



HAL
open science

Gestational cholestyramine treatment protects adult offspring of ApoE -deficient mice against maternal-hypercholesterolemia-induced atherosclerosis

Marina Habib, Mikael Croyal, Bertrand Kaeffer, Isabelle I. Grit, Blandine Castellano, Mathilde Gourdel, Cédric Le May, Chantal Thorin, Hassan Nazih, Khadija Ouguerram

► To cite this version:

Marina Habib, Mikael Croyal, Bertrand Kaeffer, Isabelle I. Grit, Blandine Castellano, et al.. Gestational cholestyramine treatment protects adult offspring of ApoE -deficient mice against maternal-hypercholesterolemia-induced atherosclerosis. *Acta Physiologica*, 2024, 240 (5), pp.e14133. <10.1111/apha.14133>. <hal-04797680>

HAL Id: hal-04797680

<https://hal.science/hal-04797680v1>

Submitted on 24 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.


L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

RESEARCH PAPER

Gestational cholestyramine treatment protects adult offspring of ApoE-deficient mice against maternal-hypercholesterolemia-induced atherosclerosis

Marina Habib¹  | Mikael Croyal^{2,3,4} | Bertrand Kaeffer¹ | Isabelle Grit¹ | Blandine Castellano¹ | Mathilde Gourdel³ | Cédric Le May⁴ | Chantal Thorin⁵ | Hassan Nazih⁶ | Khadija Ouguerram¹

¹UMR1280 Pathophysiology of Nutritional Adaptations, Nantes Université, INRAE, Nantes, France

²Mass Spectrometry Core Facility, CRNH-Ouest, Nantes, France

³Institut du thorax, Nantes Université, CNRS, INSERM, Nantes, France

⁴UMS 016, UMS 3556, Nantes Université, Inserm, CNRS, Nantes, France

⁵UMR0703 PAnTher, École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation, Nantes, France

⁶UR2160 ISOMer, Nantes Université, Nantes, France

Correspondence

Khadija Ouguerram, Nantes Université, UMR 1280 PHAN, CHU Hôtel Dieu, Place Alexis Ricordeau, 44093 Nantes, France.
Email: khadija.ouguerram@univ-nantes.fr

Funding information

Departement Alimentation Humaine, Institut National de la Recherche Agronomique Conseil Régional des Pays de la Loire

Abstract

Aim: Perinatal hypercholesterolemia exacerbates the development of atherosclerotic plaques in adult offspring. Here, we aimed to study the effect of maternal treatment with cholestyramine, a lipid-lowering drug, on atherosclerosis development in adult offspring of hypercholesterolemic ApoE-deficient (ApoE^{-/-}) mice.

Methods: ApoE^{-/-} mice were treated with 3% cholestyramine (CTY) during gestation (G). After weaning, offspring (CTY-G) were fed control diet until sacrificed at 25 weeks of age. Atherosclerosis development in the aortic root of offspring was assessed after oil-red-o staining, along with some of predefined atherosclerosis regulators such as LDL and HDL by high-performance liquid chromatography (HPLC), and bile acids (BA) and trimethylamine N-oxide (TMAO) by liquid chromatography-mass spectrometry (LC-MS/MS).

Results: In pregnant dams, cholestyramine treatment resulted in significantly lower plasma total- and LDL-cholesterol as well as gallbladder total BA levels. In offspring, both males and females born to treated dams displayed reduced atherosclerotic plaques areas along with less lipid deposition in the aortic root. No significant change in plasma total cholesterol or triglycerides was measured in offspring, but CTY-G males had increased HDL-cholesterol and decreased apolipoproteins B100 to A-I ratio. This latter group also showed reduced gallbladder total and specifically tauro-conjugated bile acid pools, whereas for CTY-G females, hydrophilic plasma tauro-conjugated BA pool was significantly higher. They also benefited from lower plasma TMAO.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. *Acta Physiologica* published by John Wiley & Sons Ltd on behalf of Scandinavian Physiological Society.

Conclusion: Prenatal cholestyramine treatment reduces atherosclerosis development in adult offspring of ApoE^{-/-} mice along with modulating the plaques' composition as well as some related biomarkers such as HDL-C, bile acids and TMAO.

KEYWORDS

atherosclerosis, cholestyramine, hypercholesterolemia, perinatal

1 | INTRODUCTION

Despite the progress made in the treatment of cardiovascular diseases (CVDs), they have remained the leading cause of death worldwide.¹ Atherosclerosis (ATS) is a progressive complex vascular disease leading to CVDs. It launches with the accumulation of lipid-laden macrophages which maintain a local inflammatory response in the arterial wall.² High plasma cholesterol level is a major risk factor for atherosclerosis and specifically, ApoB-containing low-density lipoproteins (LDL)-cholesterol,² whereas ApoA-I-rich high-density lipoproteins (HDL) play an anti-atherogenic role. These latter mediate the reverse cholesterol transport from the peripheral tissues to the liver for its elimination, essentially after its conversion into bile acids (BA) and conjugation to taurine or glycine.³ Of note, distinct conjugated bile acids display various biological activities.⁴

Both epidemiological and experimental studies evidenced the link between pathological events during fetal life and the development of atherosclerosis-related diseases later in life.⁵⁻⁸ In this respect, Napoli et al. firstly reported larger atherosclerotic plaques,⁹ with faster progression of lesions in offspring born to hypercholesterolemic mothers.¹⁰ Then, different animal models of genetic and/or nutritional hypercholesterolemia underpinned this observation.¹¹⁻¹³ Moreover, maternal hypercholesterolemia exposes the fetus to high levels of cholesterol, which is accompanied by an important oxidative stress due to lipid peroxidation.¹⁴ Thus, potential underlying mechanisms of early atherosclerosis programming by maternal hypercholesterolemia include placental and fetal endothelial dysfunction due to increased oxidative stress,^{15,16} epigenetic programming¹⁷ and dysregulation of the expression of aortic^{12,18} and cholesterol metabolism-related genes.¹⁹ In ApoE^{-/-} mouse model, we highlighted²⁰ a positive correlation between aortic root lesion area and plasma trimethylamine-N-oxide (TMAO) concentration, a biomarker of atherosclerosis originating from gut bacteria metabolism,²¹ as well as a modulation of gallbladder bile acid pool, a regulator of reverse cholesterol transport.³

In parallel, maternal cholesterol-lowering and antioxidant interventions, using cholestyramine and vitamin E, respectively, were able to successfully reduce atherosclerosis in rabbit offspring born to dams fed high-cholesterol diet during gestation.^{11,22} We have also reported the beneficial effect of perinatal administration of antioxidant C-Phycocyanin rich extract against atherosclerosis development in ApoE^{-/-} mice offspring.¹³

