Perinatal maternal high-fat diet promotes alterations in hepatic lipid metabolism and resistance to the hypolipidemic effect of fish oil in adolescent rat offspring

Lorraine S. Oliveira1*, Luana L. Souza1*, Aline F. P. Souza1, Aline Cordeiro1, George E. G. Kluck2, Georgia C. Atella2, Isis H. Trevenzoli1 and Carmen C. Pazos-Moura1

1 Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ, Brazil
2 Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, RJ, Brazil

Scope: Maternal high-fat diet (HFD) promotes obesity and metabolic disturbances in offspring at weaning and adult life. We investigated metabolic consequences of maternal HFD in adolescent rat offspring and the potential benefic effects of fish oil (FO) (n-3 polyunsaturated fatty acid source).

Methods and results: Female rats received isocaloric, standard diet (STD: 9% fat) or HFD (28.6%) before mating, and throughout pregnancy and lactation. After weaning, male offspring received standard diet and, from 25th to 45th day, received oral administration of soybean oil (SO) or FO. HFD offspring showed higher body weight and adiposity, which was not attenuated by FO. In STD offspring, FO reduced serum triglyceride and cholesterol, as expected, but not in HFD offspring. Liver of HFD offspring groups showed increased free cholesterol and FO-treated HFD group showed lower expression of Abcg8, suggesting decreased cholesterol biliary excretion. HFD offspring presented higher hepatic expression of lipogenic markers, Srebf1 mRNA and acetyl CoA carboxylase (ACC). Serum n-3 PUFA were decreased in FO-treated HFD compared to FO-treated STD offspring, which may explain the reduced hypolipidemic FO effect.

Conclusion: Maternal HFD impaired the ability of FO to reduce adiposity and serum lipids in adolescent offspring, suggesting a potential predisposition to future development of metabolic disorders.

Keywords:
Adolescence / Fish oil / Hepatic cholesterol metabolism / Hepatic triglycerides metabolism / Liver / Maternal high-fat diet / Metabolic programming

1 Introduction

Obesity and obesity-related disorders are reaching epidemic proportions worldwide [1]. The lifestyle and dietary behaviors, characterized by excess of calories, sugar and lipids contribute to this phenotype [2]. Besides the deleterious impact on individual physiology, these nutritional choices can affect future generations [3], which requires attention regarding the increased prevalence of obesity among women in reproductive age [4].

*These authors contributed equally to this work.

Abbreviations: ABCA1, ATP-binding cassette subfamily A member; ACAT, sterol O-acyltransferase 1; ACC, acetyl CoA carboxylase; ABCG8, ATP-binding cassette subfamily member 8; ChREBP, carbohydrate-responsive element-binding protein; CPT-1, carnitine palmitoyl transferase 1; CYP7A1, cytochrome P450, family 7, subfamily a, polypeptide 1; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; FFA, free fatty acids; FO, fish oil; HFD, high-fat diet; HMG CoA, 3-hidroxi-3-methyl-glutaril-CoA; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid;
Maternal obesity induced by different high-fat diets (HFD) has been shown to generate offspring with elevated susceptibility to obesity [5,6], insulin resistance [7], and lipid disorders such as nonalcoholic fatty liver disease [8], due to alterations in gene expression pattern involved in the regulation of energy metabolism [9,10]. Our group has established a model of maternal obesity induced by a moderate HFD that has the same amount of calories as the standard diet. These offspring from HFD dams exhibited increased body weight and adiposity, and hormonal changes at weaning [5] and at adult life [6].

The offspring’s response to maternal behavior during the perinatal period is known as metabolic programming [5,7,11]. It is an adaptive response to environmental changes that involves epigenetic mechanisms [12]. The epigenetic plasticity is intense during the perinatal period, but it decreases gradually throughout life [3]. Besides early life, adolescence is also a period with high plasticity [3] when interventions may be effective in preventing or attenuating the long-term metabolic disturbances in the offspring from obese mothers [13].

