

SUPPLEMENTAL MATERIAL

Cholestatic pregnancy programmes metabolic disease in the offspring

Supplemental Tables

Supplemental Table 1: Morphometric features of mouse cholestatic pregnancy

Maternal diet	Litter size	Fetal body weight (g)
NC	7.2 ± 1.4	0.83 ± 0.2
CA	6.5 ± 1.2	0.76 ± 0.2

NC: normal chow, CA: cholic acid; n=17 animals per group; ± values represent standard error of the mean (SEM)

Supplemental Table 2: Transcriptomic profile in livers of western diet (WD)-fed females from cholestatic mothers as predicted with Ingenuity Pathway Analysis of microarray data.

Gene ID	Gene name	fold-change	Pathway
BC006872	Cel	169	Fatty acid synthesis and cleavage
AK133952	Myc	169	Fatty acid synthesis and cleavage
AK171155	Dpep2	66	Fatty acid synthesis and cleavage
BC145908	Pla2g1b	42	Fatty acid synthesis and cleavage
AK152189	Il6	2.5	Fatty acid synthesis and cleavage
BC027742	Clec7a	2.4	Fatty acid synthesis and cleavage
AF359558	Il4	1.9	Fatty acid synthesis and cleavage
AK079235	Ggt1	1.9	Fatty acid synthesis and cleavage
BC094923	Pnliprp1	1.6	Fatty acid synthesis and cleavage
BC062902	Gpc1	1.5	Fatty acid synthesis and cleavage
BC145867	Ccl2	1.5	Fatty acid synthesis and cleavage
U49110	Lepr	-1.5	Fatty acid synthesis and cleavage
AB005909	Dmbt1	386	Inflammation
BC119057	Mmp7	9	Inflammation
AK171435	Mmp12	3.6	Inflammation
AK007645	Gal3st1	3.6	Inflammation
BC003780	Chi3l1	3	Inflammation
BC132069	Lcn2	2.7	Inflammation
BC061154	Chi3l3	2.4	Inflammation
BC061126	Ccl7	2.3	Inflammation
BC141556	Il8ra	2.3	Inflammation
BC002063	Lgals1	2.2	Inflammation
AF359558	Il4	2	Inflammation
BC006783	Ctgf	1.9	Inflammation
BC003480	Capg	1.8	Inflammation
BC010726	Pla2g7	1.6	Inflammation
BC033485	Trem2	1.6	Inflammation
X16834	Lgals3	1.6	Inflammation
BC132022	Raet1b	1.6	Inflammation
BC146516	Ear10	1.5	Inflammation
AK152189	Il6	1.5	Inflammation
BC145867	Ccl2	1.5	Inflammation

Fold-change of liver gene expression of WD-fed female offspring from cholestatic mothers compared to hepatic gene expression of WD-fed females from normal mothers. Significant changes ($p \leq 0.05$) with a fold-change ≥ 1.5 are shown. Gene expression profile is consistent with increased fatty acid synthesis and cleavage and inflammation in the WD-fed female from CA-fed mothers.

Supplemental Table 3: Inflammation-related genes that significantly change in white adipose tissue (WAT) of western diet (WD)-fed females from cholestatic mothers as predicted with Ingenuity Pathway Analysis

Gene ID	Gene name	fold-change
AK132915	Itgad	15
BC054091	Serpine1	4.7
BC055885	Saa3	3.5
BC109158	Selp	3.2
BC094009	Krt8	3.1
BC006783	Ctgf	3.1
BC012650	Cldn3	3
AK137169	Itga1	2.8
BC054530	Podxl	2.6
BC096586	Prlr	2.6
BC012690	Vtn	2.5
AK133483	Gnaz	2.4
AK143562	Lbp	2.3
BC020530	Kdr	2.3
BC007125	Aqp1	2.2
BC019460	Esam	2.2
AK133933	Sept4	2.1
BC062378	Nos2	2.1
BC050824	Tek	2.1
AK159653	Itga6	2
AK169431	Pecam1	2
AK028157	Mrc2	2
BC020532	Rapgef3	2
BC053430	Pdgfb	2
BC036175	Agtr1a	2
BC065077	Lhx6	2
AK172072	Sh2d3c	1.9
BC016505	Ephb4	1.9
BC004656	Scarb1	1.9
AF114266	Tgm2	1.9
AK145864	Lifr	1.9
BC156151	Myo10	1.9
AF469622	Arap3	1.9
BC022107	Cdh2	1.8
BC075716	Kit	1.8
BC021655	Akr1b3	1.8
AK140530	Plcl1	1.7
S75867	Itpr1	1.7
BC021876	F11R	1.7
BC156169	Dock4	1.7
U28151	Bcar1	1.7
BC060129	Nrp1	1.5
BC030478	Mylk	1.5

