SUPPLEMENTARY MATERIAL

Methyl-donor supplementation in obese mice prevents the progression of NAFLD, activates AMPK and decreases acyl-carnitine levels[a]

Christoph Dahlhoff [1,2], Stefanie Worsch [3], Manuela Sailer [1], Björn A. Hummel [4,5], Jarlei Fiamoncini [1], Kirsten Uebel [3], Rima Obeid [4], Christian Scherling [1], Jürgen Geisel [4], Bernhard L. Bader [2,3,,*], Hannelore Daniel [1]

[a]Bernhard L. Bader and Hannelore Daniel contributed equally to this work

SUPPLEMENTAL FIGURES

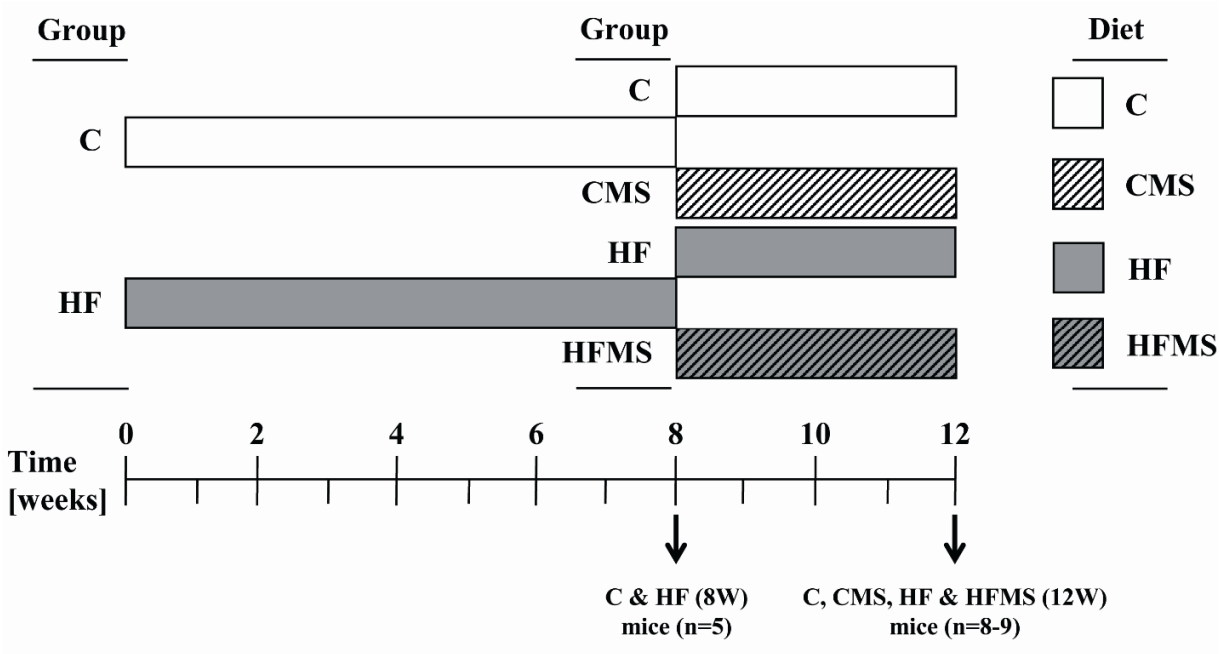

Figure S1: Experimental design of the MDS feeding trial. Open and gray boxes represent feeding of control (C) and high-fat (HF) diet, respectively, and open-lined and gray-lined boxes represent methyl-donor supplemented control (CMS) and methyl-donor supplemented high-fat (HFMS) dietary feeding, respectively. C and HF mice sacrificed after 8 weeks are depicted as 8W, and C, CMS, HF and HFMS mice sacrificed after 12 weeks are depicted as 12W.

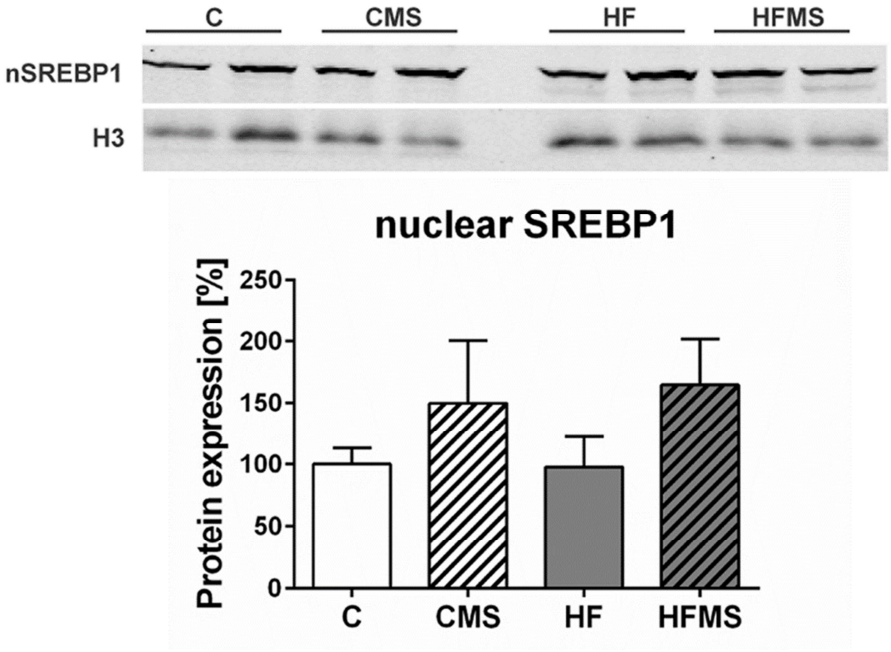

Figure S2: Western blot analysis of hepatic nSREBP1 after 4 weeks of MDS. Data are presented as mean ± SEM (n=4-9). Open and gray columns represent control (C) and high-fat (HF) dietary feeding, respectively, and open-lined and gray-lined columns represent methyl-donor supplemented control (CMS) and methyl-donor supplemented high-fat (HFMS) dietary feeding, respectively. nSREBP1, nuclear sterol regulatory element-binding protein-1; H3, histone 3.

