Supporting Information

Analysis of *Drosophila* Lipids by MALDI-MS Imaging

Ann-Christin Niehoff[1,2,3,4], Hans Kettling[4,5], Alexander Pirkl[4], Yin Ning Chiang[1], Klaus Dreisewerd[4,5], Joanne Y. Yew[1,6,7*]

[1]Temasek Life Sciences Laboratory, 1 Resarch Link, Singapore 117604

[2]NRW Graduate School of Chemistry, University of Münster, Wilhelm-Klemm-Strasse 10, 48149 Münster, Germany

[3]Institute of Inorganic and Analytical Chemistry, University of Münster, Corrensstrasse 30, 48149 Münster, Germany

[4]Institute for Hygiene, University of Münster, Robert-Koch-Strasse 41, 48149 Münster, Germany

[5]Interdisciplinary Center for Clinical Research (IZKF), Domagkstrasse 3, 48149 Münster, Germany

[6]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543

[7]Pacific Biosciences Research Center, University of Hawai'i at Mānoa, 1993 East-West Road Honolulu, HI 96822, USA

*: corresponding author

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Figure S-1. Brightfield microscope images of matrix-covered fly tissue sections.

Figure S-2. Representative mass spectra of signals corresponding to phosphatidylcholines and triacylglycerides and MS images acquired with a QSTAR instrument.

Table S-1. Lipid classes detected from *Drosophila* tissue analyzed in positive ion mode with the Synapt G2-S mass spectrometer.

Class and Adduct Ion Type[1,2]	Composition[3]	*m/z* theoretical	DHB, 35 µm[4] *m/z* observed	DHB 10 µm[4]	Dithranol 10 µm[4]
PC $[M+H]^+$	C28:1	676.492	676.485	-	-
	C28:0	678.507	678.496	-	-
	C30:4	698.401	698.401	-	-
	C30:3	700.417	700.421	-	-
	C32:3	728.530	728.528	+	+
	C32:2	730.539	730.544	+	+
	C32:1	732.554	732.551	+	+
	C34:3	756.554	756.551	+	+
	C34:2	758.570	758.570	+	+
	C34:1	760.586	760.591	+	+
	C36:4	782.570	782.573	+	+
	C36:3	784.586	784.577	+	+
	C36:2	786.601	786.608	+	+
	C38:2	814.632	814.632	+	+
	C38:1	816.648	816.652	+	+
PC-N(CH3)$_3$ $[M+H]^+$	C28:1	617.418	617.421	-	-
	C28:0	619.434	619.430	-	-
	C30:4	639.403	639.404	-	-
	C30:3	641.418	641.415	-	-
	C32:3	669.449	669.452	-	-
	C32:2	671.465	671.464	-	-
	C32:1	673.480	673.479	+	+
	C32:3	697.481	697.479	+	+
	C32:2	699.496	699.498	-	-
	C32:1	701.512	701.508	-	-
	C36:4	723.496	723.494	+	+
	C36:3	725.512	725.515	-	-
	C36:2	727.528	727.515	-	-
	C38:1	757.575	757.572	+	+
PS $[M+Na]^+$	C28:0	702.432	702.433	-	-
	C30:2	726.432	726.432	-	-
	C30:1	728.448	728.446	-	-
	C32:4	750.432	750.428	-	-
	C32:3	752.448	752.451	-	-
	C32:2	754.463	754.463	-	-
	C32:1	756.479	756.479	-	-
	C34:4	778.463	778.463	-	-

PS $[M+Na]^+$	C34:3	780.479	780.474	-	-
	C34:2	782.495	782.500	-	-
	C36:6	802.463	802.468	-	-
	C36:5	804.479	804.485	-	-
	C36:4	806.495	806.492	-	-
	C36:3	808.510	808.514	-	-
	C36:2	810.526	810.526	-	-
	C38:2	838.557	838.552	-	-
PE $[M+H]^+$	C32:2	688.492	688.493	-	-
	C32:1	690.507	690.499	-	-
	C34:4	712.492	712.488	-	-
	C34:3	714.507	714.505	-	-
	C34:2	716.523	716.525	-	-
	C34:1	718.539	718.536	-	-
	C36:6	736.492	736.491	-	-
	C36:5	738.507	738.506	-	-
	C36:4	740.523	740.524	+	-
	C36:3	742.539	742.533	+	-
	C36:2	744.554	744.557	+	+
	C36:1	746.570	746.571	+	+
	C38:6	764.523	764.520	-	-
	C38:5	766.539	766.537	-	-
	C38:4	768.554	768.545	-	-
	C38:3	770.570	770.579	-	-
	C38:2	772.586	772.592	-	-
	C38:1	774.601	774.608	-	-
PG $[M+Na]^+$	C32:2	741.468	741.463	-	-
	C32:1	743.484	743.485	-	-
	C32:0	745.500	745.498	-	-
	C34:4	765.468	765.462	-	-
	C34:3	767.484	767.487	-	-
	C34:2	769.500	769.501	-	-
	C34:1	771.515	771.513	-	+
	C34:0	773.531	773.539	-	+
	C36:2	797.531	797.530	+	+
	C36:1	799.546	799.540	-	+
TAG $[M+Na]^+$	C40:0	715.584	715.579	-	-
	C40:1	717.601	717.600	-	-
	C42:1	743.617	743.616	+	-
	C42:0	745.632	745.629	+	-
	C44:2	769.632	769.634	+	-
	C44:1	771.648	771.646	+	-
	C46:3	795.648	795.645	+	-

