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Excessive early-life cholesterol exposure may have later-life consequences for nonalcoholic fatty liver disease

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Review

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Abstract

The *in utero* and immediate postnatal environments are recognized as critical windows of developmental plasticity where offspring are highly susceptible to changes in the maternal metabolic milieu. Maternal hypercholesterolemia (MHC) is a pathological condition characterized by an exaggerated rise in maternal serum cholesterol during pregnancy which can program metabolic dysfunction in offspring, including dysregulation of hepatic lipid metabolism. Although there is currently no established reference range MHC, a loosely defined cutoff point for total cholesterol >280 mg/dL in the third trimester has been suggested. There are several unanswered questions regarding this condition particularly with regard to how the timing of cholesterol exposure influences hepatic lipid dysfunction and the mechanisms through which these adaptations manifest in adulthood. Gestational hypercholesterolemia increased fetal hepatic lipid concentrations and altered lipid regulatory mRNA and protein content. These early changes in hepatic lipid metabolism are evident in the postweaning environment and persist into adulthood. Further, changes to hepatic epigenetic signatures including microRNA (miR) and DNA methylation are observed *in utero*, at weaning, and are evident in adult offspring. In conclusion, early exposure to cholesterol during critical developmental periods can predispose offspring to the early development of nonalcoholic fatty liver disease (NAFLD) which is characterized by altered regulatory function beginning *in utero* and persisting throughout the life cycle.

Introduction

The developmental origins of health and disease postulates that early-life environmental exposures, including over and under-nutrition, influence both *in utero* and early postnatal development to alter the trajectory of disease risk in adulthood.[1] The *in utero* and postnatal environments are recognized as critical windows of developmental plasticity where offspring are highly susceptible to changes in the maternal metabolic milieu.[1–3] One of the earliest recognitions of a link between early-life nutrient exposure and the later development of non-communicable diseases was the Thrifty Gene Hypothesis established by Hales and Barker.[4] In this paradigm, nutrient restriction during gestation was hypothesized to program offspring metabolism to preferentially favor a nutrient restrictive postnatal environment. Alternatively, "mismatch" to an energy-rich postnatal environment was hypothesized to lead to metabolic adaptations that would increase susceptibility to chronic disease risk. Perhaps more pertinent to a "western" lifestyle of excess nutrient, there is also ample evidence that supports a "second hit" hypothesis whereby early-life exposure to an energy-rich environment (e.g., maternal obesity) followed by consumption of a postnatal energy-rich diet would also lead to metabolic adjustments that favor disease predisposition in adulthood.[3]

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease among pediatric and adult populations.[5,6] NAFLD is traditionally thought to be the hepatic manifestation of metabolic syndrome[7] resulting in impaired metabolic function characterized by insulin resistance, deposition of hepatic free fatty acids (FFAs), increased *de novo* lipogenesis, and dysregulated triglyceride (TG) and very low-density lipoprotein (VLDL) production and secretion.[5,8,9] Although hypercholesterolemia is typically associated with cardiovascular disease (CVD), it is also strongly associated with NAFLD and progression to nonalcoholic steatohepatitis (NASH).[10–12]

Although the origins of NAFLD are often attributed to lifestyle factors (e.g., lack of exercise and poor diet), the alarming rise in pediatric NAFLD suggests that metabolic abnormalities experienced *in utero* and in the immediate postnatal life may program liver dysfunction in developing offspring.[13] Previous human reports have linked infant hepatic fat accumulation with gestational diabetes[14,15] and maternal obesity[16,17]; however, there is little evidence linking

maternal hypercholesterolemia (MHC) to NAFLD in human offspring. Alternatively, data from a range of animal models support a role for excessive early cholesterol exposure in NAFLD development. Therefore, drawing largely from animal model evidence, this review will assess what is known about the role of excessive early cholesterol exposure in malprogramming hepatic lipid metabolism in offspring during different developmental stages and the potential mechanisms involved. Importantly, we have limited this review to studies that have examined MHC in isolation, without the potential confounding effects of maternal high-fat feeding and/or maternal obesity, both of which have been shown to independently program offspring metabolic dysfunction.