SUPPLEMENTAL TABLES

Table S1	C	CMS	HF	HFMS
Dry matter	952	952	971	971
Crude protein	208	208	241	241
Crude fat	42	42	340	340
Butter fat	-	-	-	-
Beef tallow	-	-	310	310
Soybean oil	40	40	30	30
Coconut fat	-	-	-	-
Cocoa butter	-	-	-	-
Crude fiber	50	50	60	60
Crude ash	56	56	61	61
Starch	468	468	22	22
Sugar/dextrine	108	108	224	224
N-free extracts	594	594	270	270
GE [MJ/kg]	18	18	25.2	25.2
ME [MJ/kg]	15	15	21.4	21.4
% carbohydrate	66	66	21	21
% protein	23	23	19	19
% fat	11	11	60	60
Amino acids				
Lysine	17.1	17.1	19.8	19.8
Methionine	7.3	14.8	8.3	15.8

Table S1

Continued

	C	CMS	HF	HFMS
Cystine	0.9	0.9	4.6	4.6
Met+Cys	8.2	15.7	12.8	20.3
Threonine	9.3	9.3	10.7	10.7
Tryptophan	2.7	2.7	3.1	3.1
Arginine	7.6	7.6	8.8	8.8
Histidine	6.6	6.6	7.6	7.6
Valine	14.2	14.2	16.4	16.4
Isoleucine	10.9	10.9	12.5	12.5
Leucine	20.5	20.5	23.6	23.6
Phenylalanine	11.1	11.1	12.9	12.9
Phe+Tyr	22.2	22.2	25.7	25.7
Glycine	4.3	4.3	5.0	5.0
Glutamic acid	46.9	46.9	54.1	54.1
Aspartic acid	15.5	15.5	17.9	17.9
Proline	23.9	23.9	27.6	27.6
Alanine	6.8	6.8	7.9	7.9
Serine	12.4	12.4	14.3	14.3
Vitamins				
Vitamin A	15000 IU	15000 IU	15000 IU	15000 IU
Vitamin D3	1500 IU	1500 IU	1500 IU	1500 IU
Vitamin E	150 mg/kg	150 mg/kg	150 mg/kg	150 mg/kg
Vitamin K (as menadione)	20 mg/kg	20 mg/kg	20 mg/kg	20 mg/kg
Vitamin C	30 mg/kg	30 mg/kg	30 mg/kg	30 mg/kg

Table S1

Continued

	C	CMS	HF	HFMS
Thiamin (B1)	16 mg/kg	16 mg/kg	16 mg/kg	16 mg/kg
Riboflavin (B2)	16 mg/kg	16 mg/kg	16 mg/kg	16 mg/kg
Pyridoxine (B6)	18 mg/kg	18 mg/kg	18 mg/kg	18 mg/kg
Cobalamin (B12)	30 µg/kg	1530 µg/kg	30 µg/kg	1530 µg/kg
Nicotinic acid	49 mg/kg	49 mg/kg	45 mg/kg	45 mg/kg
Pantothenic acid	56 mg/kg	56 mg/kg	55 mg/kg	55 mg/kg
Folic acid	19 mg/kg	34 mg/kg	19 mg/kg	34 mg/kg
Biotin	310 µg/kg	310 µg/kg	310 µg/kg	310 µg/kg
Choline chloride	1.04 g/kg	16.04 g/kg	2.3 g/kg	17.3 g/kg
Inositol	80 mg/kg	80 mg/kg	80 mg/kg	80 mg/kg
Betaine	-	15 g/kg	-	15 g/kg
Trace elements				
Iron	166 mg/kg	166 mg/kg	139 mg/kg	139 mg/kg
Manganese	98 mg/kg	98 mg/kg	82 mg/kg	82 mg/kg
Zinc	65 mg/kg	65 mg/kg	56 mg/kg	56 mg/kg
Zinc sulfate	-	150 mg/kg	-	150 mg/kg
Copper	14 mg/kg	14 mg/kg	12 mg/kg	12 mg/kg
Iodine	1.2 mg/kg	1.2 mg/kg	0.97 mg/kg	0.97 mg/kg
Selenium	0.14 mg/kg	0.14 mg/kg	0.13 mg/kg	0.13 mg/kg
Cobalt	0.15 mg/kg	0.15 mg/kg	0.13 mg/kg	0.13 mg/kg

Table S1

Continued

	C	CMS	HF	HFMS
Fatty acids [% in diet]				
C12:0	-		0.03	
C14:0	0.02		1.03	
C16:0	0.45		8.06	
C17:0	-		0.38	
C18:0	0.19		5.61	
C20:0	0.02		0.04	
C16:1	0.02		0.78	
C18:1	1.07		12.13	
C20:1	-		0.01	
C18:2	2.12		2.37	
C18:3	0.26		0.33	
C20:4	-		0.07	

Table S1: Compositions of experimental diets.

Nutrient composition is expressed as [g/kg], except it is delineated differently.

Table S2	C	HF
Food intake [g]	225.4 ± 1.6[a]	196.6 ± 2.9[b]
Energy intake [MJ]	3.38 ± 0.02[a]	4.21 ± 0.06[b]
Methionine intake [g]	1.65 ± 0.01	1.62 ± 0.03
Cystine intake [g]	0.20 ± 0.001[a]	0.90 ± 0.001[b]
Choline chloride intake [g]	0.23 ± 0.002[a]	0.45 ± 0.007[b]
Folate intake [mg]	4.28 ± 0.03[a]	3.74 ± 0.05[b]
Vitamin B_{12} intake [µg]	6.76 ± 0.05[a]	5.90 ± 0.09[b]
Vitamin B_6 intake [mg]	4.06 ± 0.03[a]	3.54 ± 0.05[b]

Table S2: Total nutrient and energy intake in the C and HF group after the 8-weeks feeding period (DIO-phase).

Data are presented as mean ± SEM (n=8-9). Different superscript letters indicate statistical significance ($p < 0.05$).

