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Maternal folic acid supplementation does not counteract the deleterious impact of prenatal exposure to environmental pollutants on lipid homeostasis in male rat descendants

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Abstract

Prenatal exposure to persistent organic pollutants (POPs) has been associated with the development of metabolic syndrome-related diseases in offspring. According to epidemiological studies, father's transmission of environmental effects in addition to mother's can influence offspring health. Moreover, maternal prenatal dietary folic acid (FA) may beneficially impact offspring health. The objective is to investigate whether prenatal FA supplementation can overcome the deleterious effects of prenatal exposure to POPs on lipid homeostasis and inflammation in three generations of male rat descendants through the paternal lineage. Female Sprague-Dawley rats (F0) were exposed to a POPs mixture (or corn oil) +/− FA supplementation for 9 weeks before and during gestation. F1 and F2 males were mated with untreated females. Plasma and hepatic lipids were measured in F1, F2, and F3 males after 12-h fast. Gene expression of inflammatory cytokines was determined by qPCR in epididymal adipose tissue. In F1 males, prenatal POPs exposure increased plasma lipids at 14 weeks old and hepatic lipids at 28 weeks old and prenatal FA supplementation decreased plasma total cholesterol at 14 weeks old. Prenatal POPs exposure decreased plasma triglycerides at 14 weeks old in F2 males. No change was observed in inflammatory markers. Our results show an impact of the paternal lineage on lipid homeostasis in rats up to the F2 male generation. FA supplementation of the F0 diet, regardless of POPs exposure, lowered plasma cholesterol in F1 males but failed to attenuate the deleterious effects of prenatal POPs exposure on plasma and hepatic lipids in F1 males.

Introduction

Persistent organic pollutants (POPs) are a group of chemicals characterized by their persistence in the environment, their lipophilicity, their bioaccumulation, and their biomagnification in the food chain.[1,2] International agreements at the 2001 Stockholm Convention were intended to prohibit the further release of some of these contaminants.[3] Nevertheless, POPs are still present in our environment and can be found as far north as the Arctic, which has become a repository for these pollutants following long-distance transport through the atmosphere and ocean currents.[4,5] As a result, wildlife and humans from the Arctic areas are highly exposed to POPs.[6,7]

Metabolic syndrome represents a cluster of at least three of the five following characteristics: abdominal obesity, hyperglycemia, hypertension, hypertriglyceridemia, and low high-density lipoprotein (HDL) cholesterol. Several systemic inflammation markers, including C-reactive protein, interleukin-6, and tumor necrosis factor-α (TNF-α), are also increased with metabolic syndrome.[8,9] If not treated, this medical condition exposes patients to a high risk of cardiovascular disease, type-2 diabetes (T2D), and non-alcoholic fatty liver disease.[10,11] According to the International Diabetes Federation, the prevalence of metabolic syndrome is estimated to be approximately 25% of the world's population;[12] thus, it is becoming an emerging global

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Table 1. Composition of the POPs mixture used in this study

Compound	CAS no.	Source[a]	% Weight	Dose (µg/kg body weight)
Aroclor and congener neat mix[b]		AccuStandard	32.4	500
Technical chlordane	57-74-9	AccuStandard	21.4	330.3
p,p'-Dichlorodiphenyldichloroethylene (*p,p'*-DDE)	72-55-9	Sigma-Aldrich	19.3	297.8
p,p'-Dichlorodiphenyltrichloroethane	50-29-3	SigmaAldrich	6.8	104.9
Technical toxaphene	8001-35-2	AccuStandard	6.5	100.0
α-Hexachlorocyclohexane (α-HCH)	319-84-6	Sigma-Aldrich	6.2	95.7
Aldrin	309-00-2	Sigma-Aldrich	2.5	38.6
Dieldrin	60-57-1	Sigma-Aldrich	2.1	32.4
1, 2, 4, 5-Tetrachlorobenzene	95-94-3	Sigma-Aldrich	0.9	13.9
p,p'-dichlorodiphenyldichloroethane (*p,p'*-DDD)	72-54-8	Sigma-Aldrich	0.5	7.7
β-Hexachlorocyclohexane (β-HCH)	319-85-7	Sigma-Aldrich	0.4	6.2
Hexachlorobenzene (HCB)	118-74-1	AccuStandard	0.4	6.2
Mirex	2385-85-5	Sigma-Aldrich	0.2	3.1
Lindane	58-89-9	Sigma-Aldrich	0.2	3.1
Pentachlorobenzene	608-93-5	Sigma-Aldrich	0.2	3.1

[a]AccuStandard Inc (New Haven, Connecticut); Sigma-Aldrich Inc (St Louis, Missouri).
[b]PCBs mix : Aroclor 1260 (58.9% of total); Aroclor 1254 (39.3% of total); 2,4,4'-trichlorobiphenyl (PCB 28; 1% of total); 2,2',4,4'-tetrachlorobiphenyl (PCB47; 0.8% of total); 3,3',4,4',5 pentachlorobiphenyl (PCB 126; 0.02% of total), and 3,3',4,4'-tetrachlorobiphenyl (PCB 77; 0.004% of total).

public health problem.[13] Two major environmental contributors are a greater consumption of high-fat, high-sugar, low-fiber food, which promotes atherogenic lipid abnormalities,[14] and a sedentary lifestyle, but these factors only account for part of the alarming cardiometabolic diseases.[13] In Inuit population, Singh *et al.*[15] have shown that polychlorinated biphenyl (PCB) levels in the plasma were associated with higher levels of serum triglycerides, total cholesterol, and low-density lipoprotein (LDL) cholesterol.

The Developmental Origins of Health and Disease theory proposes that specific environmental factors experienced by the fetus will impact the risk of chronic diseases later in life.[16,17] Furthermore, the endocrine-disrupting capabilities of some POPs have been associated with adverse reproductive outcomes[18] and metabolic diseases, such as obesity and T2D.[19]Although maternal transmission of environmental effects has already been observed,[19,20] paternal contributions to metabolic phenotypes in immediate offspring or across multiple generations have only recently been reported.[21] Therefore, the impact of paternal contribution to lipid metabolism and inflammation in the descendants has not yet been established. Hence, in the current study, we hypothesize that prenatal exposure to POPs at levels equivalent to those observed in the Northern Quebec Inuit population leads to metabolic disorders in lipid metabolism and inflammation in subsequent generations of male rat descendants through the paternal lineage.

