SUPPLEMENTAL INFORMATION:

Insulin resistance dysregulates CYP7B1 leading to oxysterol accumulation: a pathway for NAFL to NASH transition

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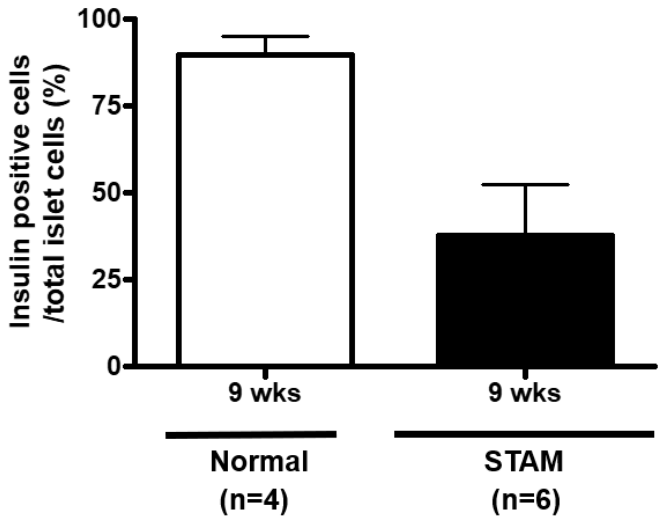
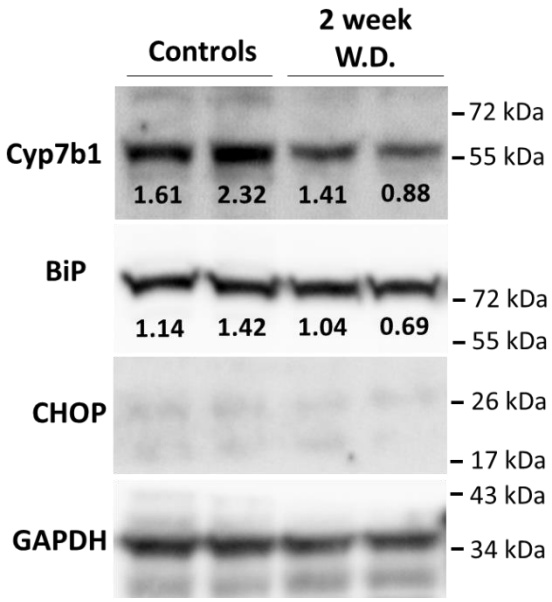
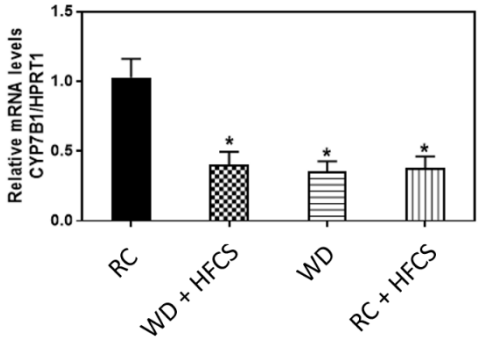


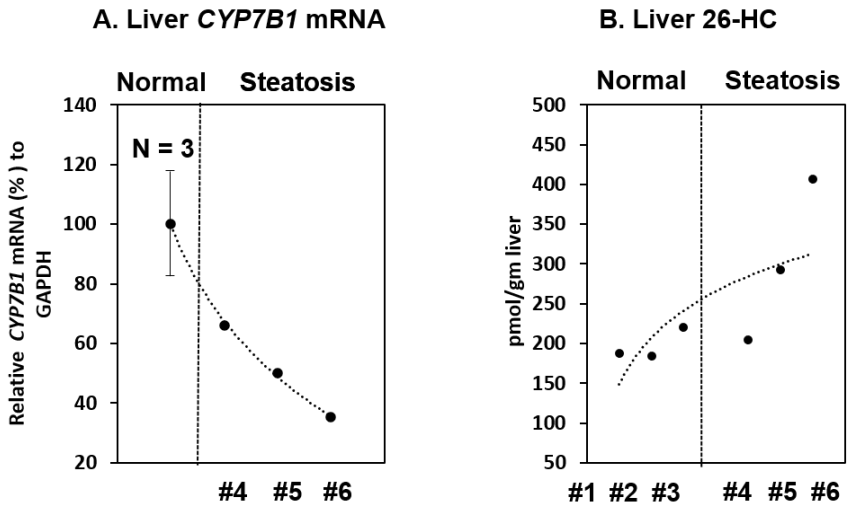
Supplemental Figure S1: Insulin positive cells of the 9-week-old STAM™ mice. Control was age matched non-injected C57Bl/6J mice fed a RC diet. Mean ± SD.