Table S3	C	CMS	HF	HFMS
Food intake [g]	343.7 ± 4.0^{a}	343.0 ± 4.1^{a}	283.0 ± 3.2^{b}	282.6 ± 5.6^{b}
Energy intake [MJ]	5.16 ± 0.06^{a}	5.15 ± 0.06^{a}	6.06 ± 0.07^{b}	6.05 ± 0.12^{b}
Methionine intake [g]	2.51 ± 0.03^{a}	5.08 ± 0.06^{b}	2.35 ± 0.03^{c}	4.47 ± 0.09^{d}
Cystine intake [g]	0.31 ± 0.004^{a}	0.31 ± 0.004^{a}	1.30 ± 0.01^{b}	1.30 ± 0.03^{b}
Choline chloride intake [g]	0.36 ± 0.004^{a}	5.50 ± 0.07^{b}	0.65 ± 0.01^{c}	4.89 ± 0.10^{d}
Folate intake [mg]	6.53 ± 0.08^{a}	11.66 ± 0.14^{b}	5.38 ± 0.06^{c}	9.61 ± 0.19^{d}
Vitamin B_{12} intake [µg]	10.31 ± 0.12^{a}	524.90 ± 6.33^{b}	8.49 ± 0.10^{a}	432.40 ± 8.55^{c}
Vitamin B_6 intake [mg]	6.19 ± 0.07^{a}	6.18 ± 0.07^{a}	5.09 ± 0.06^{b}	5.09 ± 0.10^{b}

Table S3: Total nutrient and energy intake in the C, CMS, HF and HFMS group after the 12-weeks feeding experiment (DIO- and MDS-phase).

Data are presented as mean ± SEM (n = 8-9). Different superscript letters indicate statistical significance ($p < 0.05$).

Table S4

Metabolite [µM]	C	CMS	HF	HFMS
3MHis	3.58 ± 0.29^{ac}	3.51 ± 0.30^{a}	5.80 ± 0.49^{b}	4.86 ± 0.30^{bc}
Aad	10.04 ± 0.49^{a}	8.93 ± 0.56^{ab}	7.30 ± 0.42^{b}	8.31 ± 0.40^{ab}
Abu	8.12 ± 0.68	8.83 ± 0.84	6.46 ± 0.32	8.73 ± 0.55
Ala	559.00 ± 27.16	641.11 ± 42.98	661.43 ± 45.84	765.22 ± 79.53
Arg	93.22 ± 3.89	100.60 ± 6.93	87.10 ± 6.07	101.97 ± 6.20
Asn	56.41 ± 2.38^{a}	68.93 ± 5.97^{a}	77.70 ± 4.76^{ab}	92.31 ± 7.79^{b}
Asp	9.61 ± 0.60	9.81 ± 1.03	7.88 ± 0.56	8.10 ± 0.50
Cit	48.41 ± 2.88^{a}	50.18 ± 3.24^{ac}	61.31 ± 3.36^{bc}	63.01 ± 2.34^{b}
Cys	15.99 ± 2.69	24.66 ± 2.63	21.24 ± 4.22	32.36 ± 6.26
EtN	13.12 ± 0.70	13.47 ± 0.70	13.50 ± 0.85	12.27 ± 0.44
Gln	707.44 ± 18.49	775.89 ± 18.63	793.86 ± 38.77	812.56 ± 27.98
Glu	63.79 ± 4.66	61.28 ± 3.37	51.57 ± 3.56	61.19 ± 3.68
Gly	233.56 ± 5.09^{a}	169.56 ± 5.83^{b}	218.43 ± 9.95^{a}	174.67 ± 6.59^{b}
His	74.67 ± 2.54	71.24 ± 5.27	74.63 ± 2.33	85.48 ± 3.05
Hyp	16.18 ± 0.70	16.17 ± 0.55	16.71 ± 0.91	16.01 ± 0.49
Ile	138.00 ± 7.41	155.01 ± 16.82	130.57 ± 6.86	151.56 ± 9.03
Leu	222.44 ± 11.95	249.00 ± 28.86	203.43 ± 11.61	234.22 ± 12.69
Lys	329.11 ± 12.50	331.11 ± 36.25	349.00 ± 17.98	345.11 ± 30.40
Met	48.60 ± 3.30	86.68 ± 19.73	53.10 ± 3.93	101.07 ± 11.58
Orn	55.20 ± 3.79^{a}	52.04 ± 5.18^{a}	82.53 ± 7.09^{b}	69.70 ± 3.38^{ab}
PEtN	4.94 ± 0.63	6.67 ± 0.72	6.15 ± 0.91	6.88 ± 0.71
Phe	71.97 ± 3.68	75.58 ± 5.65	69.04 ± 4.08	80.79 ± 3.57

Table S4

Continued

	C	CMS	HF	HFMS
Pro	128.72 ± 10.92	157.10 ± 33.78	160.27 ± 15.85	191.23 ± 19.21
Sar	1.43 ± 0.15^{a}	13.34 ± 3.04^{b}	1.13 ± 0.09^{a}	8.03 ± 0.95^{ab}
Ser	143.22 ± 5.83^{a}	203.56 ± 19.71^{b}	182.71 ± 10.15^{ab}	235.00 ± 15.39^{b}
Tau	310.78 ± 20.69	370.67 ± 35.22	386.86 ± 34.04	425.11 ± 32.00
Thr	191.67 ± 11.45	197.56 ± 24.32	228.71 ± 13.00	232.33 ± 13.78
Trp	74.01 ± 4.15	86.22 ± 5.94	70.53 ± 3.40	79.93 ± 3.20
Tyr	92.47 ± 4.79	106.26 ± 9.39	97.84 ± 7.32	118.34 ± 7.00
Val	346.00 ± 17.98	342.11 ± 35.50	312.86 ± 13.21	345.67 ± 15.98

Table S4: Blood plasma metabolite levels of C, CMS, HF and HFMS mice after 4-weeks dietary MDS.

Data are presented as mean ± SEM (n=8-9). Different superscript letters indicate statistical significance ($p < 0.05$). 3MHis, 3-methyl-L-histidine; Aad, L-α-aminoadipic acid; Abu, L-α-amino-n-butyrate; Ala, L-alanine; Arg, L-arginine; Asn, L-asparagine; Asp, L-asparic acid; Cit, L-citrulline; Cys, L-cystine; EtN, ethanolamine; Gln, L-glutamine; Glu, L-glutamate; Gly, glycine; His, L-histidine; Hyp, hydroxyproline; Ile, L-isoleucine; Leu, L-leucine; Lys, L-lysine; Met, L-methionine; Orn, L-ornithine; PEtN, O-phosphoethanolamine; Phe, L-phenylalanine; Pro, L-proline; Sar, sarcosine; Ser, L-serine; Tau, taurine; Thr, L-threonine; Trp, L-tryptophane; Tyr, L-tyrosine; Val, L-valine.

