

SUPPLEMENTARY DATA

Supplementary Table 1. Mean values and standard deviation (SD) of sphingolipid species and sphingomyelins (SMS) in serum, liver, skeletal muscle, visceral (visc) and subcutaneous (subcut) adipose tissue.

Sphingolipid species								
	CON		NAFL-		NAFL+		NASH	
Serum (ng/ml)	mean	SD	mean	SD	mean	SD	mean	SD
Sphingosine	5.31	2.22	9.57	6.53	7.28	3.63	13.9	10.6
Sphinganine	2.14	0.42	2.51	0.79	2.93	1.47	3.22	0.5
Sphingosine 1P	495.	149.	616.	241.	630.	229.	577.	89.9
Sphinganine 1P	68.3	28.9	77.5	26.8	85.3	41.2	78.7	15.6
Lactosyl14	71.2	30.	64.2	34.8	59.4	30.2	33.3	15.19
Hexosyl140	8.41	3.57	5.24	1.08	5.93	2.28	3.51 ***	1.02
Lactosyl16	1289.	243.	1371.	595.	1374.	497.	1212.	484.
Ceramide140	1.51	0.38	1.9	0.74	2.28	1.49	1.98	0.56
Hexosyl16	262.	84.7	299.	60.7	294.	98.2	362.	128.
Lactosyl18	9.68	2.24	10.6	4.15	12.2	4.16	9.3	2.7
Hexosyl18	6.35	2.32	8.37	2.64	8.71	2.7	8.16	2.6
Ceramide160	5.03	1.77	6.89	2.32	5.96	1.47	8.65 ***	2.4
Dihydro160	0.66	0.22	0.87	0.24	1.23	0.62	2.57 ***\$\$\$+++	1.3
Lactosyl20	1.92	0.64	2.2	0.8	1.91	0.73	2.53	1.
Ceramide18	4.51	1.69	7.53	5.29	8.16	4.31	7.87	3.3
Hexosyl20	14.9	4.33	16.4	4.3	17.8	4.7	16.5	3.
Dihydro180	1.75	0.56	3.3	1.99	4.95	2.68	7.55	7.8
Lactosyl22	33.5	8.2	33.7	11.8	33.5	9.7	35.8	10.
Lactosyl241	645.	113.	586.	225.	691.	271.	537.	188.
Hexosyl22	597.	142.	635.	233.	616.	292.	760.	131.
Ceramide20	11.8	4.03	14.7	4.93	15.3	3.77	13.9	3.9
Hexosyl241	379.	79.1	399.	113.	434.	113.	391.	81.2
LactosylC24	58.2	15.2	46.6	12.9	55.9	12.3	70.5 §	13.5
Dihydro200	0.59	0.19	1.3	0.7	1.6 #	0.82	1.19	0.09
Hexosyl240	516.	92.3	525.	170.	418.	58.8	457.	23.4
Ceramide22	195.	44.1	251.	73.8	252.	104.	178.	39.9
Ceramide241	306.	98.2	361.	92.1	449.	146.	389.	108.