Dysregulated cholesterol metabolism in NAFLD

Although NAFLD is most often discussed in the context of increased hepatic fat (TG), dysregulated cholesterol metabolism is characteristic of NAFLD and in the transition to NASH.[10–12] Hepatic cholesterol homeostasis is tightly regulated through feedback mechanisms which control synthesis through 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCoAr) and tissue clearance through low-density lipoprotein (LDL) receptor (LDLr). At the transcriptional level, the balance between cholesterol synthesis and uptake is controlled through the actions of the sterol regulatory element-binding protein (SREBP) 2, SREBP-cleavage activating protein (SCAP), and insulin-induced gene (Insig) signaling pathway.[18] NAFLD is associated with disruptions in sterol signaling resulting in elevated hepatic-free cholesterol,[19] increased cholesterol synthesis,[20] and differential modulation of cholesterol regulatory gene expression[21] that contributes to or exacerbates disease severity. Further, cholesterol can be oxidized through enzyme-mediated or nonenzymatic processes to form a wide-range of oxygenated derivatives, more commonly referred to as oxysterols. These oxysterols act as bioactive signaling molecules that facilitate cross talk between lipids and inflammatory mediators to influence hepatic oxidative damage and lipotoxicity in the development and progression of NAFLD.[22,23]

Oxysterols can independently regulate cholesterol homeostasis by inhibiting SREBP2 processing by binding to SCAP in addition to exerting control over the transcription of cholesterol regulatory genes through activation of the Liver X receptor (LXR). LXR isoforms, LXRα and LXRβ, are nuclear hormone receptors that are activated by oxysterol ligand binding. LXR forms a heterodimer with retinoid X receptor (RXR) and binds to the LXR responsive element (LXRE) in the promotor regions of genes known to regulate cholesterol homeostasis. Upon activation by oxysterol binding, the LXR–RXR heterodimer activates the transcription of the cholesterol efflux transporters adenosine triphosphate-binding cassette transporter G5/G8 (ABCG5/G8), ABCA1/G1, cholesterol 7-alpha-hydroxylase (CYP7A1), cholesteryl ester transfer protein (CETP), and the scavenger receptor type B1 (SR-B1), all of which act to facilitate the removal of excess cholesterol by increasing bile acid synthesis and reverse cholesterol transport. The net result is the direct inhibition of the SCAP/SREBP/Insig pathway coupled with increased cholesterol efflux as a result of LXR-induced transcription of cholesterol efflux genes. However, LXR activation also increases the transcription of SREBP1 which controls the transcription of the rate-limiting enzymes controlling *de novo* fatty acid synthesis, acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS), which has been proposed as the mechanistic link between hypercholesterolemia and hepatic TG accumulation.[24–27]

Although dysregulated sterol metabolism is a hallmark of NAFLD, it is currently unclear whether cholesterol accumulation is a direct initial lipotoxic trigger for NAFLD or if excessive hepatic cholesterol accumulation is secondary to TG accumulation.[10] However, direct animal model evidence of cholesterol-induced NAFLD in the absence of dietary fat[28,29] as well as reports of increased dietary cholesterol intake in lean NAFLD patients without obesity-associated insulin resistance[30] suggests a potential causative role of cholesterol in NAFLD development.

Maternal hypercholesterolemia

Although there is a normal physiological increase in maternal blood cholesterol (+25–50%) during pregnancy, an excessive increase in maternal serum cholesterol can manifest during pregnancy, particularly in women with elevated cholesterol prior to conception.[31,32] Further, a number of pathological complications encountered during pregnancy, including glucose intolerance,[33] gestational diabetes,[34] and preeclampsia[35] are associated with excessive hypercholesterolemia. It has been estimated that 10%–25% of US women of childbearing years are hypercholesterolemic, having blood total cholesterol concentrations >240 mg/dL.[36,37] This is concerning as maternal prepregnancy cholesterol concentration has been shown to influence the absolute increase in serum cholesterol during gestation[38] and is correlated to offspring LDL-C.[39] There is currently no established reference range for MHC or a biological cutoff point at which fetal health is knowingly compromised.[40] In the few human studies conducted, different cutoff points of maternal total cholesterol in the third trimester have been used in the arbitrary designation of MHC.[41–44] Based on limited work in humans, a loosely defined cutoff point for total cholesterol has been suggested in late pregnancy (i.e., >280 mg/dL in the third trimester), as concentrations above this level have been associated with fetal fatty streak development[45] and altered placental vasoreactivity.[46]

