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Malprogramming of hepatic lipid metabolism due to excessive early cholesterol exposure in adult progeny

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Abstract

Scope—The programming of hepatic lipid dysfunction in response to early cholesterol exposure and the influencing effects of postnatal diet was evaluated in apoE^{-/-} mice.

Methods and Results—In two separate studies, female mice were assigned to a standard chow (S) or a cholesterol-enriched chow (C) diet during gestation and lactation. Male offspring from each dam were weaned on a postnatal S or a hypercaloric western (W) diet resulting in four experimental groups: S-S and C-S (Experiment 1) and S-W and C-W (Experiment 2). At weaning, litters from hypercholesterolemic mothers weighed less ($p<0.05$) and pups had higher blood lipids, glucose, and hepatic cholesterol compared with pups from S-fed mothers. Adult C-S offspring demonstrated an atherogenic lipid profile and increased ($p<0.05$) hepatic cholesterol and triglyceride content with altered lipid regulatory mRNA expression and protein content compared with S-S offspring. Alternatively, no difference ($p>0.05$) was observed between S-W and C-W in serum and hepatic lipid profiles, however, serum AST and ALT was higher ($p<0.05$) in C-W vs. S-W offspring.

Conclusion—The degree of hepatic lipid deposition observed in adult offspring exposed to excessive early cholesterol is influenced by the postnatal diet.

Keywords

Maternal hypercholesterolemia; NAFLD; developmental programming; adult offspring

Introduction

A number of pathological complications encountered during pregnancy, including glucose intolerance [1], gestational diabetes [2], polycystic ovary syndrome [3], and preeclampsia

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[4] are associated with excessive hypercholesterolemia that may increase cardiovascular disease (CVD) risk for both mother and fetus. Furthermore, although there is a normal physiological increase in maternal cholesterol (+25%–50%) during pregnancy which is vital for embryogenesis and early organ development [5, 6], an excessive increase in maternal serum cholesterol can manifest during pregnancy, particularly in women with elevated cholesterol prior to conception [7, 8]. Such an exaggerated rise in early cholesterol exposure has been associated with pre-term delivery and low birth weight [9–11], increased childhood body mass index and adiposity [12–14], increased blood lipids in the neonatal, adolescent, and adult periods [15–18], and fetal fatty streaks that develop faster in childhood [19, 20]. It has been estimated that 10%–25% of US women of childbearing years are hypercholesterolemic, having blood total cholesterol (TC) concentrations >240 mg/dL [21, 22]. This is concerning as maternal pre-pregnancy cholesterol concentration has been shown to influence the absolute increase in serum cholesterol during gestation [19] and is correlated to offspring lipid status [17].

Hypercholesterolemia, although well-known as a critical modulator of CVD risk, is also strongly linked to the development of nonalcoholic fatty liver disease (NAFLD) and progression to non-alcoholic steatohepatitis (NASH) [23–25]. Hypercholesterolemic induced NAFLD is characterized by the accumulation of hepatic free cholesterol [26], increased cholesterol synthesis [27], and differential modulation of cholesterol regulatory gene expression [28] that contributes to or exacerbates disease severity. With NAFLD now the most prevalent form of chronic liver disease amongst pediatric and adult populations [29, 30], there is growing belief that early-life events encountered *in utero* or during the immediate postnatal period may program a predisposition to abnormalities in hepatic lipid metabolism [30]. Although maternal obesity and accompanying fetal fat exposure have been implicated in programming NAFLD in adult progeny [31–33], there has been very little work that has explored the potential role of excessive early cholesterol exposure in malprogramming hepatic lipid metabolism in later life. One previous report by Tsuduki et al. reported that newly-weaned and adult offspring exposed to excessive cholesterol throughout the lactation period only had increased hepatic lipid infiltration [34]. However, the influence of maternal hypercholesterolemia throughout both gestation and lactation, a scenario more reflective of this condition, is incompletely understood. Moreover, the impact of postnatal diet quality as an influential environmental factor in the metabolic health of pre-programmed offspring from hypercholesterolemic mothers has not been adequately explored. The introduction of a postnatal dietary cholesterol or high fat challenge following early cholesterol exposure has been shown to alter arterial pressure [35] and renal function [36] as well as induce dyslipidemia [37] and accelerate the progression of atherosclerosis [38] in adult offspring. However, we are not aware of previous studies that have directly compared how pre-programmed offspring exposed to maternal hypercholesterolemia throughout gestation and lactation respond to different postnatal dietary environments.