Maternal intake of folic acid (FA), the synthetic form of folate, has recently been shown to modulate deleterious metabolic effects transmitted to offspring and to prevent the onset of T2D and cardiovascular diseases.[22,23] Additionally, FA nutritional status in males has been reported to have an impact on offspring health.[24] Folate is required in DNA synthesis as a methyl donor through one-carbon metabolism and could thus influence methylation mechanisms during gestation[25,26] that could transmit to the next generation via the sperm epigenome.[24] Moreover, folate status is slightly deficient in Inuit population.[27,28]

Therefore, we aim to investigate whether prenatal FA supplementation can overcome the deleterious effects of prenatal exposure to POPs on lipid metabolism and inflammation in three generations of male rat descendants at 14 and 28 weeks old, which are approximately equivalent to young adult and adult men, respectively.

Methods

Arctic POPs mixture

Chemical suppliers are indicated in Table 1. As described previously,[29] the pollutants were weighed, dissolved in corn oil (Aldrich-Sigma, Oakville, Canada) to yield a stock solution of 5 mg PCB mixture/ml and mixed with the other components at the relative proportions shown in Table 1, and shielded from light at room temperature. The POPs mixture was designed to represent the pollutant composition of ringed seal blubber from Northern Quebec,[30] which continues to be part of the Inuit population's traditional diet. The experimental dose was adjusted by diluting the stock solution with corn oil to 500 µg PCBs/ml to reflect metabolite levels found in members of the Nunavik Inuit community.[29]

Treatments of the F0 founder females

Animal care and all treatment procedures were in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Université Laval Animal Research Ethics Committee (certificate #2015010-2). Twenty-four female Sprague-Dawley rats (≈160 g, 5-week-old) plus twelve males (≈220 g, 10-week-old) (Charles River Laboratories, Saint Constant, QC, Canada) were acclimated to their new environment for 10 days and fed an AIN-93G-purified diet (#110700 Dyets Inc., Bethlehem, PA, USA).[31] Animals were housed in standard cages under controlled temperature (22°C) and relative humidity (50%) with a 12 h day/night cycle with food and water *ad libitum*.

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Table 2. Composition of the diets

Ingredients	1X Diet[a] g/kg of diet	3X Diet[b] g/kg of diet
Casein[c]	200	200
L-Cystine	3	3
Sucrose	100	100
Cornstarch	397.486	396.286
Dyetrose[d]	132	132
Soybean Oil	70	70
t-Butylhydroquinone	0.014	0.014
Cellulose	50	50
Mineral Mix #210025	35	35
Vitamin Mix #310025	10	0
Vitamin Mix #317761 (no folate)	0	10
Folic Acid Premix (5 mg/g)	0	1.2
Choline Bitartrate	2.5	2.5
Total	1000	1000

[a]DYET #110700, AIN-93G Purified Rodent Diet – 2 mg/kg FA.
[b]DYET #117819GI, Modified AIN-93G Purified Rodent Diet – 6 mg/kg FA.
[c]Casein high nitrogen for 1X diet and sterile casein for 3X diet.
[d]Food grade depolymerized cornstarch.

After the adaptation period, the 7-week-old founder (F0) dams were randomly assigned to four treatment groups ($n = 6$/group) as follows:

1. Control (CTRL): F0 dams were fed the AIN-93G control diet containing 2 mg FA/kg and gavaged a corn oil control 3 days/week.
2. POPs: F0 dams were fed the AIN-93G control diet containing 2 mg FA/kg and gavaged the POPs mixture (500 µg PCBs/kg) 3 days/week.
3. FA-S: F0 dams were fed the modified AIN-93G diet (#117819 Dyets Inc., Bethlehem, PA, USA) containing 6 mg FA/kg and gavaged a corn oil control 3 days/week.
4. POPs + FA-S: F0 dams were fed the modified AIN-93G diet containing 6 mg FA/kg and gavaged the POPs mixture (500 µg PCBs/kg) 3 days/week.

FA concentrations of 2 mg/kg (1X) and 6 mg/kg (3X) were used in the rodent diets in the present study, as described previously in investigations on FA supplementation.[32] These doses approximate the dietary reference intake for human adults (1X ≈ 0.4 mg/day) and the FA intake from fortified foods and prenatal supplements that would be consumed by a woman in Canada (3X ≈ 1.2 mg/day), respectively.[32] Table 2 shows the complete composition of the diets.

As shown in Figure 1, the four experimental treatments were administered to F0 dams for only 9 weeks in total, starting 5 weeks before mating and ending after parturition. For breeding, two F0 females were mated with one untreated F0 male per night. The following morning, a vaginal smear was prepared and observed under a microscope to confirm gestation. The date of parturition for each dam was designated postnatal day 0 (PND 0) for the litter. All F0 postpartum females were fed a 1X diet throughout the lactation period.

Male descendants

For each generation, 12 males ($n = 12$) were kept for reproduction and 10 males ($n = 10$) were used for experiments. As described in Figure 1, male offspring of the first generation (F1) were weaned at 3 weeks old and fed the 1X diet *ad libitum*. Then, on PND 90, they were mated with 10-week-old, untreated females to yield a second generation (F2). Similarly, F2 males were mated to untreated females to yield a third generation (F3). All F1, F2, and F3 males used for experiments were weighed at 3 weeks old, coded using a nontoxic marker on the tail, and then selected to ensure equal weights within each group and between groups. Animals were housed two per cage for 2 weeks and then one per cage until the end of the study for food intake and body weight measurements (twice a week). At 14 weeks old, animals were anesthetized with 3% isoflurane to obtain blood samples from the jugular vein. At 28 weeks old, all animals were anesthetized using 3% isoflurane and sacrificed by exsanguination via cardiac puncture. Plasma samples were collected after centrifugation (950 g, 10 min). Liver, retro-peritoneal, and epididymal adipose tissues (ATs) were removed, weighed, directly snap-frozen in liquid nitrogen, and then stored at −80 °C until further analysis. Retro-peritoneal and epididymal fat pads were used to determine relative AT weight. After emptying the gastrointestinal tract, animal carcasses were autoclaved, lyophilized, ground, and then stored at −20 °C for body composition analyses. Observing effects at 14 weeks old is relevant since male rats (F1 and F2) were mated with females at 13 weeks old.