Nutritional intervention during adolescence is an important mechanism to reduce early obesity and its persistence until adulthood [14,15]. Moreover, the contribution of some diet components with antiobesogenic effects has been investigated [16]. Fish oil (FO), a source of n-3 polyunsaturated fatty acids (n-3 PUFA), has potent and well-characterized hypolipidemic action, reducing adiposity and serum lipids in adult rodents [17,18] and humans [19]. However, there is little information about n-3 PUFA’s potential beneficial effects during adolescence. In human adolescents, n-3 PUFA intervention promoted a mild improvement in serum triglyceride (TG) profile [20]. Additionally, mice fed a n-3 PUFA-rich diet from early in life exhibited reduced obesity induced by high-fat ingestion during young adult life [21].

A classical mechanism through which n-3 PUFA exerts hypolipidemic effect involves transcriptional factors that are master regulators of the hepatic metabolism of lipids, such as peroxisome proliferator activated receptor (PPAR), sterol regulatory element binding protein (SREBP) and liver X receptor (LXR) [22, 23]. Most of these transcriptional factors have their expression or activity in the offspring modulated by maternal HFD [24, 25]. These alterations early in life could contribute to a higher predisposition to metabolic syndrome in the offspring from HFD dams. Therefore, in the present study, we evaluated the impact of perinatal maternal HFD on the metabolic profile of adolescent offspring, and the potential benefic effects of FO intervention during adolescence, focusing on the hepatic lipid metabolism.

2 Material and methods

2.1 Animal care and treatments

All procedures were approved by the Ethics Committee on Animal Care of the Health Sciences Center, Federal University of Rio de Janeiro (protocol: IBCCF 140/13). Animals were maintained with free access to water and food in a room with controlled temperature (23 ± 2°C) and with 12 h light:12 h darkness cycles (lights on from 7 a.m. to 7 p.m.). Female Wistar rats, 60 days old, received a standard diet (STD) for rodents (9% of the calories as fat) or a HFD (28.6% of the calories as fat) for eight weeks before mating and during gestation and lactation [5]. Our HFD is moderately high fat compared to most experimental models, which generally use diets with 50% of the calories as fat [26, 27]. This is an important aspect, because the diet was formulated to obtain an increased percent of lipid without characteristics of low-protein or low-carbohydrate diet, and conserving equal calories with standard diet.

In the HFD, lard was used as the major fat source (mainly saturated fat). Both diets contained approximately 3.9 kcal/g (isocaloric) and followed the AIN-93G recommendations for micronutrients [28]. Diet composition is described in Supporting Information Table 1. At birth, all pups were weighed, and litters were adjusted to six males for each dam. STD and HFD offspring received standard diet from weaning (21 days old) until 46 days old (adolescence period).

During the adolescence period [33], from 25 to 45 days of age, the offspring from STD or HFD dams were divided in two groups: one that received soybean oil (SO, source n-6 PUFA; Liza, Cargill, SP, Brazil) and other that received FO (source of n-3 PUFA—19.1% eicosapentaenoic acid (EPA) and 12.2% docosahexaenoic acid (DHA); Fagron, SP, Brazil). Oil administration was given daily by gavage (0.4 mL/100 g body weight). SO was used as control to exclude lipid load effect, ensuring that the results are derived from different types of fatty acids used. Furthermore, SO is a lipid source recommended for rodents according to the American Institute of Nutrition [28], also representing the major fatty acid present in the Western diet (n-6 PUFA) [29]. The total lipid intake (food intake plus gavage administration) was similar among experimental groups (approximately 10.9 Kcal/day).

At 43 days of age, we performed an oral glucose tolerance test (OGTT). After 12 h fasting, blood glucose was measured from a tail incision. Immediately afterwards, glucose (0 (+) – Glucose; VETEC, RJ, Brazil) was orally administered (2 mg/g of body weight). Glycemia was also measured 15, 30, and 60 min after glucose administration (One Touch Ultra Mini device, Johnson and Johnson, NJ, USA).

