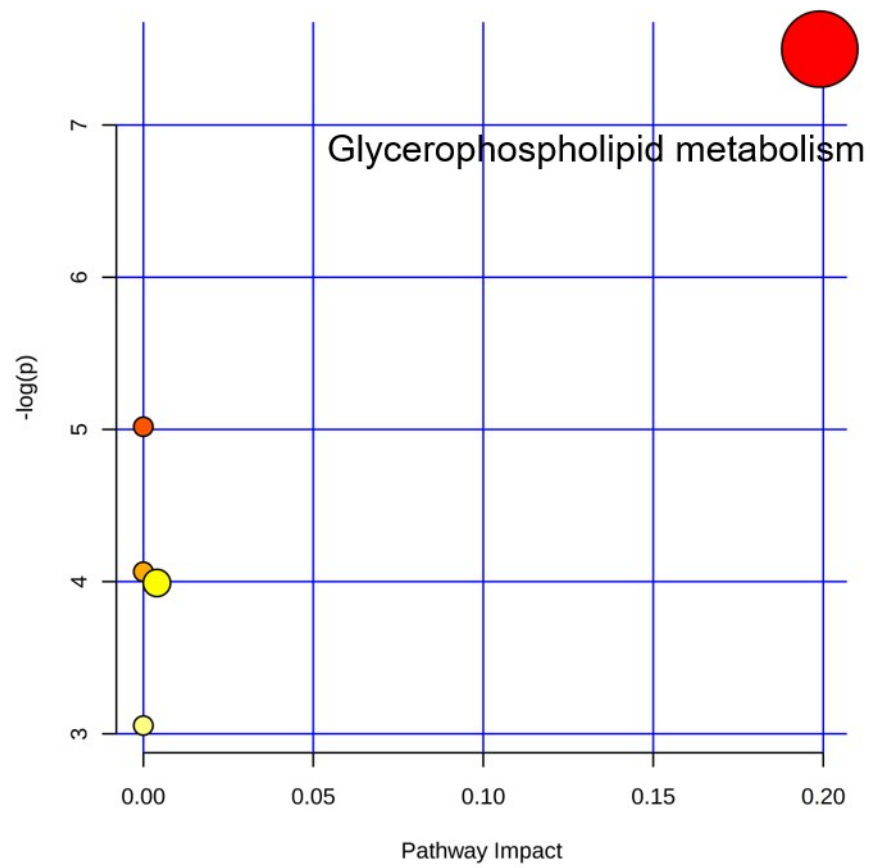


Fig. S3 Summary of pathway analysis.

Table S1. The MRM transitions for the lipid standards.

ID	Q1 Mass (Da)	Q3 Mass (Da)	CE (Volts)
PI (16:0_18:2)	835.5	575.5	26.0
PE (17:0_17:0)	720.6	579.5	32.0
Ceramide (d18:1/17:0)	552.5	264.2	43.0
LPC (12:0)	440.3	184.0	32.0
PC (19:0_19:0)	818.6	184.0	41.0

Table S2. The detected metabolites in mouse liver dialysates sampled by OFM and MD using UPLC-ESI HR MS.

□	Compound	Experimental m/z	Theoretical	Adduct	Error/ppm
			m/z		
Detected by MD and OFM	Histidine	156.0763	156.0767	[M+H] ⁺	-2.6
	Carnitine	162.1122	162.1125	[M+H] ⁺	-1.9
	Phenylalanine	166.0858	166.0862	[M+H] ⁺	-2.4
	Tyrosine	182.0807	182.0812	[M+H] ⁺	-2.7
	Glucose	203.0521	203.0526	[M+Na] ⁺	-2.5
	Acetyl-L-carnitine	204.1225	204.1230	[M+H] ⁺	-2.4
	Tryptophan	205.0966	205.0972	[M+H] ⁺	-2.9
	Cytidine	244.0920	244.0928	[M+H] ⁺	-3.3
	N-Ribosylnicotinamide	255.0968	255.0975	[M+H] ⁺	-2.7
	Adenosine	268.1033	268.1040	[M+H] ⁺	-2.6
Only Detected by OFM	Inosine	269.0873	269.0881	[M+H] ⁺	-3.0
	Lactose	343.1228	343.1235	[M+H] ⁺	-2.0
	DG(18:1_18:3)	617.5148	617.5140	[M+H] ⁺	1.3
	DG(18:1_18:1)	643.5288	643.5272	[M+Na] ⁺	2.5
	DG(42:5)	721.5792	721.5741	[M+Na] ⁺	6.9
	DG(32:0e)	577.5202	577.5166	[M+Na] ⁺	6.2
	DG(32:1e)	575.5043	575.5010	[M+Na] ⁺	5.7
	DG(34:1e)	603.5353	603.5323	[M+Na] ⁺	5.0
	DG(34:2p)	577.5204	577.5190	[M+H] ⁺	2.4
	DG(36:2p)	605.5518	605.5503	[M+H] ⁺	2.4
	DG(36:4p)	601.5177	601.5196	[M+H] ⁺	-3.2
	LPC(16:0)	496.3409	496.3398	[M+H] ⁺	2.3
	LPC(18:0)	524.3720	524.3711	[M+H] ⁺	1.7
	LPC(18:1)	522.3565	522.3554	[M+H] ⁺	2.1
	LPC(18:2)	520.3410	520.3398	[M+H] ⁺	2.4
	PC(16:0_16:0)	734.5705	734.5694	[M+H] ⁺	1.5
	PC(32:0e)	720.5918	720.5902	[M+H] ⁺	2.2
	PC(32:1)	732.5555	732.5538	[M+H] ⁺	2.4
	PC(33:0)	748.5867	748.5851	[M+H] ⁺	2.2
	PC(34:0)	762.6035	762.6013	[M+H] ⁺	2.9
	PC(16:0_18:1)	760.5863	760.5851	[M+H] ⁺	1.5
	PC(34:1e)	746.6033	746.6063	[M+H] ⁺	-4.1
	PC(16:0_18:2)	758.5707	758.5694	[M+H] ⁺	1.6
	PC(34:3)	756.5556	756.5538	[M+H] ⁺	2.4
	PC(36:1)	788.6182	788.6164	[M+H] ⁺	2.3
	PC(18:0_18:2)	786.6024	786.6007	[M+H] ⁺	2.1
	PC(36:3)	784.5866	784.5851	[M+H] ⁺	1.9
	PC(36:4)	782.5706	782.5694	[M+H] ⁺	1.5