SUPPLEMENTARY DATA

Dihydro220	38.	6.5	48.7	10.1	66.8	37.5	94.	**§	27.2
Dihydro241	45.	6.5	74.6	22.4	95.6	49.1	121.	*	77.4
Ceramide240	1598.	385.	1534.	355.	1813.	864.	2161.	§	788.
Dihydro240	87.8	26.1	105.5	22.5	129.	55.5	175.	**§	57.5
Liver (ng/mg tissue)									
Sphingosine	3.1	0.62	3.1	1.2	3.3	0.88	2.1		1.05
Sphinganine	0.55	0.16	0.58	0.26	0.97	0.43	0.96		0.33
Deoxysphingosine	0.001	0.001	0.001	0.001	0.002	0.002	0.02		0.05
Sphingosine1P	0.84	0.38	0.72	0.24	0.78	0.26	0.38		0.21
Deoxysphinganine	0.03	0.02	0.03	0.01	0.05	0.02	0.05		0.03
Sphinganine1P	0.07	0.02	0.09 ¶¶	0.02	0.08	0.03	0.04	§§*	0.03
Lactosyl14	0.16	0.03	0.16	0.07	0.12	0.03	0.09	*§	0.04
Hexosyl140	0.018	0.004	0.017	0.009	0.015	0.004	0.024		0.012
Lactosyl16	3.2	0.33	4.2	1.3	3.9	1.2	4.7		1.75
Ceramide140	0.009	0.003	0.016	0.015	0.011	0.004	0.019		0.01
Hexosyl16	0.18	0.06	0.28	0.16	0.23	0.08	0.41		0.26
Lactosyl18	0.04	0.01	0.1	0.1	0.05	0.02	0.04		0.03
Hexosyl18	0.003	0.001	0.016	0.021	0.005	0.001	0.008		0.004
Ceramide160	0.03	0.01	0.062 ¶	0.03	0.04	0.01	0.04		0.03
Dihydro160	0.003	0.001	0.006	0.004	0.006	0.002	0.011	**	0.005
Lactosyl20	0.012	0.004	0.019	0.013	0.015	0.006	0.017		0.013
Ceramide18	0.04	0.01	0.15	0.21	0.07	0.02	0.07		0.04
Hexosyl20	0.014	0.004	0.024	0.019	0.016	0.007	0.032		0.019
Dihydro180	0.024	0.011	0.043	0.025	0.049	0.049	0.052		0.02
Lactosyl22	0.63	0.15	0.79	0.47	0.64	0.2	0.85		0.25
Lactosyl241	2.8	0.45	3.8	1.5	3.4	1.3	4.7	*	1.2
Hexosyl22	1.7	0.35	2.4	1.7	2.6	2.2	7.2	§§§***++	2.9
Ceramide20	0.27	0.07	0.31	0.14	0.36	0.07	0.45		0.23
Hexosyl241	0.71	0.17	0.99	0.6	0.82	0.26	1.5	*+	0.53
LactosylC24	1.4	0.42	1.8	0.88	1.5	0.46	2.4		0.95
Dihydro200	0.006	0.001	0.011	0.006	0.019	0.018	0.012		0.003
Hexosyl240	1.2	0.26	1.5	1.	1.3	0.52	2.7		0.93

SUPPLEMENTARY DATA

Ceramide22	2.7	0.36	2.9	0.7	3.1	0.4	2.7		0.54
Ceramide241	4.9	0.89	6.1	2.5	6.9	1.4	5.7		0.87
Dihydro220	0.07	0.02	0.11	0.04	0.15	0.08	0.17	*	0.11
Dihydro241	0.12	0.04	0.23	0.15	0.34	0.24	0.43	*	0.21
Ceramide24	8.5	1.1	8.1	2.2	9.5	3.1	17.4	§§§***++	4.8
Skeletal muscle (ng/mg tissue)									
Sphingosine	0.13	0.06	0.13	0.02	0.15	0.08	0.18		0.03
Sphinganine	0.04	0.02	0.04	0.01	0.04	0.02	0.05		0.02
Sphingosine1P	0.07	0.21	0.07	0.16	0.04	0.16	0.12		
Sphinganine1P	0.04	0.04	0.06	0.02	0.03	0.02	0.03		0.03
Lactosyl14	0.05	0.02	0.05	0.02	0.04	0.02	0.03		0.02
Hexosyl140	0.004	0.001	0.006	0.003	0.004	0.002	0.006		0.006
Lactosyl16	1.8	0.75	2.1	0.73	1.4	0.47	1.2		1.3
Ceramide140	0.05	0.02	0.03	0.02	0.04	0.02	0.04		0.03
Hexosyl16	0.22	0.15	0.22	0.11	0.15	0.08	0.15		0.13
Lactosyl18	0.37	0.15	0.2	0.06	0.25	0.13	0.15		0.3
Hexosyl18	0.07	0.04	0.22	0.36	0.07	0.04	0.33		0.75
Ceramide160	0.17	0.1	0.09	0.05	0.12	0.08	0.14		0.2
Dihydro160	0.011	0.008	0.004	0.002	0.005	0.004	0.01		0.013
Ceramide18	1.3	1.3	0.58	0.33	0.92	0.59	0.44		0.54
Hexosyl20	0.041	0.023	0.15	0.25	0.023	0.013	0.24		0.581
Dihydro180	0.08	0.075	0.034	0.015	0.05	0.042	0.021		0.033
Lactosyl22	0.28	0.16	0.19	0.09	0.17	0.1	0.14		0.19
Lactosyl241	3.5	2.4	3.2	1.3	2.4	1.1	1.7		2.9
Hexosyl22	0.94	0.25	2.8	3.5	0.81	0.37	4.8		10.1
Ceramide20	0.23	0.12	0.14	0.04	0.16	0.06	0.16		0.11
Hexosyl241	0.61	0.2	2.4	3.3	0.45	0.2	2.5		5.4
LactosylC24	0.77	0.43	0.68	0.42	0.51	0.26	0.4		0.45
Dihydro200	0.015	0.011	0.012	0.006	0.009	0.008	0.049		0.105
Hexosyl240	0.87	0.27	3.9	5.3	0.42	0.24	2.2		4.3
Ceramide22	0.65	0.15	0.86	0.19	0.66	0.13	0.86		0.18