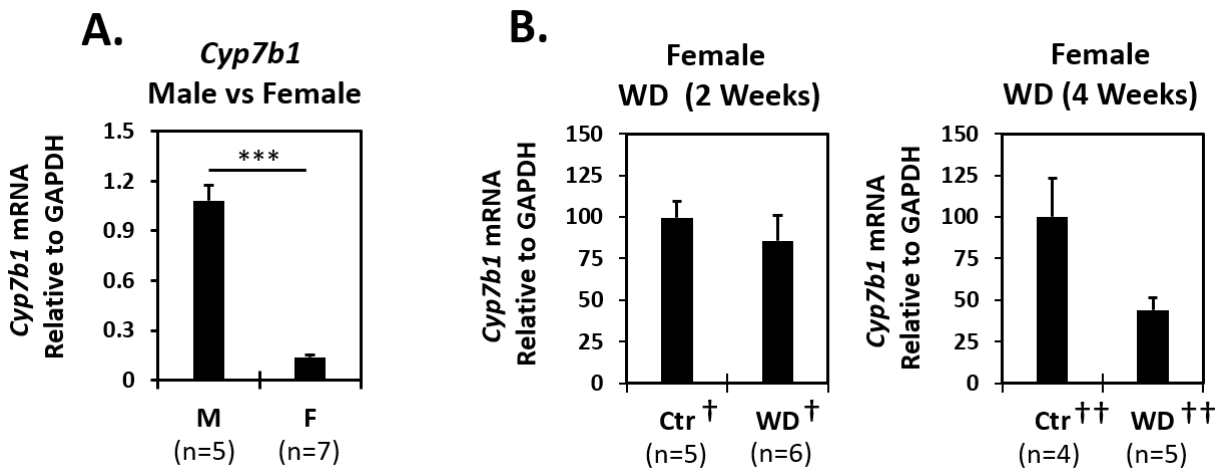
Supplemental Figure S2: Effect of WD feeding on protein levels of hepatic *Cyp7b1, BiP, and CHOP* in B6/129 mice. Male B6/129 mice fed *ad libitum* a WD with 42% of calories from fat and 43% from carbohydrates for 2 weeks to induce fatty liver. All mice were 12 week-old at time of sacrifice. Controls were age-matched B6/129 mice feed with RC. Shown are representative liver protein samples. Band intensity was determined by laser densitometry, and relative levels of Cyp7b1 and BiP expression were normalized by the intensity of GAPDH.



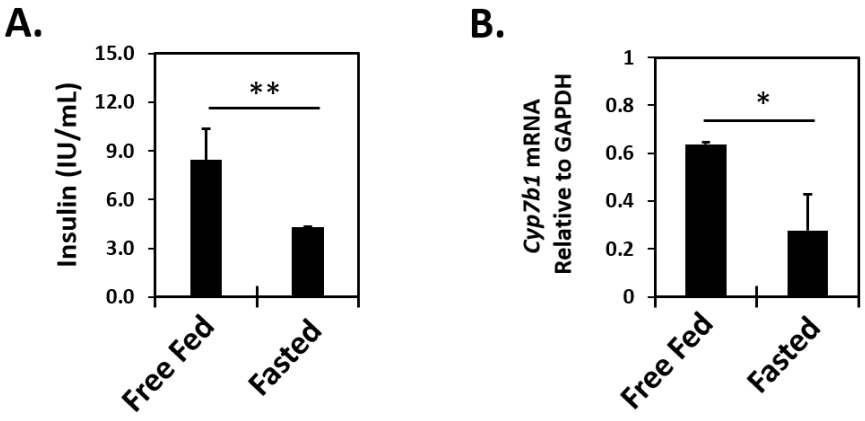
Supplemental Figure S3: High fructose corn syrup (HFCS) and Western diet (WD) suppressed hepatic *Cyp7b1* mRNA in C57Bl/6J mice. Male mice were fed one of indicated four diets for 4 weeks: A) RC: standard rodent chow (4% fat and 0.05% cholesterol); B) WD+HFCS: HFCS equivalent combined to WD at final concentration of 2%. C) WD (21% fat and 0.2% cholesterol with casein protein and sucrose/cornstarch carbohydrate); D) RC+HFCS: HFCS equivalent (water containing 55% fructose was combined to with RC at a final concentration of 2%). n=4 for all groups. Student's t-tests were performed between RC fed mice and the mice fed each diet, and significance indicated by *P<0.01.