One of the earliest recognitions that a hypercholesterolemic maternal environment during pregnancy could influence disease risk in offspring came from the work of Palinski et al.[38,47] In these studies, maternal cholesterol concentration during pregnancy was strongly associated with fetal (<6 months) cholesterol status, and perhaps more strikingly, fetal aortas from hypercholesterolemic mothers possessed an increased number of oxidized LDL-containing lesions.[38] Their follow-up study examining the evolution of early childhood lesions in autoptic children (1–14 years of age) reported that arterial lesions developed "strikingly" faster in children whose mothers were hypercholesterolemic during pregnancy versus mothers with normal cholesterol.[45] Since these initial studies, pregnancy total cholesterol concentrations exceeding 280 mg/dL[48] have been associated with preterm delivery and low birth weight,[49–51] increased childhood body mass index and adiposity,[52–54] and increased blood lipids in the neonatal, adolescent, and adult periods.[39,55–57] Further, it has been suggested that at least a portion of fetal cholesterol in early pregnancy is obtained by maternal transfer through a network of placental lipid transport proteins, including LDLr, VLDL receptor (VLDLr), SR-B1, and ABCA1/G1.[58,59] Previous work also suggests that MHC is associated with placental dysfunction,[46,60] altered placental lipid transporter expression,[41,61,62] and excessive fetal cholesterol transfer.[63]

As the vast majority of previous animal and human studies have primarily studied the programming effects of MHC in the context of atherosclerosis and CVD risk, the potential impact of

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Table 1. Effects of maternal hypercholesterolemia throughout gestation and lactation on offspring hepatic lipid metabolism in newly weaned and adult stages

Author	Model	Maternal diet	Postnatal diet	Offspring age	Offspring hepatic phenotype			
					CH	TG	Lipid regulatory Protein & mRNA	Additional outcomes
Gestation + Lactation Exposure								
Liu et al.[73]	Golden Syrian hamster	Chow or CH (0.5%)	N/A	3 WOA	↑	↔	↑ HMGCoAr & LDLr PT ↓ LXR, PCSK9, SREBP2, CPT1, DGAT mRNA	-
Dumolt et al.[75]	ApoE $^{-/-}$	Chow or CH (0.15%)	N/A	3 WOA	↑	N/A	↑ ABCG1 & CYP7A1 mRNA ↓ ABCA1 & ABCG8 mRNA	↑ Oxysterol 7βHC ↑ TNFα mRNA
Goharkhay et al.[68]	ApoE$^{+/-mat}$; ApoE$^{+/-pat}$; ApoE$^{-/-}$; ApoE$^{+/+WT}$	Chow	Chow	35 WOA	N/A	N/A	apoE$^{+/-mat}$: ↑ SCAP, SREBP1a, SREBP2, HMGCoAr & LDLr mRNA	-
Trenteseaux et al.[71]	ApoE $^{-/-}$; ApoE$^{+/-}$	Chow	Chow	18 & 25 WOA	M: ↑ 25 WOA F: ↔	M: ↑18 & 25 WOA F: ↔	18 WOA M: ↑ SR-B1& LDLr mRNA F: ↑ FXR; ↓ SR-B1 mRNA	M & F: Altered DNA methylation
Dumolt et al.[76]	ApoE $^{-/-}$	Chow or CH (0.15%)	Chow or W	12 WOA	PN Chow: ↑ PN W: ↔	PN Chow: ↑ PN W: ↔	PN Chow: ↓ ACC, FAS, SREBP1c mRNA; PN W: ↓ ACC, CPT1α, ApoB mRNA	Fatty acid profile PN Chow: ↔ PN W: ↔

Abbreviations: HMGCoAr, 3-hydroxy-3-methylglutaryl-CoA reductase; 7βHC, 7-beta hydroxycholesterol; ACC, acetyl-CoA carboxylase; apoE, apolipoprotein E; CPT1α, carnitine palmitoyltransferase 1α; CH, cholesterol; DGAT, diglyceride acyltransferase; FAS, fatty acid synthase; F, female; LXRα, liver X receptor α; LDLr, low-density lipoprotein receptor; M, male; MOA, months of age; PN, postnatal; PT, protein; PCSK9, proprotein convertase subtilisin/kexin type 9; PS, phytosterol; SREBP1; SREBP2, sterol regulatory element binding protein; SCAP, SREBP-cleavage activating protein; TC, total cholesterol; TG, triglyceride; WOA, weeks of age; W, western.

early cholesterol exposure on offspring hepatic lipid homeostasis and regulatory function has been largely overlooked. Further, the programming influence of excessive cholesterol exposure in different periods of developmental plasticity (e.g., gestation vs. immediate postnatal) has been under researched. To support the discussion of previous animal-related work conducted to date, we have organized the liver-specific effects of MHC according to exposure window including gestation and lactation (Table 1) and gestation or lactation only (Table 2).