SUPPLEMENTARY DATA

Ceramide241	2.7	0.67	3.1	0.69	3.1		0.79	2.8		0.81
Dihydro220	0.048	0.018	0.062	0.013	0.038		0.011	0.047		0.026
Dihydro241	0.061	0.04	0.083	0.034	0.056		0.019	0.083		0.06
Ceramide240	2.9	0.4	3.1	0.4	3.		1.2	5.6	***§§§+++	1.2
Dihydro240	0.082	0.036	0.096	0.017	0.062		0.022	0.108		0.063
Visc fat (ng/mg tissue)										
Sphingosine	0.131	0.039	0.092	0.037	0.094		0.023	0.098		0.022
Sphinganine	0.032	0.011	0.023	0.007	0.024		0.008	0.023		0.005
Sphingosine1P	0.053	0.023	0.04	0.012	0.03	#	0.011	0.018	**	0.005
Deoxysphinganine	0.001	0.001	0.001	0.001	0.002		0.001	0.002		0.001
Sphinganine1P	0.013	0.005	0.017	0.011	0.01		0.006	0.005	§	0.002
Lactosyl14	0.036	0.03	0.017	0.005	0.021		0.006	0.018		0.005
Hexosyl140	0.004	0.002	0.003	0.001	0.003		0.001	0.002		0.001
Lactosyl16	1.64	2.37	0.71	0.35	0.73		0.39	0.48		0.13
Ceramide140	0.15	0.05	0.17	0.04	0.17		0.04	0.14		0.03
Hexosyl16	0.51	0.1	0.44	0.13	0.46		0.13	0.234	**§+	0.11
Lactosyl18	0.06	0.05	0.08	0.07	0.07		0.04	0.04		0.02
Hexosyl18	0.021	0.005	0.029	0.011	0.03		0.016	0.009	§++	0.004
Ceramide160	0.66	0.22	0.82	0.12	0.79		0.19	0.57		0.16
Dihydro160	0.043	0.01	0.04	0.008	0.038		0.014	0.042		0.016
Ceramide181	0.004	0.001	0.006	0.003	0.007		0.003	0.006		0.002
Lactosyl20	0.049	0.02	0.069	0.024	0.067		0.045	0.023	§	0.009
Ceramide18	0.118	0.025	0.2	0.105	0.163		0.072	0.091	§	0.026
Hexosyl20	0.084	0.013	0.084	0.021	0.092		0.026	0.047	*§++	0.018
Dihydro180	0.015	0.006	0.019	0.008	0.014		0.007	0.012		0.005
Lactosyl22	0.4	0.17	0.47	0.21	0.39		0.18	0.41		0.13
Lactosyl241	2.4	3.78	1.34	0.69	1.55		1.03	1.03		0.37
Hexosyl22	1.14	0.28	0.97	0.32	0.87		0.13	0.96		0.27
Ceramide20	0.41	0.11	0.41	0.06	0.39		0.08	0.31		0.07
Hexosyl241	0.28	0.08	0.21	0.07	0.25		0.06	0.2		0.08
LactosylC24	0.44	0.31	0.37	0.15	0.32		0.11	0.32		0.08
Dihydro200	0.01	0.01	0.01	0.001	0.01		0.01	0.003	§	0.001