In the current experimental design, since the treatments were administered to gestating F0 founder dams, we consider the F1 generation fetus (*in utero*) and the F1 germline that would generate the F2 generation directly exposed. F1 and F2 phenotypic variations are called intergenerational inheritance. The F3 generation is the first generation with indirect exposure, and phenotypic variations in this generation are thus called transgenerational inheritance.[33]

Body composition

As previously described,[34] body composition analyses were performed for the F1 and F2 male lineages. Total energy content was determined by an automatic adiabatic calorimeter (Model 1241; Parr Instruments). Nitrogen content was measured using a Leco FP-528 (St. Joseph, MI, USA) according to the Dumas method. Protein concentration was calculated by multiplying the nitrogen content of the carcass by 6.25. Then, energy from protein (protein concentration × 23.51 kJ/g) was subtracted from total carcass energy to obtain energy from fat that was then used to calculate carcass fat concentration (coefficient: 39.29 kJ/g).[34]

Plasma lipid analyses

Plasma triglycerides (TGs), HDL, and total cholesterol concentrations were determined using enzymatic assay kits (ab65336, ab65390, and ab65359, respectively) according to the manufacturer's protocols (Abcam, Cambridge, MA, USA). Non-HDL cholesterol concentration was calculated (non-HDL cholesterol = total cholesterol − HDL-cholesterol).

Liver lipid extraction and measurements

Total liver lipid extraction was performed from approximately 50 mg of frozen liver according to the modified Folch method.[35,36] Briefly, the liver was homogenized in 900 µL of a chloroform:

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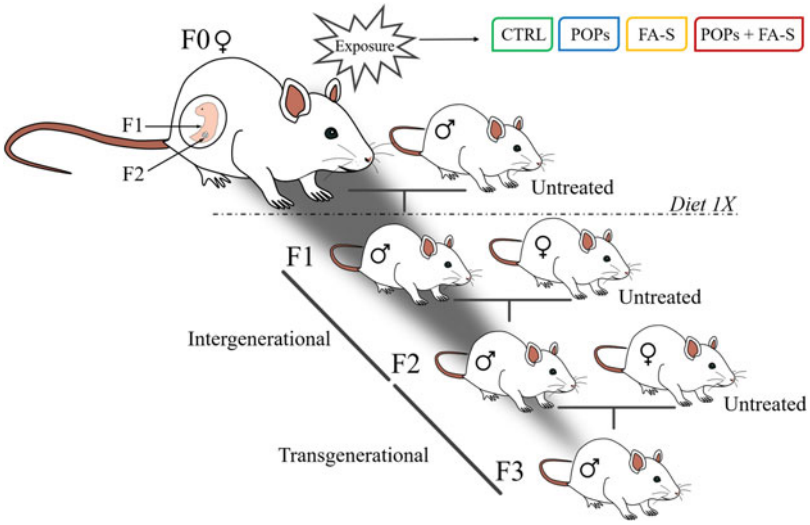

Fig. 1. Experimental design.

methanol solution (2:1). An aqueous solution of NaCl (0.73%) was then added, and after shaking, the lower phase containing total lipids was extracted. The solvent was evaporated under nitrogen, and hepatic lipids were stored at −20 °C until analysis.

As previously described,[37] lipid extract was reconstituted with isopropanol. Hepatic total cholesterol and TG concentrations were determined using the enzymatic kits from Randox (RDX-CH200, Laboratories, Crumlin, UK) and Infinity kit (TR22421, Thermo Scientific, Waltham, USA), respectively.

RNA extraction, quantification, and cDNA synthesis

To evaluate the expression of TNF-α, a cytokine involved in systemic inflammation, and F4/80, a major macrophage marker, total RNA was extracted from frozen epididymal AT. Total RNA was also extracted from frozen liver to evaluate the expression of sterol regulatory element-binding protein 1 (SREBP-1c), a transcription factor that regulates fatty acid and lipid production, of HMG-CoA reductase, the key enzyme regulating cholesterol biosynthesis and of SREBP-2, a transcription factor that regulates cholesterol homeostasis. Total RNA was extracted using Trizol for lysis (Invitrogen, Carlsbad, USA) and GeneJET RNA purification columns according to the manufacturer's protocol (Thermo Fisher Scientific, Waltham, USA). The purity and the concentration of the total RNA were determined using a BioDrop Duo (BioDrop, UK), and RNA integrity was confirmed by agarose gel electrophoresis. cDNA was synthesized from RNA (500 ng) by reverse transcription using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, USA) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction

The oligonucleotide primers were purchased from IDT (Skokie, USA). Quantitative real-time polymerase chain reaction (RT-qPCR) analysis on a CFX384 real-time system from BIO-RAD was performed to determine the mRNA expression of TNF-α, F4/80, SREBP-1c, SREBP-2, and HMG-CoA reductase. Each reaction contained 4 µL of cDNA plus 6 µL of the reaction mixture that was made with the advanced qPCR mastermix with supergreen lo-rox (Saint-Jean-Baptiste, QC, Canada). The levels of relative mRNA expression were calculated using the ΔΔCt method. All reactions were performed in triplicate for each animal, and RNA data for target genes were normalized to reference genes peptidylprolyl isomerase A and TATA box binding protein. Data analysis was performed using Maestro (BioRAD).

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM) for each treatment group. Three-way analysis of variance (ANOVA) for a 2 × 2 × 3 factorial design (POPs, FA, generation) was performed. Given that significant differences were observed between the three generations, a two-way ANOVA for a 2 × 2 factorial design (POPs and FA) was performed for each generation, at 14 and 28 weeks old, using the MIXED procedure (SAS Institute Inc., Cary, USA) to test the main effects of POPs and FA as well as their interaction (POPs*FA) on fasting variables. The model included a random effect for F0 founder female. Variables with a non-normal distribution were transformed to their natural logarithm to obtain normality. When a significant interaction POPs*FA was observed, the Tukey honest significant difference test was performed to determine between-group differences. qPCR data were analyzed using the Tukey test. Pearson correlation coefficients were calculated between variables, using the CORR procedure. Differences were significant at $P \leq 0.05$.

Results

Food consumption, body weight gain, and composition

Data for food consumption, weight gain, and body composition are shown in Table 3. The body weights of male offspring were comparable among the four experimental groups at 3, 13, and 26 weeks old for the three generations. As expected, in all the groups, body weights were significantly higher at 26 weeks old than at 13 weeks old ($P < 0.0001$) than at 3 weeks old ($P < 0.0001$). At the end of the experimental protocol, neither POPs exposure nor FA supplementation in F0 females affected the weight gain or food intake of F1, F2, and F3 males. Body composition did not differ among the groups of male rats in generations F1 and F2. Indeed, animals had similar lean and fat masses. We observed no changes in relative liver weight (ratio of liver weight to final body weight) among the F1, F2, and F3 male groups. In F1 male rats, only those from FA-S dams tended to have lower relative AT weight ($P = 0.09$).

