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# Influence of Maternal Hypercholesterolemia and Phytosterol Intervention During Gestation and Lactation on Dyslipidemia and Hepatic Lipid Metabolism in Offspring of Syrian Golden Hamsters

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## Abstract

Although there is a normal physiological rise in maternal lipids during pregnancy, excessive maternal hyperlipidemia during pregnancy increases cardiovascular disease risk for both the mother and offspring. There are limited safe lipid-lowering treatment options for use during pregnancy, therefore, we evaluated the influence of maternal phytosterol (PS) supplementation on lipid and lipoprotein metabolism in mothers and progeny. Female Syrian golden hamsters were randomly assigned to three diets throughout pre-pregnancy, gestation, and lactation (n=6/group): (i) Chow (Chow), (ii) chow with 0.5% cholesterol (CH), and (iii) chow with 0.5% cholesterol and 2% PS (CH/PS). Compared with newly-weaned pups from Chow dams, pups from dams fed the cholesterol-enriched diet demonstrated increases ($p$<0.05) in total-C, LDL-C, HDL-C, and total LDL and VLDL particle number. Pups from cholesterol-fed mothers also exhibited higher hepatic cholesterol concentration and differential mRNA expression pattern of cholesterol regulatory genes. Pups from PS-supplemented dams demonstrated reductions ($p$<0.05) in serum total-C, non-HDL-C, and LDL-C but also increased triglycerides compared with pups from CH-fed dams. Maternal PS supplementation reduced (p<0.05) hepatic cholesterol and increased the abundance of HMG-CoAr and LDLr protein in newly-weaned pups compared with the CH group. Results suggest that maternal PS supplementation is largely effective in normalizing cholesterol in pups

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**Author Contributions**

JL, AI, and AR assisted with the animal trial and lab analysis; RWB assisted with serum lipid and tissue analysis; MSP assisted with experimental design and data interpretation; TCR designed the experiment, interpreted the data, and wrote the initial manuscript draft. All authors read and approved the final manuscript.

**Conflicts of Interest Statement**

None

born to mothers with hypercholesterolemia, however, the cause and long-term influence of increased TG is not known.

**Keywords**

Maternal hypercholesterolemia; phytosterols; dyslipidemia; offspring

## Introduction

The tremendous impact of cardiovascular diseases (CVD) on the health and well-being of Americans cannot be over-emphasized as it presents major challenges to society due to increased disability, death, and a substantial financial burden on the health-care system. CVD is largely preventable as >75% of cases are attributed to preventable risk factors including dyslipidemia, smoking, physical inactivity, obesity, diabetes, and high blood pressure. As an established CVD risk factor, blood cholesterol levels are particularly troublesome as greater than 100 million US adults have total blood cholesterol levels that exceed the desirable target of ≤200 mg/dl [1].

Adding to the urgency of CVD as a global health issue is the rise in dyslipidemic risk factors amongst women of childbearing age, particularly hypertriglyceridemia and hypercholesterolemia. According to the Centers for Disease Control, 9.4% of females of childbearing age (20–44) have elevated total cholesterol (>240 mg/dl) [2] that is likely associated with numerous contributing factors including diet-induced obesity and underlying genetic influences including familial hypercholesterolemia (FH) [3]. Fetal exposure to excessive fat and cholesterol during pregnancy has been shown to increase fetal plasma cholesterol concentrations and aortic fatty streak formation and predispose adult offspring to diet-induced obesity, hyperlipidemia, and arterial plaque development [4, 5]. Women with FH who wish to become pregnant are advised to cease the use of lipid lowering medications (statins, ezetimbe, niacin) at least 4 weeks prior to discontinuing contraception [3]. The resulting increase in the absolute plasma cholesterol concentration, due to the cessation of medication as well as the typical increase in plasma total and LDL-cholesterol that is observed during a normal pregnancy (+25–70%), puts women with FH and their offspring at substantial risk of CVD [6, 7]. Therefore, limitations in acceptable therapeutic options result in a treatment dilemma and an acknowledged increase in CVD risk for dyslipidemic mothers.

Phytosterols (PS) are plant-based bioactive compounds that carry a health claim by the US Food and Drug Administration and are recommended by the National Cholesterol Education Program at a dose of 2 g/d for cholesterol lowering and CVD prevention. Over 50 years of cell culture, animal model, and human clinical investigations have repeatedly demonstrated the ability of PS to reduce cholesterol by up to 15% by limiting intestinal cholesterol absorption [8]. Furthermore, recent data suggest that PS may also be lower blood triglyceride (TG) concentrations through multiple mechanisms including interruption of intestinal fatty acid absorption [9] and modulation of hepatic lipogenesis and VLDL packaging and secretion [10].

Although a number of pre-clinical studies [11–13] and a human investigation [14] have suggested that PS supplementation during pregnancy is safe, the potential application of PS as a cholesterol-lowering therapy for use in diet-induced hypercholesterolemic pregnancies has not yet been investigated. Therefore, this study was undertaken with two aims: 1) to characterize the effect of maternal cholesterol feeding on blood lipid/lipoprotein responses and hepatic cholesterol metabolism in dams and their newly-weaned offspring and 2) to establish if maternal PS supplementation to a cholesterol-enriched diet could modulate these responses. We used the Syrian Golden hamster as it is an established model of human lipid and lipoprotein metabolism, is highly susceptible to diet-induced hypercholesterolemia, and demonstrates a well-characterized cholesterol-lowering responses to dietary PS supplementation.