SUPPLEMENTARY DATA

Hexosyl240	0.41	0.18	0.244 ¶	0.08	0.225 #	0.04	0.26	0.07
Ceramide22	2.33	0.71	2.09	0.5	1.73	0.34	1.54 *	0.19
Ceramide241	4.42	0.68	4.4	0.69	4.64	1.31	3.08 +	0.77
Dihydro220	0.12	0.04	0.09	0.03	0.07 #	0.03	0.053 **	0.01
Dihydro241	0.1	0.04	0.1	0.02	0.09	0.03	0.07	0.04
Ceramide240	6.13	1.6	4.306 ¶	1.04	3.998 ##	0.67	4.3 *	0.76
Dihydro240	0.08	0.03	0.05	0.02	0.05	0.02	0.05	0.01
Subcut fat (ng/mg tissue)								
Sphingosine	0.149	0.094	0.137	0.06	0.179	0.089	0.181	0.03
Sphinganine	0.034	0.023	0.029	0.015	0.062	0.069	0.036	0.009
Sphingosine1P	0.101	0.073	0.071	0.031	0.096	0.054	0.066	0.047
Deoxysphinganine	0.001	0.001	0.002	0.001	0.003 ####	0.001	0.002 *	0.001
Sphinganine1P	0.024	0.021	0.02	0.01	0.024	0.013	0.019	0.019
Lactosyl14	0.036	0.01	0.03	0.016	0.03	0.013	0.032	0.017
Hexosyl140	0.006	0.002	0.004	0.001	0.006	0.002	0.005	0.001
Lactosyl16	1.51	1.25	1.21	0.73	1.07	0.52	0.77	0.3
Ceramide140	0.12	0.03	0.14	0.05	0.14	0.03	0.09	0.02
Hexosyl16	0.5	0.15	0.51	0.11	0.6	0.14	0.32 ++	0.13
Lactosyl18	0.09	0.06	0.11	0.04	0.08	0.02	0.05 §	0.03
Hexosyl18	0.13	0.25	0.05	0.02	0.07	0.06	0.06	0.1
Ceramide160	0.66	0.26	0.66	0.27	0.56	0.21	0.25 *§	0.07
Dihydro160	0.04	0.02	0.03	0.02	0.03	0.01	0.02	0.01
Lactosyl20	0.07	0.02	0.07	0.03	0.05	0.02	0.02 ***§§§	0.01
Ceramide18	0.08	0.02	0.12	0.04	0.12	0.04	0.06 §§++	0.01
Hexosyl20	0.11	0.1	0.07	0.02	0.11	0.06	0.09	0.11
Dihydro180	0.02	0.01	0.02	0.01	0.02	0.01	0.01	0.01
Lactosyl22	0.59	0.23	0.54	0.23	0.37	0.11	0.36	0.17
Lactosyl241	2.4	1.92	2.03	1.27	2.2	1.35	1.21	0.42
Hexosyl22	1.23	0.76	0.96	0.21	1.47	1.27	1.84	2.04
Ceramide20	0.17	0.04	0.18	0.06	0.16	0.04	0.11 §	0.03
Hexosyl241	0.55	0.87	0.23	0.09	0.64	0.86	0.97	1.77