Therefore, using the apoE-deficient mouse model, the objectives of this study were to access hepatic lipid metabolism in adult offspring exposed to excessive early cholesterol as a result of maternal hypercholesterolemia and examine the influence of postnatal diet on these effects. Given the metabolic dysfunction we previously observed in newly-weaned offspring from hypercholesterolemic mothers [39, 40], we anticipated that adult offspring born from

hypercholesterolemic mothers would possess altered hepatic lipid metabolism and that these responses would be exacerbated upon consumption of a postnatal western-style diet versus a standard chow diet.

Materials and Methods

Animals and Diets:

Two separate experiments were conducted to examine malprogramming of early cholesterol exposure and the influence of postnatal diet on the metabolic phenotype of adult progeny. In each experiment, sixteen mature (2-month old) female mice homozygous for disruption of the *apoE* gene (*apoE2/2*, strain B6.129P2-Apoetm1Unc > /J, stock no. 002052) were purchased from Jackson Laboratory (Bar Harbor, ME) and housed at the Animal Care Facility at the University at Buffalo in individual cages with shavings and enrichment housing with free access to water in a temperature-controlled room (20°C, 12h light/dark cycle). Female mice were randomly assigned ($n = 8/\text{group}$) to either a standard chow diet (**S**, Teklad 2019 Harlan Laboratories) or the chow diet enriched with 0.15% cholesterol (**C**, TD. 140285) (Supplementary Table 1) and mated with chow-fed male *apoE*^{-/-} breeders. Females were placed with male breeders (1 male per female) for >1 week and returned to their individual cages following confirmation of pregnancy based on daily body weight gain. Feed intake and body weights were collected throughout pregnancy. At birth, litters were randomly culled to 6 pups per dam to minimize variability in postnatal pup growth influenced by litter size (33). Throughout the suckling period the dams remained on their respective diets. To prevent the pups from consuming the maternal diet as they matured, cages were changed daily and food pellets were provided on raised caged platforms that were only accessible by the mother.

In Experiment 1, male pups from each litter were weaned onto the **S** diet creating two experimental groups, S-S ($n=8$) and C-S ($n=8$). In Experiment 2, male pups from each litter were weaned onto a high calorie western-style diet (**W**, AIN76A with 40% kcal fat, 44% kcal carbohydrate, 16% kcal protein, and 0.15% (w/w) cholesterol) (Supplementary Table 2), generating S-W ($n=8$) and C-W ($n=8$) groups. In both experiments, male offspring remained on their assigned post-weaning diet (S or W) until postnatal day 84. Food intake and body weights were monitored throughout the post-weaning period. On postnatal day 84, fasted (8hrs) offspring were anesthetized with isoflurane for blood collection by cardiac puncture and then euthanized by exsanguination and heart removal. Livers were quickly excised, weighed, flash frozen in liquid nitrogen, and stored at -80°C for further processing and analyses. The mice used in this experiment were cared for in accordance with the guidelines established by the Institutional Animal Care and Use Committee. All procedures were reviewed and approved by the Animal Care Committee at the University at Buffalo (protocol no. PTE16082N).