At 46 days of age rats were killed by decapitation. Serum was obtained from trunk blood (after centrifugation at 1000× g, 4°C, 20 min) and kept frozen at −20°C for measurements of hormone levels and biochemical parameters. HOMA-IR (homeostasis model assessment of insulin resistance) was calculated to infer the insulin resistance profile in the offspring [30]. Representative depots of visceral and subcutaneous white adipose tissue (retroperitoneal and inguinal, respectively) were dissected and weighed to evaluate the adiposity in the adolescent offspring. Liver was harvested, snap frozen in liquid nitrogen, and stored at −70°C prior to extraction of total lipid, protein, and RNA.
2.2 Serum lipid profile

Serum total cholesterol, VLDL cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and TGs were evaluated using colorimetric commercial kits (Biosystems S.A., BCN, Spain), with an automated A15 spectrophotometer (Biosystems S.A.), following recommendations of the manufacturer.

2.3 Hormone assays

Serum leptin and insulin were measured using specific commercial RIA kits from Linco Research (MO, USA). Total serum thyroxine (T4), total serum triiodothyronine (T3), and serum corticosterone concentrations were determined using specific commercial RIA kits from ICN Pharmaceuticals (CA, USA). Serum adiponectin was measured by specific rat Adiponectin ELISA Kit from Merck Millipore (MA, USA). All samples were evaluated in duplicate within the same assay according to the manufacturer's instructions.

2.4 Real time PCR analysis

Real time PCR assay was used to detect mRNA relative expression of genes encoding the PPARα (Ppura), SREBP-1 (Srebf1), SREBP-2 (Srebf2) 3-hidroxi-3-methyl-glutaril-CoA (HMG CoA: Hmgcr), hepatic lipase (Lipc), LDL-receptor (LDL-R: Ldrl), sterol O-acyltransferase 1 (ACAT: Scoat1), ATP-binding cassette subfamily G member 8 (ABCG8: Abcg8), ATP-binding cassette subfamily A member 1 (ABCA1: Abcg1), cytochrome P450, family 7, subfamily a, polypeptide 1 (CYP7A1: Cyp7a1), diacylglycerol O-acyltransferase 2 (DGAT: Dgat2), carnitine palmitoyl transferase 1 (CPT-1: Cpt1a), carbohydrate-responsive element-binding protein (ChREBP: Mxipl), thyroid hormone receptor beta (TRβ: Thrb), and LXR (Nr1h3).

Total RNA was isolated from liver samples using SV Total RNA Isolation System (Promega, WI, USA). Total RNA was reverse transcribed using 1 μg of total RNA and a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). Products were amplified on Eppendorf MasterCycler RealPlex (Eppendorf, Hamburg, Germany) using Maxima SYBR Green qPCR Master Mix (Thermo Scientific, MA, USA). Cycle parameters were 50°C for 2min and 95°C for 10min, followed by 40 cycles at 95°C for 15s, 60°C for 30s, and 72°C for 45s. The intrain spanning primers sequence used in this manuscript are described in the Supporting Information Table 1. Primers were synthesized and tested by Integrated DNA Technologies (IO, USA). Samples and negative controls were evaluated in duplicate in the same assay. Efficiency of each reaction was calculated using a serial cDNA dilution (standard curve, and varied from 98 to 100%). Product purity was confirmed by agarose gel analysis and by a single peak in the melting curve analysis. Relative mRNA levels were calculated using standard curve method and normalized by Rplp0 mRNA levels. Results are expressed relative to values of control group (maternal standard diet offspring that received SO).