AK202858	Pkd1	1.5
BC005452	Bgn	1.5
BC132544	Rgs18	-2.5
AF033112	Siva1	-1.6
BC006763	Cbfb	-1.5

Fold-change of WAT gene expression of WD-fed female offspring from cholestatic mothers compared to WAT gene expression of WD-fed females from normal mothers. Significant changes ($p \leq 0.05$) with a fold-change ≥ 1.5 are shown. Gene expression profile is consistent with increased inflammation in the WD-fed female from cholic acid (CA)-fed mothers.

Supplemental Table 4: Fetal metabolic pathways in mouse pregnancy

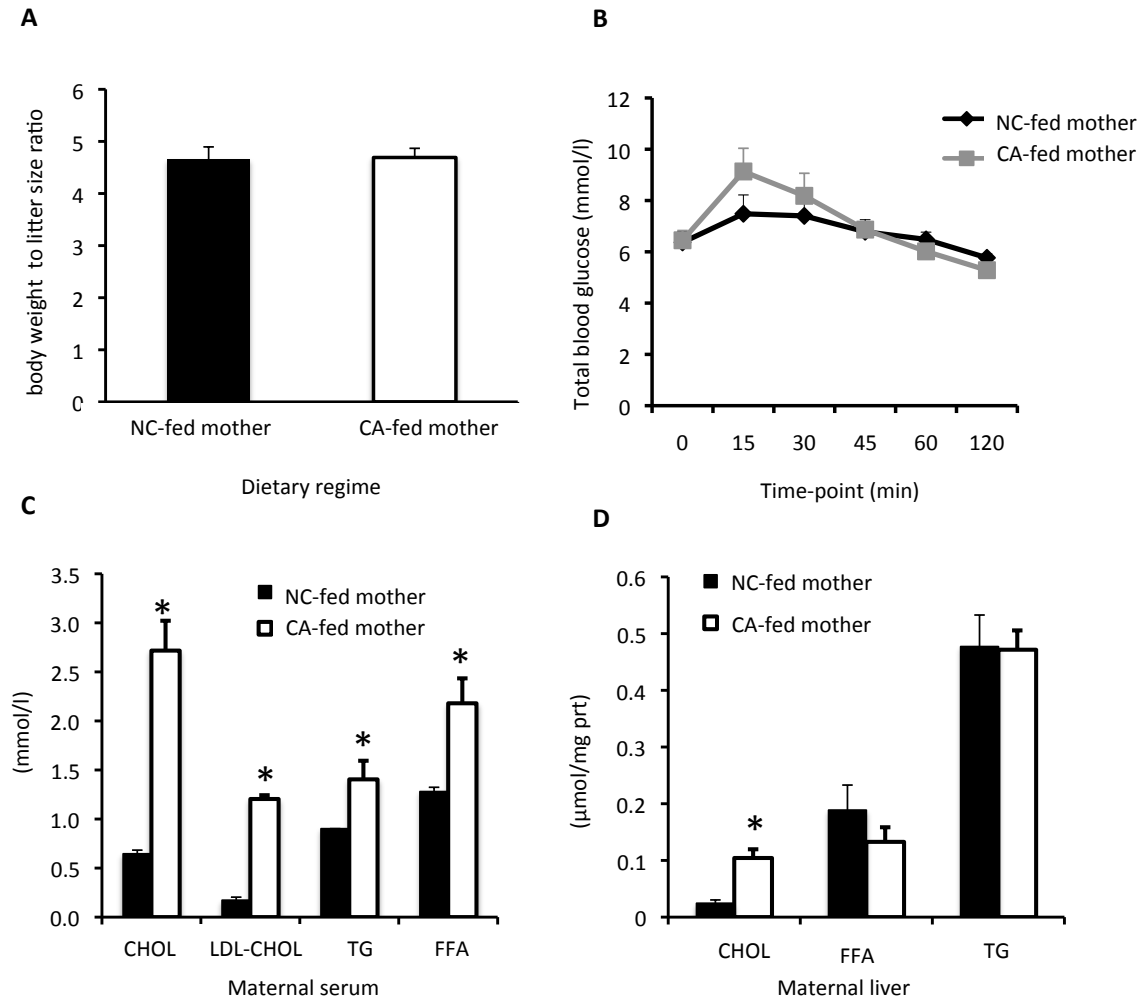
	from NC-fed mother	from CA-fed mother
Serum total bile acids ($\mu\text{mol/l}$)	18 ± 10	$72 \pm 20^*$
Bile acid homeostasis pathways (mRNA; relative expression to cyclophilin)		