Blood biochemistry:

Serum TC, high-density lipoprotein cholesterol (HDL-C), and LDL/VLDL cholesterol were determined with enzymatic analysis (BioAssay, EHDL-100). Serum LDL-C was measured by direct automated enzymatic assay (Liposcience). Non-HDL-C was calculated by

subtracting HDL-C from TC. Serum TG were determined by automated enzymatic analysis (Zenbio, STG-1-NC). Serum insulin was analysed by ELISA (ThermoScientific, EMINS) and glucose was measured by colorimetric analysis (Invitrogen, EIAGLUC). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using an Element DC Chemistry Analyzer (Heska) in a pooled subset of animals.

Hepatic lipid analysis:

Hepatic cholesterol was extracted and analyzed according to our previously published procedures [41, 42]. Approximately 0.5 mL of serum or 0.5 g of pulverized liver was spiked with α -cholestane as internal standard and saponified in freshly prepared KOH-methanol at 100°C for 1 h. The non-saponifiable sterol fraction was extracted with petroleum diethyl ether and dried under N₂ gas. Sterol fractions were analyzed on a Shimadzu GC-17A gas chromatograph fitted with a flame ionization detector using a SAC-5 capillary column (30m $\times$ 0.25mm $\times$ 0.25 mm, Supelco, Bellefonte, CA). This methodology was recently validated through an international 'Survey for Sterols and Oxysterols ST1/14' which included total cholesterol and phytosterol external reference materials from Referenzinstitut für Bioanalytik (Bonn, Germany). For hepatic triglyceride (TG) analysis, liver tissue (100 mg) was homogenized in 1 mL of an aqueous 5% NP-40 solution, heated at 90°C for 10 minutes, and spun at top speed in a microcentrifuge for 2 minutes. TG concentration in extracts were measured with a commercial kit (Zenbio, STG-1-NC) according to manufacturer's instructions.

mRNA extraction and real time RT-PCR:

RNA extraction and real-time RT-PCR were conducted according to previously published procedures system according to previously established protocols [43]. Total RNA was isolated from whole liver tissue using RNeasy mini Kit (Qiagen). RNA concentration and integrity was determined with spectrophotometry (260 nm) and agarose gel electrophoresis, respectively. RNA preparation and real-time RT-PCR were conducted using a one-step QuantiFast SYBR Green RT-PCR kit (Qiagen) with a Biorad CFX96 Touch real time PCR system. Gene expression was analyzed using the 2(-delta delta Ct) method. Sequences of sense and antisense primers for target and housekeeping genes were based on previously published reports for β -actin, fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), carnitine palmitoyltransferase 1a (CPT1a), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAr), sterol regulatory element-binding protein (SREBP) 1c, SREBP2, microsomal triglyceride transfer protein (MTP), diglyceride acyltransferase (DGAT), and apolipoprotein B (apoB) [44].

Immunoblot analysis:

Immunoblots were prepared as previously described [45]. Nuclear and cytoplasmic extracts were prepared on ice using protease inhibitors to limit protein degradation using a commercial kit (ThermoScientific, 78833). An aliquot of each fraction was taken for the determination of protein concentration for each sample. Then, equal volume of 2X sodium dodecyl sulfate loading buffer was added to each fraction for Western analysis. Samples were boiled for 5 minutes, then Western analysis was performed. Nuclear extracts were probed with commercial antibodies specific for SREBP1 (abcam, ab28481) and liver X

receptor (LXR) (abcam, ab176323). Cytoplasmic extracts were probed for ACC (Cell Signaling C83B10) and FAS (Cell Signaling C20G5). Target proteins were normalized to β -actin and quantified using Image lab (version 4.1, Biorad Laboratories, Hercules, CA).

Statistical analyses:

Litters from each dam were considered as a single observation. As the two experiments were run independently, endpoint measures were compared within postnatal diet groups based on the prenatal treatments; S-S vs. C-S and S-W vs. C-W using a one-way general linear model ANOVA with SPSS 16 for Mac (SPSS Inc, Chicago, IL). Data was checked for normality using the Shapiro-Wilk test and are presented as means $\pm$ SE. Differences were considered significant at $p < 0.05$.