Table S5

Metabolite [µmol/g protein]	C	CMS	HF	HFMS
1MHis	0.43 ± 0.03	0.41 ± 0.02	0.46 ± 0.02	0.45 ± 0. 04
Aad	1.15 ± 0.23	0.91 ± 0.19	0.56 ± 0.05	0.87 ± 0.13
Abu	0.96 ± 0.12^{a}	0.97 ± 0.12^{a}	0.50 ± 0.04^{b}	0.74 ± 0.11^{ab}
Ala	99.31 ± 5.10	106.94 ± 4.30	104.34 ± 5.76	101.60 ± 4.32
Asn	4.65 ±0.15^{ab}	4.96 ± 0.20^{a}	3.91 ± 0.17^{b}	4.43 ± 0.29^{ab}
Asp	4.23 ± 0.27^{a}	5.86 ± 0.55^{b}	3.66 ± 0.11^{a}	5.12 ± 0.34^{ab}
bAib	0.15 ± 0.01^{a}	0.14 ± 0.01^{ac}	0.08 ± 0.01^{b}	0.10 ± 0.01^{bc}
bAla	1.22 ± 0.11^{a}	1.22 ± 0.07^{a}	1.19 ± 0.14^{a}	1.90 ± 0.22^{b}
Cit	0.42 ± 0.04^{a}	0.24 ± 0.02^{bd}	0.36 ± 0.01^{ac}	0.31 ± 0.02^{cd}
EtN	0.55 ± 0.05^{a}	0.82 ± 0.07^{b}	0.49 ± 0.04^{a}	0.67 ± 0.05^{ab}
GABA	0.52 ± 0.06	0.54 ± 0.04	0.44 ± 0.03	0.45 ± 0.0 3
Gln	73.93 ± 4.62	88.54 ± 3.54	86.57 ± 4.35	85.77 ± 4.80
Glu	28.89 ± 3.20	23.89 ± 1.56	27.96 ± 0.66	29.19 ± 2.05
Gly	48.20 ± 2.19^{a}	29.49 ± 2.51^{bc}	37.94 ± 1.14^{b}	26.38 ± 2.53^{c}
His	10.65 ± 0.59	10.66 ± 0.47	10.07 ± 0.40	10.61 ± 0.45
Hyp	0.50 ± 0.03^{a}	0.44 ± 0.02^{ac}	0.38 ± 0.02^{bc}	0.38 ± 0.02^{bc}
Ile	6.55 ± 0.29^{a}	6.68 ± 0.36^{a}	5.08 ± 0.31^{b}	5.52 ± 0.37^{ab}
Leu	11.63 ± 0.61^{ab}	12.22 ± 0.61^{a}	9.22 ± 0.49^{b}	10.10 ± 0.64^{ab}
Lys	12.32 ± 0.48^{ac}	13.94 ± 0.77^{a}	9.90 ± 0.39^{b}	11.06 ± 0.49^{bc}
Met	0.64 ± 0.05	0.70 ± 0.09	0.55 ± 0.03	0.73 ± 0.05
Orn	6.18 ± 0.38^{a}	5.30 ± 0.29^{ab}	4.55 ± 0.21^{b}	4.28 ± 0.31^{b}
PEtN	4.58 ± 0.37	4.77 ± 0.19	4.49 ± 0.13	4.68 ± 0.3 3

Table S5

Continued

	C	CMS	HF	HFMS
Phe	4.21 ± 0.15^{ab}	4.33 ± 0.19^{a}	3.52 ± 0.19^{b}	3.76 ± 0.22^{ab}
Pro	8.19 ± 0.63	8.68 ± 0.90	6.50 ± 0.35	8.08 ± 0.55
Sar	1.76 ± 0.40^{a}	9.22 ± 1.89^{b}	0.75 ± 0.10^{a}	6.90 ± 0.52^{b}
Ser	7.53 ± 0.25^{ac}	8.78 ± 0.65^{a}	5.85 ± 0.28^{bc}	7.08 ± 0.30^{bc}
Tau	187.45 ± 6.09^{ab}	174.97 ± 4.81^{b}	208.79 ± 5.18^{a}	202.04 ± 6.10^{a}
Thr	7.78 ± 0.34^{ac}	9.09 ± 0.63^{a}	7.16 ± 0.32^{bc}	7.25 ± 0.37^{bc}
Trp	1.16 ± 0.04^{a}	1.26 ± 0.06^{a}	0.95 ± 0.04^{b}	1.08 ± 0.06^{ab}
Tyr	4.70 ± 0.14^{ab}	5.41 ± 0.25^{a}	4.08 ± 0.23^{b}	4.54 ± 0.19^{b}
Val	12.72 ± 0.51^{a}	12.47 ± 0.71^{ac}	9.56 ± 0.52^{b}	10.20 ± 0.65^{bc}

Table S5: Selected metabolite levels in C, CMS, HF and HFMS liver tissues after 4 weeks of dietary MDS.

Data are presented as mean ± SEM [µmol/g protein] (n=8-9). Different superscript letters indicate statistical significance ($p < 0.05$). 1MHis, 1-methyl-L-histidine; Aad, L-α-aminoadipic acid; Abu, L-α-amino-n-butyrate; Ala, L-alanine; Asn, L-asparagine; Asp, L-asparic acid; bAib, D,L-β-aminoisobutyrate; bAla, β-alanine; Cit, L-citrulline; EtN, ethanolamine; GABA, γ-amino-n-butyrate; Gln, L-glutamine; Glu, L-glutamate; Gly, glycine; His, L-histidine; Hyp, hydroxyproline; Ile, L-isoleucine; Leu, L-leucine; Lys, L-lysine; Met, L-methionine; Orn, L-ornithine; PEtN, O-phosphoethanolamine; Phe, L-phenylalanine; Pro, L-proline; Sar, sarcosine; Ser, L-serine; Tau, taurine; Thr, L-threonine; Trp, L-tryptophane; Tyr, L-tyrosine; Val, L-valine.