Bsep	1.15 ± 0.3	$5.20 \pm 0.8^*$
Cyp7a1	0.5 ± 0.3	$0.3 \pm 0.08^*$
Shp	1.1 ± 0.2	$12.6 \pm 1.1^*$
Mrp3	1.2 ± 0.2	$3.7 \pm 0.5^*$
Mrp4	1.9 ± 0.4	$6.8 \pm 1.1^*$
Sult2a1	1.0 ± 0.1	$4.7 \pm 0.7^*$
Hepatic lipid profiles ($\mu\text{mol/mg prt}$)		
Cholesterol	0.09 ± 0.004	$0.13 \pm 0.006^*$
TG	0.29 ± 0.01	$0.36 \pm 0.02^*$
FFA	0.13 ± 0.01	0.18 ± 0.03
Cholesterol biosynthesis pathway (mRNA; relative expression to cyclophilin)		
Dhcr-7	0.7 ± 0.09	$1.7 \pm 0.1^*$
Hmgcr	1.4 ± 0.3	$7.4 \pm 0.8^*$
Srebp2	1.4 ± 0.3	$5.8 \pm 0.9^*$
Fatty acid/triglyceride synthesis pathway (mRNA; relative expression to cyclophilin)		
Fas	1.1 ± 0.1	$3.5 \pm 0.4^*$
Lxr- α	1.3 ± 0.1	$5.7 \pm 0.5^*$
Scd-2	1.0 ± 0.2	$2.6 \pm 0.6^*$
Srebp1c	1.3 ± 0.16	$3.1 \pm 0.2^*$