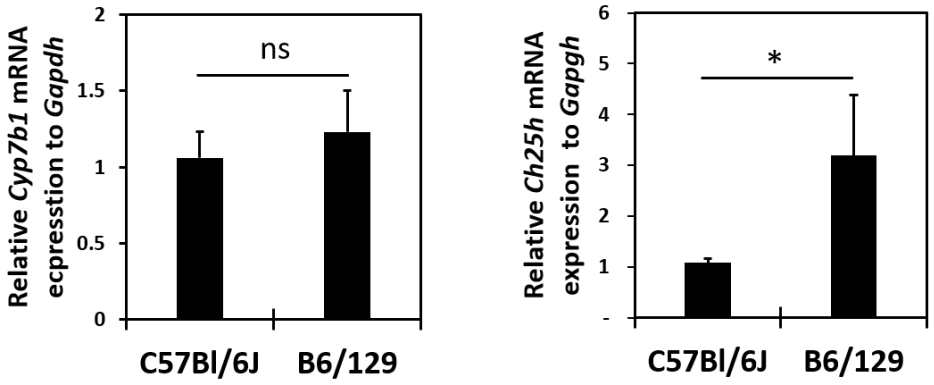
Supplemental Figure S4: Suppression of CYP7B1 mRNA levels in human steatosis (fatty liver) inversely correlates with increases in (25R)-26-Hydroxycholesterol (26-HC). 6 male human liver samples were obtained from NIH sponsored Liver Tissue Cell Distribution System (LTCDS) at University of Minnesota. Three of normal histologic appearance and three with steatosis as determined by LTPDS and by our own review of H&E stained liver slides. *CYP7B1* mRNA expression level was lower in steatosis livers than normal livers. 26-HC level in 2 out of three of the steatosis livers was increased. Greatest increases in 26-HC levels were correlated to greatest decreases in *CYP7B1* mRNA liver samples (*i.e.* patients #5 & #6).



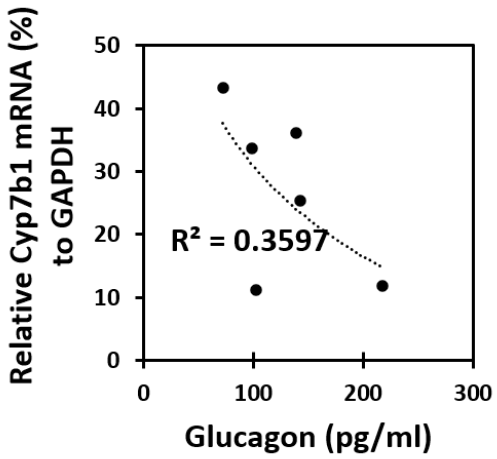
Supplemental Figure S5: Gender difference in hepatic *Cyp7b1* mRNA expression. A) Gender difference in the hepatic *Cyp7b1* mRNA expression levels of 12-week-old B6/129 mice fed a RC diet (n=5 for male mice; n=7 for female mice). B) Effect of WD feeding (2 and 4 weeks) on hepatic *Cyp7b1* mRNA expression levels of female B6/129 mice. Control was age-matched female B6/129 mice fed RC. †12-week-old at sacrifice. ††14-week-old at sacrifice. By Welch's t-tests, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 vs Control.



Supplemental Figure S6: Serum insulin and hepatic *Cyp7b1* mRNA expressions of fasted and non-fasted B6/129 mice. Male mice were fed a WD for two weeks, and were 12-week-old at sacrifice. A) Comparison of serum insulin levels between fasted (n=3) and free WD fed mice (n=3). B) Comparison of hepatic *Cyp7b1* mRNA expressions between fasted (n=3) and free WD fed mice (n=3). *P ≤0.05, **P ≤ 0.01 by Welch's t-test. Refer to Figure 4 for reference to relative *Cyp7b1* mRNA levels in RC fed mice.



Supplemental Figure S7: Comparison of hepatic *Cyp7b1* and *Ch25h* mRNA expression levels between C57Bl/6J and B6/129 mice. All mice were male fed a regular chow diet. At sacrifice, mice were 8-week (C57/Bl/6J) and 11-week (B6/129) old, respectively. Refer to Figure 4 and Figure 6 for relative 25-hydroxycholesyerol and other oxysterol levels of these mice. n=6 for C57Bl/6J; n=4 for B6/129. By Welch's t-test, *P $\leq$ 0.05.