Period-specific effects of MHC on offspring lipid status

Excessive cholesterol exposure throughout both gestation and lactation

As both the gestation and lactation periods represent two important but distinct biologically sensitive windows of developmental plasticity, it is critical to examine the period-specific programming responses to MHC. The majority of previous preclinical work has characterized the effects of MHC throughout both the gestation and lactation periods,[64–71] including work from our own lab in Golden Syrian hamster and apolipoprotein E-deficient (apoE$^{-/-}$) mice[72,73] (Table 1). In both models, newly weaned offspring exposed to MHC through gestation and lactation demonstrated increased hepatic cholesterol deposition[72,73] (Table 1). Further, in apoE$^{-/-}$ mice, in addition to increased hepatic cholesterol and TG, newly weaned offspring from MHC dams demonstrated increased hepatic 7-β hydroxycholesterol (7β-HC), an oxysterol marker of oxidative stress.[74,75] These maladaptations in hepatic lipid status were accompanied by alterations in the mRNA and protein expression of cholesterol and fatty acid regulatory targets. In addition to increased hepatic lipid content,[72] newly weaned offspring from hypercholesterolemic apoE$^{-/-}$ mice showed a reduction in hepatic *de novo* lipogenic gene expression.[74] Similarly, newly weaned pups from hypercholesterolemic hamsters demonstrated increased hepatic cholesterol accompanied with elevated LDLr and HMGCoAr protein content despite no change in SREBP2 protein levels.[73]

The adaptations in newly weaned offspring described above can be linked to direct exposure to excessive cholesterol, whether *in utero* or through the suckling of maternal milk with modified nutrient (e.g., cholesterol, TG) or hormonal composition. However, the degree to which early-life alterations in hepatic lipid metabolism persist into adulthood and by what mechanisms remain incompletely understood. However, we have recently assessed the programming effects of MHC on hepatic lipid phenotype in adulthood and examined if the postnatal diet modulates these responses.[76] Male apoE$^{-/-}$ mice offspring from control or hypercholesterolemic mothers were weaned onto either a postnatal chow (3.5 kcal/g; 19.9% energy (E) from fat, 58.7% E from carbohydrate, 21.4% E from protein) or a Western diet (4.5 kcal/g; 40% E from fat, 44% E from carbohydrate, 15.8% E from protein) until 12 weeks of age. Adult offspring from MHC mothers fed the postnatal chow diet had increased hepatic TG which was characterized by a decreased hepatic SREBP1c, ACC, and FAS mRNA, indicative of reduced *de novo* lipogenesis and possibly a negative feedback mechanism in response to increased lipid stores. Perhaps surprisingly, consumption of the postnatal Western diet attenuated the metabolic effects attributed to early cholesterol exposure but was associated with elevations of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicative of reduced hepatic function. Rather than supporting a heightened

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Table 2. Effects of maternal hypercholesterolemia during gestation or lactation on offspring hepatic lipid metabolism in fetal, newly weaned, and adult stages

Author	Model	Maternal diet	Postnatal diet	Offspring age	Offspring hepatic phenotype			
					CH	TG	Lipid regulatory Protein & mRNA	Additional outcomes
Gestation exposure								
Yao et al.[77]	Golden Syrian hamster	Chow or CH (0, 0.12,0.5, 2.0%)	N/A	GD 11.5	↑	N/A	↔ SREBP2, HMGCoAr, HMGCoAs PT	-
Montoudis et al.[78]	New Zealand White rabbit	Chow or CH (0.2%)	N/A	GD 30	↑	N/A	N/A	↓ ACAT & HMGCoAr activity
Marseille-Tremblay et al.[79]	New Zealand White rabbit	Chow or CH (0.2%)	N/A	At birth	↑	↓	↓ FAS PT ↔ HMGCoAr, SREBP1, & SREBP2 PT	-
Dumolt et al.[80]	ApoE $^{-/-}$	Chow or CH (0.15%)	N/A	GD 18	↔	↑	↓ ACC, FAS, SREBP1c mRNA	↑ miR-27a ↓ miR-200c
Lactation exposure								
Yao et al.[91]	Golden Syrian hamster	Chow or CH (2.0%) (from GD14)	Chow	3 & 10 WOA	3 WOA: ↑ 10 WOA: ↔	N/A	N/A	Sterol synthesis: 3 WOA: ↑ 10 WOA: ↔
Tsuduki et al.[92]	C57BL/6 J	Chow or CH (0.2%) (from birth)	Chow	3 & 10 WOA	3 WOA: ↑ 11 WOA: ↑	3 WOA: ↔ 11 WOA: ↑	3 WOA: ↑ LRP1, LXRα & Insig2 mRNA ↓ HMGCoAr & Insig1 mRNA 10 WOA: ↑ LRP1, HL, ACO mRNA ↓ SREBP1c mRNA	-