In the current study, we aimed to explore the effects of gestational cholestyramine treatment on atherosclerosis development in adult offspring of genetically hypercholesterolemic ApoE^{-/-} mice. In fact, cholestyramine is a routinely used and effective lipid-lowering drug that can be considered during pregnancy.²³ For this purpose, the area and the lipid content of atherosclerotic plaques were analyzed in offspring. Furthermore, we provide a description of the metabolic parameters involved at different stages of the disease as total cholesterol, LDL-C, HDL-C, apolipoproteins B100 to A-I ratio, triglycerides, and some microbiota-related metabolites as BA²⁴ and TMAO.²⁵

2 | RESULTS

2.1 | Gestational cholestyramine treatment reduces plasma cholesterol and gallbladder total BA pool in dams

As shown in Table 1, TC, LDL-C and TG levels were similar in all dams at gestational day 0.5 (GD 0.5). As expected, TC and LDL-C were significantly reduced in cholestyramine-treated mothers at GD 11.5, and remained significantly lower at GD 17.5 in CTY-G dams compared to untreated CONT dams. In contrast, no significant change was observed in TG concentrations upon cholestyramine treatment during gestation. Besides, cholestyramine treatment effectively altered BA pool size of treated dams, as measured by the significant decrease of BA total pool concentration in their gallbladders.

TABLE 1 Plasma total cholesterol (TC), LDL-cholesterol (LDL-C) and triglycerides (TG) (mg/dL) of pregnant dams were measured by colorimetric assays at GD 0.5, GD 11.5, and GD 17.5. Total bile acids (BA) (mg/mL) were measured by LC-MS/MS in gallbladder of dams on GD 18.5.

Groups	Plasma						Gallbladder		
	TC (mg/dL)		LDL-C (mg/dL)		TG (mg/dL)		Total BA (mg/mL)		
	GD 0.5	GD 11.5	GD 17.5	GD 0.5	GD 11.5	GD 17.5	GD 17.5	GD 11.5	GD 18.5
CONT	268 ± 18	125 ± 7.9	187 ± 18	165 ± 10	73 ± 2.8	79 ± 6.3	107 ± 8.7	85 ± 7.5	67 ± 3.5
CTY-G	291 ± 26	73 ± 13 [†]	122 ± 20*	155 ± 9.5	52 ± 3.6 [†]	92 ± 10	128 ± 22	79 ± 10	45 ± 7.1*

Note: Values represent the mean ± SEM of the data from a total number of 4–7 dams per experimental group.

Abbreviations: CONT, control/untreated mothers; CTY-G, cholestyramine-treated mothers during gestation; GD, gestational day.

* $p < 0.05$ and [†] $p < 0.01$ as determined by unpaired *t*-test to compare the CONT versus CTY-G groups at the same GD, for each variable.

2.2 | Gestational cholestyramine treatment protects against the development of atherosclerotic plaques in offspring

Offspring born to cholestyramine-treated dams (CTY-G) benefited from a significant reduction of the total atherosclerotic plaque area in the aortic root. Indeed, CTY-G males showed a significant decrease in the total plaque area at 600 and 800 μm from the heart compared to CONT males (Figure 1A,B). Similarly, total atherosclerotic plaque area was significantly decreased at 800 μm in CTY-G females compared to CONT females, and a tendency to decrease was measured at 600 μm from the heart (Figure 1A,C).

Regarding atherosclerotic plaque composition, CTY-G males displayed lower neutral lipids accumulation in the atherosclerotic lesions than CONT males, as measured by reduced ORO staining at 600 μm ($2.7 \pm 0.7\%$ vs. $6.3 \pm 0.6\%$, respectively), and also at 800 μm from the heart ($2 \pm 0.7\%$ vs. $4.4 \pm 0.6\%$ respectively) (Figure 1A,D,E). CTY-G females also benefited from lower ORO-positive area compared to CONT females at 600 μm ($3.4 \pm 0.6\%$ vs. $6.1 \pm 0.4\%$, respectively) and at 800 μm ($4 \pm 0.7\%$ vs. $6.7 \pm 0.6\%$, respectively, Figure 1A,F,G).

To better interpret these observations, we investigated some predefined contributory factors and metabolic parameters related to the development of atherosclerosis.

Plasma total cholesterol concentration remained unchanged between groups (Figure 2A,E). Nevertheless, in CTY-G males, HDL-cholesterol was significantly higher compared with CONT males (Figure 2B,C). CTY-G males also showed a significant decrease in plasma ApoB100 to ApoA-I ratio (ApoB/ApoA-I) (Figure 2D). Regarding female offspring, no significant changes were observed in HDL-cholesterol, lipoproteins fractions or ApoB/ApoA-I between groups (Figure 2F–H). In offspring of both sexes, no significant differences were observed either in plasma triglyceride-rich lipoprotein (TRL-cholesterol) or in the low-density lipoprotein cholesterol (LDL-cholesterol) (Figure S1A,B,D,E). Total plasma triglycerides concentrations did not differ between groups (Figure S1C,F).

2.3 | Gestational cholestyramine treatment modulates the concentrations of bile acids in offspring

Maternal cholestyramine treatment reduced the development of atherosclerotic plaques along with the modulation of bile acids (BA) metabolism. In CTY-G male offspring, the gallbladder total pool of BA and in particular of tauro-conjugated BA were significantly reduced compared to