Supplemental Figure S8: Plasma glucagon concentration inversely correlates to hepatic *Cyp7b1* mRNA expression level in STZ injected C57Bl/6J mice fed RC diet. 8-week-old C57Bl/6J male mice fed a RC diet were harvested, and hepatic mRNA and plasma glucagon levels were determined. The mice had free access to food until being sacrificed. The dots in the graphs represents each mouse (n=6).

Supplemental Table S1: Complete list of abbreviations and names of oxysterols and bile acids analyzed in this study.

Abbreviations	Name	MRM (*m/z*)	CE (eV)	R.T. (min)
Oxysterols				
4β-HC	4β-Hydroxycholesterol	613.5 → 124.1	26	19.0
7α-HC	7α-Hydroxycholesterol	613.4 → 124.1	24	17.4
7β-HC	7β-Hydroxycholesterol	613.5 → 367.4	19	17.8
7-KC	7-Oxocholesterol	547.4 → 383.2	23	16.9
20-HC	20(S)-Hydroxycholesterol	508.4 → 124.1	26	14.6
22S-HC	22(S)-Hydroxycholesterol	654.4 → 613.3	15	15.9
22R-HC	22(R)-Hydroxycholesterol	654.4 → 613.3	15	15.4
24-HC	24(S)-Hydroxycholesterol	613.5 → 124.0	33	16.7
25-HC	25-Hydroxycholesterol	508.4 → 124.0	24	13.3
26-HC	(25R)-26-Hydroxycholesterol (27-Hydroxycholesterol)	613.4 → 490.2	20	18.1
7α,24-diHC	7α,24(S)-Dihydroxycholesterol	488.4 → 124.0	30	8.4
7α,25-diHC	7α,25-Dihydroxycholesterol	629.5 → 124.0	26	6.5
7α,26-diHC	(25R)-7α,26-Dihydroxycholesterol	488.4 → 124.0	38	9.4
Cholic acid (3α,7α,12α-Trihydroxy-5β-cholanoic acid) related BAs				
CA	Cholic acid	407.3 → 343.3	33	41.8
GCA	Glycocholic acid	464.5 → 74.2	52	28.5
TCA	Taurocholic acid	514.3 → 124.0	56	30.3
CA-3S	Cholic acid 3-sulfate	487.3 → 97.0	46	31.6
CA-7S	Cholic acid 7-sulfate	487.3 → 97.0	46	30.6
CA-3G	Cholic acid-3-glucuronide	583.3 → 113.1	45	22.5
GCA-3S	Glycocholic acid 3-sulfate	544.4 → 464.1	25	14.5
TCA-3S	Taurocholic acid 3-sulfate	594.4 → 514.0	46	15.1
TCA-7S	Taurocholic acid 7-sulfate	594.3 → 514.1	25	14.4
norCA	Nor-cholic acid (24-Nor-3α,7α,12α-trihydroxy-5β-cholan-23-oic acid)	393.3 → 329.2	34	34.2
G-norCA	Glyco norcholic acid	450.3 → 74.1	37	17.3
T-norCA	Tauro norcholic acid	500.3 → 124.1	52	18.7