2.5 Western blotting analysis

We used the Western blotting assay to detect hepatic fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), CPT-1a, and glyceroldehyde-3-phosphate dehydrogenase (GAPDH) protein abundance. Liver samples were homogenized in lysate buffer pH 6.4 (50mM HEPES, 1mM MgCl2, 10mM EDTA, and 1% Triton X) with protease inhibitor cocktail complete (Roche Diagnostics, IN, USA). After centrifugation, the total protein content of the supernatant was quantified using a Pierce™ BCA Protein assay kit (Thermo Scientific). Total protein was resolved by SDS–PAGE on a 10% gel and transferred onto a polyvinylidene difluoride membrane (Hybond-P 0.45μm PVDF; Amershams Biosciences, BKM, England). Membrane was blocked with 5% nonfat dry milk (Moloco, Nestle, SP, Brazil) and incubated overnight at 4°C with primary antibodies: anti-FAS (Cell signaling, MA, USA; 1:1000 dilution), anti-ACC (Cell signaling; 1:10000 dilution), anti-CPT-1a (Abcam, MA, USA; 1:3000 dilution), and loading control anti-GAPDH (Cell signaling; 1:3000 dilution). Membranes were washed and incubated with peroxidase-labeled anti-rabbit IgG antibody (Amershams Biosciences, Inc.; 1:10 000 dilution) or anti-mouse IgG antibody (Cell signaling; 1:10 000) for 3h at room temperature. All blots were then washed and incubated with a luminogen detection reagent (Amershams ECL Prime Western Blotting Detection reagent; Amershams Biosciences). Chemiluminescent signal was detect by ImageQuant LAS 4000 equipment followed by densitometric analyses (GE Healthcare Life Sciences).

2.6 Thin-layer chromatography

Liver total lipids (10 mg of tissue) were extracted by the method of Bligh and Dyer [31] with modifications. After incubation in chloroform-methanol-water solution (2:1:0.8, v/v), samples were centrifuged (1500 × g for 20 min at 4°C). Then, the chloroform was added to the collected supernatant. After centrifugation (1500 × g for 20 min) the organic phase was removed and dried in nitrogen. The extracted lipids were analyzed by thin-layer chromatography (TLC) for neutral lipids, using a DC Silicagel 60 plate (Merck Millipore, HE, Germany). The plates were submerged for 10 s in Charring solution (3% CuSO4 and 8% H3PO4, v/v), then they were dried and heated to 110°C for 10 min. TLC plates were analyzed by densitometry (ImageMaster software from TotalLab, Auckland, New Zealand).

2.7 GC–MS analysis

The analysis of the fatty acids fractions by GC–MS was carried out as described [32]. The lipid sample was dissolved in
Figure 1. FO effects in adolescent male offspring of dams that received standard or HFD. Offspring received daily oral intervention with SO or FO from 25 to 45 days of age. (A) Body weight change during intervention. (B) Inguinal adipose tissue mass. (C) Retroperitoneal adipose tissue mass. (D) Serum leptin after intervention. (E) Oral glucose tolerance test at 43 days of age. (F) Insulin resistance evaluation by HOMA-IR. Data are expressed as mean ± SEM (n = 8–10). Statistical analysis was performed using two-way ANOVA followed by Tukey’s post-test. Maternal diet effect and interaction were described at the bottom of the graph. STD SO versus HFD SO *p < 0.05.

3 Results and discussion

Adolescent offspring from HFD dams showed increased body weight gain (Fig. 1A) with a higher inguinal (21%, p < 0.05) (Fig. 1B) and retroperitoneal adipose tissue mass (21%, p = 0.06) (Fig. 1C), compared with standard diet (STD) offspring. Increased adiposity was associated with hyperleptinemia (Fig. 1D) and increased levels of corticosterone (Table 1). FO treatment was not effective in reversing this phenotype, since FO-treated groups did not differ from the SO-treated ones regardless of the diet. The HFD offspring that received SO, presented elevated glycemia at 30 min after OGTT (Fig. 1E), and a trend toward elevated HOMA-IR (approximately 45%, p = 0.07) (Fig. 1F), without statistically significant changes in glycemia and insulinemia in fed state (Table 1). The same trend to elevated HOMA-IR was not observed in HFD offspring treated with FO, which raises the possibility that FO may attenuate this phenotype. Other studies have shown that maternal HFD promotes robust glucose intolerance in the offspring at weaning and adult life [26, 27]. The mild phenotype observed regarding body weight and fat mass in the present study, as well as glucose homeostasis, may be related to the young age of the offspring or to the moderate fat diet content used in our model compared with other HFD models [26, 27].