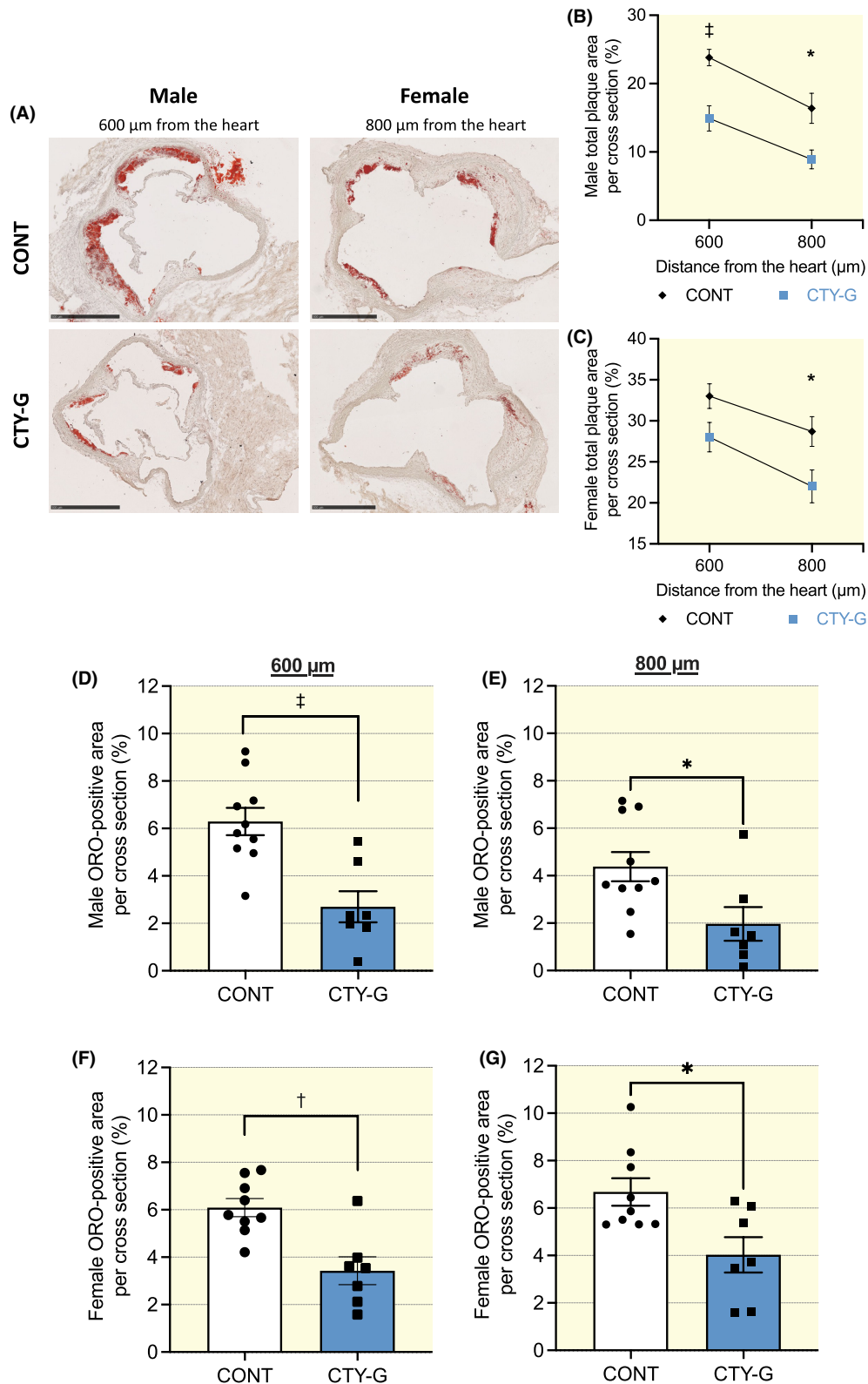


FIGURE 1 Quantification of atherosclerotic lesions in offspring. (A) Representative oil red O (ORO)/hematoxylin staining in aortic root cross sections from adult $\text{ApoE}^{-/-}$ born to control or treated mothers. Atherosclerotic plaques in aortic cross sections were quantified (%) in males (B) and females (C) at 600 and 800 μm from the heart. Quantification of lipid deposition after ORO coloration (%) at 600 and 800 μm from the heart in male (D, E) and female (F, G) offspring. Scale Bar: 500 μm * $p < 0.05$, † $p < 0.01$ and ‡ $p < 0.001$ as determined by unpaired t -test to compare the CONT versus CTY-G groups of same sex. $N=7-10$ males and $N=6-9$ females per group. CONT, offspring of control/untreated mothers; CTY-G, offspring of cholestyramine-treated mothers during gestation.

