Ethanol and C2 ceramide activate fatty acid oxidation in human hepatoma cells

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Supplementary Figures

Supplementary Table 1: *Ceramide Quantitation in whole cell extracts by mass spectrometry.* VL-17A cells were treated for 48 h with control- or 100 mM ethanol-containing media or supplemented with BSA (vehicle control),100 µM oleate, 40 µM palmitate or 10 µM C2 ceramide. Protein normalized samples were loaded onto LC-MS/MS. Listed concentrations are in nM. Listed p values compare control and ethanol samples within same lipid treatment. *: $p<0.05$, N=3

	BSA			Oleate			Palmitate			C2 Ceramide		
	Control	Ethanol		Control	Ethanol		Control	Ethanol		Control	Ethanol	
	AVG ± SEM	AVG ± SEM	*p*	AVG ± SEM	AVG ± SEM	*p*	AVG ± SEM	AVG ± SEM	*p*	AVG ± SEM	AVG ± SEM	*p*
Cer C2:0	13.3 ± 8.8	16.7 ± 3.3	0.74	23.3 ± 3.3	26.7 ± 12.0	0.80	20.0 ± 5.8	6.7 ± 6.7	0.21	60.0 ± 5.8*	73.3 ± 37.6	0.74
Cer C4:0	13.3 ± 8.8	10.0 ± 10.0	0.81	13.3 ± 3.3	13.3 ± 3.3	1.00	13.3 ± 8.8	6.7 ± 3.3	0.52	26.7 ± 6.7	13.3 ± 3.3	0.15
Cer C6:0	23.3 ± 3.3	16.7 ± 8.8	0.52	53.3 ± 8.8*	26.7 ± 13.3	0.17	36.7 ± 3.3*	40.0 ± 20.8	0.88	26.7 ± 3.3	20.0 ± 15.3	0.69
Cer C8:0	23.3 ± 3.3	16.7 ± 8.8	0.52	53.3 ± 8.8*	26.7 ± 13.3	0.17	36.7 ± 3.3*	40.0 ± 20.8	0.88	26.7 ± 3.3	20.0 ± 15.3	0.69
Cer C10:0	20.0 ± 5.8	26.7 ± 6.7	0.49	13.3 ± 3.3	20.0 ± 0.0	0.12	13.3 ± 3.3	16.7 ± 3.3	0.52	33.3 ± 8.8	20.0 ± 5.8	0.27
Cer C12:0	3.3 ± 3.3	6.7 ± 3.3	0.52	13.3 ± 3.3	10.0 ± 0.0	0.37	30.0 ± 11.5	23.3 ± 8.8	0.67	16.7 ± 8.8	16.7 ± 6.7	1.00
Cer C14:0	0.0 ± 0.0	16.7 ± 8.8	0.13	6.7 ± 3.3	10.0 ± 0.0	0.37	23.3 ± 18.6	10.0 ± 5.8	0.53	10.0 ± 5.8	16.7 ± 6.7	0.49
Cer C14:1	20.0 ± 5.8	16.7 ± 6.7	0.72	16.7 ± 6.7	10.0 ± 0.0	0.37	3.3 ± 3.3	6.7 ± 3.3	0.52	10.0 ± 5.8	16.7 ± 3.3	0.37
Cer C16:0	1363.3 ± 150.7	853.3 ± 67.7	0.04	1163.3 ± 66.9	730.0 ± 32.1*	0.00	1786.7 ± 117.2*	823.3 ± 39.3*	0.00	1426.7 ± 112.0	730.0 ± 30.6*	0.00
Cer C16:1	120.0 ± 25.2	70.0 ± 10.0	0.14	60.0 ± 32.1	80.0 ± 11.5	0.59	83.3 ± 27.3	83.3 ± 18.6	1.00	100.0 ± 11.5	100.0 ± 10.0	1.00
Cer C18:0	113.3 ± 13.3	93.3 ± 14.5	0.37	153.3 ± 50.4	110.0 ± 0.0	0.44	166.7 ± 13.3*	80.0 ± 20.0	0.02	196.7 ± 16.7*	110.0 ± 10.0	0.01
Cer C18:1	23.3 ± 8.8	16.7 ± 8.8	0.62	40.0 ± 5.8	20.0 ± 11.5	0.20	53.3 ± 12.0	16.7 ± 3.3	0.04	30.0 ± 10.0	26.7 ± 6.7	0.80