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Table 3. Body weight of F1, F2, and F3 male rats at 3, 13, and 26 weeks old, total weight gain, food intake, and body composition

	F1			
	CTRL	POPs	FA-S	POPs + FA-S
Weight at 3 weeks old (g)	51 ± 2	52 ± 1	48 ± 2	51 ± 1
Weight at 13 weeks old (g)	386 ± 9	400 ± 10	384 ± 9	387 ± 12
Weight at 26 weeks old[a] (g)	493 ± 12	521 ± 14	499 ± 12	493 ± 18
Total weight gain[a,b] (g)	441 ± 12	469 ± 14	451 ± 11	442 ± 18
Food intake[a] (g/day)	18 ± 0.3	19 ± 0.4	18 ± 0.4	19 ± 0.6
Body carcass composition[c]				
Total energy (KJ/g)	266 ± 2	260 ± 4	254 ± 4	261 ± 5
Body fat mass (mg/g)	360 ± 17	336 ± 23	294 ± 14	337 ± 23
Lean body mass (mg/g)	529 ± 20	543 ± 21	588 ± 10	544 ± 21
Liver and AT[a]				
Relative liver weight (%)	2.7 ± 0.2	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Relative AT weight[d] (%)	4.1 ± 0.2	4.3 ± 0.4	3.3 ± 0.2†	4.0 ± 0.3
	F2			
	CTRL	POPs	FA-S	POPs + FA-S
Weight at 3 weeks old (g)	51 ± 2	50 ± 2	50 ± 1	51 ± 1
Weight at 13 weeks old (g)	410 ± 11	408 ± 11	415 ± 11	408 ± 13
Weight at 26 weeks old[a] (g)	538 ± 15	522 ± 15	544 ± 15	520 ± 18
Total weight gain[a,b] (g)	486 ± 15	472 ± 14	494 ± 15	470 ± 18
Food intake[a] (g/day)	20 ± 0.6	19 ± 0.5	19 ± 0.6	19 ± 0.6
Body carcass composition[a]				
Total energy (KJ/g)	264 ± 6	255 ± 8	253 ± 9	266 ± 4
Body fat mass (mg/g)	347 ± 29	332 ± 28	307 ± 33	353 ± 21
Lean body mass (mg/g)	542 ± 26	530 ± 19	562 ± 20	542 ± 23
Liver and AT[a]				
Relative liver weight (%)	2.5 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.4 ± 0.1
Relative AT weight[d] (%)	4.0 ± 0.3	4.2 ± 0.3	3.9 ± 0.3	4.6 ± 0.3
	F3			
	CTRL	POPs	FA-S	POPs + FA-S
Weight at 3 weeks old (g)	52 ± 2	53 ± 2	52 ± 1	53 ± 2
Weight at 13 weeks old (g)	411 ± 7	419 ± 8	403 ± 8	401 ± 9
Weight at 26 weeks old[a] (g)	537 ± 12	547 ± 15	526 ± 11	512 ± 17
Total weight gain[a,b] (g)	485 ± 12	494 ± 15	474 ± 10	461 ± 17
Food intake[a] (g/day)	19 ± 0.3	20 ± 0.3	20 ± 0.6	19 ± 0.6
Liver and AT[a]				
Relative liver weight (%)	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
Relative AT weight[d] (%)	4.1 ± 0.5	5.2 ± 0.2	4.6 ± 0.3	4.2 ± 0.4

There were no significant differences between treatment groups. We only noticed a trend for the FA-S group regarding relative AT weight in F1 males (†$P \leq 0.10$).
[a]Results are expressed as mean ± SEM, $n = 9$ or 10.
[b]Difference between body weights measured at 28 and 3 weeks old.
[c]Results are expressed as the mean ± SEM, $n = 8$ to 10.
[d]Retro-peritoneal and epididymal fat pads were used to determine relative AT weight.

Plasma lipids

Figures 2 and 3 illustrate plasma total, HDL, and non-HDL cholesterol and TG levels in F1, F2, and F3 male rats at 14 and 28 weeks old after a 12-h fast.

In F1 males, at 14 weeks old, prenatal exposure to POPs increased plasma total cholesterol (0.91 ± 0.06 vs. 0.79 ± 0.06 µg/µl, POPs vs. non-POPs exposure, $P = 0.02$) (Fig. 2a) and non-HDL cholesterol levels (0.48 ± 0.05 vs. 0.39 ± 0.04 µg/µl, POPs vs. non-POPs exposure; $P = 0.01$) (Fig. 3b). The offspring of FA-S dams had lower plasma total cholesterol (0.80 ± 0.06 vs. 0.89 ± 0.04 µg/µl, FA-S vs. non-FA-S; $P = 0.05$) (Fig. 2a) and non-HDL cholesterol (0.40 ± 0.05 vs. 0.47 ± 0.04 µg/µl, FA vs. non-FA supplementation; $P = 0.05$) (Fig. 3b) than the offspring of non FA-S dams. No interaction between POPs and FA was observed on plasma lipids at 14 weeks old. Total cholesterol and TG levels did not change among the four male groups at 28 weeks old (Fig. 2c and 2d).

At 14 weeks old, F2 male descendants of POPs-treated lineages tended to have lower plasma total and non-HDL cholesterol levels ($P = 0.10$ and $P = 0.06$, respectively), and plasma TG levels were lower ($P = 0.05$) compared to non-POPs-exposed lineages (1.00 ± 0.12 vs. 1.42 ± 0.23 µg/µl; POPs vs. non-POPs exposure) (Figs. 2a, 2b and 3b). We found positive correlation between plasma TG levels and food consumption ($r = 0.35$, $P = 0.03$). No effects were noted at 28 weeks old (Fig. 2c and 2d).

In F3 males, we observed no changes in plasma total cholesterol or TG levels at 14 weeks or 28 weeks old (Fig. 2a, 2b, 2c and 2d).

In F1, F2, and F3 males, we observed no difference in plasma HDL cholesterol (Fig. 3a and 3c).

Hepatic lipids

Figure 4 illustrates hepatic total cholesterol and TG levels at 28 weeks old after a 12 h fast.

In F1 males, prenatal exposure to POPs increased hepatic total cholesterol ($P = 0.04$) (5.0 ± 0.25 and 4.39 ± 0.21; POPs vs. non-POPs) and TG ($P = 0.03$) (27.2 ± 3.3 and 19.1 ± 1.9; POPs vs. non-POPs) (Fig. 4a and 4b). We noted relevant correlations between hepatic TG and relative liver weight ($r = 0.31$, $P = 0.05$) and plasma total cholesterol ($r = -0.33$, $P = 0.04$).

In F2 and F3 males, hepatic total cholesterol and TG did not differ among groups (Fig. 4a and 4b).