isoCA	Isocholic acid (3β,7α,12α-Trihydroxy-5β-cholan-24-oic acid)	407.3 → 343.3	33	33.7
7-epiCA	7-Epicholic acid (3α,7β,12α-Trihydroxy-5β-cholan-24-oic acid)	407.3 → 343.2	34	26.9
Chenodeoxycholic acid (3α,7α-Dihydroxy-5β-cholanoic acid) related BAs				
CDCA	Chenodeoxycholic acid	437.3 → 391.2	23	51.1
GCDCA	Glycochenodeoxycolic acid	448.3 → 74.2	34	38.2
TCDCA	Taurochenodeoxycholic acid	498.3 → 80.0	76	39.2
CDCA-3S	Chenodeoxycholic acid 3-sulfate	471.3 → 97.0	56	40.5
GCDCA-3S	Glycochenodeoxycolic acid 3-sulfate	528.4 → 448.1	31	22.3
TCDCA-3S	Taurochenodeoxycholic acid 3-sulfate	578.4 → 498.1	25	23.1
CDCA-3G	Chenodeoxycholic acid 3-glucuronide	567.3 → 391.1	41	35.1
Ursodeoxycholic acid (3α,7β-Dihydroxy-5β-cholanoic acid) related BAs				
UDCA	Ursodeoxycholic acid	437.3 → 391.2	23	44.0
GUDCA	Glycourosodeoxycholic acid	448.3 → 74.2	40	26.6
TUDCA	Tauroursodeoxycholic acid	498.3 → 80.0	71	28.7
UDCA-3S	Ursodeoxycholic acid 3-sulfate	471.3 → 97.0	46	31.4
UDCA-3G	Ursodeoxycholic acid 3-glucuronide	567.3 → 391.1	39	22.8
GUDCA-3S	Glycourosodeoxycholic acid 3-sulfate	528.4 → 448.3	32	12.5
TUDCA-3S	Tauroursodeoxycholic acid 3-sulfate	578.4 → 498.1	25	13.1
UDCA-7GlcNAc	Urosodeoxycholic acid 7-*N*-acetylglucosaminide	594.4 → 391.2	38	32.8
GUDCA-7GlcNAc	Glycoursodeoxychlic acid 7-*N*-acetylglucosaminide	651.4 → 100.1	55	17.0
TUDCA-7GlcNAc	Taurourosodeoxycholic acid 7-*N*-acetylglucosaminide	701.4 → 480.2	62	18.7
UDCA-3S-7GlcNAc	Ursodeoxycholic acid 3-sulfate,7-*N*-acetylglucosaminidde	674.4 → 96.8	69	20.3
GUDCA-3S-7GlcNAc	Glycourosodeoxycholic acid 3-sulfate,7-*N*-acetylglucosaminide	365.2 → 74.0	26	9.4
TUDCA-3S-7GlcNAc	Taurourosodeoxycholic acid 3-sulfate, 7-*N*-acetylglucosaminide	390.2 → 97.0	56	9.8
Deoxycholic acid (3α,12α-Dihydroxy-5β-cholanoic acid) related BAs				
DCA	Deoxycholic acid	391.3 → 345.3	34	51.8
GDCA	Glycodeoxycholic acid	448.3 → 74.1	38	39.7
TDCA	Taurodeoxycholic acid	498.3 → 124.0	52	40.6

DCA-3S	Deoxycholic acid 3-sulfate	471.3 → 97.1	65	41.0
GDCA-3S	Glycodeoxycholic acid	528.4 → 448.0	30	23.8
TDCA-3S	Taurodeoxycholic acid 3-sulfate	578.4 → 498.1	30	24.6
DCA-3G	Deoxycholic acid 3-glucuronide	567.3 → 391.3	40	34.1
Lithocholic acid (3α-hydroxy-5β-cholanoic acid) related BAs				
LCA	Lithocholic acid	375.3 → 375.3*	10	61.2
GLCA	Glycolothocholic acid	432.3 → 74.1	40	46.6
TLCA	Taurolithocholic acid	482.3 → 80.0	76	47.4
LCA-3S	Lithocholic acid 3-sulfate	455.3 → 97.1	63	47.5
LCA-3G	Lithocholic acid 3-glucuronide	551.3 → 375.3	39	41.2
GLCA-3S	Glycolithocholic acid 3-sulfate	512.4 → 432.0	30	33.3
TLCA-3S	Taurolithocholic acid 3-sulfate	562.4 → 482.2	23	33.8
isoLCA	Isolithocholic acid (3β-Hydroxy-5β-cholan-24-oic acid)	375.3 → 375.3*	30	59.8
alloLCA	Allolithocholic acid (3β-Hydroxy-5α-cholan-24-oic acid)	375.4 → 375.4*	10	61.8
iso-alloLCA	Isoallolithocholic acid (3β-Hydroxy-5α-cholan-24-oic acid)	375.3 → 375.3*	10	58.9
Hyo- and muricholic acid (3α,6ξ,7ξ-Trihydroxy-5β-cholanoic acid) related BAs				
HCA	Hyocholic acid (3α,6α,7α-Trihydroxy-5β-cholan-24-oic acid)	407.3 → 407.3*	10	40.2
GHCA	Glycohyodeoxycholic acid	464.3 → 74.1	38	22.6
THCA	Taurohyocholic acid	514.3 → 124.1	54	24.3
HCA-3G	Hyocholic acid 3-glucuronide	583.3 → 407.2	44	24.2
αMCA	α-Muricholic acid (3α,6β,7α-Trihydroxy-5β-cholan-24-oic acid)	453.3 → 407.2	21	35.9
αMCA-3S	α-Muricholic acid 3-sulfate	487.3 → 97.0	45	24.8
T-αMCA	Tauro α-muricholic acid	514.4 → 80.1	73	18.3
T-αMCA-3S	Tauro α-muricholic acid 3-sulfate	594.4 → 514.1	35	10.3
βMCA	β-Murocholic acid (3α,6β,7β-Trihydroxy-5β-cholan-24-oic acid)	453.3 → 407.3	26	37.5
T-βMCA	Tauro β-muricholic acid 3-sulfate	487.3 → 97.0	52	25.8
βMCA-3S	β-Muricholic acid 3-sulfate	514.4 → 124.2	53	19.0
T-βMCA-3S	Tauro β-muricholic acid 3-sulfate	594.4 → 514.3	35	10.3
ωMCA	ω-Muricholic acid (3α,6α,7β-Trihydroxy-5β-cholan-24-oic acid)	453.3 → 407.2	20	34.3