SUPPLEMENTARY DATA

LactosylC24	0.54	0.2	0.44	0.17	0.38	0.12	0.33	0.13
Dihydro200	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
Hexosyl240	0.83	1.44	0.29	0.11	0.62	0.81	0.92	1.48
Ceramide22	1.24	0.22	1.5	0.37	1.1	0.24	0.84	§§
Ceramide241	2.68	0.96	3.43	0.8	3.17	1.2	2.01	0.89
Dihydro220	0.11	0.04	0.1	0.02	0.09	0.03	0.05	*
Dihydro241	0.09	0.05	0.1	0.04	0.09	0.04	0.06	0.05
Ceramide240	3.06	0.76	2.62	0.45	2.46	0.56	2.9	0.45
Dihydro240	0.09	0.05	0.07	0.02	0.09	0.08	0.07	0.02
Serum (ng/ml)								
SMS161	7897.	1586.	9038.	2272.	8510.	1635.	7498.	1355.
SMS140	4504.	521.	4473.	1073.	4316.	585.	4167.	1007.
SMS160	55442.	9127.	54043.	9669.	51358.	9472.	43728.	10064.
SMS181	5280.	1892.	6217.	1632.	6606.	1725.	5887.	2736.
SMS18	11441.	3375.	12918.	3823.	14148.	3904.	11842.	6365.
SMS20	8719.	1872.	8155.	1338.	8059.	959.	7337.	1263.
SMS221	16571.	2586.	16519.	3334.	15872.	2561.	13783.	3877.
SMS22	17245.	3018.	16798.	3045.	15993.	2045.	15516.	2864.
SMS241	44300.	11249.	42571.	12063.	44424.	11713.	38779.	14609.
SMS24	13739.	2854.	12287.	2621.	11412.	1793.	11046.	3166.
Liver (ng/mg tissue)								
SMS161	9.87	2.5	10.5	3.78	11.5	3.21	9.29	1.3
SMS140	8.46	0.68	7.95	2.71	8.15	1.26	8.64	1.32
SMS160	164.	19.24	145.	49.7	161.	42.1	167.	37.7
SMS181	7.19	2.63	8.08	2.46	8.98	1.67	10.68	3.62
SMS18	49.	13.	51.	13.	56.	11.	66.	27.
SMS20	44.	5.13	33.	12.	38.	5.32	37.	12.
SMS221	34.9	3.75	29.3	9.54	31.6	2.91	30.3	4.01
SMS22	129.	15.7	97.5	32.3	110.	20.9	126.	24.6
SMS241	180.	36.7	146.1	51.4	168.	40.8	176.	45.
SMS24	141.	20.6	96.8	¶¶	31.4	103.	#	21.6
						118.		18.7

SUPPLEMENTARY DATA

Skeletal muscle (ng/mg tissue)								
SMS161	3.36	1.4	4.16	1.04	3.75	0.91	4.59	2.04
SMS140	2.96	0.63	3.31	0.74	3.19	1.18	3.74	1.21
SMS160	54.5	22.6	56.	10.1	57.1	20.5	62.6	27.5
SMS181	6.47	0.74	6.73	1.52	6.5	0.98	7.74	0.81
SMS18	46.7	6.89	45.2	6.73	44.7	6.97	48.1	12.7
SMS20	6.74	2.61	8.43	4.73	5.79	1.95	10.7	9.11
SMS221	8.26	2.26	10.1	3.34	9.27	4.05	9.75	7.58
SMS22	22.4	4.08	26.4	7.51	22.1	7.23	31.1	14.1
SMS241	50.9	20.2	62.2	26.9	59.4	16.1	78.9	49.2
SMS24	29.6	8.37	31.9	10.6	27.5	6.93	40.5	23.
Visc fat (ng/mg tissue)								
SMS161	3.69	1.2	4.32	2.18	4.31	1.37	5.46	1.14
SMS140	3.33	0.87	2.93	1.07	2.83	0.87	3.69	0.84
SMS160	60.2	13.2	45.6	20.5	45.8	16.4	55.9	10.1
SMS181	1.61	0.41	1.65	0.61	1.81	0.74	2.48	1.05
SMS18	8.44	1.65	6.63	1.66	7.4	2.61	8.98	2.66
SMS20	9.19	1.92	6.85	2.8	7.55	2.29	9.35	1.68
SMS221	8.68	3.33	7.65	3.75	7.81	2.67	9.04	4.71
SMS22_	21.8	7.02	15.2	7.7	14.5	4.77	19.8	4.83
SMS241	29.4	5.43	21.1	10.1	25.3	12.1	36.3	10.1
SMS24	16.6	6.2	9.45	4.36	9.71	4.13	13.4	3.68
Subcut fat (ng/mg tissue)								
SMS161	7.54	2.42	8.73	5.06	10.	3.5	11.07	3.46
SMS140	4.87	1.37	4.55	2.16	5.33	1.8	5.73	1.05
SMS160	68.7	17.4	59.7	34.1	67.1	21.6	73.1	12.6
SMS181	2.61	1.35	2.97	1.97	3.54	1.74	4.52	1.9
SMS18	9.56	4.86	8.42	4.61	10.1	4.51	12.9	3.37