Hepatic marker gene expression levels related to lipid homeostasis

To explain the previous phenotype related to TG and cholesterol levels that we observed in F1 males, we quantified the mRNA expression of key genes of lipid (SREBP-1c) and cholesterol (SREBP-2 and HMG-CoA reductase) homeostasis in F1 male rats. No change was observed between the four groups in F1 males (Fig. 5a, 5b and 5c).

Inflammatory marker gene expression levels in AT

We next assessed the impact of maternal POPs exposure and FA supplementation expression of genes encoding markers of inflammation in epididymal AT. No change was noted in the mRNA expression of the pro-inflammatory cytokine TNF-α in AT of F1, F2, or F3 males (Fig. 5d). We quantified the mRNA expression level for one macrophage marker (F4/80) in the epididymal AT. No difference was observed between the four groups in F1, F2, or F3 males (Fig. 5e).

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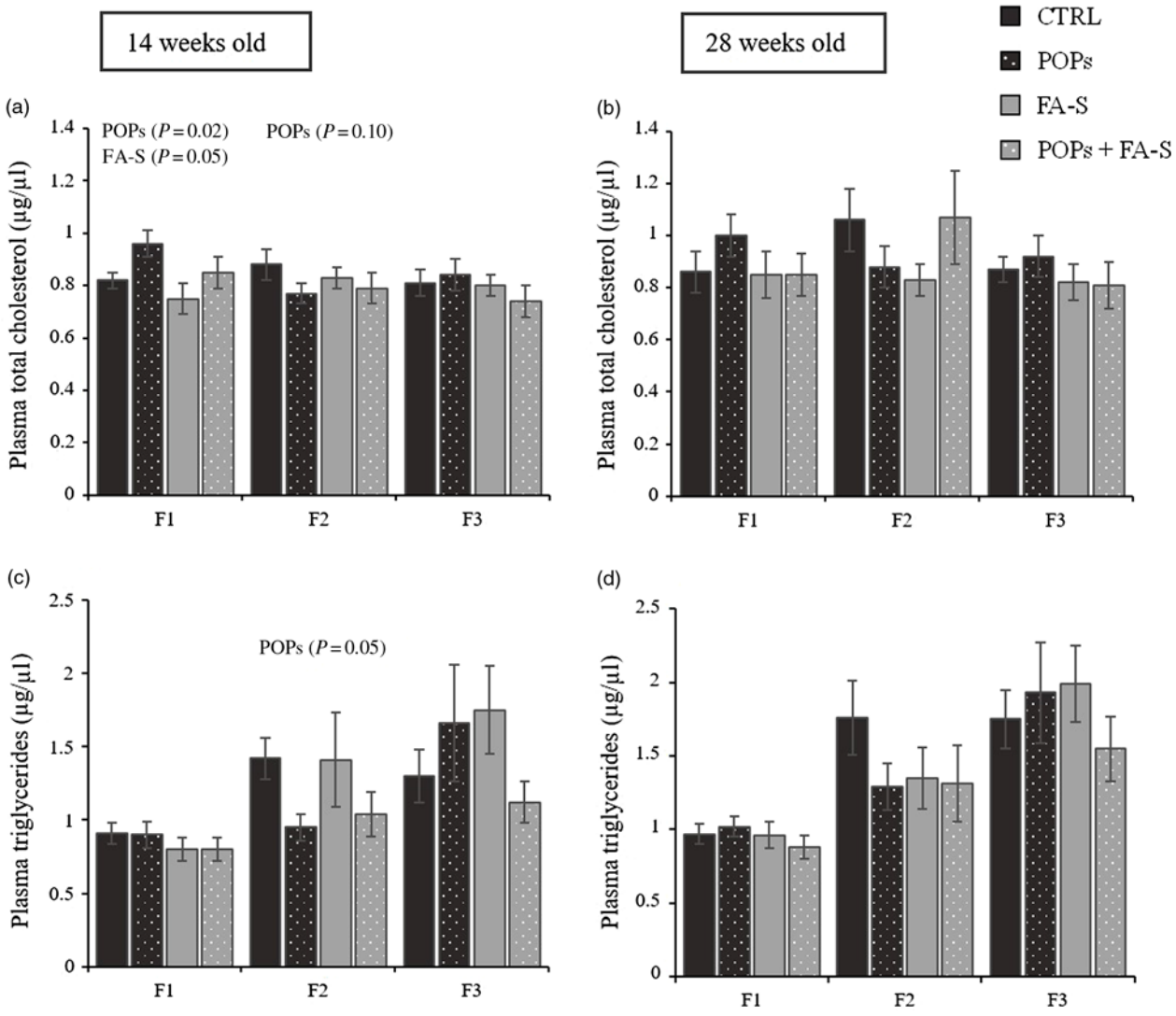

Fig. 2. Impact of POPs exposure and/or FA-S of F0 female rats on plasma total cholesterol and triglycerides in F1, F2 and F3 male rats aged 14 weeks old (*a*–c) and 28 weeks old (*b*–*d*). Results are expressed as mean ± SEM ($n = 9$ or 10). For plasma total cholesterol, there were significant POPs and FA-S main effects in F1, at 14 weeks old. There were no significant differences between treatment groups at 28 weeks old. For plasma triglycerides, there were no significant differences between treatment groups except a POPs main effect for F2 at 14 weeks old.

Discussion

The current study investigated the impact of prenatal Arctic POPs exposure, prenatal dietary FA supplementation, or a combination of POPs exposure plus FA supplementation on plasma lipid response across three generations of male rats. The key finding of this study is that prenatal exposure to an environmentally relevant mixture of Arctic POPs leads to increased hepatic and plasma lipid levels in male offspring of the first generation (F1) at 14 and 28 weeks old. Our results also show that FA supplementation of the F0 dam diet does not counteract the deleterious impact of prenatal POPs exposure observed in F1 males. We did not observe effects of prenatal POPs and FA supplementation exposure on AT mRNA expression of inflammatory genes in male offspring of the F1, F2, or F3 generations.

Exposure to environmental pollutants, such as POPs, has been recently found to impact lipid homeostasis and to promote metabolic diseases in human and animal studies.[38–40] In the present analysis, we demonstrated that F1 males directly exposed to POPs through the F0 dams had higher plasma total and non-HDL cholesterol at 14 weeks old. Consistent with our results, La Merrill *et al.*[19] showed that prenatal dichlorodiphenyltrichloroethane exposure provoked higher circulating total cholesterol in offspring fed a high-fat diet (HFD), which may in part be due to higher hepatic cholesterol synthesis.[41] In humans, Lee *et al.*[39,40] showed that long-term exposure to low doses of POPs (dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), PCBs) is associated with higher plasma TG and lower plasma HDL-cholesterol levels, two features of the metabolic syndrome, and T2D.[42] In our study, however, we found no significant difference for plasma TG and HDL-cholesterol concentrations in F1 males.