Cer C18:2	6.7 ± 3.3	16.7 ± 3.3	0.10	13.3 ± 3.3	3.3 ± 3.3	0.10	10.0 ± 10.0	13.3 ± 3.3	0.77	20.0 ± 5.8	10.0 ± 10.0	0.44
Cer C20:0	1880.0 ± 251.5	1506.7 ± 59.3	0.22	1583.3 ± 21.9	1420.0 ± 23.1	0.01	2130.0 ± 167.7	1630.0 ± 147.3*	0.09	1436.7 ± 718.4	2050.0 ± 240.1	0.46
Cer C20:1	46.7 ± 46.7	53.3 ± 27.3	0.91	23.3 ± 23.3	70.0 ± 5.8	0.12	76.7 ± 39.3	43.3 ± 21.9	0.50	100.0 ± 5.8	16.7 ± 16.7	0.01
Cer C20:2	20.0 ± 15.3	20.0 ± 5.8	1.00	16.7 ± 8.8	6.7 ± 3.3	0.35	33.3 ± 13.3	16.7 ± 3.3	0.29	3.3 ± 3.3	20.0 ± 10.0	0.19
Cer C22:0	356.7 ± 23.3	233.3 ± 26.0	0.02	253.3 ± 31.8	253.3 ± 29.6	1.00	420.0 ± 15.3	206.7 ± 24.0	0.00	263.3 ± 24.0*	206.7 ± 21.9*	0.16
Cer C22:1	80.0 ± 34.6	76.7 ± 3.3	0.93	86.7 ± 12.0	70.0 ± 5.8	0.28	103.3 ± 8.8	63.3 ± 14.5	0.08	116.7 ± 27.3	96.7 ± 31.8	0.66
Cer C22:2	36.7 ± 6.7	33.3 ± 8.8	0.78	30.0 ± 5.8	23.3 ± 6.7	0.49	53.3 ± 8.8	43.3 ± 3.3	0.35	20.0 ± 5.8	20.0 ± 10.0	1.00
Cer C22:3	13.3 ± 3.3	13.3 ± 3.3	1.00	16.7 ± 6.7	3.3 ± 3.3	0.15	36.7 ± 6.7*	20.0 ± 5.8	0.13	13.3 ± 3.3	16.7 ± 12.0	0.80
Cer C22:4	6.7 ± 3.3	10.0 ± 5.8	0.64	20.0 ± 10.0	6.7 ± 3.3	0.27	10.0 ± 5.8	6.7 ± 3.3	0.64	3.3 ± 3.3	3.3 ± 3.3	1.00
Cer C24:0	583.3 ± 27.3	420.0 ± 15.3	0.01	436.7 ± 28.5*	330.0 ± 43.6*	0.11	496.7 ± 31.8	323.3 ± 18.6*	0.01	523.3 ± 29.6	320.0 ± 25.2*	0.01
Cer C24:1	1070.0 ± 25.2	656.7 ± 57.0	0.00	1163.3 ± 31.8	643.3 ± 60.6*	0.00	1296.7 ± 121.3	616.7 ± 54.6*	0.01	1006.7 ± 127.2	660.0 ± 80.0*	0.08
Cer C24:2	73.3 ± 14.5	53.3 ± 12.0	0.35	73.3 ± 16.7	50.0 ± 5.8	0.26	103.3 ± 14.5	53.3 ± 14.5	0.07	50.0 ± 10.0	46.7 ± 17.6	0.88
Cer C24:3	16.7 ± 6.7	3.3 ± 3.3	0.15	26.7 ± 13.3	3.3 ± 3.3	0.16	26.7 ± 6.7	13.3 ± 6.7	0.23	16.7 ± 12.0	20.0 ± 15.3	0.87
Cer C24:4	6.7 ± 6.7	3.3 ± 3.3	0.68	10.0 ± 5.8	6.7 ± 6.7	0.72	10.0 ± 0.0	0.0 ± 0.0	0.00	3.3 ± 3.3	0.0 ± 0.0	0.37
Cer C26:0	26.7 ± 14.5	26.7 ± 12.0	1.00	40.0 ± 15.3	16.7 ± 3.3	0.21	36.7 ± 12.0	20.0 ± 11.5	0.37	23.3 ± 8.8	50.0 ± 15.3	0.21
Cer C26:1	33.3 ± 13.3	20.0 ± 5.8	0.41	26.7 ± 3.3	20.0 ± 10.0	0.56	40.0 ± 5.8	13.3 ± 13.3	0.14	30.0 ± 5.8	26.7 ± 6.7	0.72
Cer C26:2	6.7 ± 3.3	20.0 ± 5.8	0.12	16.7 ± 3.3	13.3 ± 8.8	0.74	16.7 ± 8.8	6.7 ± 3.3	0.35	16.7 ± 3.3	10.0 ± 5.8	0.37
Cer C26:3	6.7 ± 3.3	3.3 ± 3.3	0.52	13.3 ± 3.3	6.7 ± 3.3	0.23	20.0 ± 10.0	13.3 ± 6.7	0.61	6.7 ± 6.7	20.0 ± 11.5	0.37
Cer C26:4	60.0 ± 11.5	33.3 ± 12.0	0.18	23.3 ± 12.0	43.3 ± 12.0	0.30	60.0 ± 17.3	26.7 ± 3.3*	0.13	40.0 ± 10.0	56.7 ± 3.3	0.19