SUPPLEMENTARY DATA

SMS20	9.05	4.48	7.6	4.02	7.96	3.	10.5	1.43
SMS221	11.6	3.2	11.9	6.54	12.2	4.59	15.7	3.84
SMS22	24.2	7.45	21.3	9.91	20.2	6.47	24.9	4.18
SMS241	32.3	14.7	28.3	17.4	36.	14.5	51.1	12.
SMS24	18.9	8.27	13.4	7.3	14.8	5.03	20.1	4.72

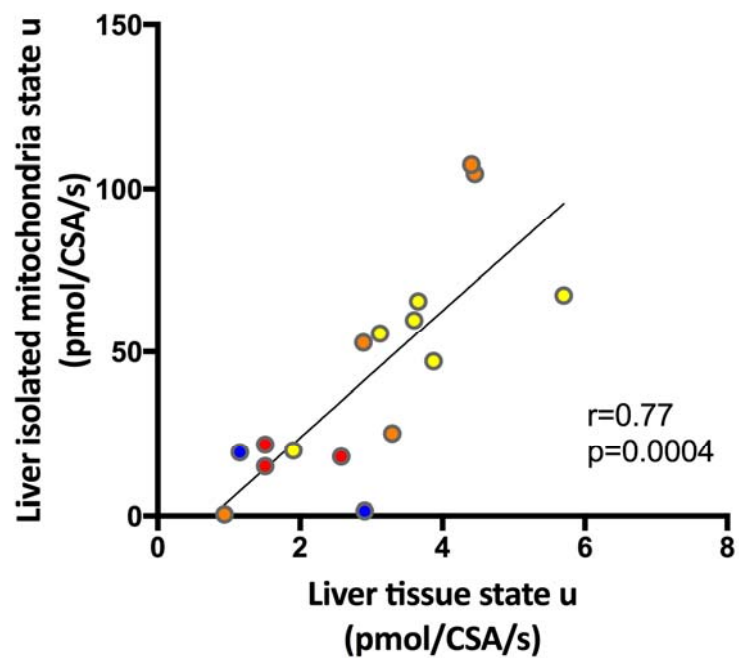
$p \leq 0.05$. ¶¶ $p \leq 0.01$. ¶¶¶ $p < 0.001$ CON vs NAFL-
 # $p \leq 0.05$. ## $p \leq 0.01$. ### $p < 0.001$ CON vs NAFL+
 * $p \leq 0.05$. ** $p \leq 0.01$. *** $p < 0.001$ CON vs NASH
 † $p \leq 0.05$. †† $p \leq 0.01$. ††† $p < 0.001$ NAFL- vs NAFL+
 § $p \leq 0.05$. §§ $p \leq 0.01$. §§§ $p < 0.001$ NAFL- vs NASH
 + $p \leq 0.05$. ++ $p \leq 0.01$. +++ $p < 0.001$ NAFL+ vs NASH

Supplementary Table 2. Associations between hepatic ceramide species and total hepatic ceramides and state u respiration and TBARS in all groups.

	State u respiration		TBARS
	Hepatic lactosylceramide 14:0	Hepatic sphinganine	Liver total ceramides
CON	t= -0.40 p=0.69	t= 0.24 p=0.81	t= 0.75 p=0.46
NAFL –	t=2.90 p=0.01	t= 0.75 p=0.46	t= -0.45 p=0.65
NAFL+	t= 0.49 p=0.62	t= 0.44 p=0.65	t= 0.30 p=0.76
NASH	t= 0.49 p=0.63	t= 0.31 p=0.75	t= 2.78 p=0.01