Abbreviations: HMGCoAr, 3-hydroxy-3-methylglutaryl-CoA reductase; ACAT, acetyl-CoA acetyltransferase; ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; apoE, apolipoprotein E; CH, cholesterol; FAS, fatty acid synthase; GD, gestational day; HL, hepatic lipase; Insig1; Insig2, insulin-induced gene; LXRα, liver X receptor α; LRP1, low-density lipoprotein-like receptor 1; miR, microRNA; PN, postnatal; PND, postnatal day; PT, protein; SREBP1; SREBP2, sterol regulatory element binding protein; TC, total cholesterol; TG, triglyceride; WOA, weeks of age.

adverse lipid response to a secondary dietary insult in adult life, these unexpected findings were likely related to an overriding influential effect of the postnatal Western diet that effectively masked any malprogramming response to early cholesterol exposure observed in the postnatal chow-fed group.

Although our previous work utilized a model of genetic (apoE$^{-/-}$) and diet-induced (cholesterol-enriched) hypercholesterolemia, two other studies using the apoE$^{-/-}$ model employed selective breeding to produce offspring born to genetically determined hypercholesterolemic mothers (Table 1). Goharkhay et al.[68] used cross-breeding of male and female apoE$^{-/-}$ and apoE$^{+/+}$ to produce four genetic offspring variants (apoE$^{+/-\ \text{paternal}}$, apoE$^{+/-\ \text{maternal}}$, apoE$^{-/-}$, and apoE$^{+/+}$) which were suckled by their respective birth mothers and then weaned onto a postnatal chow diet until 8 months of age. Compared to apoE$^{+/+}$ adult offspring, amalgamated apoE$^{+/-\ \text{maternal}}$ male and female offspring showed increased expression of hepatic SCAP, SREBP1a, SREBP2, HMGCoAr, and LDLr indicative of increased hepatic *de novo* lipid synthesis and uptake; however, hepatic lipid concentrations were not measured in this study.

Trenteseaux et al.[71] crossbred male apoE$^{+/-}$ mice with either apoE$^{+/-}$ or apoE$^{-/-}$ females to produce male and female apoE$^{-/-}$ offspring from normocholesterolemic (apoE$^{+/-}$) or hypercholesterolemic (apoE$^{-/-}$) mothers which were also weaned onto a postnatal chow diet for phenotyping at 18 and 25 weeks of age. At 18 weeks of age, male offspring from hypercholesterolemic mothers showed increased hepatic TG with concurrent increases in SREBP1 and LDLr mRNA compared to control males. At 25 weeks of age, males from hypercholesterolemic dams had increased hepatic cholesterol and TG compared to male controls. No difference in hepatic lipid status was seen between females from normocholesterolemic or hypercholesterolemic mothers at either age, but they did show reduced SREBP1 mRNA and increased LXR mRNA compared to female controls at 18 weeks of age. Taken together, these studies demonstrate that MHC throughout gestation and lactation is capable of altering newly weaned and adult hepatic lipid metabolism; however, the developmental window in which these maladaptations occur is less clear.

Excessive cholesterol exposure in gestation only

Few studies have determined the effects of excessive gestational cholesterol exposure only on fetal hepatic lipid metabolism (Table 2). In response to incremental increases in maternal dietary cholesterol, Golden Syrian hamster fetuses accrued hepatic cholesterol despite no change in mature SREBP2, HMGCoAr, and HMGCoA synthase (HMGCoAs) protein concentrations.[77] Further, fetal hepatic sterol synthesis rates were only partially suppressed in response to high maternal cholesterol intake,[77] likely due to a negative fetal sterol balance as a result of rapid cell growth.