In STD offspring, FO administration reduced serum total cholesterol (22%), TGs (22%), VLDL (16%), HDL (28%),
Table 1. Evaluation of FO effects on serum glucose and serum hormones involved in metabolism control of adolescent male offspring from dams that received control or HFD

<table>
<thead>
<tr>
<th>Serum measurements</th>
<th>Maternal Standard diet</th>
<th>Maternal HFD</th>
<th>Maternal Diet effect</th>
<th>Interaction</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SO</td>
<td>FO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>180 ± 6</td>
<td>187 ± 7</td>
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<tr>
<td>Insulin (ng/mL)</td>
<td>0.56 ± 0.08</td>
<td>0.75 ± 0.05</td>
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<tr>
<td>Adiponectin (ng/mL)</td>
<td>24.289 ± 2965</td>
<td>32.829 ± 1904</td>
<td>No</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>T3 (ng/dL)</td>
<td>44.1 ± 1.1</td>
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<td>No</td>
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<tr>
<td>T4 (μg/dL)</td>
<td>5.45 ± 0.35</td>
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<td>No</td>
</tr>
<tr>
<td>Corticosterone (ng/mL)</td>
<td>98.805 ± 19.029</td>
<td>79.064 ± 9.888</td>
<td>No</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 8–10). Statistical analysis was performed using two-way ANOVA.

and LDL (23%) (Fig. 2), confirming its potent hypolipidemic action observed in many experimental models [17, 33, 34], which is conserved during the adolescence period. It has been reported that maternal HFD affects offspring lipid metabolism, promoting increased serum lipids and hepatic lipid accumulation [35, 36], even in early life [37, 38]. In this study, we observed normal serum lipids in HFD adolescent offspring (Fig. 2), higher liver weight (Fig. 3A), 1.2-fold higher hepatic total lipid content (Fig. 3B), higher free cholesterol (Fig. 3C), and reduced cholesterol ester (Fig. 3D). Interestingly, even without maternal diet effect on serum lipids, the classical effect of FO in reducing serum total cholesterol (Fig. 2A), serum TGs (Fig. 2B), VLDL (Fig. 2C), and HDL (Fig. 2D) was abolished in HFD offspring. In addition, the effect of FO to decrease hepatic free cholesterol content in the STD offspring was lost in HFD offspring (Fig. 2E). However, FO partially reduced serum LDL in HFD offspring (Fig. 2E), probably due to the lack of VLDL reduction in these rats (Fig. 2C) [39]. Altogether, our data showed that maternal HFD induced resistance to the hypolipidemic effects of FO.

In rodents, n-3 PUFA supplementation promotes decreased serum cholesterol [23] and hepatic cholesterol.