## Materials and Methods

### Animals and Diets

3 month-old female (n=18) and male (n=18) Syrian golden hamsters were purchased from Harlan Laboratories (Indianapolis, Indiana). Animals were housed in the Animal Care Facility at the University at Buffalo in a temperature-controlled room (20°C, 12h light/dark cycle) in individual cages with shavings and enrichment housing with free access to water. For the following 2 week pre-pregnancy period, the animals (n=6/group) were randomly assigned to 1 of 3 standard chow-based diets (Teklad 2019, 19% protein, 51% carbohydrate, 8% fat): (i) chow only (Chow), (ii) chow with 0.5% cholesterol (CH), and (iii) chow with 0.5% cholesterol and 2% PS (CH/PS, PS sourced from Forbs Medi-Tech Corp, Kearny, NJ). All diets were prepared by Envigo (Madison, WI) (Suppl. Table 1). As a percent of total sterols, in-house analysis confirmed that the PS supplement was composed largely of β-sitosterol (>75%) with minor amounts of sitostanol (<15%) and campesterol (<7%). Following the 2-week pre-pregnancy period, females were mated with male breeders (1 male per female) and returned to their individual cages following confirmation of pregnancy based on daily weight gain. Following parturition, dams and pups were left undisturbed until postnatal (PN) day 5 as dams are prone to cannibalism of their young in the first few days after birth if disturbed. On PN day 5, litters were culled to 6 pups per dam by random selection to minimize variability in postnatal pup development influenced by litter size [15]. Throughout the suckling period the dams remained on their respective diets. To prevent the pups from consuming the diet as they matured, food pellets were provided on raised caged platforms that were only accessible by the mother. At weaning (d21), the dams and pups were anesthetized with isoflurane for blood collection. Fasting (15 h) blood (serum) was collected by cardiac puncture and stored at −80°C until further processing and analyses. The animals used in this experiment were cared for in accordance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC). All procedures were reviewed and approved by the Animal Care Committee at the University at Buffalo (protocol # PTE16082N).

### Serum Biochemistry

Serum cholesterol panel (total-C, HDL-C, and direct LDL-C) was measured by direct automated enzymatic assay while lipoprotein particle number and size was conducted by

nuclear magnetic resonance spectroscopy (Liposcience, Raleigh, NC) [16]. Non-HDL cholesterol was calculated by subtracting HDL-C from the total cholesterol fraction. Serum activity of cholesterol-ester-transfer protein (CETP) in offspring was measured with a commercial kit fluorometric assay kit (Kamiya, KT-782).

PS (from diet and serum samples) and hepatic cholesterol were extracted and analyzed according to our previously published procedures [17, 18]. Approximately 0.5 mL of serum or 0.5 g of pulverized liver or diet 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 $N_2$ 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 × 0·25mm × 0·25 mm, Supelco, Bellefonte, CA).

## Hepatic lipids

Hepatic TG were analyzed with a commercial kit (ab65336, Abcam, Cambridge, MA, USA). Hepatic total fat was extracted by homogenization in aqueous Triton-X buffer (2%) and measured at $OD_{570nm}$ according to manufacturer instructions.

## RNA preparation and real-time RT-PCR

Total RNA was isolated from whole liver tissue using TRIzol reagent (Invitrogen Inc., Grand Island, NY). RNA concentration and integrity was determined with spectrophotometry (260 nm) and agarose gel electrophoresis, respectively. RNA preparation and real-time RT-PCR was conducted using a one-step QuantiFast SYBR Green RT-PCR kit (Qiagen Inc., Valencia, CA) on a Biorad MyiQ real time PCR system according to previously established protocols [19]. Gene expression was analyzed using the 2(-delta delta Ct) method [20]. Sequences of sense and antisense primers for target and housekeeping genes were based on previously published reports for β-actin [21], ABCA1, ABCG1, ABCG5, ABCG8 [22], HMG-CoAr, LDLr, LXR, PCSK9, SREBP2, SREBP1C, ACC, FAS, CPT1 [23], PPARα [24], MTTP, DGAT [25], CD36 [26], and FABP2 [27], and CETP [28].

Hepatic total protein and nuclear/cytoplasmic fractions were prepared and extracts were probed for the abundance of target proteins with commercial antibodies for HMG-CoAr (sc-27578, Santa Cruz Biotechnology), LDLr (ab30532, abcam), and SREBP2 (ab30682, abcam) according to previously published procedures [29]. Target proteins were normalized to b-actin and quantified using Image lab (version 4.1, Biorad Laboratories, Hercules, CA).

## Statistical analyses

Litters from each female were considered as a single observation. Data were analyzed with a general linear model ANOVA and multiple comparisons between treatment groups were analyzed with a Bonferonni post-hoc test [30]. Data were analyzed with SPSS 16 for Mac (SPSS Inc, Chicago, IL). Data are presented as mean ± SEM. Differences were considered significant at $p \leq 0.05$.

# Results

## Maternal response

Maternal feed intake and body weight between the treatment groups did not differ ($p>0.05$) throughout the pre-pregnancy period, gestation, and lactation. Maternal cholesterol feeding (CH) during the 2-wk pre-pregnancy phase increased ($p<0.05$) serum total cholesterol (+178% compared with Chow), however dietary PS supplementation prevented this hypercholesterolemic response (−62% compared with the CH group, Fig 1a). No difference ($p>0.05$) was observed in pre-pregnancy serum TG concentrations between the treatment groups (Fig 1b).