Using New Zealand white rabbits, Montoudis et al.[78] reported that term fetuses from diet-induced hypercholesterolemic mothers had increased hepatic-free cholesterol but not total cholesterol compared to control term fetuses (Table 2). Interestingly, the rise in free cholesterol corresponded to reduced hepatic acetyl-CoA acetyltransferase (ACAT) activity likely contributing to reduced cholesterol esterification; however, the reported reduction in HMGCoAr activity did not affect total hepatic cholesterol concentrations in cholesterol-exposed fetuses. Despite the increase in free cholesterol in cholesterol-exposed fetuses, there was no change in CYP7A1 activity between groups, again, likely due to no net change

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in total hepatic cholesterol stores. Using the same animal model, Marseille-Tremblay et al.[79] observed increased hepatic-free cholesterol, FFA, and TG in cholesterol-exposed term fetuses compared to control offspring (Table 2). The authors also reported no change in the protein concentrations of the hepatic sterol regulating enzymes HMGCoAr and SREBP1 and 2, but did note reduced FAS protein content in fetuses from hypercholesterolemic mothers, possible due to a negative feedback mechanism in response to elevated FFA concentrations. In support of these findings, we have recently shown that MHC during the gestation period is associated with hepatic TG accumulation in fetal apoE$^{-/-}$ mice[80] which is similarly linked to a reduction in hepatic lipogenic mRNA expression. This response was similar to what we observed in newly weaned and adult males exposed to MHC throughout gestation and lactation described earlier[74,76] (Table 2), suggesting that the programming of hepatic dysfunction in adulthood may originate *in utero*.

Excessive cholesterol exposure in lactation only

Maternal milk composition may be sensitive to changes in maternal diet and ultimately influence offspring disease risk status[81,82]; however, a previous systematic review reported that there was weak causal evidence and a lack of well-conducted studies to show a direct link between maternal diet and breast milk nutrient composition.[83] That being said, maternal disease state factors, including obesity has been shown to alter the nutrient profile of maternal milk including the composition of bioactive nonnutritive components such as hormones and inflammatory mediators,[84,85] suggesting that milk-dependent mechanisms may partly underlie the transmission of disease risk in newborns. However, few studies have characterized the independent effects of neonatal exposure to breast milk from hypercholesterolemic dams.

Although they did not specifically examine MHC or hepatic-specific endpoints, previous baboon studies by Mott and McGill provide interesting insight in how early cholesterol exposure in the preweaning period only may alter sterol metabolism throughout life. In a series of publications, the authors reported the influence of preweaning cholesterol-enriched infant formula (2, 30, or 60 mg cholesterol/100 mL) versus breast-feeding on sterol metabolism in juvenile (3–6 years of age) and young adult (7–8 years of age) baboons. In the juvenile-adolescent period, the level of cholesterol in infant formula did not influence cholesterol metabolism[86] or serum lipid concentrations, including TC, VLDL/LDL-C, or apolipoprotein B.[87] Adult offspring fed a cholesterol-enriched formula demonstrated higher HDL-C and TG (borderline significance for both), higher rates of cholesterol production and neutral sterol excretion, lower bile cholesterol saturation, but no difference in arterial fatty streaks.[88,89] Interestingly, a previous human infant study examining breast milk versus regular formula with and without added cholesterol (133 mg/L) reported minimal differences in lipid profile in 4-month old infants and little impact on cholesterol synthesis.[90]

Yao et al.[91] reported that neonatal hamsters (born to normal mothers) which suckled from hypercholesterolemic mothers had increased hepatic cholesterol and decreased sterol synthesis rates at postnatal day 10 and 20 (Table 2). However, the authors reported no change in hepatic sterol metabolism in adult animals (Table 2). Further, as a direct examination of the effects of postnatal cholesterol exposure through maternal milk, this study is limited as MHC was induced near the end of gestation (day 14 of a 15.5-day gestation period) and solid food ingestion was detected in 5-day old neonates.

In a C57BL/6 J mouse model, Tsuduki et al.[92] reported that MHC increased the cholesterol concentration in maternal milk and enhanced hepatic cholesterol and low-density lipoprotein-like receptor 1 (LRP1) mRNA and protein content in newly weaned female offspring (Table 2). Further, a reduction in HMGCoAr mRNA with no change in the transcripts of CYP7A1, ABCG5, or ABCA1 suggests the accumulation of hepatic cholesterol was a result of increased clearance of serum cholesterol without the expected increase in bile acid synthesis or cholesterol efflux. These early changes persisted into adulthood with increases in hepatic cholesterol, TG, and phospholipids in 11-week-old female offspring (vs. control) (Table 2). Overall, this study suggests that the lactation period is a critical cholesterol exposure window that can influence hepatic lipid metabolism in adulthood.