Table S6

Metabolite [µmol/mg tissue]	C	CMS	HF	HFMS
C0	57.561 ± 2.219[a]	42.271 ± 1.819[b]	63.116 ± 2.457[a]	47.757 ± 2.405[b]
C10	0.318 ± 0.017[a]	0.294 ± 0.010[a]	0.347 ± 0.014[ab]	0.385 ± 0.012[b]
C10:1	0.095 ± 0.004[a]	0.093 ± 0.004[a]	0.164 ± 0.005[b]	0.162 ± 0.005[b]
C10:2	0.079 ± 0.005	0.082 ± 0.004	0.084 ± 0.002	0.085 ± 0.003
C12	0.062 ± 0.004[a]	0.052 ± 0.002[ab]	0.046 ± 0.001[b]	0.048 ± 0.001[b]
C12-DC	0.159 ± 0.010	0.149 ± 0.008	0.128 ± 0.004	0.154 ± 0.007
C12:1	0.177 ± 0.013[a]	0.240 ± 0.011[b]	0.183 ± 0.011[a]	0.202 ± 0.007[ab]
C14	0.135 ± 0.008[a]	0.114 ± 0.005[ab]	0.117 ± 0.003[ab]	0.099 ± 0.004[a]
C14:1	0.051 ± 0.003	0.048 ± 0.002	0.054 ± 0.003	0.049 ± 0.002
C14:1-OH	0.040 ± 0.003[ab]	0.034 ± 0.003[a]	0.046 ± 0.001[b]	0.045 ± 0.002[b]
C14:2	0.025 ± 0.002	0.021 ± 0.001	0.022 ± 0.001	0.023 ± 0.001
C14:2-OH	0.041 ± 0.003	0.038 ± 0.001	0.039 ± 0.001	0.040 ± 0.002
C16	0.614 ± 0.050[ac]	0.438 ± 0.036[b]	0.726 ± 0.040[a]	0.545 ± 0.037[bc]
C16-OH	0.039 ± 0.003[a]	0.036 ± 0.002[ab]	0.032 ± 0.002[ab]	0.029 ± 0.001[b]
C16:1	0.164 ± 0.011[a]	0.125 ± 0.010[ab]	0.164 ± 0.013[a]	0.120 ± 0.007[b]
C16:1-OH	0.037 ± 0.002[a]	0.029 ± 0.001[a]	0.082 ± 0.004[b]	0.071 ± 0.004[b]
C16:2	0.019 ± 0.001	0.018 ± 0.001	0.018 ± 0.001	0.016 ± 0.001
C16:2-OH	0.024 ± 0.002[a]	0.017 ± 0.001[b]	0.038 ± 0.001[c]	0.033 ± 0.002[c]
C18	0.322 ± 0.029[a]	0.197 ± 0.008[b]	0.561 ± 0.030[c]	0.441 ± 0.027[d]
C18:1	0.756 ± 0.061[a]	0.469 ± 0.050[a]	1.217 ± 0.097[b]	0.913 ± 0.092[b]
C18:1-OH	0.055 ± 0.004[a]	0.046 ± 0.002[a]	0.074 ± 0.003[b]	0.060 ± 0.002[ac]
C18:2	0.174 ± 0.013[a]	0.122 ± 0.006[b]	0.143 ± 0.005[ab]	0.119 ± 0.006[b]

Table S6

Continued

	C	CMS	HF	HFMS
C2	18.413 ± 0.858^{a}	12.340 ± 0.474^{b}	14.566 ± 0.841^{b}	10.685 ± 0.827^{c}
C3	4.772 ± 0.362^{a}	3.363 ± 0.302^{b}	5.396 ± 0.264^{a}	4.129 ± 0.272^{b}
C3-DC (C4-OH)	0.907 ± 0.062^{ab}	0.759 ± 0.053^{bc}	1.042 ± 0.045^{a}	0.666 ± 0.052^{c}
C3-OH	0.049 ± 0.003^{a}	0.050 ± 0.003^{a}	0.037 ± 0.002^{b}	0.044 ± 0.002^{ab}
C3:1	0.017 ± 0.001	0.016 ± 0.001	0.014 ± 0.0005	0.016 ± 0.001
C4	2.344 ± 0.100^{a}	1.238 ± 0.088^{b}	1.773 ± 0.105^{c}	1.078 ± 0.088^{d}
C4:1	0.198 ± 0.011^{a}	0.196 ± 0.008^{a}	0.160 ± 0.005^{b}	0.174 ± 0.006^{ab}
C5	1.179 ± 0.064^{a}	0.733 ± 0.080^{b}	1.453 ± 0.095^{a}	1.033 ± 0.089^{b}
C5-DC (C6-OH)	0.281 ± 0.012^{a}	0.207 ± 0.009^{b}	0.557 ± 0.021^{c}	0.482 ± 0.023^{d}
C5-M-DC	0.188 ± 0.018^{a}	0.112 ± 0.006^{b}	0.264 ± 0.021^{c}	0.181 ± 0.010^{a}
C5-OH (C3-DC-M)	0.426 ± 0.013^{a}	0.362 ± 0.013^{b}	0.464 ± 0.014^{a}	0.350 ± 0.015^{b}
C5:1	0.044 ± 0.004	0.039 ± 0.003	0.033 ± 0.001	0.036 ± 0.001
C5:1-DC	0.164 ± 0.010^{a}	0.119 ± 0.010^{b}	0.156 ± 0.010^{ab}	0.129 ± 0.011^{ab}
C6 (C4:1-DC)	0.749 ± 0.041^{a}	0.399 ± 0.028^{bc}	0.497 ± 0.028^{b}	0.341 ± 0.030^{c}
C6:1	0.047 ± 0.004	0.043 ± 0.003	0.037 ± 0.002	0.042 ± 0.002
C7-DC	0.255 ± 0.027^{a}	0.354 ± 0.043^{ab}	0.459 ± 0.085^{b}	0.519 ± 0.025^{b}
C8	0.276 ± 0.014^{a}	0.217 ± 0.009^{a}	0.973 ± 0.047^{b}	0.862 ± 0.050^{b}
C9	0.094 ± 0.005^{a}	0.091 ± 0.007^{a}	0.279 ± 0.015^{b}	0.277 ± 0.015^{b}

Table S6: Concentrations of selected hepatic carnitines and acyl-carnitines in C, CMS, HF and HFMS liver tissue after 4 weeks of dietary MDS.