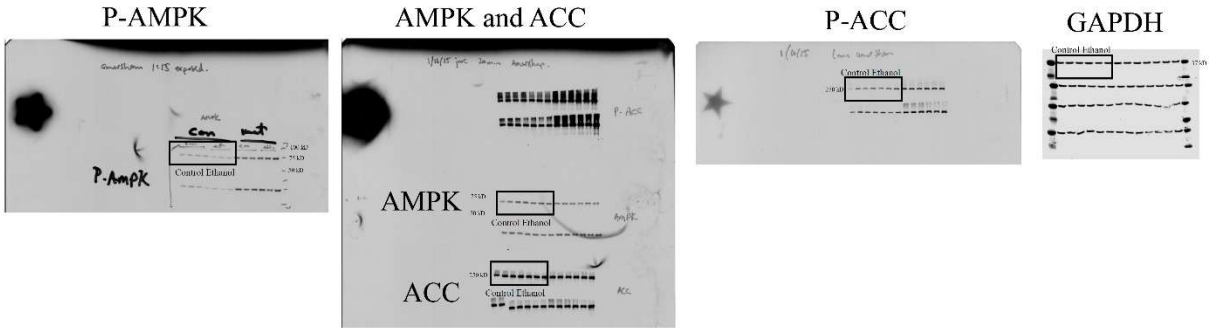
Supplementary Table 2: *Ceramide Quantitation in isolated lipid droplets by mass spectrometry.* VL-17A cells were treated for 48 h with control- or 100 mM ethanol-containing media or supplemented with BSA (vehicle control),100 μM oleate, 40 μM palmitate or 10 μM C2 ceramide. Lipid droplets were isolated, protein normalized and loaded onto LC-MS/MS. Listed concentrations are in nM.