Sex-specific programming of NAFLD in MHC

On a population basis, NAFLD disproportionately affects a greater number of males than females.[93] In response to this sex-divergent prevalence of NAFLD, a growing number of preclinical and human studies have sought to uncover the underlying protective mechanisms in females versus males.[94,95] While sex-specific effects in offspring body weight and glucose tolerance have been reported in response to altered maternal nutrient and metabolic status,[96] whether early cholesterol exposure alters hepatic lipid metabolism in a sex-specific manner is not known. To date, we are only aware of one study that has directly compared divergent sex effects of MHC in male and female offspring. As previously discussed, Trenteseaux et al.[71] demonstrated that adult male apoE$^{-/-}$ offspring exposed to excessive maternal cholesterol had hepatic lipid accumulation compared to control males. However, compared with their male counterparts, adult female offspring had lower hepatic TG, TC, and bile acids (total unconjugated, tauro-conjugated, and glycol-conjugated BA). Although this result may suggest that cholesterol-exposed males may be more susceptible to NAFLD programming than females, the authors further demonstrated that adult females had more atherosclerotic plaques than male offspring with increased serum trimethylamine-N-Oxide,[71] a potential marker of CVD risk.[97]

Epigenetic mechanism of MHC

Epigenetics refers to heritable changes in gene expression and translation that do not involve modifications in the underlying DNA sequence[98] that influence metabolism and phenotype.[99] Further, epigenetic modifications have been implicated as key modulators in the malprogramming of offspring metabolic health.[100,101] DNA methylation, the most extensively studied epigenetic mechanism, silences gene transcription through the methylation of CpG-rich islands in the promoter or enhancer regions of the gene of interest.[102]

Although previous work has suggested that early cholesterol exposure may enhance later-life atherosclerosis through epigenetic events in vascular tissue,[103,104] liver-specific epigenetic modifications in cholesterol-exposed offspring have received less attention. In addition to changes in hepatic lipid status described above,[71] Trenteseaux et al. reported that adult males from hypercholesterolemic mothers had reduced percent methylation in several CpG sites of the LDLr promotor region, which corresponded to

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increased LDLr gene expression. While no programming effect of methylation status was found between normocholesterolemic and hypercholesteremic female offspring, both female groups had reduced methylation of the flavin-containing monooxygenase 3 (FMO3) gene compared to their male counterparts.

Among the epigenetic mechanisms that have been shown to influence lipid phenotype, microRNAs (miRs) have emerged as significant mediators of lipid homeostasis. miRs are small, 22 nucleotides long, non-coding RNAs that control gene expression and translation through posttranscriptional degradation of mRNA and have been shown to influence NAFLD development and progression.[105] Recent work has linked hepatic lipid accumulation and severe steatosis in offspring from obese pregnancies with altered hepatic miRNA profiles in baboon fetal livers.[106] Further, in our previous study demonstrating MHC-induced hepatic lipid deposition and reduced lipogenic mRNA expression in fetal livers discussed above, we have also observed altered hepatic miR-27a and miR200c expression.[80] While a direct link between increased expression of fetal miR27a and target gene FAS in response to MHC cannot be made at this time, changes to fetal hepatic miR expression in MHC warrant further consideration. Despite limited evidence, it appears elevated maternal cholesterol can alter offspring epigenetic signatures that can originate *in utero* and persist into adulthood.

Summary and conclusions

Maternal cholesterol status during pregnancy may have profound consequences to offspring metabolic health. Although cholesterol has been shown to be intricately linked with NAFLD development and progression, the animal model studies presented in this review demonstrate that excessive perinatal cholesterol exposure during critical developmental periods can predispose offspring to early-life development of NAFLD which can persist throughout the life cycle. However, we are not aware of any human study that has examined this link. Therefore, a critical next step will be to explore if these associations can explain the alarming rise in pediatric NAFLD in a human pregnancy cohort. Beyond this important translation step, mechanistic studies are required to more fully tease out the mechanisms (epigenetic modifications and otherwise), the potential sex-divergent offspring effects, and how MHC *per se* may interact with other maternal conditions including maternal obesity and gestational diabetes.

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