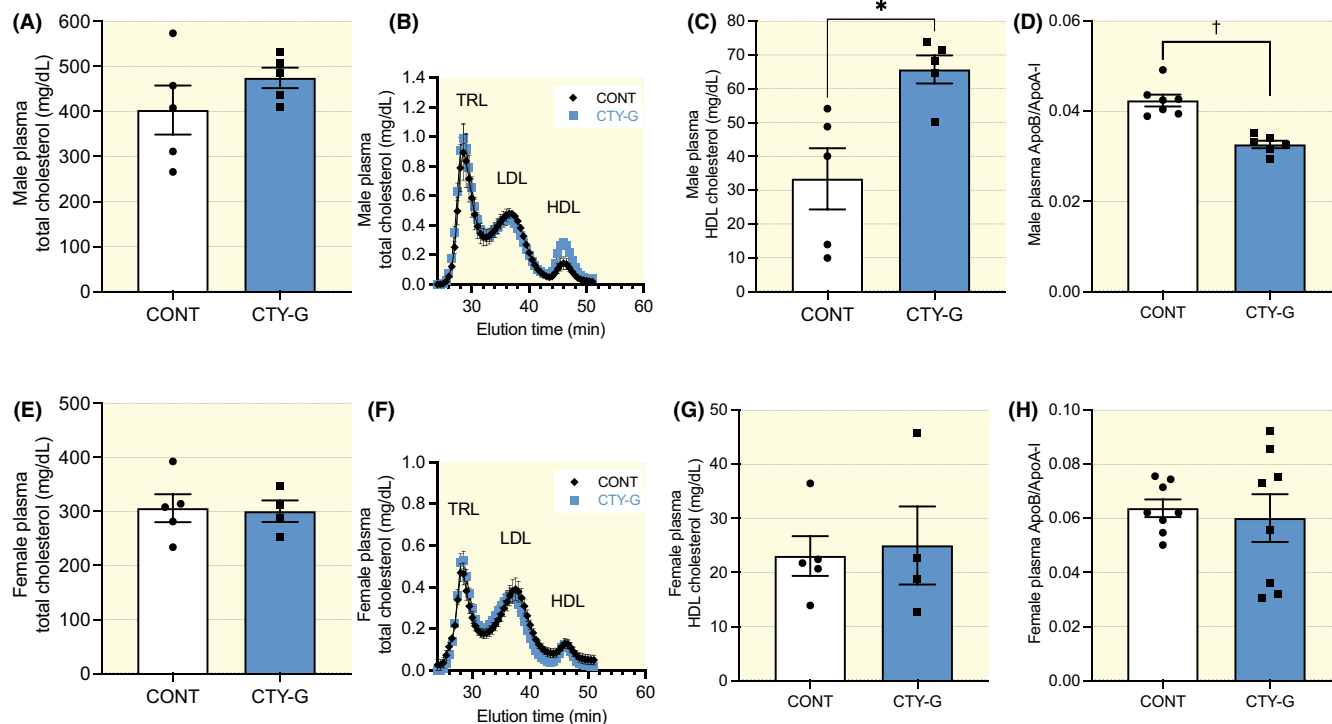


FIGURE 2 Plasma cholesterol, lipoprotein fractions, HDL-cholesterol, and apolipoprotein B-to-apolipoprotein A-I ratio of offspring. Plasma total cholesterol in male (A) and female (E) and cholesterol repartition (B, F) after high-performance liquid chromatography (HPLC) enabling TRL (triglyceride-rich lipoprotein), LDL (low-density lipoprotein), and HDL (high-density lipoprotein) separation, and the calculation of HDL (C, G). * $p < 0.05$ as determined by unpaired *t*-test. $N=5$ males and $N=4-5$ females per group. Plasma apolipoprotein B100-to-apolipoprotein A-I ratio (ApoB/ApoA-I) in male (D) and female (H) offspring. † $p < 0.01$ as determined by Mann-Whitney *U*-test. $N=6-7$ males per group and $N=8$ females per group. CONT, offspring of control/untreated mothers; CTY-G, offspring of cholestyramine-treated mothers during gestation.

CONT males (Figure 3A). In female offspring, maternal cholestyramine treatment did not significantly impact gallbladder total BA pool (Figure 3B).

In plasma, maternal cholestyramine treatment had no significant effect on total or tauro-conjugated BA concentrations of CTY-G males (Figure 3C). In females, plasma total BA concentrations were not significantly changed upon maternal treatment with cholestyramine, while tauro-conjugated BA concentrations were significantly higher in CTY-G group (Figure 3D).

2.4 | Gestational cholestyramine treatment modulates plasma concentrations of microbiota-derived metabolite

Lately, studies have suggested a correlation between trimethylamine-N-oxide (TMAO) levels and atherosclerosis. Interestingly, CTY-G females benefited from a significant reduction of plasma TMAO levels compared to CONT females (Figure 4D), but not male offspring (Figure 4B). Trimethylamine (TMA), precursor of TMAO,

was not significantly modified in male or female offspring born to treated dams compared to those born to untreated dams (Figure 4A,C).

3 | DISCUSSION

We showed that maternal treatment with cholestyramine protected against atherosclerosis development in adult offspring of ApoE^{-/-} mice, along with a sex-dependent modulation of several physiological parameters involving neutral lipids deposition in the aortic root, plasma level of HDL-cholesterol, ApoB100/ApoA-I ratio, BA metabolism and TMAO.

Our study was conducted in accordance with the “Developmental Origins of Health and Disease” (DOHaD) theory. Given that maternal hypercholesterolemia constitutes a risk factor for CVD in offspring,²⁶ we aimed to investigate the impact of early prenatal lipid-lowering interventions on atherosclerosis prevention in adulthood.

For this purpose, cholestyramine, a non-absorbable BA sequestrant, was administered to ApoE^{-/-} mice, characterized by elevated plasma cholesterol concentration,

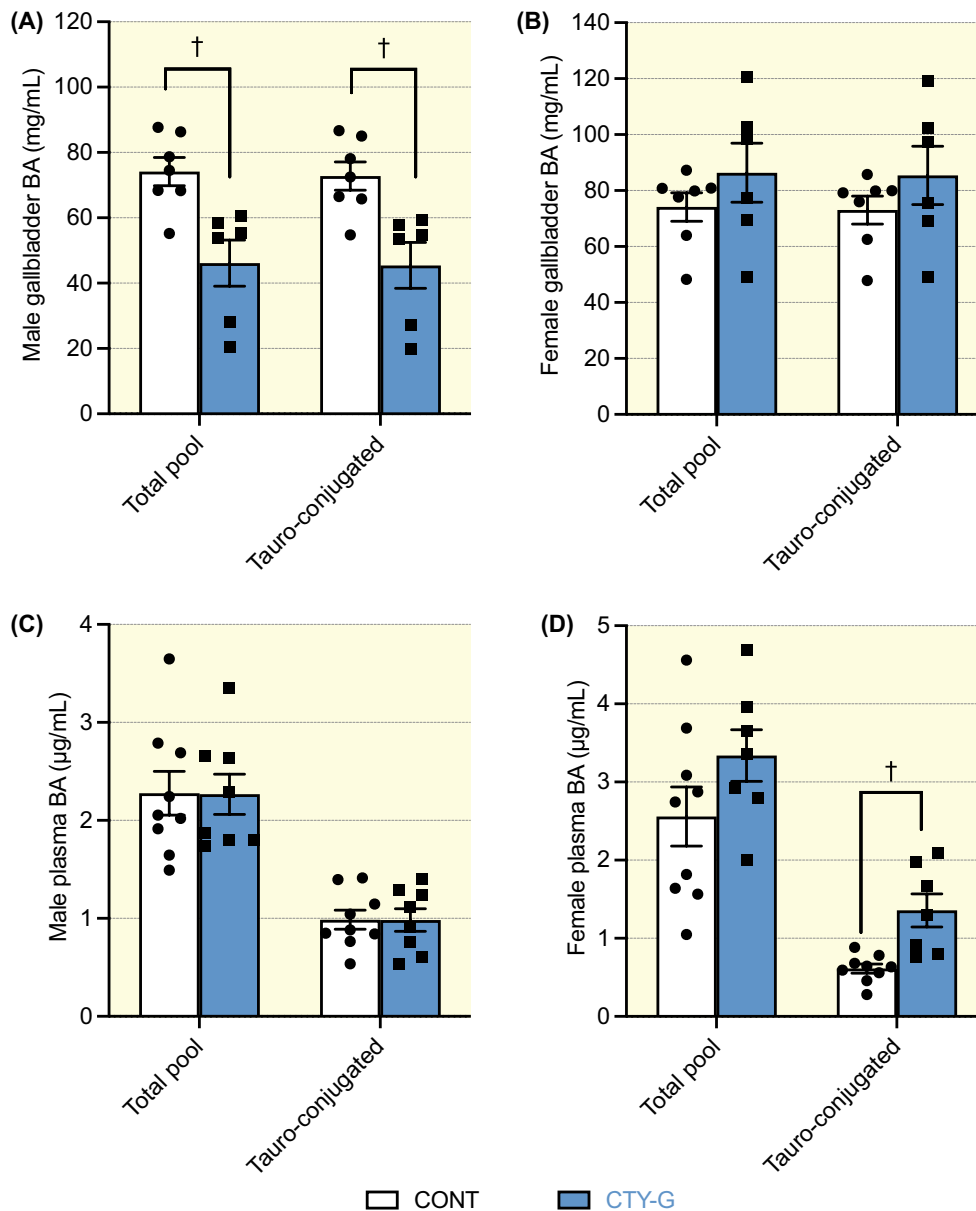


FIGURE 3 Bile acid concentrations in gallbladder and plasma of offspring. Gallbladder total and tauro-conjugated bile acids of male (A) and female (B) offspring. * $p < 0.01$ and † $p < 0.01$ as determined by unpaired *t*-test. $N = 6-7$ males and females per group. Plasma total and tauro-conjugated bile acids of male (C) and female (D) offspring. † $p < 0.01$ as determined by Mann-Whitney *U*-test. $N = 8-9$ males and $N = 7-9$ females per group. BA, bile acid; CONT, offspring of control/untreated mothers; CTY-G, offspring of cholestyramine-treated mothers during gestation.