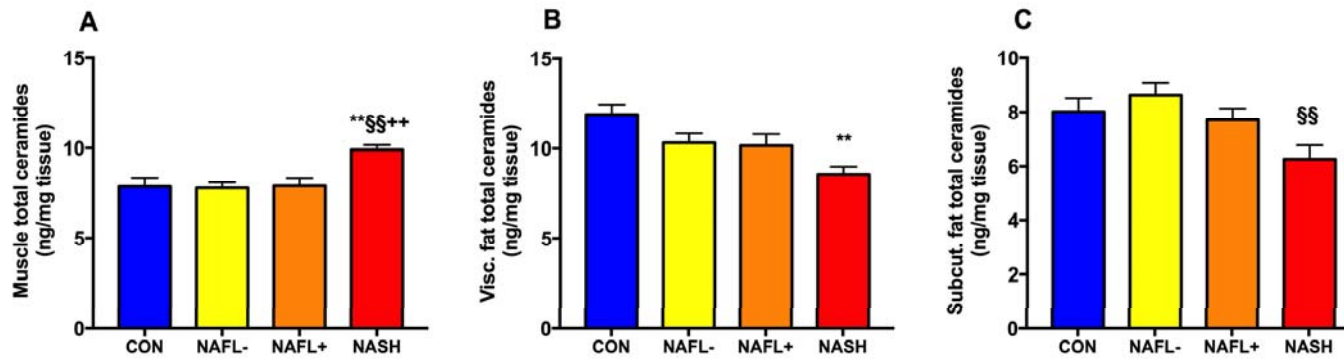
SUPPLEMENTARY DATA

Supplementary Figure 1. Correlation between maximal uncoupled (state u) respiration in hepatic isolated mitochondria and in liver tissue (CON: blue circles. NAFL-: yellow circles. NAFL+: orange circles. NASH: red circles).



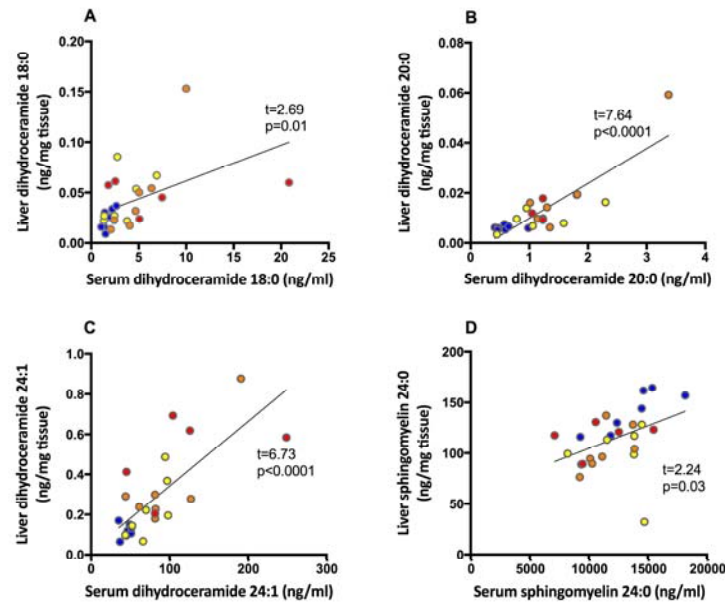
SUPPLEMENTARY DATA

Supplementary Figure 2. Correlations between liver and serum levels of dihydroceramide species (DHC) and sphingomyelins (SMS) (CON: blue circles. NAFL-: yellow circles. NAFL+: orange circles. NASH: red circles)



SUPPLEMENTARY DATA

Supplementary Figure 3. Total ceramides in skeletal muscle, subcutaneous (subcut fat) and visceral fat (visc fat) in lean individuals (CON) and obese participants without NAFL- and with NAFL+ and NASH.



Data are presented as mean \pm standard error of mean.

¶ $p \leq 0.05$. ¶¶ $p \leq 0.01$. ¶¶¶ $p < 0.001$ CON vs NAFL-
$p \leq 0.05$. ## $p \leq 0.01$. ### $p < 0.001$ CON vs NAFL+
* $p \leq 0.05$. ** $p \leq 0.01$. *** $p < 0.001$ CON vs NASH
‡ $p \leq 0.05$. †† $p \leq 0.01$. ††† $p < 0.001$ NAFL- vs NAFL+
§ $p \leq 0.05$. §§ $p \leq 0.01$. §§§ $p < 0.001$ NAFL- vs NASH
+ $p \leq 0.05$. ++ $p \leq 0.01$. +++ $p < 0.001$ NAFL+ vs NASH