Results

Maternal and offspring phenotype at the end of lactation

Mothers consuming the C diet during gestation and lactation had elevated ($p < 0.05$) serum total and non-HDL-C, and lower ($p < 0.05$) HDL-C compared S-fed mothers (Table 1). No difference ($p > 0.05$) in gestational weight gain or feed intake (data not shown) was observed between the two groups (Table 1).

Litters from C-fed mothers weighed less ($p < 0.05$) on postnatal day 21 compared with litters from S-fed mothers (Table 1). Newly-weaned pups from C-fed mothers had increased ($p < 0.05$) serum TC, LDL-C, and TG ($p = 0.07$) with no change in HDL-C compared with offspring from S-fed dams (Table 1). Newly-weaned offspring from C mothers demonstrated increased ($p < 0.05$) glucose with no change in insulin, suggestive of lower insulin sensitivity. Hepatic cholesterol concentration was higher ($p < 0.05$) in newly-weaned offspring from C versus S-fed mothers (Table 1).

Adult offspring Phenotype

Experiment 1: No change in postnatal growth trajectory was observed between S-S and C-S offspring (Fig 1A). C-S progeny demonstrated lower ($p < 0.05$) serum TC, mainly stemming from a reduction in HDL-C that increased the nonHDL:HDL ratio compared with S-S offspring. C-S offspring further exhibited a reduction ($p < 0.05$) in serum TG compared with S-S offspring (Fig 2A). No change ($p > 0.05$) in serum glucose and insulin concentrations were observed between S-S and C-S offspring, however, C-S progeny tended ($p = 0.07$) to have a lower glucose to insulin ratio (Fig 3A). We observed no change ($p > 0.05$) in serum AST (351.3 ± 66.1 U/L vs. 364.0 ± 57.3 U/L) and ALT (68.5 ± 13.7 vs 59.0 ± 13.7) in S-S and C-S offspring, respectively.

Compared with the S-S progeny, C-S progeny had higher ($p < 0.05$) hepatic cholesterol and TG concentrations (Fig 4A), however, no change ($p > 0.05$) in FA profile was detected (Fig 4B). Relative to the S-S group, C-S progeny demonstrated lower ($p < 0.05$) hepatic mRNA expression of lipogenic targets [FAS (-3.2 fold), ACC (-1.6 fold), HMG-CoAr (-2.1 fold), SREBP1c (-3.3 fold), and SREBP2 (-1.7 fold)] and higher ($p < 0.05$) expression of genes involved in triglyceride synthesis (DGAT ($+2.3$ fold) and VLDL packaging [MTP ($+1.9$

fold) and apoB (+2.2 fold)] (Fig 5A). No change ($p>0.05$) in hepatic nuclear SREBP1c and LXR protein content was observed between S-S and C-S offspring (Fig 6A & B). Compared with S-S progeny, C-S offspring exhibited reduced ($P<0.05$) ACC abundance (~0.60 fold of S-S, Fig 6C) but no change ($p>0.05$) in cytosolic FAS (Fig 6D).

Experiment 2: No change in postnatal growth trajectory was observed between S-W and C-W offspring (Fig 1B). Serum lipids were not different ($p>0.05$) between S-W and C-W offspring (Fig. 2B), however C-W offspring tended ($p=0.08$) to have a lower non-HDL:HDL ratio. While no change in glucose, insulin, or G/I ratio was observed between the offspring groups, a tendency ($p=0.06$) for lower serum insulin in C-W versus S-W offspring was noted (Fig 3B). Lastly, C-W offspring demonstrated increased ($p<0.05$) serum AST (535.5 ± 2.5 U/L vs. 313.3 ± 33.4 U/L) and ALT (88.5 ± 7.9 U/L vs. 46.5 ± 5.55 U/L) compared to S-W offspring, respectively, suggesting that early cholesterol exposure detrimentally impacts hepatic injury when challenged with a postnatal western diet.