T-ωMCA	Tauro ω-muricholic acid	514.4 → 80.0	71	17.2
Δ^{22}-βMCA	3α,6β,7β-Trihydroxychol-5,22-diene-24-oic acid	405.3 → 323.2	32	34.9
T-Δ^{22}-βMCA	Tauro 3α,6β,7β-trihydroxychol-5,22-diene-24-oic acid	512.4 → 80.1	60	20.0
Hyo- and Muri- deoxycholic (3α,6ξ-Dihydroxy-5β-cholanoic acid) related BAs				
HDCA	Hyodeoxycholic acid (3α,6α-Dihydroxy-5β-cholan-24-oic acid)	391.3 → 391.3*	10	44.6
MDCA	Murideocycholic acid (3α,6β-Dihydroxy-5β-cholan-24-oic acid)	437.3 → 391.3	22	41.8
GHDCA	Glycohyodeoxycholic acid	448.3 → 74.1	40	27.6
THDCA	Taurohyodeoxycholic acid	498.3 → 80.0	72	29.8
HDCA-3G	Hyodeoxycholic acid 3-glucuronide	567.3 → 391.2	41	30.2
Tetrahydroxy bile acids				
CA-1β-ol	1β-Hydroxycholic acid (1β,3α,7α,12α-Tetrahydroxy-5β-cholan-24-oic acid)	423.3 → 263.0	35	17.6
GCA-1β-ol	Glyco 1β-hydroxycholic acid	480.3 → 74.1	40	9.3
TCA-1β-ol	Tauro 1β-hydroxycholic acid	530.4 → 124.1	53	9.8
CA-6α-ol	6α-Hydroxycholic acid (3α,6α,7α,12α-Tetrahydroxy-5β-cholan-24-oic acid)	423.3 → 313.3	35	25.0
GCA-6α-ol	Glyco 6α-hydroxycholic acid	480.3 → 74.1	39	12.9
TCA-6α-ol	Tauro 6α-hydroxycholic acid	530.4 → 124.1	55	13.9
CDCA-1β-ol	1β-Hydroxychenodeoxycholic acid (1β,3α,7α-Trihydroxy-5β-cholan-24-oic acid)	407.2 → 389.1	33	29.4
BA with 3β-hydroxy-Δ^5 structure				
isoLCA-Δ^5	3β-Hydroxy-5-cholen-24-oic acid	373.3 → 373.3*	30	57.3
G-isoLCA-Δ^5	Glyco 3β-hydroxy-5-cholen-24-oic acid	430.3 → 73.9	31	39.1
T-isoLCA-Δ^5	Tauro 3β-hydroxy-5-cholen-24-oic acid	480.3 → 80.0	63	40.3
isoLCA-Δ^5-3S	3β-Hydroxy-5-cholen-24-oic acid 3-sulfate	453.3 → 97.0	40	43.6
G-isoLCA-Δ^5-3S	Glyco 3β-hydroxy-5-cholen-24-oic acid 3-sulfate	510.4 → 97.1	50	23.5
T-isoLCA-Δ^5-3S	Tauro 3β-hydroxy-5-cholen-24-oic acid 3-sulfate	279.7 → 97.0	35	24.5
isoCDCA-Δ^5	3β,7α-Dihydroxy-5-cholen-24-oic acid	389.3 → 389.3*	10	41.7
G-isoCDCA-Δ^5	Glyco 3β,7α-dihydroxy-5-cholen-24-oic acid	446.3 → 74.1	32	23.8
T-isoCDCA-Δ^5	Tauro 3β,7α-dihydroxy-5-cholen-24-oic acid	496.3 → 80.0	75	25.8
isoCDCA-Δ^5-3S	3β,7α-Dihydroxy-5-cholen-24-oic acid 3-sulfate	469.3 → 97.0	35	36.4