Maternal cholesterol feeding throughout pre-pregnancy, gestation, and lactation increased ($p<0.05$) in total-C (+316%), non-HDL cholesterol (+1135%), HDL-C (116%), and TG (+112%) compared with the chow fed mothers (Fig 2a). Furthermore, dams consuming the CH diet displayed an increased the total-C:HDL-C ratio (+103%) compared with the chow-fed dams. Supplementation of the maternal diet with PS (CH/PS) reduced total-C (-71%), non-HDL cholesterol (−83%), HDL-C (−50%), TG (−36%), and the total-C:HDL-C ratio (−40%) compared with the CH diet. Maternal cholesterol feeding also increased ($p<0.05$) total LDL (+2661%) and VLDL particles (+3678%) compared with the chow group, however, supplementation of PS largely protected ($p<0.05$) against this response by reducing total LDL (−87%) and VLDL particle number (−89%) compared with the CH group (Fig 2b). No difference ($p>0.05$) in HDL particle number (Fig 2b) was observed between the treatment groups. Although dietary treatment did no influence ($p>0.05$) LDL and HDL particle size, CH-fed dams demonstrated a reduction ($p<0.05$) in VLDL size compared with CH dams. Maternal PS-supplementation ($p<0.05$) increased VLDL size in relation to the CH-fed moms, however, not to chow levels (Fig 2c).

Compared with the chow-fed dams, cholesterol feeding increased ($p<0.05$) hepatic cholesterol concentrations, however, supplementation with PS normalized this response (Fig 3a). Hepatic TG concentrations were lower ($p<0.05$) in the CH and PS-supplemented dams compared with chow (Fig 3b).

## Pup response

Litter size on PN day 0 and the fraction of males and females per litter (40–60%) were not affected ($p>0.05$) by maternal diet. Litter weights on PN day 5 did not differ with respect to maternal diet, however, litters from the CH and CH/PS dams weighed less (~13%, $p<0.05$) than litters from the chow-fed dams on PN day 10. Litter weights by PN day 20 were similar ($p>0.05$) among the treatment groups.

Compared with pups from chow-fed dams, pups from dams fed the cholesterol-enriched diet displayed increased ($p<0.05$) total-C (+68%), non-HDL-C (+123%), LDL-C (+154%), HDL-C (30%) but no change ($p>0.05$) in TG on PN day 21 (Fig 4a). The total-C:HDL-C ratio increased (+29%, $p<0.05$) in pups from cholesterol-fed dams compared with chow pups. Pups from PS-supplemented dams exhibited reductions ($p<0.05$) in total-C (−26%), non-HDL-C (−32%), LDL-C (−29%), and HDL-C (−19%), but increased TG (+62%,

$p$=0.05) compared with pups from cholesterol-supplemented dams (Fig 4a). The total-C:HDL-C ratio decreased (−9%, $p$<0.05) in the CH/PS pups compared with the CH pups.

Although increases ($p$<0.05) were observed in total LDL particle number (+216%) and VLDL particle number (+254%) in pups from dams consuming the cholesterol-enriched diet compared with pups from chow-fed dams, maternal PS-supplementation was not effective ($p$>0.05) in ameliorating this response (Fig 4b). Total HDL particle number did not differ ($p$>0.05) among the treatment groups (Fig 4b). Maternal diet did not alter ($p$>0.05) LDL, HDL or VLDL particle size in pups (Fig 4c). Serum activity of CETP (pmol/μL/hr) was measured as a potential contributor to the observed lipoprotein responses, however, activity measurements did not different (p>0.05) between the Chow (33.2±9.0), CH (32.3±12.1), and CH/PS (37.3±14.5) groups.

Hepatic cholesterol concentration was increased ($p$<0.05) in pups reared from CH-fed mothers compared with chow-fed dams but was normalized ($p$<0.05) to chow levels in pups from mothers supplemented with PS (Fig 5a). No difference ($p$<0.05) in hepatic TG concentrations was noted between pups in any treatment group (Fig 5b).

The expression of a host of hepatic cholesterol and TG regulatory targets were examined in pups from each treatment group (Table 1). In comparison with the chow group, pups from cholesterol-supplemented dams displayed a differential mRNA expression pattern with increased ($p$<0.05) expression of ABCG1 (1.6 fold) and LDLr (1.6 fold) and decreased expression in a number of regulatory targets including LXR (0.60 fold), PCSK9 (0.56 fold), SREBP2 (0.43 fold), CPT1 (0.60 fold), DGAT (0.61 fold), and CETP (0.43 fold). Surprisingly, no differences were noted in the mRNA expression patterns between the CH and CH/PS groups.

Hepatic nuclear SREBP2 protein expression did not differ between the treatment groups (Fig 6a). Compared with chow pups, pups from CH-fed dams demonstrated increased protein expression of both the LDLr (~2 fold, Fig 6b) and HMG-CoAr (~1.8 fold, Fig 6c) that was normalized to chow levels in pups from PS-supplemented dams.