At 28 weeks old, F1 males with direct POPs exposure showed higher hepatic TG and cholesterol levels. Our results are in good agreement with those of Wu *et al.*[43] who studied the specific effect of PCB–153 on the metabolic profile of C57BL/6J mice and reported higher hepatic TG levels and mRNA expression of SREBP-1, which is involved in lipogenesis activation,[44] in mice exposed to PCB-153 fed a normal diet. In our study, the absence

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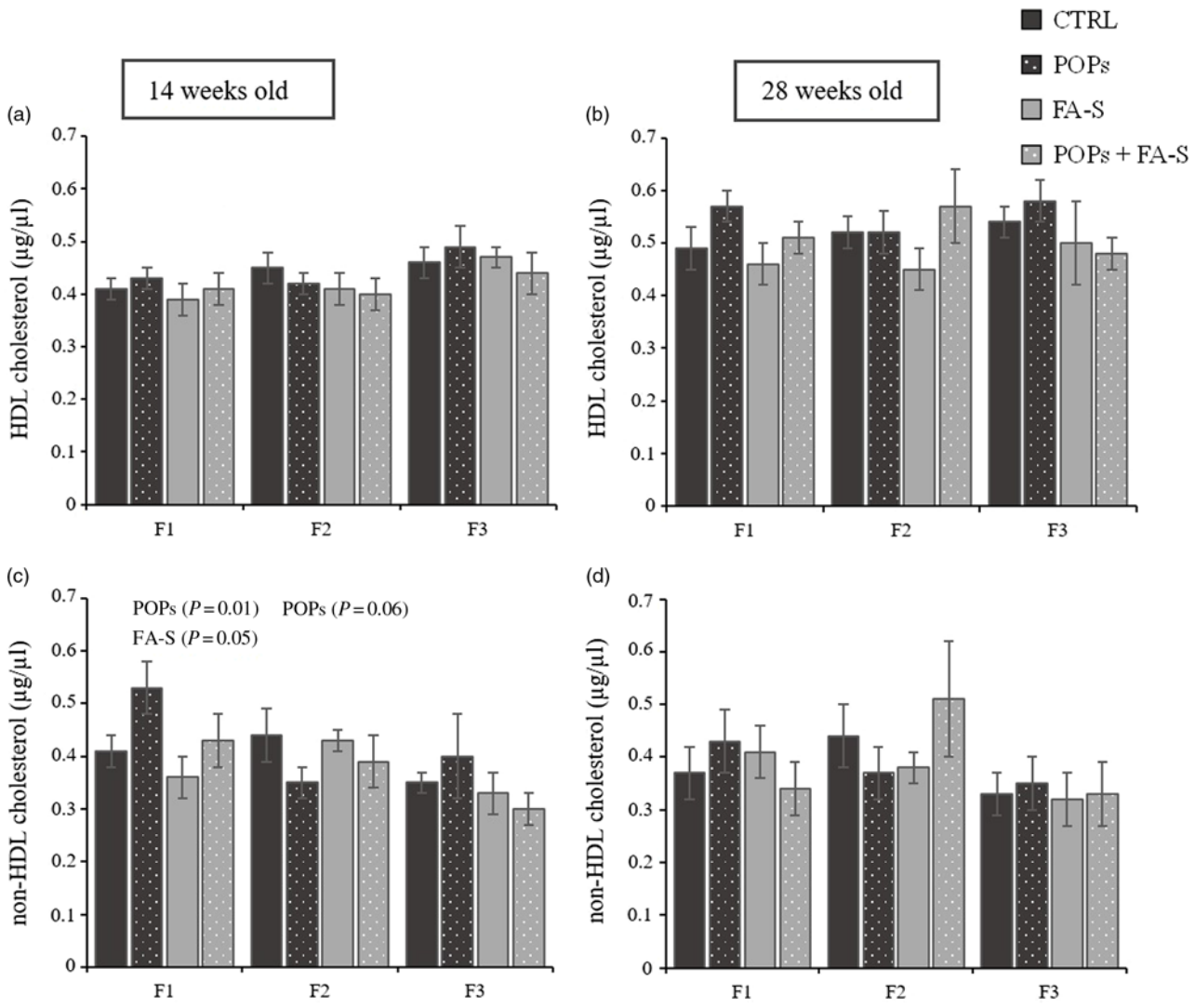

Fig. 3. Impact of POPs exposure and/or FA-S of F0 female rats on plasma HDL cholesterol and non-HDL cholesterol in F1, F2 and F3 male rats aged 14 weeks old (*a–c*) and 28 weeks old (*b–d*). Results are expressed as mean ± SEM ($n = 9$ or 10). For plasma non-HDL cholesterol, there were significant POPs and FA-S main effects in F1, at 14 weeks old. There were no significant differences between treatment groups at 28 weeks old. For plasma HDL cholesterol, there were no significant differences between treatment groups.

of differences of SREBP-1c and HMG-CoA reductase expression levels suggests that lipogenesis and cholesterol synthesis were not responsible for the higher hepatic TG and cholesterol concentrations. Moreover, our results suggest that SREBP-2, that controls LDL cholesterol receptor, was probably not involved in the increase of hepatic cholesterol level. However, other potential mechanisms have been proposed. Mailloux *et al.*[45] reported that exposure to a northern contaminant mixture (with PCBs and DDE) of genetically obese JCR rats promoted lipid accumulation in the liver by decreasing hepatic cholesterol export. The authors found that the protein levels of ABCA1, which is required for cholesterol efflux from hepatic tissue, were lower in hepatic tissue in JCR rats treated with the contaminant mixture. They also reported[45] that exposure to the POPs mixture altered ATP homeostasis in rats. Furthermore, in F1 males, at 28 weeks old, correlations between hepatic TG and relative liver weight and plasma total cholesterol suggest that prenatal POPs exposure is linked to non-alcoholic fatty liver disease and higher cholesterol secretion by the liver. Although F1 rats from POPs lineage have not been directly treated with our POPs mixture, they were directly exposed through the placenta during gestation and through lactation.[46–48] Indeed, the study of Anas *et al.*[29] shows that POPs levels in plasma of F0 dams and F1 male pups are representative of the POPs mixture that were given by gavage to the F0 dams. Surprisingly, in F2 males, at 14 weeks old, POPs exposure of F0 dams was associated with decreased TG levels. The positive correlation between plasma TG levels and food consumption indicates that hypotriglyceridemia in the present study was associated with lower food intake, although non-significant. Unlike previous studies, we did not observe either higher mRNA expression levels of genes encoding pro-inflammatory cytokines (TNF-α) or higher mRNA expression of genes encoding macrophage infiltration markers (F4/80) in F1, F2, or F3 males. Although recent studies demonstrated that the father can transmit diseases to his offspring through sperm,[49,50] we did not observe such effects on F3 male descendants following POPs exposure of F0 dams.