TAG $[M+Na]^+$	C46:2	797.663	797.665	+	-
	C46:1	799.679	799.676	+	-
	C48:3	823.679	823.678	+	-
	C48:2	825.695	825.696	+	-
	C48:1	827.710	827.717	+	-
	C50:3	851.710	851.712	+	-
	C50:2	853.726	853.726	+	-
	C50:1	855.742	855.742	+	-
	C52:3	879.742	879.749	-	-
	C52:2	881.757	881.757	-	-
cVA $[M+Na]^+$	$C_{20}H_{38}O_2$	333.277	333.279	-	-
CH503 $[M+Na]^+$	$C_{30}H_{56}O_3$	487.417	487.421	-	-

[1]PC: phosphatidylcholine; PS: phosphatidylserine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; TAG: triacylglyceride; male sex pheromones: cVA (cis-vaccenyl acetate) and CH503 ((3*R*,11*Z*,19*Z*)-3-acetoxy-11,19-octacosadien-1-ol).
[2]Most prominent adduct detected.
[3]Notation indicates the number of carbons followed by number of double bonds in the fatty acyl chains of each lipid species.
[4]Laser spot size.

Table S-2. Lipid classes detected from *Drosophila* tissue analyzed in positive ion mode with 50 µm lateral resolution using the QSTAR mass spectrometer.

Class[1,2]	Composition[3]	*m/z* theoretical	*m/z* observed
PC $[M+H]^+$	C32:2	730.539	730.536
	C32:1	732.554	732.561
	C34:3	756.554	756.551
	C34:2	758.570	758.577
	C34:1	760.601	760.613
	C36:3	784.592	784.586
	C36:2	786.620	786.620
PC-N(CH3)$_3$ $[M+H]^+$	C32:1	673.481	673.461
	C34:3	697.481	697.477
	C34:2	699.496	699.471
	C36:4	723.496	723.482
	C36:3	725.512	725.522
	C36:2	727.528	727.534
PE $[M+Na]^+$	C34:3	736.492	736.480
	C34:2	738.507	738.505
	C36:4	740.523	740.526
	C36:3	742.539	742.537
	C36:2	744.554	744.567
	C36:1	746.560	746.573
PG $[M+Na]^+$	C32:1	743.484	743.496
	C32:0	745.500	745.515
	C34:4	765.468	765.476
	C34:3	767.484	767.499
	C34:2	769.500	769.504
	C34:1	771.515	771.513
	C36:4	793.500	793.490
	C36:3	795.515	795.507
	C36:2	797.531	797.554
	C36:1	799.546	799.559
	C36:0	801.562	801.571
TAG $[M+Na]^+$	C40:0	717.601	717.617
	C42:1	743.617	743.624
	C42:0	745.632	745.642
	C44:2	769.632	769.637
	C44:1	771.648	771.648
	C44:0	773.663	773.679
	C46:2	797.663	797.675
	C46:1	799.679	799.695
	C46:0	801.695	801.707

TAG $[M+Na]^+$	C48:2	825.695	825.691
	C50:2	853.726	853.749
	C50:1	855.742	855.753
	C52:3	879.742	879.738
	C52:2	881.757	881.770
cVA $[M+Na]^+$	$C_{20}H_{38}O_2$	333.277	333.269
CH503 $[M+Na]^+$	$C_{30}H_{56}O_3$	487.417	487.427

[1]PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; TAG: triacylglyceride; male sex pheromones: cVA (cis-vaccenyl acetate) and CH503((3*R*,11*Z*,19*Z*)-3-acetoxy-11,19-octacosadien-1-ol).
[2]Most prominent adduct detected.
[3]Notation indicates the number of carbons followed by number of double bonds in the fatty acyl chains of each lipid species.

Table S-3. Lipid classes detected from *Drosophila* tissue analyzed in negative ion mode with 10 µm lateral resolution using the Synapt G2-S mass spectrometer.

Class and Adduct Ion Type[1]	**Composition**[2]	***m/z* theoretical**	***m/z* observed**
PI $[M-H]^-$	C32:2	805.487	805.487
	C32:1	807.502	807.496
	C34:4	829.487	829.491
	C34:3	831.502	831.506
	C34:2	833.518	833.522
	C36:6	853.487	853.491
	C36:5	855.502	855.509
	C36:4	857.518	857.516
	C36:3	859.534	859.538
PE $[M-H]^-$	C32:2	686.476	686.479
	C32:1	688.492	688.494
	C34:2	714.507	714.510
	C34:1	716.523	716.518
	C36:4	738.507	738.502
	C36:3	740.522	740.521
	C36:2	742.539	742.542

[1]PI: phosphatidylinositol; PE: phosphatidylethanolamine.
[2]Notation indicates the number of carbons followed by number of double bonds in the fatty acyl chains of each lipid species.

Figure S-1. (A) Bright field microscope image of a fly tissue section covered with DHB crystals, prior to MS analysis. Red arrowheads indicate underlying cuticle (dark spots) and testes (yellow-tinged tissue) or (B) muscle fibers of the underlying fly tissue. Testes are identified by the distinct yellow color. Muscle fibers are identified by striated and repeating arrangement. (C) Brightfield microscope image of a dithranol-covered fly tissue section, prior to MS analysis.