	BSA		Oleate		Palmitate		C2 Ceramide	
	Control	Ethanol	Control	Ethanol	Control	Ethanol	Control	Ethanol
Cer C2:0	0.0	0.0	25.0	0.0	28.6	28.6	33.3	33.3
Cer C4:0	0.0	50.0	25.0	33.3	28.6	28.6	33.3	33.3
Cer C6:0	0.0	0.0	0.0	0.0	28.6	0.0	0.0	0.0
Cer C8:0	0.0	0.0	0.0	33.3	28.6	0.0	0.0	0.0
Cer C10:0	40.0	0.0	0.0	0.0	28.6	0.0	33.3	0.0
Cer C12:0	40.0	50.0	25.0	66.7	28.6	28.6	33.3	33.3
Cer C14:0	200.0	200.0	300.0	400.0	342.9	428.6	300.0	366.7
Cer C14:1	40.0	100.0	25.0	33.3	57.1	57.1	100.0	233.3
Cer C16:0	40.0	0.0	25.0	33.3	57.1	28.6	33.3	33.3
Cer C16:1	40.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0
Cer C18:0	40.0	50.0	25.0	0.0	57.1	28.6	33.3	0.0
Cer C18:1	120.0	100.0	200.0	233.3	171.4	200.0	166.7	133.3
Cer C18:2	0.0	0.0	25.0	33.3	28.6	28.6	33.3	33.3
Cer C20:0	40.0	0.0	0.0	0.0	0.0	28.6	0.0	0.0
Cer C20:1	0.0	0.0	0.0	0.0	28.6	28.6	33.3	0.0
Cer C20:2	40.0	0.0	75.0	33.3	85.7	28.6	33.3	33.3
Cer C22:0	120.0	0.0	125.0	133.3	57.1	85.7	0.0	66.7
Cer C22:1	360.0	400.0	650.0	1133.3	628.6	628.6	466.7	433.3
Cer C22:2	120.0	150.0	200.0	300.0	285.7	285.7	133.3	200.0
Cer C22:3	40.0	0.0	75.0	0.0	0.0	57.1	0.0	33.3
Cer C22:4	40.0	0.0	0.0	33.3	0.0	0.0	33.3	0.0
Cer C24:0	40.0	0.0	0.0	33.3	28.6	28.6	33.3	0.0
Cer C24:1	40.0	50.0	50.0	100.0	57.1	85.7	100.0	33.3
Cer C24:2	920.0	1000.0	1850.0	2400.0	1400.0	1514.3	1200.0	1100.0
Cer C24:3	560.0	700.0	1175.0	1733.3	1342.9	1628.6	700.0	733.3
Cer C24:4	80.0	100.0	150.0	133.3	142.9	85.7	133.3	100.0
Cer C26:0	280.0	250.0	325.0	400.0	285.7	428.6	266.7	300.0
Cer C26:1	40.0	50.0	50.0	33.3	28.6	57.1	33.3	33.3
Cer C26:2	0.0	0.0	25.0	0.0	0.0	28.6	0.0	0.0
Cer C26:3	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0
Cer C26:4	40.0	50.0	75.0	66.7	28.6	57.1	33.3	33.3

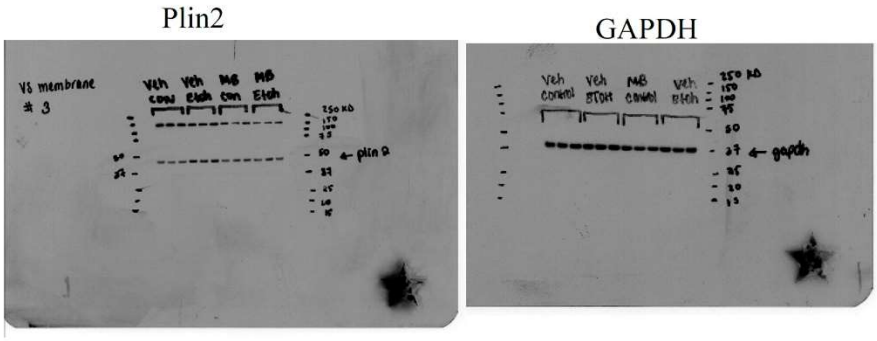
Supplementary Figure 1: *Full-length blot images for cropped western blots.* A: Figure 1E. B: Figure 3A. C: Figure 3G. D: Figure 4B.

A.

B.



C.



D.