To prevent POPs-associated metabolic diseases, we also investigated a dietary intervention with FA supplementation. As a methyl donor, FA has a key role in the maintenance of DNA stability[51] and can impact metabolic phenotypes that could

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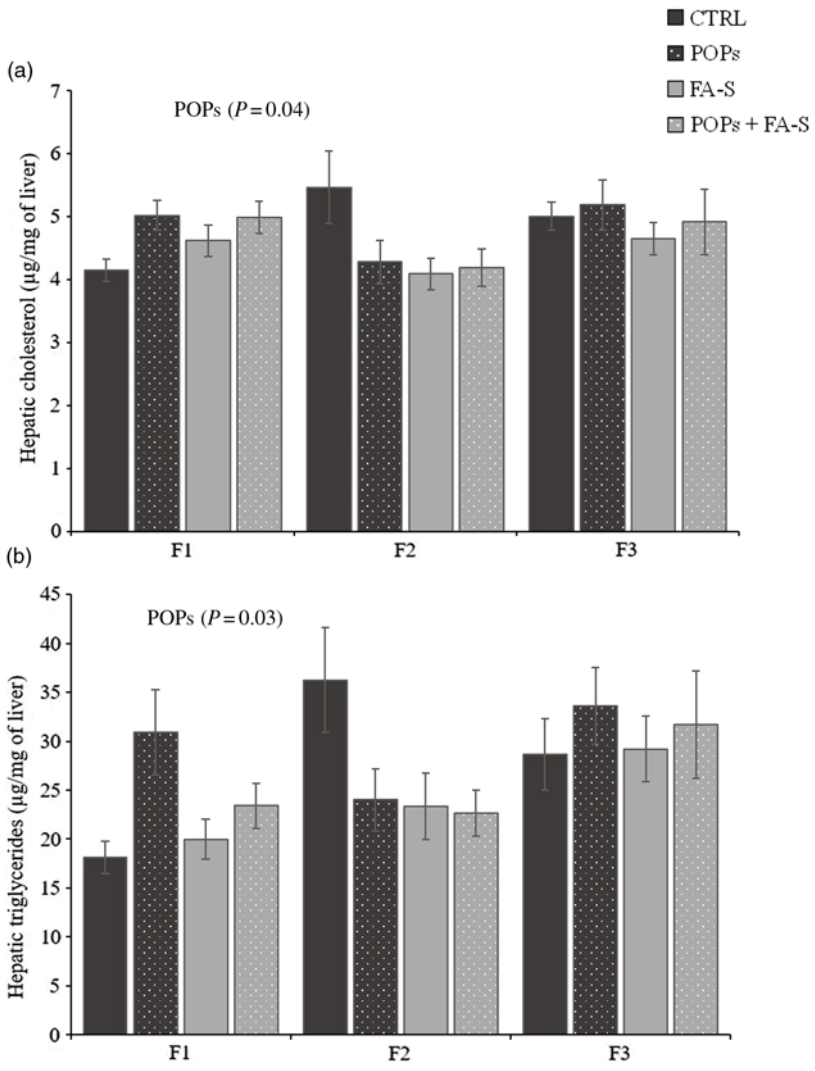

Fig. 4. Impact of POPs exposure and/or FA-S of F0 female rats on hepatic cholesterol and triglycerides in F1, F2 and F3 male rats aged 28 weeks old (*a–b*). Results are expressed as mean ± SEM (n = 9 or 10). For hepatic total cholesterol and triglycerides, there was a POPs main effect for F1 males.

be transmitted to descendants via the sperm epigenome.[24] Accordingly, previous studies reported that FA can modulate lipid metabolism.[52] In a Wistar rat model fed a HFD, high FA supplementation (40 mg/kg diet) reduced visceral AT weight and hepatic TG levels compared to rats fed a HFD without FA supplementation.[53] During the perinatal period, maternal FA restriction has been shown to increase weight, visceral adiposity, plasma total cholesterol, and TG levels as well as TNF-α in plasma and AT in FA-deficient offspring.[22] Chmurzynska *et al.*[54] reported that prenatal FA supplementation with a 5 mg FA/kg diet lowered total cholesterol in offspring. Consistently, in the present study, at 14 weeks old, we observed lower plasma total cholesterol for F1 males of FA-S dams independent of F0 POPs treatment. Therefore, FA supplementation during early life may present a beneficial outcome on plasma cholesterol levels in offspring. FA supplementation of F0 dams, however, did not induce phenotypic modifications related to lipid metabolism and inflammation through intergenerational transmission in F2 males or through transgenerational transmission in F3 males.

Although methyl donor supplementation has been shown to protect metabolic health against environmental factors,[25,55,56] our results in F1 males provide no evidence to support the hypothesis that FA supplementation in the F0 dams diet would be able to alleviate the adverse effects of prenatal POPs exposure. Huang *et al.*[57] showed that *in utero* exposure to high FA concentrations (40 mg FA/kg diet) predisposed male rat offspring to obesity, greater insulin resistance, and glucose intolerance than their control (2 mg FA/kg diet) and FA (5 mg FA/kg diet) counterparts after 8 weeks of a HFD. The authors observed that high FA concentrations exacerbated the detrimental effect of a HFD. Interestingly, the 5 mg FA/kg diet did not affect male offspring, which is consistent with our results. In contrast, Morakinyo *et al.*[58] reported that supplementation of the standard AIN 93G diet with 5 mg FA/kg of females during gestation and of their offspring induced insulin resistance, dyslipidemia, and disrupted glucose metabolism by reducing adiponectin secretion in AT. In our study, the dose of 6 mg FA/kg diet did not lead to adverse metabolic effects related to metabolic syndrome. Finally, the unchanged lipid phenotype in F3 males of the POPs-treated and FA supplemented lineages suggests that reprogramming of imprinted genes occurs during gamete development.

