

CDAHFD-induces a NASH model mimicking the clinic in the metabolism of hepatic phosphatidylcholines and acyl carnitines

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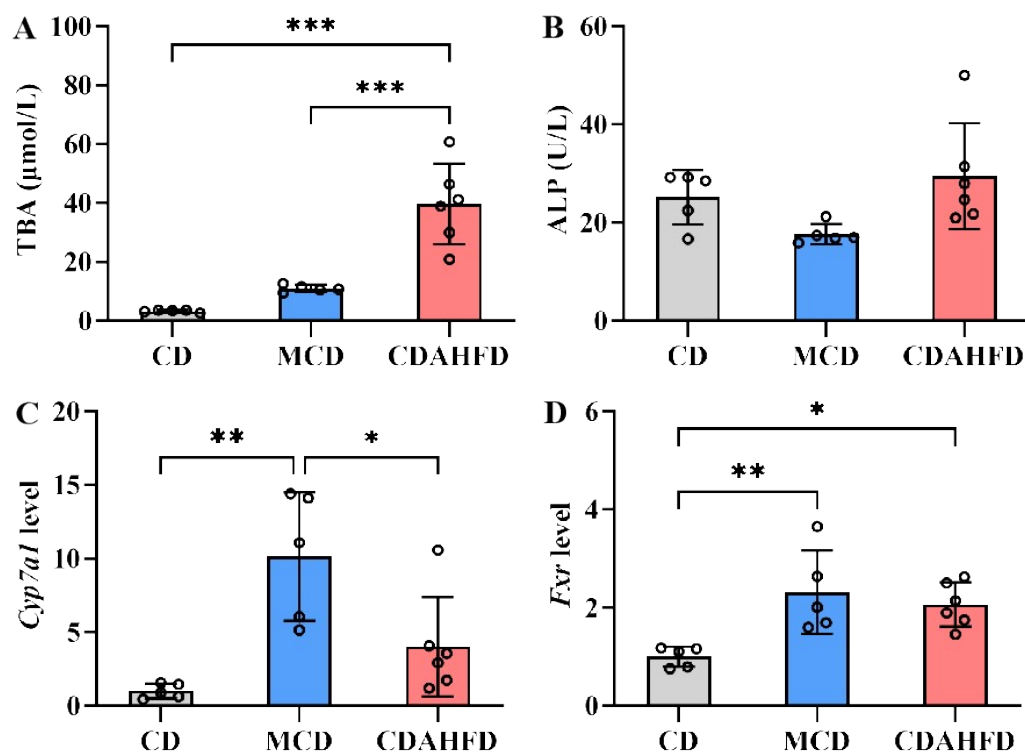


Fig. S1 The overload of bile acid and their synthesis adaptation were more pronounced in CDAHFD-induced NASH. (A) Serum TBA level in mice. (B) Serum ALP level in mice. (C, D) The relative mRNA levels of *Cyp7a1* and *Fxr* were determined by qPCR and normalized with 18S. Data was expressed as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, respectively.

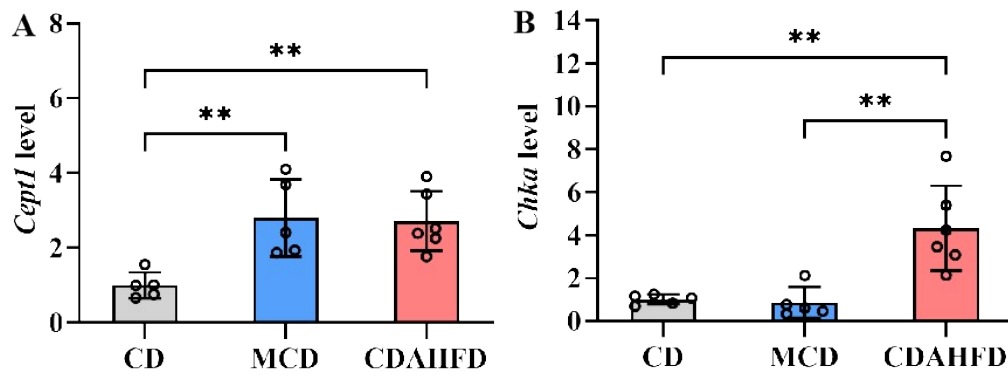


Fig. S2 Effects of the MCD and CDAHFD diets on the transcription of genes involved PC synthesis.