G-isoCDCA-Δ^5-3S	Glyco 3β,7α-dihydroxy-5-cholen-24-oic acid 3-sulfate	526.4 → 96.8	33	16.2
T-isoCDCA-Δ^5-3S	Tauro 3β,7α-dihydroxy-5-cholen-24-oic acid 3-sulfate	287.7 → 97.0	24	17.0
isoUDCA-Δ^5	3β,7β-Dihydroxy-5-cholen-24-oic acid	389.3 → 389.3*	10	43.2
isoUDCA-Δ^5-3S	3β-Sulfoxy,7β-hydroxy-5-cholen-24-oic acid	469.3 → 96.9	35	29.3
isoUDCA-Δ^5-3S-7-GlcNAc	3β-Sulfoxy,7β-(*N*-acetyglucosaminyl)-5-cholen-24-oic acid	672.4 → 97.1	45	19.8
G-isoUDCA-Δ^5-3S-7-GlcNAc	Glyco 3β-sulfoxy,7β-(*N*-acetyglucosaminyl)-5-cholen-24-oic acid	364.2 → 97.0	33	8.7
T-isoUDCA-Δ^5-3S-7-GlcNAc	Tauro 3β-sulfoxy,7β-(*N*-acetyglucosaminyl)-5-cholen-24-oic acid	389.2 → 97.0	31	9.2
isoDCA-Δ^5	3β,12α-Dihydroxy-5-cholen-24-oic acid	389.3 → 287.2	32	42.5
isoCA-Δ^5	3β,7α,12α-Trihydroxy-5-cholen-24-oic acid	405.3 → 289.2	33	29.0
isoCA-Δ^5-3S	3β,7α,12α-Trihydroxy-5-cholen-24-oic acid 3-sulfate	485.3 → 97.0	66	23.4
G-isoCA-Δ^5	Glyco 3β,7α,12α-trihydroxy-5-cholen-24-oic acid	462.4 → 74.1	43	14.2
T-isoCA-Δ^5	Tauro 3β,7α,12α-trihydroxy-5-cholen-24-oic acid	512.4 → 124.0	47	15.3
G-isoCA-Δ^5-3S	Glyco 3β,7α,12α-trihydroxy-5-cholen-24-oic acid 3-sulfate	542.4 → 97.1	67	10.7
T-isoCA-Δ^5-3S	Tauro 3β,7α,12α-trihydroxy-5-cholen-24-oic acid 3-sulfate	295.7 → 97.0	25	11.2
BAs with Δ^4-3-one structure				
DhCA-Δ^4	7α,12α-dihydroxy-3-oxo-4-cholen-24-oic acid	403.3 → 123.2	40	31.2
G-DhCA-Δ^4	Glyco 7α,12α-dihydroxy-3-oxo-4-cholen-24-oic acid	460.3 → 74.1	38	16.3
T-DhCA-Δ^4	Tauro 7α,12α-dihydroxy-3-oxo-4-cholen-24-oic acid	510.3 → 358.2	52	17.5
DhCDCA-Δ^4	7α-Hydroxy-3-oxo-4-cholen-24-oic acid	387.3 → 369.3	28	45.3
G-DhCDCA-Δ^4	Glyco 7α-hydroxy-3-oxo-4-cholen-24-oic acid	444.3 → 74.1	35	27.9
T-DhCDCA-Δ^4	Tauro 7α-hydroxy-3-oxo-4-cholen-24-oic acid	494.3 → 342.1	51	30.1
DhDCA-$\Delta^{4,6}$	12α-Hydroxy-3-oxo-4,6-choldien-24-oic acid	385.3 → 341.2	26	42.5
DhLCA-$\Delta^{4,6}$	3-Oxo-4,6-choldien-24-oic acid	369.3 → 325.3	29	56.2
DhLCA-Δ^4-3-one	3-Oxo-4-cholen-24-oic acid	371.3 → 123.1	32	59.1
Keto Bile Acids				
DhLCA	Dehydrolithocholic acid (3-Oxo-5β-cholan-24-oic acid)	373.3 → 373.3*	10	62.0
7-OxoDCA	3α,12α-Dihydroxy-7-oxo-5β-cholan-24-oic acid	405.3 → 289.2	35	35.5