C-W offspring demonstrated no change in hepatic cholesterol ($p>0.05$) but tended ($p=0.12$) to have increased hepatic TG compared to S-W offspring (Fig 4C). Further, no change in hepatic FA profile was detected between the treatment groups (Fig 4D). Compared to S-W progeny, alterations in hepatic mRNA expression remained largely unchanged in C-W offspring ($p>0.05$) with the exception of a significant down regulation ($p<0.05$) of genes known to regulate de novo lipogenesis [ACC (-2.1 fold)], β -oxidation [CPT1 α (-1.7 fold)], and VLDL synthesis [apoB (-1.7 fold)] (Fig 5B). No change ($p>0.05$) in hepatic nuclear SREBP1c and LXR protein content were observed between S-W and C-W offspring (Fig 7A & B). Despite a reduction in hepatic ACC mRNA in C-W offspring, no change ($p>0.05$) in ACC protein content was observed (Fig 7C). However, cytosolic FAS protein content was reduced ($p<0.05$) in C-W versus S-W progeny (Fig 7D).

Discussion

Results of this study suggest that offspring from hypercholesterolemic mothers have metabolic disturbances in hepatic lipid metabolism that are evident in early life and persistent into adulthood. Additionally, our results highlight the postnatal nutritional environment as an influential determinant factor in the manifestation and degree of developmental programming in response to excessive early cholesterol exposure. Early cholesterol exposure programmed a range of metabolic abnormalities including dyslipidemia and increased intrahepatic cholesterol and TG concentrations in adult offspring fed a postnatal chow diet (C-S) compared to their control counterparts (S-S). Contrary to our hypothesis, consumption of a postnatal western diet appeared to largely attenuate most, but not all, of this metabolic dysfunction. Cholesterol-exposed offspring fed a postnatal western diet (C-W) demonstrated elevations in hepatic injury markers (AST and ALT) compared with S-W offspring. These metabolic differences were supported by a more pronounced lowering of hepatic lipogenic genes (FAS, ACC, HMG-CoA, SREBP1/2) and enhanced expression of TG synthesis and VLDL packaging targets (MTP, DGAT, and ApoB) in cholesterol-exposed postnatal chow-fed offspring compared to those fed a western diet (relative to their respective controls). Further evidence of a reduced hepatic lipogenic

response was seen in both postnatal groups with reduced ACC (C-S) and FAS (C-W) protein abundance compared with their respective controls.

The buildup of intrahepatic TG concentrations in NAFLD is thought to be a consequence of enhanced hepatic fat uptake associated with adipose insulin resistance [46] and an increased rate of *de novo* lipogenesis as measured directly using stable isotopes [47] and indirectly through enhanced lipogenic gene expression [48]. Although the source of hepatic fat accumulation in cholesterol-exposed offspring is not known in the current study, a reduction in insulin sensitivity is known to influence fat delivery to the liver, however, no significant change in the G/I ratio was observed in these studies ($p=0.07$). The hepatic gene and protein expression data show a clear down-regulation of lipogenic targets, particularly evident in early-cholesterol exposed offspring fed the postnatal chow diet (C-S). Hepatic lipogenic gene expression in experimental NAFLD has been shown to vary depending on the mouse model employed. It has been reported that induction of NAFLD through consumption of a western diet results in an increase in lipogenic gene expression that is not seen when induced through a methionine-choline deficient diet [49]. In human studies, lipogenic gene targets in human fatty livers have been reported to be enhanced [47], however, this pattern appears to be inversely associated with the degree of fat accumulation with a reduction in lipogenic gene response as steatosis increases [48]. This response may be associated with hepatic insulin resistance [50] or a feedback mechanism in response to fat accumulation.