## Discussion

We noted several novel findings that warrant further discussion. First, newly-weaned offspring from dams with diet-induced hypercholesterolemia throughout pregnancy and lactation were dyslipidemic compared with pups from chow-fed mothers. Second, maternal PS supplementation to a cholesterol-enriched diet during pre-pregnancy and throughout gestation/lactation protected pups against adverse blood and hepatic cholesterol profiles but also triggered an unexpected increase in serum TG concentrations compared with pups from cholesterol-fed mothers. Finally, pups from cholesterol-fed dams demonstrated differential mRNA and protein expression patterns of hepatic lipid regulators with a notable increase in the protein abundance of the LDLr and HMG-CoA reductase that was normalized by maternal PS-supplementation.

Serum lipid and lipoprotein profiles in children have been shown to be predictive of those in adulthood [31–33] and there is strong evidence to suggest that fetal and neonatal lipid status

is influenced by that of the mother [34–36]. In a human study with aborted fetuses and premature newborns who died shortly after birth, Napoli et al. (1997) reported a strong association between fetal (<6 months) and maternal serum cholesterol and observed an increased number of oxidized LDL-containing lesions in fetal aortas from hypercholesterolemic versus normal cholesterolemic mothers [37]. Similarly, animal studies support an association between maternal cholesterol status and serum cholesterol levels in offspring [38, 39]. Burke et al. (2009) reported increased placental LDL-C fetal transfer in hypercholesterolemia dams compared with control dams [38]. Our results support the relationship between maternal and offspring cholesterol in that pups from cholesterol-supplemented dams displayed increase cholesterol in all serum lipoprotein fractions at weaning compared with pups from chow-fed dams. Moreover, offspring from cholesterol-fed dams exhibited an increased number of total LDL and VLDL particles compared with chow offspring, an important observation as very few studies have accessed how early exposure to cholesterol influences advanced lipoprotein measures even though these endpoints are considered valuable CVD predictive biomarkers [40].

Additionally, pups from cholesterol-fed mothers also had higher hepatic cholesterol concentrations compared with chow pups. Although data on cholesterol-balance in newly-weaned offspring is limited, a number of studies have shown that maternal cholesterol feeding is associated with increased cholesterol deposition in hamster fetal tissues (placenta, yolk sac) [38, 41] and in rabbit liver upon birth [42]. Considering the importance of the liver in regulating whole-body cholesterol metabolism in neonates [43], it stands to reason that this dramatic increase in liver cholesterol deposition in pups from cholesterol-fed dams was also associated with a differential mRNA expression pattern of hepatic cholesterol-regulators that included a decrease in SREPB2 mRNA compared with chow-pups. Despite this change, SREBP2 target gene expression did not follow a predicable pattern as evidenced by a reduction in PCSK9 mRNA, an increase in LDLr mRNA, and no change in HMG-CoAr mRNA expression compared with chow pups. Furthermore, the protein abundance of LDLr and HMG-CoA reductase was increased in pups from cholesterol-fed dams compared with chow-pups. Although this apparent increase in hepatic cholesterol uptake and synthesis may partially underlie the dyslipidemic response we observed in the CH offspring at weaning, it contradicts previous work suggesting that fetal sterol synthesis rates are reduced in response to maternal cholesterol feeding in hamsters [41]. However, it has been reported that maternal cholesterol feeding can program an enhanced transcriptional capacity of cholesterol synthesis genes as well as the LDLr in adult apoE offspring [44].

Similar to the results of our previous study in apoE mice [45], maternal PS supplementation was effecting in protecting pups hypercholesterolemia induced by maternal cholesterol feeding. A number of mechanisms may have contributed to this protective effect. First, by reducing hypercholesterolemia in mothers, maternal PS supplementation may have indirectly impeded excessive maternal cholesterol transfer to the offspring *in utero*. Although the developing fetus has a capacity for cholesterol synthesis, it also obtains a proportion of its cholesterol from the maternal circulation [46]. The yolk sac and placenta express a variety of receptors, including the low-density-lipoprotein receptor (LDLr) and scavenger receptor class B type 1 (SR-B1), that mediate maternal lipid and liproprotein transfer across the apical and basolaterol membranes of trophoblasts and endothelial cells

into the fetal circulation [47, 48]. Secondly, early exposure to PS during fetal development and/or through postnatal milk consumption could have directly impacted cholesterol homeostasis in offspring. Maternal PS supplementation during lactation has previously been shown to increase PS concentrations in maternal milk and result in increased circulating PS levels in infants [49]. We detected 2-fold increase in serum β-sitosterol concentrations in offspring from PS-fed dams compared with pups from the CH group (data not presented). Enhanced circulating levels of PS have been shown to directly influence the expression of cholesterol-regulatory genes, possibly by acting as ligands for liver X receptor (LXR) in the liver and peripheral tissues [50, 51]. Although we noted few differences in the transcriptional control of genes that regulate cholesterol and/or TG metabolism in PS versus CH pups, we did observe a normalization in the protein abundance of the LDLr and HMG-CoA reductase which may be associated with the protective effect of maternal PS supplementation.

Although maternal PS-supplementation protected against hypercholesterolemia in pups, we also observed an unexpected rise in serum TG compared with pups born to unsupplemented mothers. This is particularly surprising given that PS supplementation reduced serum TG (Fig 2a) and VLDL particle number (Fig 2b) in the mothers, a lesser-known effect of PS that is receiving significant research attention [52–54]. Although the cause of this TG increase is not known, we previously reported an increase in hepatic *de novo* lipogenesis in PS-fed C57BL6 mice that we interpreted to be a compensatory response to interference with intestinal fatty acid absorption by PS [17]. Therefore, it is possible that by reducing maternal TG status, PS supplementation interrupted the transfer of TG to the offspring during prenatal development or altered the TG level and/or fatty acid composition of the milk. If this is the case, the offspring may have compensated for this reduced maternal transfer with increased hepatic TG synthesis. The high demand for fatty acids to support early growth and postnatal development has been shown to derive from both maternal lipid stores and also *de novo* synthesis in the fetus and neonate [55]. However, we did not detect any change in the mRNA expression of hepatic lipid synthesis genes between the treatment groups. Regardless of the mechanism, the implications of this early increase in serum TG and whether this response causes more permanent adaptations in TG metabolism in adulthood must be investigated.