their ability to develop atherosclerosis without nutritional interventions,²⁷ and their responsiveness to cholestyramine treatment within 2 weeks.²⁸

Our results show that maternal treatment with cholestyramine effectively reduced total plasma and LDL-cholesterol during gestation as previously published in rabbit.^{11,22} In addition, we report a decrease in gallbladder BA pool in treated pregnant dams. Concomitantly, perinatal cholestyramine treatment significantly reduced atherosclerotic plaques development in offspring and more specifically reduced lipid deposition in the aortic root of both sexes.

These observations could be explained by the fact that maternal and fetal cholesterol levels correlated at least during the first two semesters of pregnancy,⁹ and that maternal BA metabolism influences the fetal one.¹³ All these parameters were modified upon maternal treatment with cholestyramine in our study. Furthermore, it is described that cholestyramine treatment could enhance both anti-inflammatory status and gut microbiota²⁹ in dams, and in turn influence inflammation and microbial composition and metabolites in offspring.^{30,31}

Then, we further examined the effect of maternal cholestyramine treatment on some predefined risk factors

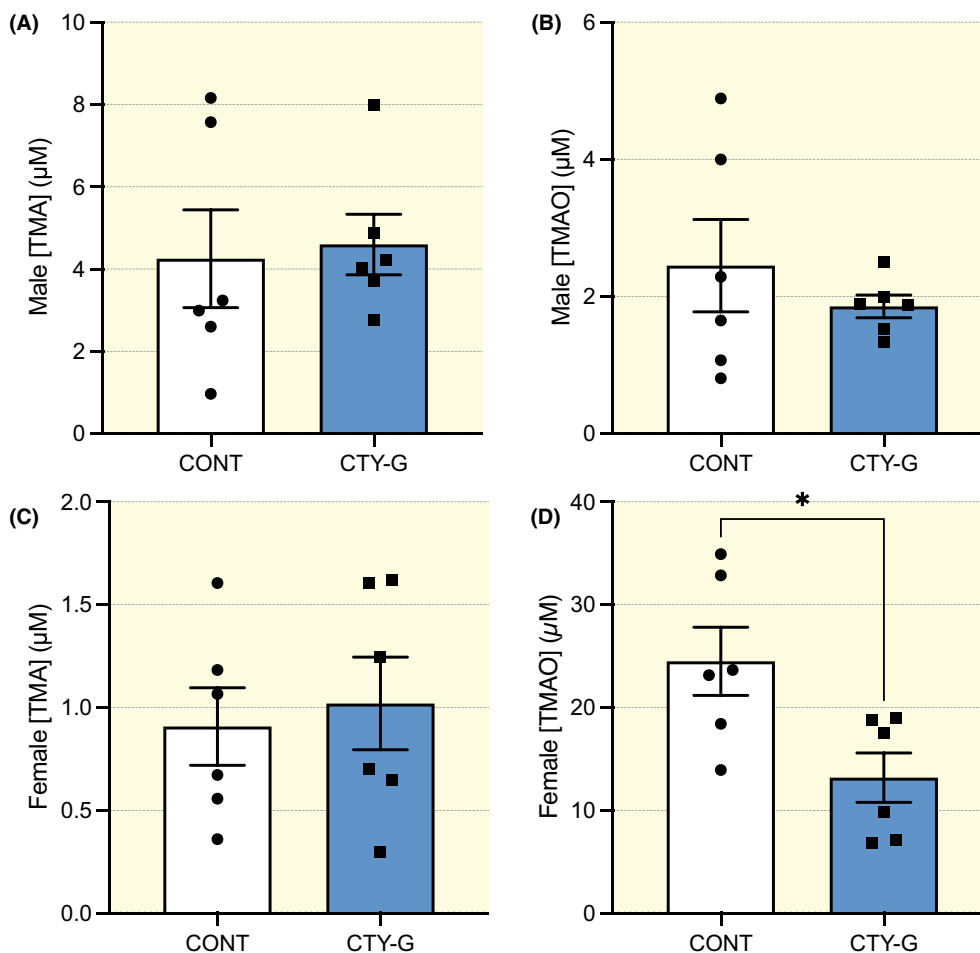


FIGURE 4 Plasma trimethylamine-N-oxide, biomarker of atherosclerosis. Plasma trimethylamine (TMA) and trimethylamine-N-oxide (TMAO) in male (A, B) and female (C, D) offspring. $p < 0.05$ as determined by unpaired t -test. $N=6$ males and females per group. CONT, offspring of control/untreated mothers; CTY-G, offspring of cholestyramine-treated mothers during gestation.

and metabolic parameters, known to be involved in atherosclerosis development in offspring.

Similarly to what was observed in rabbits,^{11,22} we showed that maternal cholestyramine treatment did not modify plasma TC or TG in offspring. Nevertheless, plasma HDL-cholesterol level significantly increased in CTY-G males along with a reduction in ApoB/ApoA-I ratio. Epidemiological studies identified low HDL-C³² and high ApoB/ApoA-I ratio as strong key risk factors for ATS. Indeed, HDL particles deploy various anti-atherogenic properties besides cholesterol efflux, namely anti-inflammatory, anti-oxidative and regulation of genes expression by miRs properties,³² whereas high ApoB/ApoA-I ratio was related to high risk of fatal myocardial infarction and intima-media thickness.³³

Increased BA synthesis rate and/or BA elimination from the enterohepatic cycle are described to protect against atherosclerosis.³ In CTY-G males' gallbladder, BA pool was significantly reduced compared to CONT males, which seems to be inconsistent with the reduction of atherosclerotic plaques. We speculate that

BA pool and metabolism were subjected to in-utero programming following maternal cholestyramine treatment. Indeed, several studies have provided programming evidences of maternal treatment on cholesterol and BA metabolism in offspring.³⁴ It was reported that BA concentration is higher in the fetal than in the healthy maternal circulation in human and mouse,³⁵ creating a fetus-to-mother gradient transfer across the placenta.³⁶ In contrast, this gradient appears to be reversed in cases of increased levels of plasma BA of intrahepatic cholestasis of pregnancy.³⁷ Therefore, we suppose that the decrease in BA pool of cholestyramine-treated dams exacerbates the fetal BA flow toward the mother, and programs a lower BA pool in the progenies, that remained irreversible in adulthood.