The fetuses of cholestatic mothers had increased serum bile acid levels as well as induced bile acid excretory pathways consistent with a cholestatic phenotype (1). Moreover, *de novo* hepatic cholesterol and fatty acid biosynthetic (2) pathways were also increased in fetuses of CA-fed mothers. NC: normal chow, CA: cholic acid; TG: triglycerides, FFA: free fatty acids; n=6 animals per group, * $p \leq 0.05$; \pm values represent standard error of the mean (SEM).

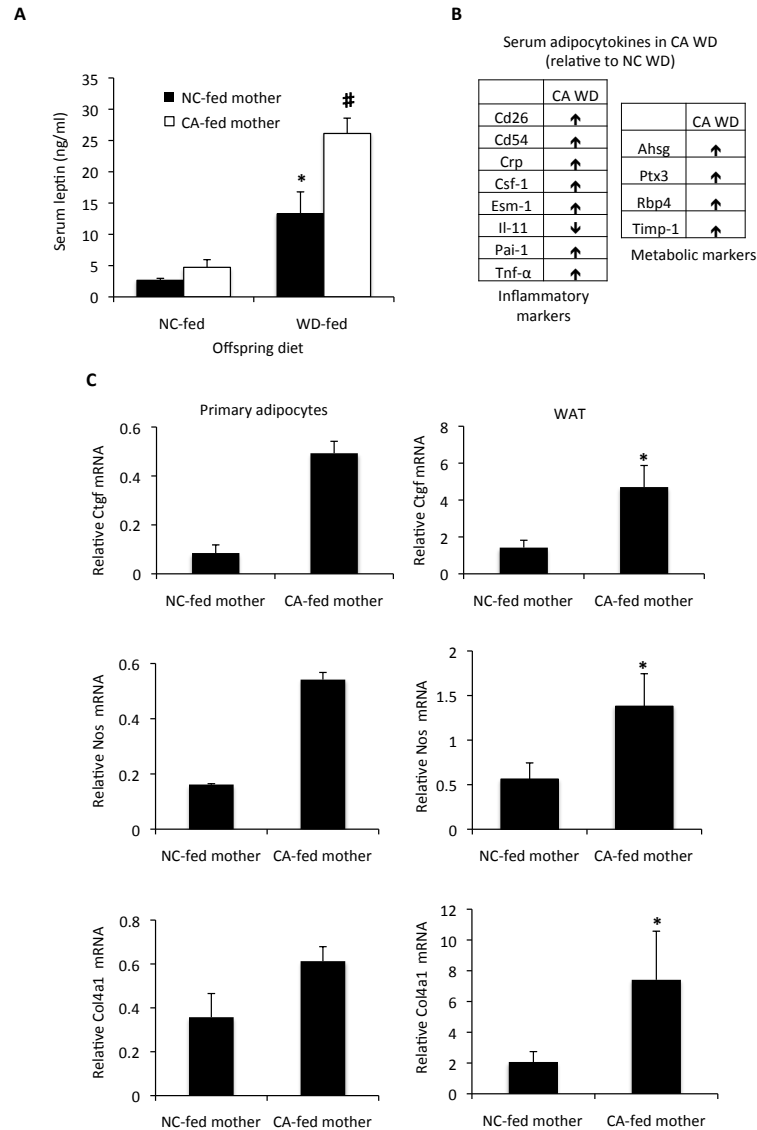
Supplemental Table 5: Primer sequences

Gene name	Forward primer	Reverse primer
Lipid storage		
Acat-2	ATATGAGCAAGGCTCCTCAC	GGTAGTTGTGAAAGGCATCTG
Adrp (Plin2)	TGGCAGCAGCAGTAGTGGAT	AGCTCACCAAGGGCAGGTT
Inflammation		
Col4a1	GCCCTTCATTAGCAGCTTTC	GCACTGCGGAATCTGAATG
Ctgf	CAGAACGCACACTGAGGTGA	TGCTATAATTGCCCTCCCCG
Nos	TCAACCTCCTGACTGAAGCA	CCAAGCCATCATTGGGAGTAGA
Bile acid homeostasis pathways		
Bsep	AAGCTACATCTGCCTTAGACACAGAA	CAATACAGGTCCGACCCTCTCT
Cyp7a1	AGCAACTAAACAACCTGCCAGTACTA	GTCCGGATATTCAAGGATGCA
Shp	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Mrp3	GCAGCAGAACCAAGCATCAAG	GACCGCATCCTCACCTGG
Mrp4	GGTTGGAATTGTGGGCAGAA	TCGTCCGTGTGCTCATTGAA
Sult2a1	GAAGGCATACCTTTTCCTGCCA	GTAACCAGACACAAGAATATCT
Cholesterol biosynthesis pathway		
Dhcr-7	GCCAAGACACCACCTGTGACAG	TGGACGCCTCCACATAACC
Hmgcr	TTGGCACCATGTCAGGCGTCC	AGCGACACACAGGCCGGGAA
Srebp2	CCTAGACCTCGCCAAAGGTG	CAGGCTGTAGCGGATCACAT
Fatty acid/triglyceride synthesis pathway		
Fas	CCCAGAGGCTTGTGCTGACT	CGAATGTGCTTGGCTTGGT
Lxr- α	AGGAGTGTGCTGCTCGCAA	CTCTTCTTGCCGCTTCAGTTT
Scd-2	AGCGGGCTGCAGAAACTTAG	GGCTGAGTAAGCGCCAGAGAT
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGAAGTCACTGT
Genes used for normalization		
Ap2	ACACCGAGATTTCTTCAAACCTG	CCATCTAGGGTTATGATGCTCTTCA
Cyclophilin	TGGAGAGCACCAAGACAGACA	TGCCGGAGTCGACAATGAT