PC(36:5)	780.5557	780.5538	[M+H] ⁺	2.5
PC(15:1_22:6)	790.5393	790.5381	[M+H] ⁺	1.5
PC(38:3)	812.6181	812.6164	[M+H] ⁺	2.1
PC(38:4)	810.6024	810.6007	[M+H] ⁺	2.1
PC(38:5)	808.5863	808.5851	[M+H] ⁺	1.4
PC(38:6)	806.5709	806.5694	[M+H] ⁺	1.8
PC(16:0_22:6)	828.5529	828.5514	[M+Na] ⁺	1.9
PC(38:7)	804.5512	804.5543	[M+H] ⁺	-3.9
PC(40:6)	834.6029	834.6007	[M+H] ⁺	2.5
PC(40:7)	832.5867	832.5851	[M+H] ⁺	1.9
PC(40:8)	830.5721	830.5700	[M+H] ⁺	2.5
PE(16:0_18:1)	740.5239	740.5201	[M+Na] ⁺	5.2
PE(18:0_18:1)	768.5557	768.5514	[M+Na] ⁺	5.6
PE(18:1_18:2)	764.5242	764.5201	[M+Na] ⁺	5.4
PE(16:0_20:4)	740.5239	740.5225	[M+H] ⁺	2.0
PE(18:1_20:4)	766.5394	766.5381	[M+H] ⁺	1.7
PE(16:0_22:6)	764.5242	764.5225	[M+H] ⁺	2.3
PE(18:1_22:6)	790.5397	790.5381	[M+H] ⁺	2.0
PE(20:4_20:4)	788.5249	788.5225	[M+H] ⁺	3.1
PE(20:4_22:6)	812.5244	812.5225	[M+H] ⁺	2.4
PS(18:1_20:4)	832.5146	832.5099	[M+Na] ⁺	5.6
PS(18:1_22:6)	856.5151	856.5099	[M+Na] ⁺	6.1
PS(20:4_20:4)	832.5143	832.5123	[M+H] ⁺	2.3
PS(20:4_22:6)	856.5148	856.5123	[M+H] ⁺	2.9
SM(d34:1)	703.5763	703.5749	[M+H] ⁺	2.1
SM(d40:1)	787.6708	787.6688	[M+H] ⁺	2.7
SM(d42:1)	815.7019	815.7001	[M+H] ⁺	2.3
SM(d42:2)	813.6866	813.6844	[M+H] ⁺	2.7
SM(d42:3)	811.6700	811.6688	[M+H] ⁺	1.5
TG(8:0_8:0_8:0)	493.3509	493.3500	[M+Na] ⁺	2.0
TG(8:0_8:0_10:0)	521.3822	521.3813	[M+Na] ⁺	1.8
TG(8:0_10:0_10:0)	549.4135	549.4126	[M+Na] ⁺	1.7
TG(38:5)	674.5363	674.5360	[M+NH ₄] ⁺	0.4
TG(16:0_14:0_14:0)	768.7079	768.7081	[M+NH ₄] ⁺	-0.3
TG(15:0_14:0_16:0)	787.6808	787.6786	[M+Na] ⁺	2.8
TG(16:0_14:0_16:0)	796.7382	796.7394	[M+NH ₄] ⁺	-1.5
TG(16:0_14:0_16:1)	794.7246	794.7232	[M+NH ₄] ⁺	1.8
TG(16:1_14:0_16:1)	792.7077	792.7081	[M+NH ₄] ⁺	-0.5
TG(15:0_16:0_16:0)	810.7558	810.7545	[M+NH ₄] ⁺	1.6
TG(15:0_16:0_16:1)	808.7411	808.7389	[M+NH ₄] ⁺	2.7
TG(15:0_16:1_16:1)	806.7253	806.7232	[M+NH ₄] ⁺	2.6