In summary, we report that newly-weaned offspring born to mothers with elevated cholesterol display a dyslipidemic plasma lipid and lipoprotein profile and increased hepatic cholesterol infiltration with a differential expression of cholesterol regulatory targets. Maternal PS-supplementation was effective in normalizing hypercholesterolemia in these newly-weaned offspring but also exacerbated serum TG concentrations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## List of Abbreviations

| | |
|---|---|
| **PS** | phytosterols |
| **CH** | cholesterol group |
| **CH/PS** | cholesterol/phytosterol group |
| **CVD** | cardiovascular disease |
| **TG** | triglyceride |
| **FH** | familial hypercholesterolemia |

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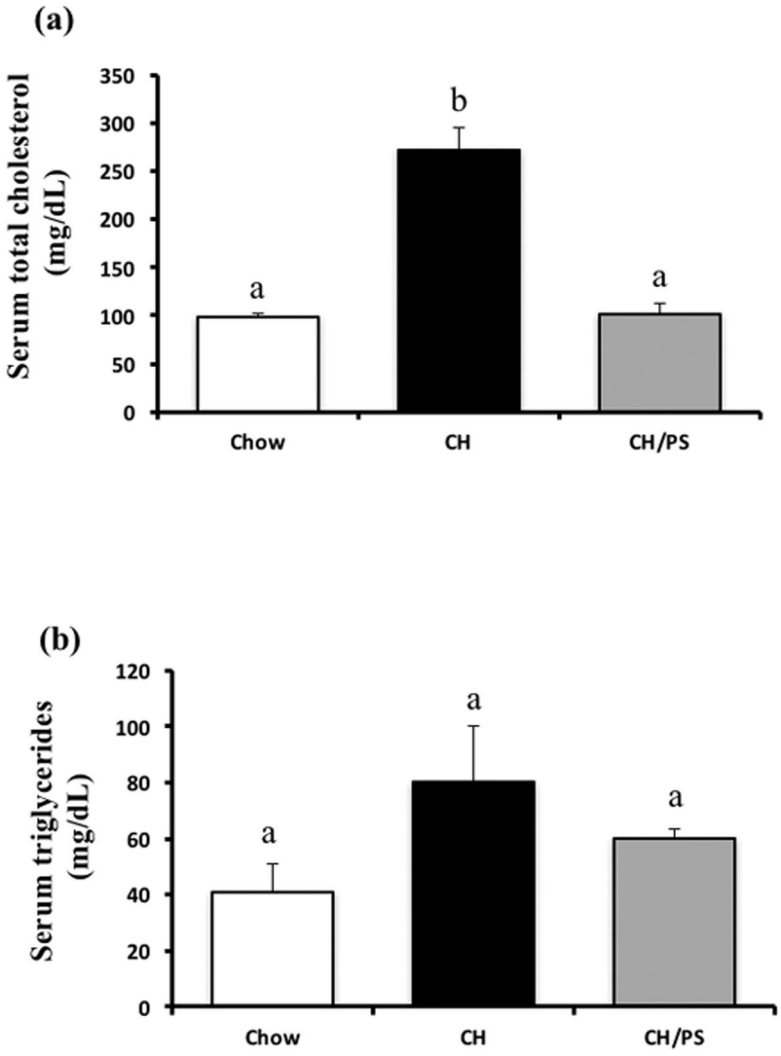


**Figure 1.**

Serum total cholesterol (a) and triglycerides (b) at the end of the pre-pregnancy period in dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS). [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