Figure 2. FO effects on serum lipids of adolescent male offspring from dams that received standard or HFD. Offspring received daily oral intervention with SO or FO from 25 to 45 days of age. (A) Serum cholesterol concentration. (B) Serum TG concentration. (C) Serum VLDL concentration. (D) Serum HDL concentration. (E) Serum LDL concentration. Data are expressed as mean ± SEM (n = 8–10). Statistical analysis was performed using two-way ANOVA followed by Tukey’s post-test. Maternal diet effect and interaction are described at the bottom of the graph. Oil effect is represented as the bars at the top of the graph (*p ≤ 0.05).
Figure 3. FO effects on hepatic cholesterol metabolism of adolescent male offspring from dams that received standard or HFD. Offspring received daily oral intervention with SO or FO from 25 to 45 days of age. (A) Absolute liver mass. (B) Total hepatic liver content. (C) Hepatic free cholesterol content. (D) Hepatic cholesterol ester content. (E) Hepatic mRNA expression of HMG CoA Reductase (Hmgcr). (F) Hepatic mRNA expression of ACAT (Soat1). (G) Hepatic mRNA expression of ABCG8 (Abcg8). (H) Hepatic mRNA expression of CYP7A1 (Cyp7a1). (I) Hepatic mRNA expression of SREBP-2 (Srebf2). Data are expressed as mean ± SEM (n = 7–9). Statistical analysis was performed using two-way ANOVA followed by Tukey’s post-test. Maternal diet effect and interaction are described at the bottom of the graph. Oil effect is represented as the bars at the top of the graph (*p < 0.05).

content [40] that has been associated or not with reduced HMG CoA reductase and SREBP-2 expression [40–42]. In our study, we observed that FO reduced serum cholesterol in the adolescent standard diet offspring (Fig. 2A) without changing the expression of the genes encoding the HMG CoA (Hmgcr) (Fig. 3E) or LDL-R (Ldlr) (data not shown). However, FO induced a decrease in serum LDL (Fig. 2E) which is probably a result of the marked decrease in serum VLDL (Fig. 2C), a LDL precursor [39]. An increased hepatic clearance of LDL independent of LDL-R has been shown by others [34, 39], and could contribute to the FO hypocholesterolemic effect in these animals. An increase in hepatic clearance of LDL would promote initially an increment of hepatic cholesterol content that would stimulate the activity and expression of ACAT, the key enzyme for cholesterol esterification, as a protective mechanism against liver injury [43]. This may explain the increased hepatic cholesterol ester content (Fig. 3D) and increased Soat1 expression (ACAT) (Fig. 3F) observed in STD offspring that received FO. N-3 PUFAs are also able to increase cholesterol output into the bile [44] and its conversion to bile acids [41]. In adolescent FO-treated STD offspring, we observed reduced free cholesterol content (Fig. 3C) that may be associated with the higher Abcg8 expression (Fig. 3G), a gene encoding an important sterol transporter to increase cholesterol output into bile [44].
HFD offspring that received FO presented decreased Abcg8 expression (Fig. 3G) that could explain at least in part the increased hepatic cholesterol content (Fig. 3C), because the cholesterol output into the bile might be decreased, disrupting an important mechanism of the hypocholesterolemic effect of FO [44]. The higher content of cholesterol in the liver may be the reason for the suppression of Srehf2 expression observed in HFD offspring that received FO (Fig. 3I), since cholesterol increment promotes a negative feedback mechanism of cholesterol synthesis mediated by LXR activation [45]. Consistent with this hypothesis, HFD offspring had increased Cyp7A1 expression (Fig. 3H), but did not change Abca1 expression (Supporting information Fig. 1). However, the effect of FO in increasing Soat1 expression (ACAT) and, consequently, hepatic cholesterol ester content, is maintained in HFD offspring (Fig. 3F and 3D). The observation of this FO effect varies depending on the experimental model, since the opposite profile was reported by others [46, 47].

Regarding fatty acid metabolism, we observed that, regardless of maternal diet, FO groups showed less hepatic TGs (Fig. 4A), probably by suppression of Dgat2 (Fig. 4D) and reduction of its substrate, diacylglycerol (Fig. 4B) [48]. This FO action is an important mechanism involved in decreasing hepatic VLDL synthesis and release, which is observed in many experimental models [49]. FO groups also showed higher hepatic free fatty acids (FFA) (Fig. 4C) without changes in monoacylglycerol content or Lipc, gene encoding the hepatic lipase expression (Supporting Information Fig. 1). Despite no changes in Lipc expression among groups (Supporting Information Fig. 1), we did not discard the possibility of changes in other lipases, such as adipose TG lipase [50]. Our observations of decreased diacylglycerol (Fig. 4B) and increased FFA content (Fig. 4C) in the liver also suggest increased activity of lipases involved in diacyl- and monoacylglycerol breakdown [48]. Another possibility is that FO treatment directs the FFA to hepatic oxidation instead of esterification.