One limitation of this study is that our animals were fed a low-fat diet, whereas most previous studies used HFDs that exacerbated the effects of POPs. Indeed, Ibrahim *et al.*[38] showed that C57BL/6J mice fed a HFD containing farmed salmon fillets contaminated with POPs presented with twice the weight of control and HFD groups. Then, HFD + POPs mice also showed a strong increase in AT weight compared to other groups. Finally, the authors found macrophage infiltration in AT of HFD + POPs mice compared to

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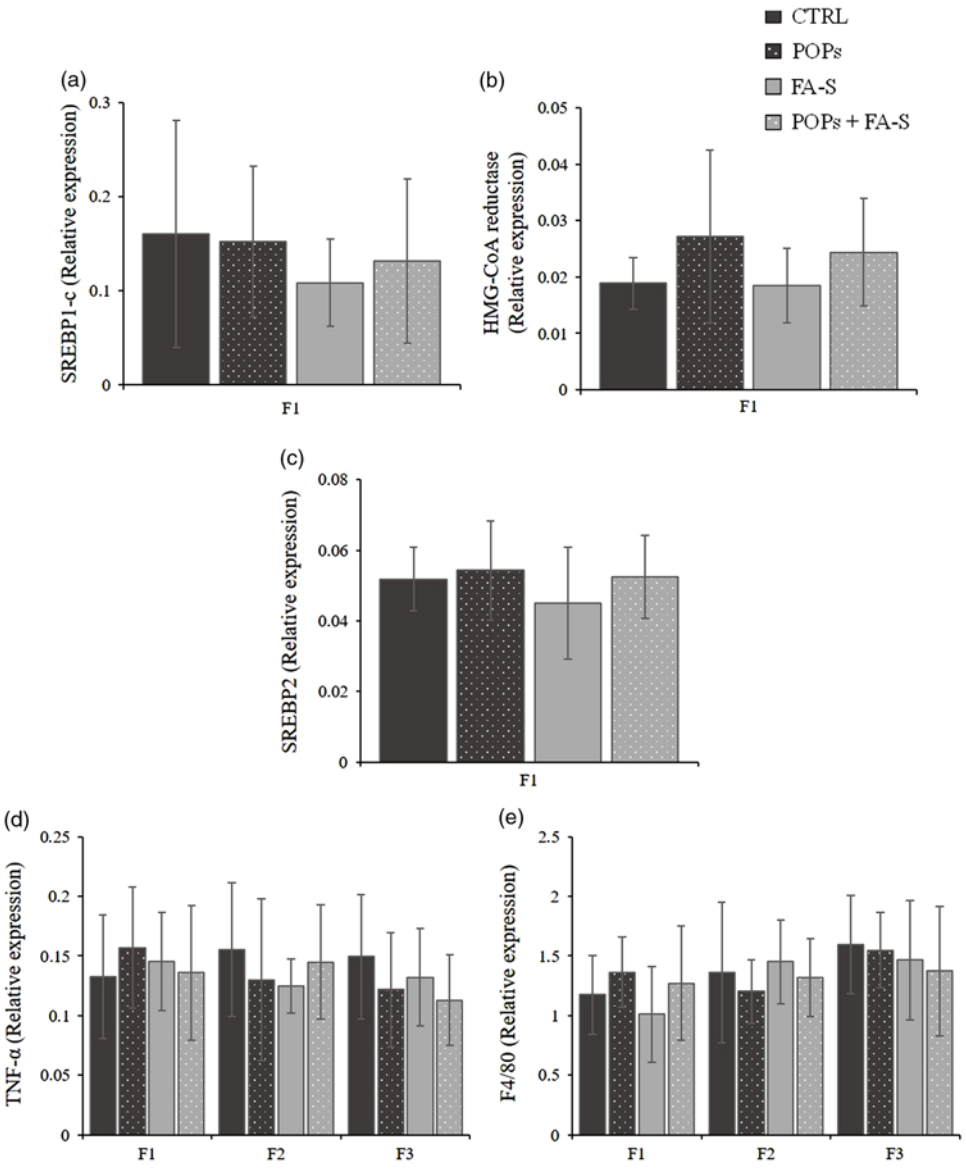

Fig. 5. Relative mRNA expression at for SREBP-1c, HMG-CoA reductase, SREBP-2 in F1 male rats aged 28 weeks old (*a–c*) and TNF-α and F4/80 in F1, F2 and F3 male rats aged 28 weeks old (*d–e*) normalized to PPIA (n = 8 or 9). Data are expressed as mean ± SEM. Relative mRNA expression for SREBP-1c, HMG-CoA reductase, SREBP-2, F4/80 and TNF-α were also normalized to TATA-binding protein (TBP). There were no significant differences between treatment groups.

HFD mice as well as strong expression of the pro-inflammatory cytokine TNF–α, suggesting the development of chronic low-grade inflammation. Taken together, these results demonstrate an exacerbating effect of POPs exposure, predisposing individuals to cardiometabolic diseases. The low-fat diet used in our study likely explains the absence of inflammatory markers in AT of rats of POPs-exposed lineage in our study. Future studies should investigate the exacerbated effect of a HFD on the detrimental metabolic mechanisms underlying POPs exposure. Moreover, the use of a rat model to investigate cholesterol homeostasis represents another limitation of the present study given that rodents carry excess cholesterol in the HDL fraction instead of LDL fraction as in humans.[59]

In conclusion, FA supplementation of the F0 dam diet failed to attenuate the adverse POPs effects on plasma and hepatic lipid parameters in F1 males. Our findings reveal, however, that FA supplementation of F0 females can reduce plasma cholesterol in F1 males regardless of POPs exposure. Although treatments of the F0 females did not affect lipid parameters in F3 males, our results show the significance of paternal health in the transmission of health outcomes until F2 males. Moreover, in this study, using a low-fat diet, FA supplementation or/and POPs exposure of the F0 females did not promote inflammation in AT. Further research is needed to better understand the mechanisms of prenatal POPs exposure on lipid metabolism in male descendants in order to find a relevant nutritional approach against such contaminants.

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Conflicts of Interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (Canadian Council on Animal Care) and has been approved by the institutional committee (Université Laval Animal Research Ethics Committee, certificate #2015010-2).

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Authors' Contributions. Authors contributed to initial conception or design (J.L.B., J.M.T., S.K., A.J.M.); final design (P.N., M.D., N.L., J.L.B., H.J.); data acquisition, analysis, or interpretation (P.N., M.D., P.M.H., M.L., N.L., B.M., P.M., A.M., J.L.B., H.J.), drafting the manuscript (P.N.); and critically revising the manuscript (P.N., M.D., P.M.H., M.L., P.L.C., B.M., P.M., N.L., S.K., J.M.T., A.J.M., A.M., J.L.B., H.J.). All authors have read and approved the final manuscript.

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