Besides their role in the entero-hepatic cycle and cholesterol elimination, BA in the systemic circulation act as signaling molecules.³⁸ CTY-G females showed higher plasma tauro-conjugated BA pool. In fact, elevated tauro-conjugated BA levels were associated with anti-atherosclerotic³⁹ and antioxidant⁴⁰ properties. Moreover,

in humans, lower circulating BA were associated with increased presence and severity of coronary artery disease.⁴¹ Thus, we suggest a role of increased tauro-conjugated BA upon perinatal cholestyramine treatment in atherosclerosis reduction in offspring.

We also measured plasma TMAO levels, as a cardiovascular disease biomarker.⁴² Female offspring benefited from less macrophage's infiltration in the aortic root and reduced plasma TMAO accumulation. A pro-atherogenic role of TMAO was attributed in part to enhanced cholesterol uptake in macrophages and BA synthesis inhibition.⁴³ Since we did not measure significant reduction of TMAO precursors, such as TMA and other methylamines (choline, carnitine and betaine (data not shown)), TMAO reduction could be attributed to a modification in its turnover.

Of note, all offspring were raised under chow diet that do not affect their genetic hypercholesterolemia, thereby demonstrating the imprint of solely prenatal exposure and modulation of maternal atherosclerotic risk factors on ATS development in offspring. It would be relevant to determine if the anti-atherosclerotic effect of prenatal cholestyramine treatment would persist in offspring exposed to additional risk factors of atherosclerosis. For instance, in cases of exacerbated hypercholesterolemia using a cholesterol-enriched diet, or unducing a lowered endothelial shear stress levels around the carotid arteries.⁴⁶

4 | MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

4.1 | Animals, diets, and tissue sampling

Seven-week-old female and male B6.129P2-Apoe/J were purchased from Charles River Laboratories (France). After 1 week of acclimation, mating was initiated and females with positive vaginal plug on the following morning were identified. These females were randomly assigned either to the control group (CONT) fed control breeding diet (A03; Safe Diets, France) or to the cholestyramine-treated group (CTY-G) (A03 + 3% Cholestyramine; customized at Safe Diets) during gestation (3 weeks). Animals had ad libitum access to water and food under constant light/dark cycles (12h/12h). Offspring of all groups were weaned at 3 weeks, fed a breeding diet until 10 weeks, and then sustained under maintenance chow diet (A04; Safe Diets, France) until sacrifice at 25 weeks of age.

4.1.1 | Dams

To collect samples from untreated and treated mothers during pregnancy without disturbing fetal development, additional female ApoE^{-/-} mice were mated as described above. Gestational day 0.5 (GD 0.5) corresponded to the morning of positive vaginal plug detection. Dams were then randomly assigned either to the control group (CONT) or to the cholestyramine-treated group during gestation (CTY-G). At GD 0.5, GD 11.5 and GD 17.5 and after 4h of fasting, blood samples were collected into K2 EDTA tubes (Sarstedt, Germany) and plasma was isolated by conventional centrifugation (4000 RPM, 4°C, 5 min). Dams were sacrificed at G18.5. for gallbladder collection.

4.1.2 | 25-week-old offspring

Adult offspring were fasted for 4 h and then anesthetized under isoflurane (5 L/min, 2%–3%). Intracardiac blood was collected on heparinized syringe into K2 EDTA tubes for plasma separation. A physiological saline solution was injected into systemic circulation to wash out remaining blood in the aorta. Then, the heart along with the linked ascending and descending aortas, the gallbladders and the livers were collected.

All tissues were rapidly frozen in liquid nitrogen and then stored at –80°C until analysis.

All experimental procedures were approved by the Committee of Ethics in Animal Experiments of Pays de la Loire, France (APAFIS 20118) and performed according to the European Union regulations for the care and use of animals for experimental procedures (2010/63/EU).

4.2 | Aortic lesion analysis and oil-red-o staining

To quantify atherosclerotic lesions at the aortic root, perivascular fat was cleaned before detaching the heart from the aortic arch and aorta. Then, 2–3 mm long aortic arch base were frozen in embedding medium for serial cryosections. The first appearance of the valve leaflets was used as a reference point (0 μm) and then 10-μm thick cryosections were collected. The disappearance of the aortic valve cusps was progressively monitored under microscope and occurred at 800 μm.

Neutral lipids of sections at 600 and 800 μm from the heart were stained as follow: fixation with 4% formol, dehydration with isopropyl alcohol, staining with oil red O (ORO), and counterstaining with hematoxylin (Sigma Aldrich, France). Stained slides were captured using Nanozoomer Hamamatsu 2.0 HT slide scanner.

Quantification of total plaque area in the aortic root, defined by the intimal elastic lamina to the luminal edge of the lesion, was done using image analysis software “Fiji”⁴⁴ by an investigator blinded to the intervention.

Quantification of neutral lipid-stained lesions were performed using image analysis software QuPath version 0.1.2 using machine learning. All the scripts developed for this analysis are available online (<https://github.com/magalieFeyeux/microPICell/tree/master/QuPathScript/AortaPlaquesegmentation>).

The plaque area in the aortic root and ORO-positive staining area were reported relatively to the luminal area of the aorta, and results were expressed as the average percentages obtained on two to five sections.

4.3 | Biochemical, chromatography, and mass spectrometry analyses

Concentrations of relevant metabolites and biomarkers in atherosclerosis development were measured in plasma and bile.

4.3.1 | Plasma

Total plasma cholesterol (TC) and triglycerides (TG) as well as LDL-cholesterol (LDL-C) concentrations were determined by enzymatic assay kits (Diasys, EURO BIO Scientific, France) according to the manufacturer's instructions.

For lipoprotein separation and lipid content calculation, plasma samples were automatically injected and carried through the Superose column (Superose™ 6 Increase 3.2/300, GE Healthcare, Sweden) by the elution buffer (NaCl 150 mM; Tris-HCl 10 mM; Na-Azide 0.02%, pH 7.4) delivered at a flow rate of 40 mL min⁻¹ for a total run-time of 65 min. Eluted lipoproteins were continuously mixed with specific cholesterol reagents in a single reaction heating chamber (37°C; 1000 reaction coil). Absorbance was measured at 500 nm with a 2998 Photodiode Array Detector. Chromatograms were analyzed using Empower3 system manager software. The cholesterol content in the respective eluted fractions was calculated as the product of the area percentage of each lipid and the lipid content determined from the injected samples.