TG(16:0_16:0_16:1)	822.7569	822.7545	[M+NH ₄] ⁺	2.9
TG(16:0_16:1_16:1)	820.7402	820.7389	[M+NH ₄] ⁺	1.6
TG(15:0_16:0_18:1)	836.7716	836.7702	[M+NH ₄] ⁺	1.7
TG(16:0_16:1_17:1)	834.7563	834.7545	[M+NH ₄] ⁺	2.2
TG(16:0_16:0_18:1)	850.7874	850.7858	[M+NH ₄] ⁺	1.9
TG(16:0_16:1_18:1)	848.7719	848.7702	[M+NH ₄] ⁺	2.1
TG(16:0_16:1_18:2)	846.7544	846.7551	[M+NH ₄] ⁺	-0.8
TG(18:4_16:0_16:1)	825.6957	825.6967	[M+H] ⁺	-1.2
TG(18:0_16:0_18:0)	880.8321	880.8333	[M+NH ₄] ⁺	-1.4
TG(18:0_16:0_18:1)	878.8195	878.8171	[M+NH ₄] ⁺	2.7
TG(16:0_18:1_18:1)	876.8028	876.8015	[M+NH ₄] ⁺	1.5
TG(16:0_18:1_18:2)	874.7876	874.7858	[M+NH ₄] ⁺	2.0
TG(16:0_18:2_18:2)	872.7718	872.7702	[M+NH ₄] ⁺	1.8
TG(16:1_18:1_18:3)	853.7274	853.7280	[M+H] ⁺	-0.7
TG(18:0_18:0_18:1)	906.8506	906.8484	[M+NH ₄] ⁺	2.4
TG(18:0_18:1_18:1)	904.8346	904.8328	[M+NH ₄] ⁺	2.0
TG(18:1_18:1_18:1)	902.8179	902.8171	[M+NH ₄] ⁺	0.9
TG(18:1_18:1_18:2)	900.8033	900.8015	[M+NH ₄] ⁺	2.0
TG(18:1_18:2_18:2)	898.7875	898.7858	[M+NH ₄] ⁺	1.8
TG(18:1_18:2_18:3)	896.7716	896.7702	[M+NH ₄] ⁺	1.6
TG(16:1_18:1_20:4)	879.7430	879.7436	[M+H] ⁺	-0.7
TG(16:0_16:1_24:0)	934.8820	934.8797	[M+NH ₄] ⁺	2.5
TG(20:0_18:1_18:1)	932.8666	932.8641	[M+NH ₄] ⁺	2.8
TG(18:0_16:0_22:4)	928.8319	928.8333	[M+NH ₄] ⁺	-1.5
TG(18:0_18:1_20:4)	909.7900	909.7906	[M+H] ⁺	-0.7
TG(18:0_18:2_20:4)	924.8023	924.8020	[M+NH ₄] ⁺	0.3
TG(18:1_18:1_20:5)	905.7588	905.7593	[M+H] ⁺	-0.5
TG(18:1_18:2_20:4)	905.7586	905.7593	[M+H] ⁺	-0.7
TG(16:1_18:1_24:0)	960.8951	960.8959	[M+NH ₄] ⁺	-0.8
TG(20:0_18:1_20:4)	937.8216	937.8219	[M+H] ⁺	-0.3
TG(18:1_18:2_22:6)	946.7856	946.7864	[M+NH ₄] ⁺	-0.8

Table S3. Evaluation of liver fibrosis model

	ALT (U/L)	AST (U/L)
Control (n=6)	41.6	96.4*
Model (n=6)	935.1	1233.3*

*: $p < 0.05$

Table S4. Results of pathway analysis.

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact
Glycerophospholipid metabolism	2/36	5.2479E ⁻⁴	7.5525	0.044083	0.044083	0.19895
Linoleic acid metabolism	1/5	0.0064433	5.0447	0.53479	0.27062	0.0
Alpha-Linolenic acid metabolism	1/13	0.016709	4.0918	1.0	0.37776	0.0
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1/14	0.017989	4.018	1.0	0.37776	0.00399
Arachidonic acid metabolism	1/36	0.045927	3.0807	1.0	0.77157	0.0