7-OxoLCA	3α-Hydroxy-7-oxo-5β-cholan-24-oic acid	435.3 → 389.2	21	47.2
12-OxoCDCA	3α,7α-Dihydroxy-12-oxo-5β-cholan-24-oic acid	447.3 → 405.3	27	38.6
12-OxoLCA	3α-Hydroxy-12-oxo-5β-cholanoic acid	389.3 → 371.3	30	47.8
7-Oxo-isoLCA-Δ^5	3β-Hydroxy-7-oxo-5-cholen-24-oic acid)	387.2 → 287.4	32	45.2
BA with Cholestan or Cholesten structures				
C27-DHCA	3α,7α-Dihydroxy-5β-cholestanoic acid	433.3 → 415.3	40	61.3
C27-THCA	3α,7α,12α-Trihydroxy-5β-cholestanoic acid	449.4 → 431.1	40	53.7
3β-HCA	(25R)-3β-Hydroxy-5-cholest-26-oic acid	415.4 → 415.4*	15	64.8
C27-Δ^5-3β,7α-diol	(25R)-3β,7α-Dihydroxy-5-cholest-26-oic acid	431.3 → 431.3*	15	56.3
C27-Δ^5-3β,24S-diol	(25R)-3β,7α,24(S)-Trihydroxy-5-cholest-26-oic acid	431.3 → 73.0	25	52.6
C27-Δ^4-3-one	(25R)-3-Oxocholest-4-en-26-oic acid	413.4 → 413.4*	15	65.2
C27-7α-OH-Δ^4-3-one	(25R)-7α-Hydroxy-3-oxocholest-4-en-26-oic acid	429.4 → 411.3	32	58.1

† Total bile acid (TBA) in this manuscript means sum of above bile acid concentrations.

* Due to lack of fragmentation, the same mass was monitored for both parent and daughter ions.

Supplemental Table S2: Primers used for mRNA analysis

	Forward	Reverse
Cyp7a1	GAG AAG GCA AAC GGG TGA AC	GGA TTG GCA CCA AAT TGC AGA
Cyp7b1	CCC TCT TTC CTC CAC TCA TA	GAA CCG ATC GAA CCT AAA TTC CT
Cyp8b1	GCC TTC AAG TAT GAT CGG TTC CT	GAT CTT CTT GCC CGA CTT GTA GA
Cyp27a1	CTA TGT GCT GCA CTT GCC C	GGG CAC TAG CCA GAT TCA CA
Ch25h	TGC TAC AAC GGT TCG GAG C	AGA AGC CCA CGT AAG TGA TGA T
Ccl24	TGC CCT AGC ATT TTG GCG A	ACC TCC TGT AGT TTG GGA TGC
Chi3l3	GAA TGA AGG AGC CAC TGA GGT CTG	TTG AGC CAC TGA GCC TTC AAC TT
Rorc	AAA GAA GAC CCA CAC CTC ACA AAT	GGT GAT AAC CCC GTA GTG GA
StarD1	ACG AGG GCT AGG GCC AAA T	CAG ACC CCT TAT GCC TCC C
Gata3	GCT CCT TGC TAC TCA GGT GAT	GGA GGG AGA GAG GAA TCC GA
Arg1	ACA AGA CAG GGC TCC TTT CAG	TGG TTA CCC TCC CGT TGA GT
Nos2	GGG ACT GAG CTG TTA GAG ACA	TGC ACT TCT GCT CCA AAT CCA
Tbp	TTT GGC TAG GTT TCT GCG GT	TGA AAT AGT GAT GCT GGG CA CT

Supplemental Table S3: Body weight, liver Lipids and serum parameters of timed WD fed female B6/129 mice

	Control (RC)	Length of WD feeding	
		2 Weeks	4 Weeks
Body Weight (gm)	27.3 ± 1.83	34.0 ± 1.96 *	42.8 ± 1.95 ***
Liver triglyceride (mg/g)	29.1 ± 4.90	188 ± 37.7 **	322 ± 70.5 **
Liver total cholesterol (mg/g)	3.69 ± 0.616	11.3 ± 0.638 ***	23.7 ± 1.33 ***
Serum triglyceride (mg/dL)	62.9 ± 3.85	61.0 ± 6.42	63.0 ± 3.34
Serum total cholesterol (mg/dL)	131 ± 7.32	188 ± 9.15 ***	252 ± 15.6 ***
ALT (U/mL)	26.9 ± 2.17	48.7 ± 4.97 **	83.7 ± 16.2 *
AST (U/mL)	79.0 ± 5.73	100 ± 4.81 *	164 ± 33.7 *
Serum glucose (mg/dL)	288 ± 20.1	283 ± 5.74	289 ± 15.1
Serum insulin (ng/mL)	0.438 ± 0.0967	0.430 ± 0.128	1.07 ± 0.240 *
HOMA-IR	0.317 ± 0.0772	0.306 ± 0.0945	0.767 ± 0.183

† B6/129 mice fed WD for two (n=6) and four (n=6) weeks. They were 12-week-old and 14-week-old at sacrifice, respectively; Control was 12-week-old B6/129 mice fed a RC (n=7).

††Values are expressed as mean ±SE

††† By Welch's t-test, *P<0.05, **P<0.01, ***p<0.001 vs Control group (RC).