Plasma apolipoproteins (A-I, AII, B, CI, C-II and C-III, M) were determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS), as described previously.⁴⁵ Briefly, the apolipoproteins were quantified in 20- μ L aliquots using trypsin proteolysis and the subsequent analysis of proteotypic peptides (see Table in the

Supplemental material). The intra- and inter-assay variabilities did not exceed 6.4%.

Bile acids were extracted from plasma samples and subsequently quantified by reversed-phase liquid chromatography–tandem mass spectrometry (LC–MS/MS) as described previously.²⁰ Methylamines, including the TMAO, were extracted from plasma samples and quantified by HILIC LC–MS/MS as previously described.²⁰

4.3.2 | Gallbladder

Bile acids were extracted from gallbladder then quantified by the same method described in plasma.

4.4 | Statistical analysis

Statistical analyses were performed with GraphPad Prism software (Version 9.4.1, GraphPad Software Inc., La Jolla, CA, USA). Experimental values were presented as the mean \pm SEM. Visual analysis of quantile-quantile plots (QQ plots) and Shapiro–Wilk test were used to determine the normal distribution of the data. Unpaired *t*-test was applied to variables meeting homoscedasticity and normality assumptions. If one of the two assumptions was not respected, Mann-Whitney *U*-test was applied. The results were considered to be significant at $p < 0.05$. A tendency to variation is considered for p values < 0.1 .

5 | CONCLUSION

In conclusion, we showed that gestational cholestyramine treatment provides anti-atherogenic effects in adult offspring in a sex-dependent manner. This study shows promising clinical results with lipid-lowering therapies using bile acid sequestrants for women of childbearing age with genetic familial hypercholesterolemia and also for their children by providing protection against the development of atherosclerosis. However, further investigations are needed to understand affected mechanisms, in particular: (1) investigate the inflammatory response and macrophages profile in the aorta, (2) measure of the kinetics of BAs turnover at least in male offspring, (3) investigation of gut microbiota activity with special focus on metabolites' production such as TMAO and bile acids, (4) analysis of the effect of cholestyramine treatment on placenta leading to reduced ATS programming in offspring, and (5) investigate if the effect of this treatment persist in offspring challenged with further atherogenic risk factors as diet enriched in cholesterol.

AUTHOR CONTRIBUTIONS

Khadija Ouguerram: Conceptualization; funding acquisition; writing - original draft, review and editing. **Marina Habib:** WInvestigation; Formal analysis; writing - original draft, review and editing; Visualization. **Mikael Croyal:** Investigation; writing - original draft; mass spectrometry methodology. **Bertrand Kaeffer:** Investigation; writing - original draft. **Isabelle Grit, Blandine Castellano and Mathilde Gourdel:** Investigation and technical assistance. **Cedric Le May** High-performance liquid chromatography (HPLC) methodology Investigation and technical assistance. **Chantal Thorin:** Statistical analysis methodology. **Hassan Nazih:** writing - original draft, review and editing.

ACKNOWLEDGMENTS

We thank the IBISA MicroPICell facility (Biogenouest, Nantes) and LVTS UMRS1148 (Paris, France) for their technical support in atherosclerotic plaques quantification. We are also grateful for Alexis Gandon and Chloe Chaîne for their technical help.

FUNDING INFORMATION

This work was supported by AlimH department of French National Research Institute for Agriculture, Food and Environment (Inrae) and research program EPERSAT by region Pays de la Loire, France.

CONFLICT OF INTEREST STATEMENT

Nothing to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Marina Habib  <https://orcid.org/0000-0002-3440-7824>

REFERENCES

- Cardiovascular diseases (CVDs). <https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-cvds>, Accessed date: 11 February 2024
- Lusis AJ. Atherosclerosis. *Nature*. 2000;407:233-241.
- Chiang JYL, Ferrell JM, Wu Y, Boehme S. Bile acid and cholesterol metabolism in atherosclerotic cardiovascular disease and therapy. *Cardiol Plus*. 2020;5:159-170.
- Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov*. 2008;7:678-693.
- Barker DJP. Fetal origins of coronary heart disease. *BMJ*. 1995;311:171-174.
- Mendelson MM, Lyass A, O'Donnell CJ, D'Agostino RB, Levy D. Association of maternal prepregnancy dyslipidemia with adult offspring dyslipidemia in excess of anthropometric, lifestyle, and genetic factors in the Framingham Heart Study. *JAMA Cardiol*. 2016;1:26-35.
- Napoli C, Pignalosa O, de Nigris F, Sica V. Childhood infection and endothelial dysfunction: a potential link in atherosclerosis? *Circulation*. 2005;111:1568-1570.
- Evelein AMV, Visseren FLJ, van der Ent CK, Grobbee DE, Uiterwaal CSPM. Excess early postnatal weight gain leads to thicker and stiffer arteries in young children. *J Clin Endocrinol Metab*. 2013;98:794-801.
- Napoli C, D'Armiento FP, Mancini FP, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*. 1997;100:2680-2690.
- Napoli C, Glass CK, Witztum JL, Deutsch R, D'Armiento FP, Palinski W. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. *Lancet*. 1999;354:1234-1241.
- Wulf P, D'Armiento FP, Witztum JL, et al. Maternal hypercholesterolemia and treatment during pregnancy influence the long-term progression of atherosclerosis in offspring of rabbits. *Circ Res*. 2001;89:991-996.
- Napoli C, de Nigris F, Welch JS, et al. Maternal hypercholesterolemia during pregnancy promotes early atherogenesis in LDL receptor-deficient mice and alters aortic gene expression determined by microarray. *Circulation*. 2002;105:1360-1367.
- Coué M, Croyal M, Habib M, et al. Perinatal administration of C-phycocyanin protects against atherosclerosis in apoE-deficient mice by modulating cholesterol and trimethylamine-N-oxide metabolisms. *Arterioscler Thromb Vasc Biol*. 2021;41:e512-e523.
- Liguori A, D'Armiento F, Palagiano A, et al. Effect of gestational hypercholesterolaemia on omental vasoreactivity, placental enzyme activity and transplacental passage of normal and oxidised fatty acids. *BJOG Int J Obstet Gynaecol*. 2007;114:1547-1556.
- Leiva A, de Medina CD, Salsoso R, et al. Maternal hypercholesterolemia in pregnancy associates with umbilical vein endothelial dysfunction: role of endothelial nitric oxide synthase and arginase II. *Arterioscler Thromb Vasc Biol*. 2013;33:2444-2453.
- Khan I, Dekou V, Hanson M, Poston L, Taylor P. Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. *Circulation*. 2004;110:1097-1102.
- de Nigris F, Cacciatore F, Mancini FP, et al. Epigenetic hallmarks of fetal early atherosclerotic lesions in humans. *JAMA Cardiol*. 2018;3:1184-1191.
- de Oliveira Cipriano Torres D, Santos ACOD, Silva AKSE, de Moura PMMF, Beltrão EIC, Peixoto CA. Influence of fatty acids in maternal diet on atherogenesis in offspring of LDL receptor-deficient mice. *Int J Clin Exp Med*. 2012;5:56-63.
- Goharkhay N, Tamayo EH, Yin H, Hankins GDV, Saade GR, Longo M. Maternal hypercholesterolemia leads to activation of endogenous cholesterol synthesis in the offspring. *Am J Obstet Gynecol*. 2008;199:273.e1-273.e6.
- Trenteseaux C, Gaston A, Aguesse A, et al. Perinatal hypercholesterolemia exacerbates atherosclerosis lesions in offspring by altering metabolism of trimethylamine-N-oxide and bile acids. *Arterioscler Thromb Vasc Biol*. 2017;37:2053-2063.