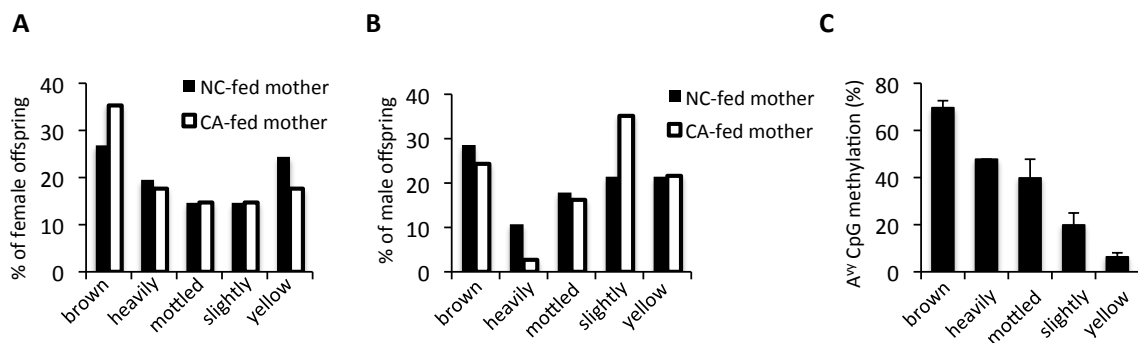
Supplemental Figures



Supplemental Figure 1: Mouse cholestatic pregnancy results in maternal dyslipidemia without affecting body weight and glucose tolerance. A) Body weight to litter size ratio in normal chow (NC)-fed and cholic acid (CA)-fed mice on day 18 of pregnancy, n=6 animals per group. B) Glucose tolerance test in NC-fed and CA-fed mice on day 18 of pregnancy after a 6-hour fast. Following an intraperitoneal injection of glucose (1g/kg body weight), total blood glucose was measured at the indicated time-points, n=6 animals per group. C, D) Lipid levels in NC-fed and CA-fed mice on day 18 of pregnancy in maternal serum (C) and liver (D). Significance of data was established by Student *t*-testing, n=6 animals per group, * $p \leq 0.05$. Chol: cholesterol, LDL-chol: low-density lipoprotein-cholesterol, TG: triglycerides, FFA: free fatty acids.



Supplemental Figure 2: Effects of cholestatic pregnancy on adipose tissue function. A) Serum leptin levels in female offspring after a 4-hour fast, $n=6$ animals per group, $*p \leq 0.05$ for differences in offspring fed a different diet, $^{\#}p \leq 0.05$ for differences in offspring exposed to a different intrauterine environment. B) Markers of inflammation and metabolic pathways in serum of WD-fed female offspring of CA-fed mothers (compared to WD-fed offspring of NC-fed mothers). \uparrow : increase, \downarrow decrease. C) Ctgf, Nos and Col4a1 mRNA expression relative to cyclophilin in differentiated primary adipocytes (left panel) isolated from WD-fed female offspring of NC-fed or CA-fed mothers. Differentiation levels were normalized to the aP2 differentiation marker. Error bars represent SD of duplicate wells of the same group of animals. WAT tissue of WD-fed offspring (right panel) was also assessed for target gene mRNA levels. $n=4$ animals per group, $*p < 0.05$, error bars represent \pm standard error of the mean (SEM). WAT: white adipose tissue, NC: normal chow, CA: cholic acid, WD: western diet, NC WD: WD-fed offspring from NC-fed mothers, CA WD: WD-fed offspring from CA-fed mothers.



Supplemental Figure 4: Cholestatic pregnancy alters the epigenome of 3-week old offspring. A) Coat colour phenotype of female offspring of normal chow (NC)- or cholic acid (CA)-fed mothers. B) Coat colour phenotype of male offspring of NC- or CA-fed mothers. C) Percentage methylation of CpG sites of the *A^y* cryptic promoter in at least 4 samples per phenotype.

References

1. Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., McKee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., et al. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 6:517-526.
2. Goldstein, J.L., DeBose-Boyd, R.A., and Brown, M.S. 2006. Protein sensors for membrane sterols. *Cell* 124:35-46.