Data are presented as mean ± SEM (n=8-9). Different superscript letters indicate statistical significance ($p < 0.05$). C0, DL-carnitine; C10, decanoyl-L-carnitine; C10:1 decenoyl-L-carnitine; C10:2, decadienyl-L-carnitine; C12, dodecanoyl-L-carnitine; C12-DC, dodecanedioyl-L-carnitine; C12:1, dodecenoyl-L-carnitine; C14, tetradecanoyl-L-carnitine; C14:1, tetradecenoyl-L-carnitine; C14:1-OH, hydroxytetradecenoyl-L-carnitine; C14:2, tetradecadienyl-L-carnitine; C14:2-OH, hydroxy-

tetradecadienyl-L-carnitine; C16, hexadecanoyl-L-carnitine; C16-OH, hydroxyhexadecanoyl-L-carnitine; C16:1, hexadecenoyl-L-carnitine; C16:1-OH, hydroxyhexadecenoyl-L-carnitine; C16:2, hexadecadienyl-L-carnitine; C16:2-OH, hydroxyhexadecadienyl-L-carnitine; C18, octadecanoyl-L-carnitine; C18:1, octadecenoyl-L-carnitine; C18:1-OH, hydroxyoctadecenoyl-L-carnitine; C18:2, octadecadienyl-L-carnitine; C2, acetyl-L-carnitine; C3, propionyl-L-carnitine; C3-DC / C4-OH, malonyl-L-carnitine / hydroxybutyryl-L-carnitine; C3-OH, hydroxypropionyl-L-carnitine; C3:1, propenyl-L-carnitine; C4, butyryl-L-carnitine; C4:1, butenyl-L-carnitine; C5, valeryl-L-carnitine; C5-DC / C6-OH, glutaryl-L-carnitine / hydroxyhexanoyl-L-carnitine; C5-M-DC, methylglutaryl-L-carnitine; C5-OH / C3-DC-M, methylmalonyl-L-carnitine / hydroxyvaleryl-L-carnitine; C5:1, tiglyl-L-carnitine; C5:1-DC, glutaconyl-L-carnitine; C6 / C4:1-DC, fumaryl-L-carnitine / hexanoyl-L-carnitine; C6:1, hexenoyl-L-carnitine; C7-DC, pimelyl-L-carnitine; C8, octanoyl-L-carnitine; C9, nonayl-L-carnitine.

Table S7 Metabolite [µmol/mg tissue]	C	CMS	HF	HFMS
PC aa C24:0	1.37 ± 0.18	1.13 ± 0.18	1.39 ± 0.23	1.66 ± 0.27
PC aa C26:0	4.59 ± 0.89	4.66 ± 0.93	3.05 ± 0.63	3.92 ± 0.72
PC aa C28:1	1.50 ± 0.22	1.37 ± 0.19	1.29 ± 0.19	1.48 ± 0.21
PC aa C30:0	**5.63 ± 0.26**	**5.42 ± 0.17**	**4.49 ± 0.21**	**4.51 ± 0.14**
PC aa C30:2	0.73 ± 0.11	0.60 ± 0.09	0.66 ± 0.11	0.78 ± 0.13
PC aa C32:0	**233.42 ± 8.66**	**190.71 ± 4.84**	**153.70 ± 9.36**	**147.61 ± 5.48**
PC aa C32:1	**320.77 ± 21.63**	**380.67 ± 19.39**	**199.79 ± 9.73**	**189.49 ± 5.64**
PC aa C32:2	**55.15 ± 2.86**	**67.79 ± 2.95**	**32.43 ± 1.58**	**32.10 ± 1.41**
PC aa C32:3	3.02 ± 0.20	3.04 ± 0.14	2.83 ± 0.20	3.03 ± 0.23
PC aa C34:1	964.50 ± 49.03	910.28 ± 50.06	860.80 ± 27.16	988.33 ± 32.59
PC aa C34:2	797.29 ± 64.95	635.77 ± 50.09	956.22 ± 30.77	1064.57 ± 34.27
PC aa C34:3	**362.64 ± 17.52**	**435.95 ± 9.22**	**212.16 ± 12.82**	**223.95 ± 9.71**
PC aa C34:4	**28.90 ± 1.25**	**30.69 ± 0.70**	**24.59 ± 1.34**	**25.10 ± 0.87**
PC aa C36:0	5.62 ± 0.33	5.22 ± 0.11	5.76 ± 0.54	4.91 ± 0.40
PC aa C36:1	**295.24 ± 10.44**	**272.08 ± 6.31**	**396.85 ± 30.12**	**378.45 ± 8.96**
PC aa C36:2	925.51 ± 34.44	882.21 ± 22.38	898.01 ± 34.67	965.12 ± 27.20
PC aa C36:3	788.50 ± 27.92	743.42 ± 16.48	850.98 ± 35.66	847.81 ± 21.22
PC aa C36:4	1023.12 ± 58.64	1027.93 ± 43.71	894.67 ± 36.13	1033.56 ± 44.18
PC aa C36:5	**223.16 ± 9.62**	**229.99 ± 7.53**	**184.63 ± 10.08**	**188.63 ± 6.34**
PC aa C36:6	16.68 ± 0.92	15.93 ± 0.67	17.57 ± 0.95	17.80 ± 0.53
PC aa C38:0	**7.35 ± 0.26**	**5.88 ± 0.20**	**12.14 ± 0.82**	**12.12 ± 0.47**
PC aa C38:1	**4.51 ± 0.35**	**3.13 ± 0.26**	**5.99 ± 0.56**	**5.75 ± 0.22**