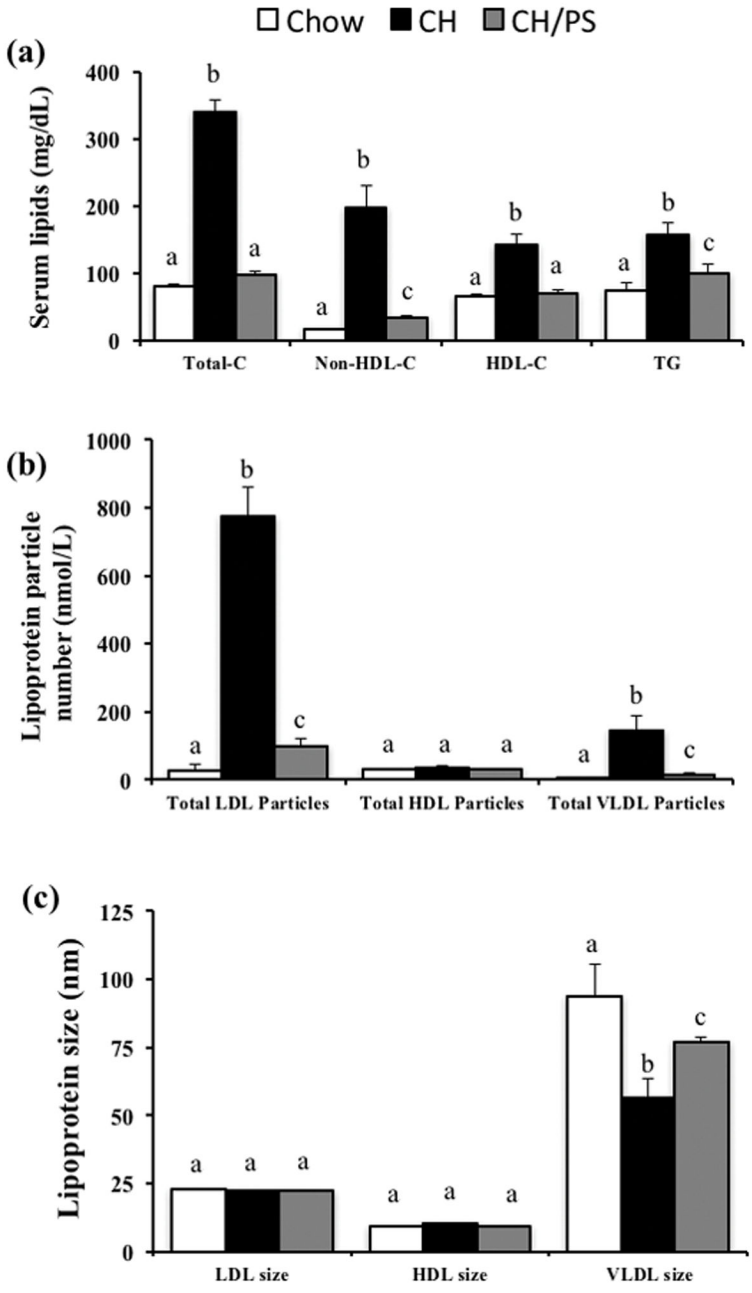


**Figure 2.**

Serum lipids (a), lipoprotein particle number (b), and lipoprotein size (c) in dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during the pre-pregnancy period and throughout gestation and lactation. Total-C, total cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and TG, triglycerides. [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

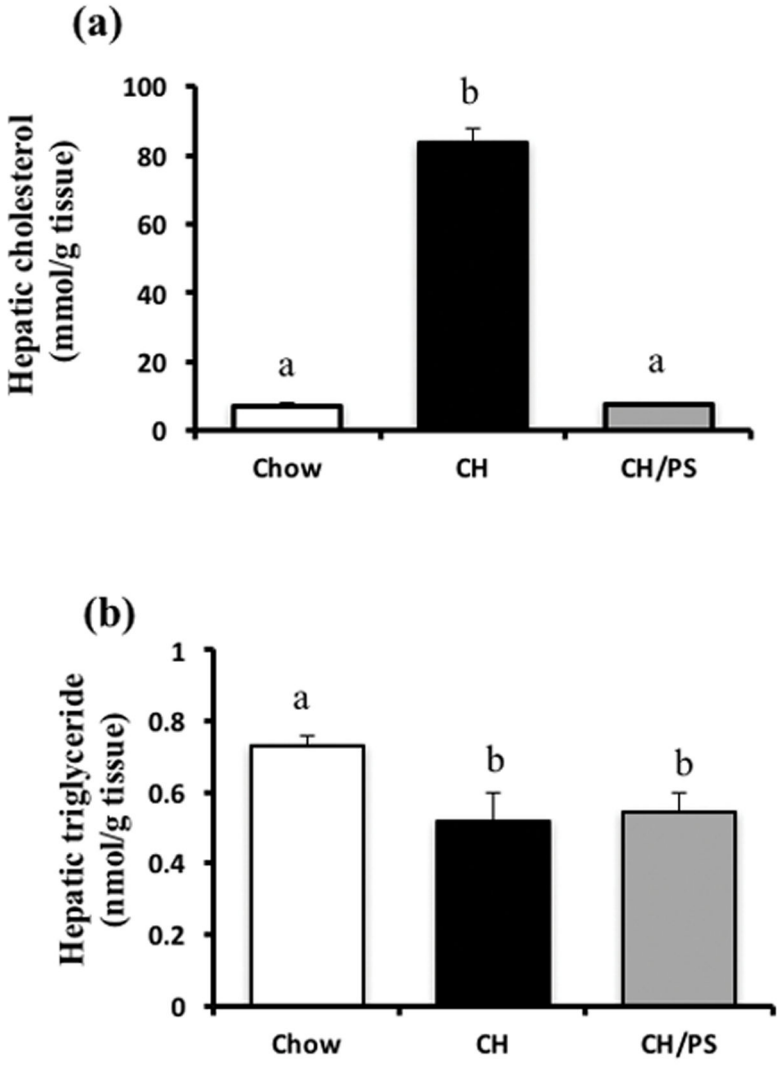


**Figure 3.**

Hepatic concentration of cholesterol (a) and triglycerides (b) in dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during the pre-pregnancy period and throughout gestation and lactation. [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