Maternal HFD promoted 1.4-fold increase in the Srehf1 expression (Fig. 4F) and 1.8-fold increase in the abundance of hepatic ACC protein (Fig. 5A), regardless of the oil treatment. These alterations may be associated with increased hepatic de novo lipogenesis that could potentially contribute to disrupt the action of FO [51]. Interestingly, the downstream enzyme in lipid synthesis, FAS, was not changed by maternal HFD (Fig. 5B). Moreover, the suppressive effect of FO in FAS expression was conserved in HFD offspring (Fig. 5B). The result of these molecular changes might promote accumulation of malonyl-CoA, the product of ACC reaction, which is able to inhibit CPT-1 activity, decreasing fatty acid oxidation in the liver of HFD offspring [52]. Although we did not evaluate CPT-1 activity, we observed a trend to reduction of...
CPT 1 protein abundance (Fig. 5C) and Cpt1a mRNA levels ($p = 0.06$; Fig. 4E) in HFD offspring, independent of the oil treatment.

HFD offspring showed hormonal changes that might disturb lipid metabolism. Previously, we showed that FO fails to reduce serum TGs in hypothyroid rats [17]; however, since total T4 is increased and T3 decreased in HFD offspring (Table 1), the participation of thyroid hormones in the mechanism leading to resistance to the effect of FO on HFD offspring is uncertain. Interestingly, in our previous studies with the same model of programming, due to maternal obesity, the HFD offspring showed increased serum thyroid hormones at weaning [5], while here at adolescence we showed decreased T3 and increased T4, suggesting changes in the deiodination process during the course of development [53]. In addition, these studies suggest that thyroid function in offspring programmed by maternal HFD changes along life, suggesting long-term adaptive mechanisms. Thus, other changes such as the rise in serum corticosterone observed in HFD offspring (Table 1) could increase hepatic lipogenesis and decrease fatty acid oxidation [54, 55], impairing FO effect.

In agreement with our observations, previous reports found that hepatic Srebf1 is upregulated in adult mice offspring from maternal HFD [24, 56], but the molecular mechanism is not fully understood. In this study, the increased serum corticosterone (Table 1) [54] and increased hepatic cholesterol content (Fig. 3C) might be involved in upregulation of Srebf1 in HFD offspring [57]. Regardless of maternal diet, FO intervention did not change hepatic Srebf1 mRNA expression (Fig. 4F), but we did not investigate FO effect on nuclear SREBP-1 content. In many experimental models, FO suppresses hepatic SREBP-1 maturation at post-translational level [51], with or without changes in mRNA expression [58, 59]. Although the PUFA effect on SREBP-1 is known to be involved in FAS suppression [51], this mechanism is not exclusive [60, 61]. PUFA suppression on FAS promoter is mediated by the coordinated action of SREBP-1c and ChREBP [62]. Besides that, mutation of SREBP-1 site in Fasn promoter abolished only 25% of PUFA suppression effect [60]. This could explain our divergent observations between Srebf1 mRNA expression (Fig. 4F) and FAS abundance (Fig. 5B). However, we did not observe changes in genes encoding the ChREBP among experimental groups (Supporting Information Fig. 1).

To further investigate the molecular mechanisms involved in resistance to the hypolipidemic FO effect, we evaluated the mRNA expression of master nuclear receptors involved in hepatic lipid metabolism. The relative expression of the genes encoding the PPAR alpha, thyroid hormone receptor beta (TRβ), and LXR alfa (LXRα) were not altered (Supporting Information Fig. 2).