Table S7

Continued

	C	CMS	HF	HFMS
PC aa C38:3	**233.14 ± 10.07**	**187.56 ± 7.92**	**396.63 ± 31.58**	**366.04 ± 12.92**
PC aa C38:4	**765.00 ± 22.69**	**673.51 ± 22.98**	**922.61 ± 33.06**	**1000.79 ± 28.57**
PC aa C38:5	**475.33 ± 21.48**	**412.81 ± 18.17**	**614.90 ± 36.32**	**617.23 ± 19.10**
PC aa C38:6	1003.86 ± 41.77	892.95 ± 23.28	995.63 ± 39.83	1067.03 ± 26.24
PC aa C40:1	1.53 ± 0.05	1.29 ± 0.04	1.68 ± 0.10	1.68 ± 0.12
PC aa C40:2	2.76 ± 0.06	2.23 ±0.05	2.49 ± 0.18	2.56 ± 0.16
PC aa C40:3	**4.74 ± 0.16**	**3.81 ± 0.09**	**4.29 ± 0.29**	**3.97 ± 0.20**
PC aa C40:4	24.01 ± 0.70	18.56 ± 0.47	25.23 ± 1.94	24.15 ± 0.83
PC aa C40:5	42.92 ± 1.70	33.73 ± 1.46	55.00 ± 4.73	48.44 ± 1.98
PC aa C40:6	**300.01 ± 8.28**	**241.98 ± 10.32**	**369.29 ± 26.56**	**383.30 ± 8.89**
PC aa C42:0	1.36 ± 0.05	1.29 ± 0.04	1.37 ± 0.09	1.47 ± 0.09
PC aa C42:1	1.25 ± 0.04	1.00 ± 0.06	1.20 ± 0.09	1.19 ± 0.07
PC aa C42:2	1.24 ± 0.04	1.01 ± 0.03	1.08 ± 0.07	1.27 ± 0.11
PC aa C42:4	1.38 ± 0.04	1.13 ± 0.03	1.27 ± 0.10	1.24 ±0.07
PC aa C42:5	**2.11 ± 0.05**	**1.69 ± 0.05**	**1.71 ± 0.11**	**1.72 ± 0.10**
PC aa C42:6	**7.20 ± 0.23**	**5.36 ± 0.15**	**5.22 ± 0.44**	**5.23 ± 0.31**
PC ae C30:0	0.50 ± 0.03	0.45 ± 0.02	0.50 ± 0.03	0.56 ± 0.05
PC ae C30:1	1.30 ± 0.30	1.21 ± 0.26	0.74 ± 0.16	0.92 ± 0.18
PC ae C30:2	0.46 ± 0.07	0.40 ± 0.06	0.41 ± 0.06	0.49 ± 0.07
PC ae C32:1	6.93 ± 0.39	6.44 ± 0.24	6.43 ± 0.47	6.76 ± 0.50
PC ae C32:2	2.13 ± 0.27	1.71 ± 0.22	1.78 ± 0.25	2.09 ± 0.28
PC ae C34:0	**6.14 ± 0.22**	**5.24 ± 0.11**	**18.21 ± 1.40**	**17.64 ± 0.67**
PC ae C34:1	**40.67 ± 1.55**	**41.59 ± 1.16**	**79.25 ± 4.93**	**78.06 ± 2.57**

Table S7

Continued

	C	CMS	HF	HFMS
PC ae C34:2	**18.06 ± 1.00**	**20.63 ± 0.66**	**24.73 ± 1.77**	**26.91 ± 1.22**
PC ae C34:3	3.43 ± 0.28	3.32 ± 0.19	3.03 ± 0.30	3.42 ± 0.36
PC ae C36:0	**2.97 ± 0.23**	**3.04 ± 0.10**	**4.01 ± 0.23**	**4.10 ± 0.23**
PC ae C36:1	**28.02 ± 1.24**	**30.19 ± 0.55**	**76.90 ± 5.91**	**75.38 ± 1.88**
PC ae C36:2	**39.69 ± 1.63**	**42.06 ± 1.15**	**104.48 ± 7.32**	**106.82 ± 3.13**
PC ae C36:3	**15.12 ± 0.59**	**16.84 ± 0.34**	**26.88 ± 1.66**	**26.56 ± 0.93**
PC ae C36:4	**21.54 ± 0.89**	**18.99 ± 0.58**	**28.81 ± 1.89**	**29.67 ± 1.54**
PC ae C36:5	7.24 ± 0.23	6.55 ± 0.13	6.99 ± 0.49	6.60 ± 0.31
PC ae C38:0	78.14 ± 5.22	71.24 ± 3.93	74.28 ± 4.79	74.44 ± 3.24
PC ae C38:1	**7.19 ± 0.26**	**6.18 ± 0.17**	**13.85 ± 0.85**	**12.97 ± 0.46**
PC ae C38:2	**16.30 ± 0.59**	**15.25 ± 0.50**	**32.31 ± 2.49**	**29.76 ± 1.35**
PC ae C38:3	**15.69 ± 0.44**	**13.89 ± 0.50**	**40.75 ± 2.66**	**38.81 ± 1.19**
PC ae C38:4	**31.11 ± 0.91**	**28.31 ± 1.26**	**115.84 ± 8.61**	**118.50 ± 3.77**
PC ae C38:5	**25.44 ± 0.90**	**22.29 ± 0.63**	**38.46 ± 2.51**	**38.22 ± 1.63**
PC ae C38:6	**11.70 ± 0.38**	**10.34 ± 0.32**	**16.96 ± 1.26**	**17.66 ± 0.77**
PC ae C40:1	**61.06 ± 4.11**	**42.70 ± 2.39**	**49.49 ± 3.38**	**45.95 ± 2.01**
PC ae C40:2	7.03 ± 0.34	6.00 ± 0.27	6.96 ± 0.49	6.67 ± 0.32
PC ae C40:3	**6.26 ± 0.17**	**5.60 ± 0.13**	**11.47 ± 0.68**	**10.46 ± 0.37**
PC ae C40:4	**12.01 ± 0.32**	**10.86 ± 0.48**	**31.02 ± 2.37**	**28.56 ± 1.19**
PC ae C40:5	**9.96 ± 0.36**	**7.67 ± 0.27**	**15.05 ± 0.93**	**14.38 ± 0.68**
PC ae C40:6	**14.49 ± 0.54**	**11.83 ± 0.58**	**51.51 ± 4.03**	**53.17 ± 1.36**
PC ae C42:0	**6.14 ± 0.32**	**4.86 ± 0.23**	**4.95 ± 0.32**	**4.78 ± 0.28**
PC ae C42:1	5.54 ± 0.40	4.22 ± 0.38	4.93 ± 0.55	5.41 ± 0.60