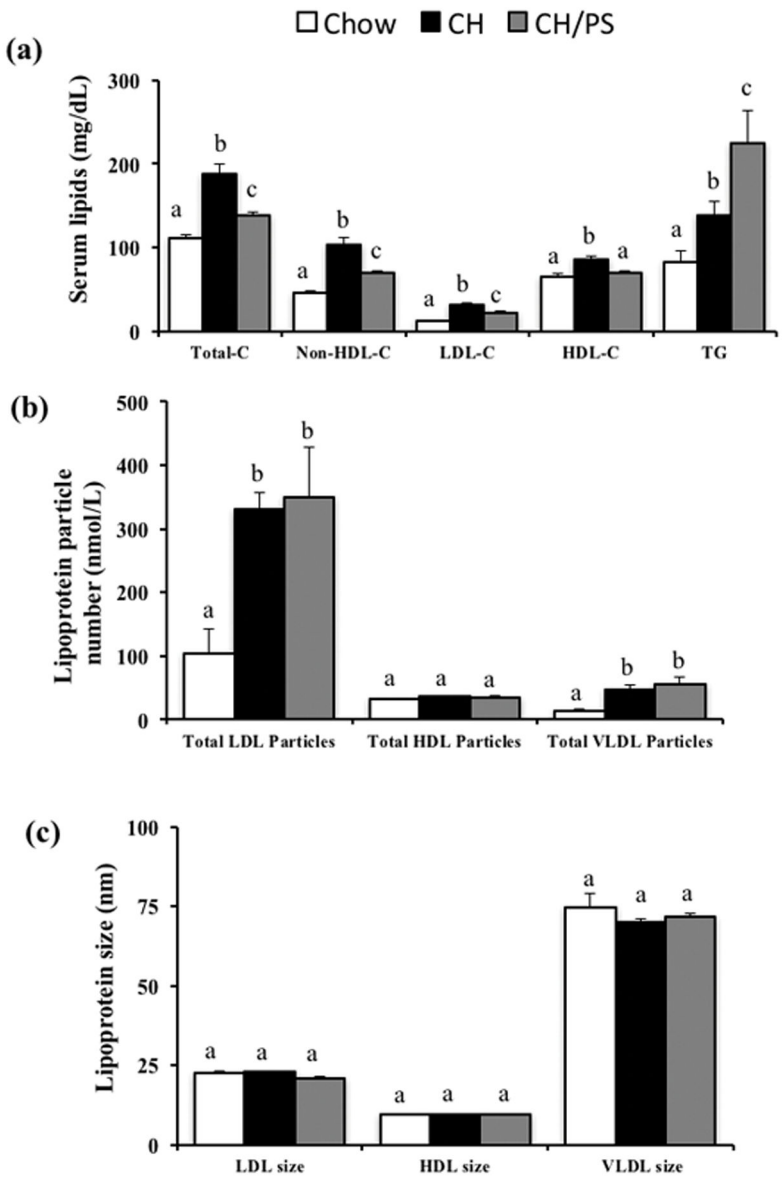


**Figure 4.**

Serum lipids (a), lipoprotein particle number (b), and (c) lipoprotein size in 21 day old offspring from dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during a 14 day pre-pregnancy period and throughout gestation and lactation. Total-C, total cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and TG, triglycerides. [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

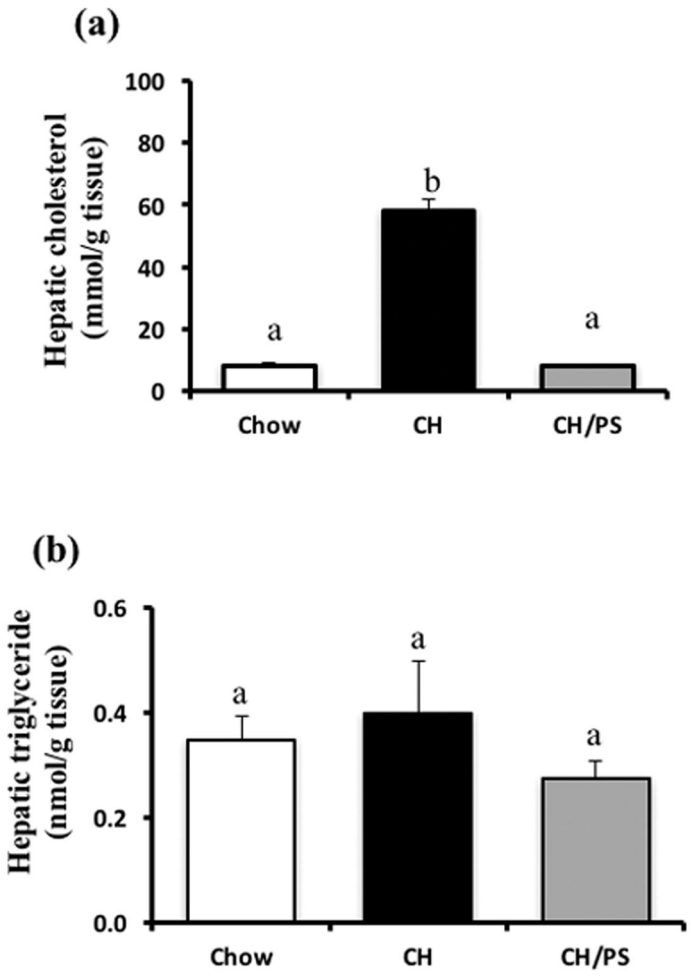


**Figure 5.**

Hepatic concentration of cholesterol (a) and triglycerides (b) in 21 day old offspring from dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during the pre-pregnancy period and throughout gestation and lactation. [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

