

Supplemental mass-spectrometry data for “Cardiomyocyte specific loss of diacylglycerol acyl transferase 1 (DGAT1) reproduces the abnormalities in lipids found in severe heart failure”

Supplemental Methods

Liquid Chromatography Tandem Mass Spectrometry (LC/MS): Lipid analyses by LC/MS were performed as described (1). Briefly, lipid extraction buffer was pre-cooled at 4°C. 0.25 mL of 0.1 M KH_2PO_4 and 0.25 mL of 2-propanol were added to the weighed, frozen tissue aliquots (15-20 mg). 20 μL of 50 μM heptadecanoyl-CoA, dissolved in 0.1 M KH_2PO_4 /2-propanol/acetonitrile (1:1: 2), was added per sample as internal control. The sample was homogenized with a bead beater (BioSpec). 30 μL of saturated aqueous ammonium sulfate and 0.5 mL of acetonitrile were added to the homogenate and the mixture was vortexed well. After centrifugation at 2,500g for 10 min, the supernatant was transferred to MS vials for analyses. All analyses were carried out on a Waters Xevo TQ MS ACQUITY UPLC system (Waters) controlled by Mass Lynx Software 4.1 as described previously (2).

Supplemental Table 1. Heart lipids from 10-month-old male mice (n=7, *p<0.05, **p<0.01)

A. Ceramides (pmol/mg tissue)		
	<i>Dgat1^{fllox/fllox}</i>	<i>hDgat1^{-/-}</i>
Total	11.78±2.734	16.65±3.790
C14	0.031±0.007	0.048±0.013
C16	5.15±1.44	7.69±1.9*
C18	4.06±0.96	5.36±1.19*
C18:1	0.032±0.18	0.47±0.17
C20	0.033±0.009	0.066±0.026*
C20:1	0.027±0.007	0.042±0.015*
C22	0.059±0.025	0.084±0.034
C22:1	0.017±0.004	0.0028±0.008**
C24	1.334±0.255	1.621±0.421
C24:1	0.0595±0.127	1.055±0.031**
C26	0.054±0.010	0.047±0.008
C26:1	0.090±0.039	0.121±0.39
B. Fatty acids (pmol/mg tissue)		
	<i>Dgat1^{fllox/fllox}</i>	<i>hDgat1^{-/-}</i>
Total	4822.863 ±1302.241	8456.95 ±3788.75*
C12	1.49±0.26	1.60 ±0.28
C14:1	1.26 ±0.23	1.28 ±0.28
C14	5.90 ±2.49	6.41 ±2.08
C16:1	24.84 ± 7.69	29.66 ±9.76
C16	584.15 ±194.14	801.76 ±329.28
C18:3	13.53 ±2.56	16.16 ±4.05
C18:2	2774.60 ±824.34	5643.45 ± 2841.94*
C18:1	1061.00 ±235	1478.73 ±451.97*
C18	181.92 ±54.49	280.22 ±113.04*
C20:5	6.90 ±0.94	7.94 ±2.65
C20:4	54.84 ±13.04	65.61 ±20.79
C20:1	33.18 ±11.07	40.77 ±10.52
C20	2.195 ± 0.88	2.54 ±0.57
C22:6	73.46 ±21.76	77.83 ±28.74
C22:1	3.17 ±1.32	2.73 ±0.46
C22	0.835 ±0.32	0.768 ±0.20
C24:1	0.86 ±0.28	0.91 ±0.19
C24	0.24 ±0.06	0.21 ±0.06
C. Fatty acyl-CoAs (pmol/mg tissue)		
	<i>Dgat1^{fllox/fllox}</i>	<i>hDgat1^{-/-}</i>
Total	59.68±17.39	45.85±12.51
C12	1.20±0.33	1.13±0.36
C14:1	3.60 ±1.05	2.79±1.08
C14	3.94 ±0.37	3.35±0.88
C16:1	4.65± 0.81	3.75±1.02

C16	9.10±2.96	7.35±2.66
C18:3	1.26 ±0.58	0.91±0.26
C18:2	14.56 ±7.03	9.53 ± 2.96
C18:1	15.33±4.88	12.10±3.44
C18	1.48±0.29	1.10 ±0.26*
C20:5	0.32±0.15	0.29±0.13
C20:4	1.97±0.45	1.77±0.19
C20:1	1.57±0.31	1.28±0.48
C20	0.066± 0.028	0.052 ±0.031
C22:6	0.60±0.16	0.42±0.12
C22:1	0.035±0.014	0.029 ±0.019

Supplemental Table 2. Heart ceramide measurements in 5- to 6-month-old vehicle or exenatide-treated *hDgat1*^{-/-} mice (n=7, *p<0.05)

Ceramides (pg/ g tissue)	<i>hDgat1</i> ^{-/-} + vehicle	<i>hDgat1</i> ^{-/-} + exenatide
Total	8.5509±1.6946	6.5173±1.463*
C14	0.0129±0.0013	0.0097±0.0042
C16:1	0.7447±0.1506	0.5837±0.1662
C16	2.457±0.2519	1.9041±0.4545*
C18:1	0.1903±0.037	0.1612±0.0395
C18	3.8017±1.0293	2.7325±0.6077*
C20:1	0.0044±0.002	0.0043±0.0016
C20	0.0775±0.0314	0.0614±0.0189
C22:1	0.0181±0.0068	0.0148±0.0046
C22	0.0898±0.0327	0.064±0.015
C24:1	0.2417±0.0672	0.1959±0.0586
C24	0.8981±0.2026	0.7751±0.2075
C26:1	ND	ND
C26	0.0128±0.0053	0.0092±0.0047

Supplemental references

1. Clugston, R. D., Jiang, H., Lee, M. X., Piantedosi, R., Yuen, J. J., Ramakrishnan, R., Lewis, M. J., Gottesman, M. E., Huang, L. S., Goldberg, I. J., Berk, P. D., and Blaner, W. S. (2011) Altered hepatic lipid metabolism in C57BL/6 mice fed alcohol: a targeted lipidomic and gene expression study. *J Lipid Res* **52**, 2021-2031
2. Clugston, R. D., Yuen, J. J., Hu, Y., Abumrad, N. A., Berk, P. D., Goldberg, I. J., Blaner, W. S., and Huang, L. S. (2014) CD36-deficient mice are resistant to alcohol- and high-carbohydrate-induced hepatic steatosis. *J Lipid Res* **55**, 239-246