Despite the alterations found in the lipid hepatic metabolism, we did not observe significant differences among experimental groups in the profile of the major fatty acid classes present in liver (Supporting Information Table 3). Serum fatty acid composition was similar among groups, except by arachidonic acid (20:4) and DHA and EPA content (Fig. 6). HFD offspring showed reduced arachidonic acid compared with STD offspring, and it was reduced by FO administration regardless of maternal diet (Fig. 6A). Interestingly, serum DHA and EPA concentrations were 47.4% lower in FO-treated HFD offspring than in FO-treated STD offspring (Fig. 6B). DHA and EPA are long chain PUFA responsible for most of beneficial effects of n-3 PUFA [22] and their lower concentrations could be responsible for reduced response to FO administration in HFD offspring. The n-3 PUFA content in lipoproteins increases lipoprotein lipase activity, increasing and accelerating triacylglycerol uptake [63], which contributes to serum TGs reduction [49]. As the FO dosage was adjusted by animal body weight daily, it is
Figure 6. FO effects on serum fatty acid composition of adolescent male offspring from dams that received standard (STD) or HFD. Offspring received daily oral intervention with (SO) or FO from 25 to 45 days of age. (A) Major fatty acid classes present in serum: myristic acid (C14:0); palmitic acid (C16:0); stearic acid (C18:0); oleic acid (C18:1); linoleic acid (18:2); arachidonic acid (C20:4). (B) Serum DHA (C22:6) and EPA (C20:5) content. (C) Serum n-6/n-3 ratio. Data are expressed as mean ± SEM (n = 3). Statistical analysis was performed using two-way ANOVA followed by Tukey’s post-test. Maternal diet effect and interaction are described at the bottom of the graph. Oil effect is represented as the bars at the top of the graph (*p < 0.05).

possible that in HFD offspring, the n-3 PUFA was reduced by other metabolic alterations such as oxidative stress [22]. This is a common finding in HFD offspring associated with reduced serum n-3 PUFA and increased n-6/n-3 PUFA ratio [38], such as we observed in our model (Fig. 6C).

HFD offspring showed hepatic molecular changes that could disrupt FO effect, but the lower n-3 PUFA serum content may be the main responsible for this phenotype. Regulatory and adaptive mechanisms seem to be activated during adolescence, a period of life with increased epigenetic plasticity [3], allowing a mild phenotype and conservation of some of the FO actions in hepatic lipid metabolism. However, under metabolic challenges, these adaptive mechanisms may fail, leading to metabolic disturbances [64] and affecting serum parameters. Indeed, the resistance to the hypolipidemic action of FO, revealed in the present study, supports this hypothesis.

4 Concluding remarks

In this study, we report that the maternal consumption of a moderate HFD induces resistance to the hypolipidemic effect of FO in adolescent offspring. The hepatic alterations in lipid content, and key transcription factors or/and enzymes controlling hepatic lipid metabolism contribute to this phenotype. Moreover, the reduced n-3 PUFA serum levels in FO-treated HFD offspring may explain, at least in part, the disruption of the hypolipidemic actions of FO. Our study highlights that the long-term changes caused by nutritional imbalance early in life due to a moderate high fat maternal diet are present already at adolescence and may interfere with classical dietary interventions. Moreover, the alterations in hepatic lipid metabolism associated with the resistance to FO suggest an early imbalance that may predispose the HFD offspring to development of metabolic disorders, such as hepatosteatosis, in response to further challenges.

L.S.O., A.F.P.S., A.C., and L.L.S. have participated in animal experiments, collection, and interpretation of data. L.S.O. and L.L.S. have performed all experimental procedures and analyses. G.E.G.K. and G.C.A. have performed TLC and gas chromatography analyses. L.S.O., C.C.P.-M., and I.H.T. have delineated experimental design and supervised the study. L.S.O., L.L.S., and C.C.P.-M. have written the manuscript.
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The authors have declared no conflicts of interest.

5 References


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