(A, B) The relative mRNA levels of *Cept1* and *Chka* were determined by qPCR and normalized with

18S. Data was expressed as mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, respectively.

Table S1. The ingredients and nutrient compositions of the diets used in this study.

Ingredient (gm)	CD	MCD	CDAHFD
L-Alanine	0	3.5	6.0
L-Arginine	19.8	12.0	7.1
L-Asparagine	0	5.9	0
L-Aspartic Acid	10	3.5	14.3
L-Cystine	6.6	3.5	5.0
L-Glutamine	0	39.6	45.0
Glycine	10	23.1	3.5
L-Histidine	8	4.5	5.4
L-Isoleucine	14	8.1	9.0
L-Leucine	28.8	11.0	18.6
L-Lysine	16.4	17.8	15.6
L-Phenylalanine	10	7.4	9.9
L-Proline	0	3.5	21.0
L-Serine	10	3.5	11.8
L-Threonine	13	8.1	8.5
L-Tryptophan	3.8	1.8	2.5
L-Tyrosine	12	5.0	10.8
L-Valine	16.8	8.1	11.0
Sucrose	140	451.1	203.7

Corn Starch	0	148.6	90.9
Maltodextrin 10	30.3	49.5	117.9
Cellulose	150	29.7	58.9
Corn Oil	20	99.1	0
Soybean Oil	0	0	29.5
Mineral Mix	24	34.7	11.8
Sodium Bicarbonate	0	7.4	8.8
Vitamin Mix	24	9.9	11.8
L-Methionine	4	0	0.9
Choline	2.5	0	0
Cholesterol	20	0	10.3
FD&C Yellow Dye #5	0	0	0.1
Potassium Citrate	10	0	19.5
Calcium Carbonate	16	0	6.5
DiCalcium Phosphate	10	0	15.3
Lard	80	0	209.2
crude ash	290	0	0
Total (gm)	1000	1000	1000
Kcal percentage (%)			
Protein	18	16	18
Carbohydrate	78	63	36

Fat	4	21	46
Total (%)	100	100	100

Table S2. The primer sequences used in the qPCR analysis in this study.

Genes	Forward primer	Reverse primer
<i>Srebf1</i>	TGACCCGGCTATTCCGTGA	CTGGGCTGAGCAATACAGTTC
<i>Plin2</i>	CTTGTGTCCTCCGCTTATGTC	GCAGAGGTCACGGTCTTCAC
<i>Abca1</i>	GCTTGTTGGCCTCAGTTAAGG	GTAGCTCAGGCGTACAGAGAT
<i>Il-2</i>	TGAGCAGGATGGAGAATTACAGG	GTCCAAGTTCATCTTCTAGGCAC
<i>Tnf-α</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Il-10</i>	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
<i>Ccl2</i>	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
<i>Cyclin D1</i>	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
<i>Cyclin E1</i>	CTCCGACCTTTTCAGTCCGC	CACAGTCTTGTCATCTTGGA
<i>Cyclin B1</i>	GCGTGTGCCTGTGACAGTTA	CCTAGCGTTTTTGCTTCCCTT
<i>Ki67</i>	ATCATTGACCGCTCCTTTAGGT	GCTCGCCTTGATGGTTCCT
<i>Pcyt1a</i>	GATGCACAGAGTTCAGCTAAAAGT	TGGCTGCCGTAAACCAACTG
<i>Pemt</i>	ATCACCATTGTGTTCAACCCAC	CCAGGGAATAGCAGGCTAGG
<i>Lpcat</i>	GGCTCCTGTTGCTGCTTT	TTCCTCCATAAGGCCAGGGG
<i>Cept1</i>	ATGAGTGGGCATCGGTCAAC	GTGGTGTCGGTAACTGAAACAA
<i>Chka</i>	CAGGGGTGGTCTCAGTAACAT	CGCAAGAGCACTTTCCGAG
<i>Cpt1</i>	TGGCATCATCACTGGTGTGTT	GTCTAGGGTCCGATTGATCTTTG
<i>Cpt2</i>	CAGCACAGCATCGTACCCA	TCCAATGCCGTTCTCAAAT
<i>Acox1</i>	CCGCCACCTTCAATCCAGAG	CAAGTTCTCGATTTCTCGACGG
<i>Cyp7a1</i>	GTCCGGATATTCAAGGATGC	GGGAATGCCATTTACTTGGA