21. Croyal M, Saulnier P-J, Aguesse A, et al. Plasma trimethylamine N-oxide and risk of cardiovascular events in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2020;105:2371-2380.
22. Napoli C, Witztum JL, Calara F, de Nigris F, Palinski W. Maternal hypercholesterolemia enhances atherogenesis in normocholesterolemic rabbits, which is inhibited by antioxidant or lipid-lowering intervention during pregnancy: an experimental model of atherogenic mechanisms in human fetuses. *Circ Res.* 2000;87:946-952.
23. Scaldaferrì F, Pizzoferrato M, Ponziani FR, Gasbarrini G, Gasbarrini A. Use and indications of cholestyramine and bile acid sequestrants. *Intern Emerg Med.* 2013;8:205-210.
24. Wang HH, Garruti G, Liu M, Portincasa P, Wang DQ-H. Cholesterol and lipoprotein metabolism and atherosclerosis: recent advances in reverse cholesterol transport. *Ann Hepatol.* 2017;16:S27-S42.
25. Wang B, Qiu J, Lian J, Yang X, Zhou J. Gut metabolite trimethylamine-N-oxide in atherosclerosis: from mechanism to therapy. *Front Cardiovasc Med.* 2021;8:723886.
26. Palinski W, Napoli C. The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB J.* 2002;16:1348-1360.
27. Emini Veseli B, Perrotta P, De Meyer GRA, et al. Animal models of atherosclerosis. *Eur J Pharmacol.* 2017;816:3-13.
28. Masuda Y, Yamaguchi S, Nishizawa T. Cholesterol-lowering pattern affects the progression of atherosclerosis in apolipoprotein E deficient mice. *J Pharmacol Sci.* 2016;132:271-274.
29. Flaig B, Garza R, Singh B, Hamamah S, Covasa M. Treatment of dyslipidemia through targeted therapy of gut microbiota. *Nutrients.* 2023;15:228.
30. Palinski W, Yamashita T, Freigang S, Napoli C. Developmental programming: maternal hypercholesterolemia and immunity influence susceptibility to atherosclerosis. *Nutr Rev.* 2007;65:S182-S187.
31. Bolte EE, Moorshead D, Aagaard KM. Maternal and early life exposures and their potential to influence development of the microbiome. *Genome Med.* 2022;14:4.
32. Kontush A. HDL-mediated mechanisms of protection in cardiovascular disease. *Cardiovasc Res.* 2014;103:341-349.
33. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy—a review of the evidence. *J Intern Med.* 2006;259:493-519.
34. Innis SM. Influence of maternal cholestyramine treatment on cholesterol and bile acid metabolism in adult offspring. *J Nutr.* 1983;113:2464-2470.
35. Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, et al. Maternal cholestasis during pregnancy programs metabolic disease in offspring. *J Clin Invest.* 2013;123:3172-3181.
36. Colombo C, Roda A, Roda E, et al. Correlation between fetal and maternal serum bile acid concentrations. *Pediatr Res.* 1985;19:227-231.
37. Geenes V, Lövgren-Sandblom A, Benthin L, et al. The reversed fetomaternal bile acid gradient in intrahepatic cholestasis of pregnancy is corrected by ursodeoxycholic acid. *PLoS ONE.* 2014;9:e83828.
38. Duboc H, Taché Y, Hofmann AF. The bile acid TGR5 membrane receptor: from basic research to clinical application. *Dig Liver Dis.* 2014;46:302-312.
39. Yang J-Y, Zhang T-T, Yu Z-L, et al. Taurine alleviates trimethylamine N-oxide-induced atherosclerosis by regulating bile acid metabolism in ApoE^{-/-} mice. *J Agric Food Chem.* 2022;70:5738-5747.
40. Maia AR, Batista TM, Victorio JA, et al. Taurine supplementation reduces blood pressure and prevents endothelial dysfunction and oxidative stress in post-weaning protein-restricted rats. *PLoS ONE.* 2014;9:e105851.
41. Chong Nguyen C, Duboc H, Rainteau D, et al. Circulating bile acids concentration is predictive of coronary artery disease in human. *Sci Rep.* 2021;11:22661.
42. Schiattarella GG, Sannino A, Toscano E, et al. Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. *Eur Heart J.* 2017;38:2948-2956.
43. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* 2011;472:57-63.
44. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012;9:676-682.
45. Blanchard V, Garçon D, Jaunet C, et al. A high-throughput mass spectrometry-based assay for large-scale profiling of circulating human apolipoproteins. *J Lipid Res.* 2020;61:1128-1139.
46. Alkemade, F. E., Gittenberger-de Groot, A. C., Schiel, A. E., VanMunsteren, J. C., Hogers, B., van Vliet, L. S. J., Poelmann, R. E., Havekes, L. M., Willems van Dijk, K., & DeRuiter, M. C. (2007). Intrauterine Exposure to Maternal Atherosclerotic Risk Factors Increases the Susceptibility to Atherosclerosis in Adult Life. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(10), 2228–2235. <https://doi.org/10.1161/01.atv.0000282193.31936.fd>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Habib M, Croyal M, Kaeffer B, et al. Gestational cholestyramine treatment protects adult offspring of ApoE-deficient mice against maternal-hypercholesterolemia-induced atherosclerosis. *Acta Physiol.* 2024;240:e14133. doi:[10.1111/apha.14133](https://doi.org/10.1111/apha.14133)