In general, the majority of maternal overnutrition models examining maladaptive programming to high fat diets during pregnancy support a ‘second hit’ phenomenon whereby prenatal and postnatal fat exposure has adverse synergistic effects on metabolic dysfunction in adult offspring [51]. Xie et al [37] reported that apoE^{-/-} adult offspring from dams fed a high fat/cholesterol diet (21% milk fat, 0.2% cholesterol) exhibited overt dyslipidemia when fed the same diet throughout the postnatal period compared to those exposed to the challenge diet during the postnatal period alone. However, in the current study, the relative lack of metabolic dysfunction in blood and hepatic lipid metabolism between cholesterol-exposed and control adult offspring consuming a postnatal western diet does not support a heightened adverse lipid response to a secondary insult in adult life. This observation may be due to the overriding influential effect of the postnatal cholesterol-supplemented western diet that effectively masked any malprogramming response to early cholesterol exposure. For instance, the relatively high intake of dietary cholesterol in those animals consuming the postnatal western diet may have sufficiently stressed the system enough to effectively override the HDL-C reducing effect observed in offspring from hypercholesterolemic dams fed a chow-based postnatal diet. Feeding such a high fat/cholesterol diet in adult apoE deficient mice is well-established to cause detrimental metabolic effects related to lipid metabolism and cardiovascular health [52, 53]. Alternatively, as a mismatch between the prenatal versus postnatal environment underscores metabolic dysfunction in offspring from undernourished mothers exposed to postnatal nutrient overload (‘thrifty’ phenotype), the opposite scenario in which an adverse *in utero* environment may protect against metabolic maladaptations upon further exposure to the same challenge in adult life has been suggested [54, 55]. This ‘predictive adaptive response’ was reported by Norman et al. [56] who demonstrated that prenatal lipid exposure in pigs offered protection from atherosclerosis development and lipid deposition in offspring

exposed to an atherogenic diet in adulthood. The authors concluded that *in utero* exposure to an atherogenic diet (1% cholesterol and 15% beef tallow) programmed an adaptive response that allowed offspring to better manage a postnatal hyperlipidemic challenge. Further, results from Couvreur et al. [55] reported that a maternal high fat diet throughout gestation and lactation had a partial sex-dependent protective effect on diet-induced weight gain in offspring when challenged with a highly palatable diet in the postnatal period. In light of these scenarios, our results could be interpreted to suggest that offspring raised in a hypercholesterolemic environment may be better adapted to a cholesterol challenge in adult life. However, the maternal chow-based diet used in this study differed substantially in the quantity and quality of macronutrients compared with the offspring semi-synthetic western diet. Although the perinatal and postnatal dietary cholesterol concentrations were matched (0.15%), we are not able to fully compare a matched prenatal versus postnatal environment due to these different diets.

In summary, our results suggest that cholesterol-induced malprogramming of metabolic dysfunction in adult progeny is largely influenced by the postnatal dietary environment. The effects of maternal hypercholesterolemia on serum lipids and hepatic lipid status were more evident in adult progeny fed a chow vs. a western diet in the postnatal period. Consumption of a postnatal western diet attenuated some of the metabolic effects attributed to early cholesterol-exposure but was associated with elevations of liver enzymes (AST and ALT) indicative of reduced hepatic function. Given the rise of NALFD in pediatric and adolescent populations, the influence of maternal hypercholesterolemia, amongst other maternal metabolic factors, in malprogramming hepatic lipid dysfunction warrants further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

(C)	cholesterol group
(C-S)	cholesterol-chow group
(C-W)	cholesterol-western diet group
(S-S)	chow-chow group
(S-W)	chow-western diet group
(NAFLD)	nonalcoholic fatty liver disease
(S)	standard chow group

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Figure 1: Postnatal growth in male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Offspring consumed a standard chow diet in experiment 1 (S-S, C-S, **panel A**) or a western diet in experiment 2 (S-W, C-W, **panel B**) throughout the postnatal period. n=8 mothers per group.