Table S7

Continued

	C	CMS	HF	HFMS
PC ae C42:2	**5.37 ± 0.36**	**3.46 ± 0.26**	**4.86 ± 0.37**	**4.41 ± 0.36**
PC ae C42:3	**19.21 ± 1.32**	**10.71 ± 0.75**	**13.83 ± 1.16**	**12.82 ± 0.87**
PC ae C42:4	**2.42 ± 0.08**	**1.89 ± 0.09**	**2.60 ± 0.18**	**2.64 ± 0.11**
PC ae C42:5	**3.02 ± 0.09**	**2.46 ± 0.07**	**3.99 ± 0.25**	**3.72 ± 0.15**
PC ae C44:3	1.46 ± 0.13	1.21 ± 0.11	1.29 ± 0.15	1.54 ± 0.19
PC ae C44:4	1.11 ± 0.11	0.83 ± 0.10	0.97 ± 0.11	1.08 ± 0.15
PC ae C44:5	1.93 ± 0.12	1.39 ± 0.10	1.52 ± 0.12	1.61 ± 0.14
PC ae C44:6	1.32 ± 0.04	1.49 ± 0.09	1.49 ± 0.09	1.61 ± 0.07
lysoPC a C14:0	3.64 ± 0.21	3.33 ± 0.17	2.98 ± 0.10	3.42 ± 0.13
lysoPC a C16:0	**113.28 ± 5.33**	**120.38 ± 2.40**	**102.26 ± 2.63**	**101.40 ± 4.01**
lysoPC a C16:1	**5.74 ± 0.42**	**6.67 ± 0.18**	**4.51 ± 0.18**	**4.20 ± 0.25**
lysoPC a C17:0	**1.43 ± 0.05**	**1.44 ± 0.05**	**4.54 ± 0.16**	**4.63 ± 0.14**
lysoPC a C18:0	**59.88 ± 1.95**	**54.78 ± 1.23**	**71.60 ± 1.76**	**73.72 ± 2.12**
lysoPC a C18:1	33.29 ± 2.49	32.95 ± 1.15	37.93 ± 1.03	34.63 ± 1.27
lysoPC a C18:2	**39.65 ± 2.20**	**37.73 ± 1.03**	**29.50 ± 1.14**	**27.36 ± 0.85**
lysoPC a C20:3	5.95 ± 0.50	4.71 ± 0.18	7.41 ± 0.38	6.33 ± 0.29
lysoPC a C20:4	26.12 ± 1.95	22.23 ± 0.86	29.05 ± 1.24	27.89 ± 1.31
lysoPC a C24:0	1.53 ± 0.15	1.30 ± 0.14	1.37 ± 0.18	1.71 ± 0.23
lysoPC a C26:0	1.62 ± 0.19	1.44 ± 0.19	1.59 ± 0.23	2.01 ± 0.29
lysoPC a C26:1	4.01 ± 0.46	3.50 ± 0.36	3.27 ± 0.44	4.07 ± 0.46
lysoPC a C28:0	0.97 ± 0.10	0.85 ± 0.10	1.16 ± 0.16	1.38 ± 0.19
lysoPC a C28:1	1.86 ± 0.29	1.58 ± 0.25	1.69 ± 0.35	2.12 ± 0.38

Table S7

Continued

	C	CMS	HF	HFMS
SM (OH) C14:1	1.44 ± 0.08	1.45 ± 0.06	1.83 ± 0.11	1.77 ± 0.07
SM (OH) C16:1	**0.38 ± 0.02**	**0.37 ± 0.01**	**1.52 ± 0.09**	**1.63 ± 0.07**
SM (OH) C22:1	6.14 ± 0.32	6.41 ± 0.27	8.39 ± 1.03	8.31 ± 0.50
SM (OH) C22:2	1.41 ± 0.09	1.58 ± 0.07	1.69 ± 0.19	1.62 ± 0.11
SM (OH) C24:1	**2.18 ± 0.13**	**1.58 ± 0.09**	**1.90 ± 0.16**	**1.87 ± 0.10**
SM C16:0	35.92 ± 1.35	37.16 ± 1.21	40.11 ± 2.07	41.25 ± 1.38
SM C16:1	1.68 ± 0.11	1.67 ± 0.09	1.67 ± 0.11	1.78 ± 0.11
SM C18:0	**4.10 ± 0.15**	**3.83 ± 0.11**	**10.05 ± 0.77**	**10.64 ± 0.42**
SM C18:1	**0.38 ± 0.02**	**0.36 ± 0.01**	**0.86 ± 0.06**	**0.93 ± 0.03**
SM C20:2	0.20 ± 0.03	0.28 ± 0.02	0.02 ± 0.01	0.04 ± 0.01
SM C22:3	2.74 ± 0.15	2.30 ± 0.25	3.07 ± 0.26	2.88 ± 0.20
SM C24:0	**18.86 ± 0.53**	**19.64 ± 0.60**	**13.53 ± 1.37**	**13.56 ± 0.74**
SM C24:1	**9.76 ± 0.73**	**15.12 ± 0.95**	**2.21 ± 0.64**	**2.66 ± 0.66**
SM C26:0	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
SM C26:1	0.13 ± 0.01	0.11 ± 0.01	0.15 ± 0.01	0.15 ± 0.02

Table S7: PC, lyso-PC, hydroxyl- and sphingomyelin metabolite concentrations in C, CMS, HF and HFMS livers after 4 weeks of dietary MDS.

Data are presented as mean ± SEM (n=8-9). Bold numbers indicate statistical significance ($p < 0.01$). PC aa, phosphatidylcholine diacyl; PC ae, phosphatidylcholine acyl-alkyl; SM (OH), hydroxysphingomyeline; SM, sphingomyeline.