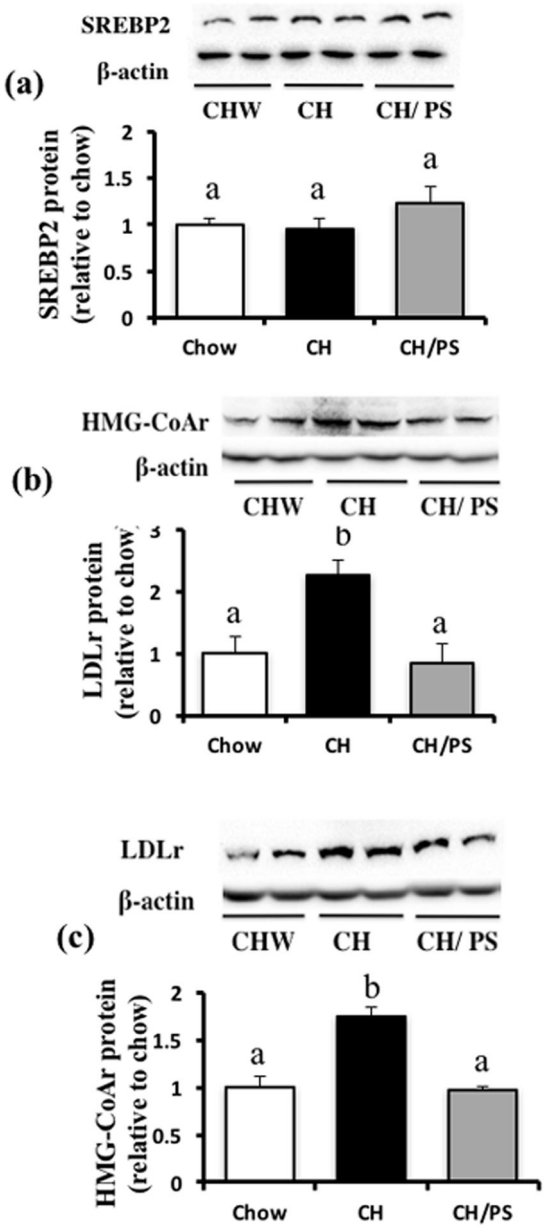


**Figure 6.**

Hepatic protein expression of cholesterol regulatory targets in 21 day-old offspring from dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during the pre-pregnancy period and throughout gestation and lactation. (a) SREBP2 in nuclear extract, (b) HMG-CoAr in total tissue fraction, (c) LDLr in total tissue fraction. [ab]Groups not sharing a superscript are significantly different ($p<0.05$).

## Table 1

Hepatic mRNA expression of cholesterol and fatty acid/triglyceride regulatory targets in offspring from dams fed a chow diet (chow) or chow diet supplemented with cholesterol (CH) or cholesterol and phytosterols (CH/PS) during a 14-day pre-pregnancy period and throughout gestation and lactation.

| Gene | Chow | CH | CH/PS |
|---|---|---|---|
| *Cholesterol regulatory targets* | | | |
| ABCA1 | 1.0 ± 0.14[a] | 0.76 ± 0.18[a] | 0.95 ± 0.17[a] |
| ABCG1 | 1.0 ± 0.08[a] | 1.68 ± 0.28[b] | 1.09 ± 0.12[ab] |
| ABCG5 | 1.0 ± 0.25[a] | 0.82 ± 0.13[a] | 1.16 ± 0.20[a] |
| ABCG8 | 1.0 ± 0.16[a] | 1.05 ± 0.32[a] | 1.18 ± 0.26[a] |
| HMG-CoAr | 1.0 ± 0.13[a] | 1.54 ± 0.54[a] | 1.42 ± 0.16[a] |
| LDLr | 1.0 ± 0.16[a] | 1.60 ± 0.14[b] | 1.48 ± 0.13[b] |
| LXR | 1.0 ± 0.11[a] | 0.60 ± 0.04[b] | 0.79 ± 0.10[ab] |
| PCSK9 | 1.0 ± 0.12[a] | 0.56 ± 0.12[b] | 0.63 ± 0.56[b] |
| SREBP2 | 1.0 ± 0.15[a] | 0.43 ± 0.04[b] | 0.52 ± 0.09[b] |
| CETP | 1.0 ± 0.14[a] | 0.43 ± 0.03[b] | 0.42 ± 0.06[b] |
| *Fatty acid/triglyceride regulatory targets* | | | |
| CD36 | 1.0 ± 0.26[a] | 1.06 ± 0.27[a] | 0.72 ± 0.08[a] |
| FABP2 | 1.0 ± 0.15[a] | 0.98 ± 0.21[a] | 1.10 ± 0.18[a] |
| SREBP1C | 1.0 ± 0.13[a] | 0.96 ± 0.12[a] | 0.78 ± 0.09[a] |
| PPARα | 1.0 ± 0.12[a] | 0.72 ± 0.08[a] | 0.65 ± 0.11[a] |
| ACC | 1.0 ± 0.23[a] | 0.64 ±0.03[a] | 0.91 ± 0.17[a] |
| FAS | 1.0 ± 0.14[a] | 0.75 ± 0.13[a] | 0.74 ± 0.19[a] |
| CPT1 | 1.0 ± 0.05[a] | 0.60 ± 0.08[b] | 0.67 ±0.08[b] |
| MTTP | 1.0 ± 0.15[a] | 0.65 ± 0.08[a] | 0.64 ± 0.14[a] |
| DGAT | 1.0 ± 0.09[a] | 0.61 ± 0.11[b] | 0.63 ± 0.09[b] |