Figure 2:

Serum lipids in 84-day old male offspring from mothers fed a standard chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Offspring consumed a chow diet in experiment 1 (S-S, C-S, **panel A**) or a western diet in experiment 2 (S-W, C-W, **panel B**) throughout the postnatal period. Data are means $\pm$ SE; n=8 mothers per group; *, denotes a significant difference ($p < 0.05$).

Figure 3: Serum glucose and insulin in 84-day old male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Offspring consumed a chow diet in experiment 1 (S-S, C-S, **panel A**) or a western diet in experiment 2 (S-W, C-W, **panel B**) throughout the postnatal period. Data are means $\pm$ SE; n=8 mothers per group.

Figure 4: Hepatic lipids (cholesterol, triglycerides, and fatty acids) in 84-day old male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Offspring consumed a chow diet in experiment 1 (S-S, C-S, **panels A & B**) or a western diet in experiment 2 (S-W, C-W, **panels C & D**) throughout the postnatal period. Data are means $\pm$ SE; $n=8$ mothers per group; *, denotes a significant difference ($p<0.05$).

Figure 5:

Hepatic mRNA expression of lipid-regulatory genes in 84-day old male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Offspring consumed a chow diet in experiment 1 (S-S, C-S, **panel A**) or a western diet in experiment 2 (S-W, C-W, **panel B**) throughout the postnatal period. Data are means $\pm$ SE and normalized to their respective control group (S-S or S-W; n=8 mothers per group; *, denotes a significant difference ($p < 0.05$)).

Figure 6:

Hepatic protein abundance of nuclear SREBP1 (A) and LXR (B) and cytosolic ACC (C) and FAS (D) in chow-fed 84-day old male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Data are means $\pm$ SE; all data normalized to β -actin; *, denotes a significant difference ($p < 0.05$).

Figure 7:

Hepatic protein abundance of nuclear SREBP1 (A) and LXR (B) and cytosolic ACC (C) and FAS (D) in western diet-fed male offspring from mothers fed a chow (S) or cholesterol-supplemented chow (C) throughout gestation and lactation. Data are means $\pm$ SE; all data normalized to β -actin; *, denotes a significant difference ($p < 0.05$).

Table I:

Maternal and offspring phenotype at the end of lactation (postnatal day 21).

Endpoint	S	C	p-value
Maternal phenotype (end of lactation period)			
Gestational weight gain (g)	13.5 ± 1.1	13.3 ± 0.5	0.89
Feed intake (g/d)	6.89 ± 0.3	6.68 ± 0.29	0.57
Serum total cholesterol (mmol/L)	10.3 ± 1.4	28.7 ± 2.2	0.01
Serum non-HDL-C (mmol/L)	8.4 ± 1.2	28.01 ± 2.2	0.00
Serum HDL-C (mmol/L)	1.90 ± 0.4	0.6 ± 0.1	0.02
Offspring phenotype (postnatal day 21)			
Litter weight	49.48 ± 1.18	43.08 ± 2.63	0.01
Serum total cholesterol (mmol/L)	10.96 ± 0.39	12.46 ± 0.46	0.04
Serum HDL-C (mmol/L)	0.61 ± 0.03	0.67 ± 0.07	0.49
Serum LDL-C (mmol/L)	2.60 ± 0.14	3.62 ± 0.13	0.00
Serum triglycerides (mmol/L)	1.13 ± 0.08	2.10 ± 0.40	0.07
Serum glucose (mmol/L)	8.75 ± 0.94	14.11 ± 1.35	0.01
Serum insulin (μIU/L)	42.41 ± 2.14	44.36 ± 0.90	0.42
Hepatic cholesterol (μmol/g)	13.13 ± 2.31	19.94 ± 2.25	0.02

Data are means ± SE; S, standard chow (n=8); C, chow diet enriched in cholesterol (0.15%) (n=8).