Fxr

GCTTGATGTGCTACAAAAGCTG

CGTGGTGATGGTTGAATGTCC

Table S3. Changed liver lipid metabolites in the MCD group versus the CDAHFD group.

NO	Molecular Formula	Compound	Rt(min)	[M+H] ⁺ m/z
1	C21H35NO8	AC(14:3;O4)	0.877	430.24179
2	C33H53NO4	AC(26:6)	1.387	528.40918
3	C39H69NO5	AC(32:4;O)	11.872	632.52179
4	C30H57NO4	Cer(30:2;O3)	2.64	496.42206
5	C36H71NO5	Cer(36:1;O4)	2.379	598.49377
6	C36H62O5	DG(33:4)	13.821	575.50781
7	C38H64O5	DG(35:5)	16.275	601.51599
8	C50H97NO13	Hex2Cer(38:0;O2)	16.271	920.69122
9	C41H75NO8	HexCer(35:3;O2)	2.979	710.60345
10	C45H81NO10	HexCer(39:4;O4)	11.464	796.56824
11	C47H83NO9	HexCer(41:5;O3)	8.975	806.61908
12	C50H97NO10	HexCer(44:1;O4)	16.527	872.76288
13	C39H68NO14P	IPC(33:5;O5)	9.033	806.44476
14	C40H80NO13P	IPC(34:0;O4)	13.822	814.54156
15	C42H84NO11P	IPC(36:0;O2)	12.05	810.58716
16	C40H80NO8P	PC(32:0)	12.656	734.57214
17	C42H80NO8P	PC(34:2)	10.527	758.54956
18	C42H78NO8P	PC(34:3)	8.719	756.55597
19	C44H86NO8P	PC(36:1)	13.75	788.60742
20	C46H80NO8P	PC(38:6)	8.608	806.56097

21	C48H84NO8P	PC(40:6)	12.06	834.60004
22	C48H82NO8P	PC(40:7)	9.228	832.57965
23	C43H84NO7P	PC(O-35:2)	10.315	758.58905
24	C41H80NO8P	PE(36:1)	13.933	746.56024
25	C46H80NO8P	PE(41:6)	8.877	806.57257
26	C43H84NO7P	PE(O-38:2)	10.378	758.60028
27	C47H84NO7P	PE(O-42:6)	8.182	806.57257
28	C43H84NO10P	PS(37:0)	9.094	806.57257
29	C44H72NO10P	PS(38:7)	8.993	806.51447
30	C45H88NO10P	PS(39:0)	12.229	834.59412
31	C45H86NO10P	PS(39:1)	8.735	832.57965
32	C46H76NO10P	PS(40:7)	9.252	834.49951
33	C41H76NO9P	PS(O-35:3)	10.86	758.54956
34	C46H93N2O6P	SM(41:1;O2)	14.643	801.68713
35	C47H95N2O6P	SM(42:1;O2)	14.839	815.69824

Abbreviations: AC, acyl carnitine; Cer, ceramide; DG, diglyceride; Hex2Cer, dihexosylceramide; HexCer, hexosylceramide; IPC, inositol phosphorylceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin.