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An early fish oil-enriched diet reverses biochemical, liver and adipose tissue alterations in male offspring from maternal protein restriction in mice☆

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Abstract

Fetal programming is linked to adulthood metabolic and chronic diseases. We hypothesized that early fish oil (FO) intake would revert the programming responses in adult offspring. Pregnant mice were fed either standard chow (SC) or a low-protein diet (LP) throughout pregnancy/lactation. At weaning, the following groups were formed: SC and SC-FO, LP and LP-FO, which were fed SC or SC+FO, respectively. The LP offspring are predisposed to becoming fat, hypercholesterolemic and hyperglycemic. In addition, during adulthood, they become hypertensive with hepatic steatosis and have a high level of sterol regulatory element binding protein (SREBP-1). However, LP offspring that were fed an FO-enriched diet have decreased body mass (BM) gain and lower final BM. In addition, with this diet, these mice have improved lipid metabolism with a decrease in total cholesterol (TC) and triacylglyceride (TG) levels, reduced fat pad masses and reduced adipocyte size. Furthermore, these LP offspring show reduced liver structural damage of alanine aminotransferase (ALT), liver steatosis with low SREBP-1 protein expression and high peroxisome proliferator activity receptor-alpha expression, and improvement of blood pressure (BP) and tumor necrosis factor (TNF)-alpha level. Early fish oil intake has beneficial effects on the programming responses that control body fat pad, glucose and lipid metabolism, and liver and adipose tissue structure in adult programmed offspring.

Keywords: Fish oil; Protein restriction; Fatty liver; Obesity; Hypertension; Insulin resistance

1. Introduction

The fetal programming hypothesis proposes that an adverse intrauterine environment alters the metabolic and hormonal milieu of the fetus, resulting in developmental adaptations to ensure fetal survival [1]. If these adaptive responses, designed for survival in a substrate-limited fetal environment, persist into postnatal life, metabolic and endocrine disorders may arise [2]. Therefore, low birth weight has been linked to adulthood hypertension [3], insulin resistance [4], vascular dysfunction [5], obesity [6], dyslipidemia [7], and the liver appears to be highly affected by fetal programming [8].

Poor nutrition in early life and the type of diet utilized during the post-weaning period appear to be critical in the determination of obesity in adulthood. In addition, studies have suggested that inflammatory mediators such as tumor necrosis factor-alpha (TNF-alpha) and interleukins play a central role in the development of cardiovascular diseases. Elevated levels of TNF-alpha have been found to be associated with obesity, insulin resistance and hypertriglyceridemia [9].

The dietary intake of fish oil (FO), rich in n-3 polyunsaturated fatty acid (PUFA), has proven to be effective in lowering both triglyceride and very low density lipoprotein concentrations in experimental animals [10]. In rats, it has been shown that FO decreases the level of mRNAs that encode several enzymes involved in *de novo* hepatic lipogenesis and enhances fatty acid (FA) oxidation [a peroxisome proliferator activity receptor (PPAR)-alpha stimulated this process] [11]. Thus, FA synthesis and beta-oxidation in the liver are suggested to be important factors that improve lipid metabolism by n-3 PUFA. In addition, n-3 PUFA can prevent steatosis in mice with dietary-induced hepatic steatosis [12,13]. FO feeding prevents abdominal fat accumulation compared to the ingestion of other types of dietary oils [14,15].

Based on these data, we hypothesized that early FO intake would help or revert the programming responses on body composition,

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carbohydrate and lipid metabolism, and liver and adipose tissue structure in programmed adult offspring.

2. Materials and methods

2.1. Sampling and diet protocols

All procedures with animals were performed according to the guidelines of the animal ethics committee of the State University of Rio de Janeiro and were carried out in accordance with conventional guidelines for experimentation with animals (NIH Publication No. 85-23, revised 1996). Animals were maintained under controlled conditions (21±2°C, humidity 60±10%, 12:12 h dark–light cycle) with free access to food and water.

Virgin Swiss female mice 3 months old weighing between 26 and 27 g were caged with males overnight. Beginning at the first day of pregnancy, mice were housed individually in cages and randomly assigned to either a group fed standard chow (SC) with 19% protein content or a group fed a 5% low-protein chow (LP) throughout the pregnancy and lactation period (21 days after birth). Litter size was adjusted at random to six pups per litter to ensure adequate and standardized nutrition until weaning [16]. At weaning, only one male pup per litter was randomly kept and assigned to form the groups, which were maintained until the age of 4 months.

The offspring were divided based on the diet they were fed before and after weaning. For the offspring, one group continued on an SC diet (SC-SC or LP-SC) and the other group was switched to an FO-enriched diet (SC-FO or LP-FO). The mineral and vitamin contents in the experimental diets were identical to all groups, in accordance with the AIN-93G recommendation [17]. From 3 months of life until euthanasia (at the end of the fourth month of life), the offspring received the maintenance diet (AIN-93M) (Table 1).

2.2. Body mass, food intake and feed efficiency

Food intake was measured daily and body mass (BM) was measured weekly. Fresh chow was provided daily and any remaining chow from the previous day was discarded. Food consumption was determined as the difference between the food supplied and the amount of food left in the grid. Energy intake was the product of the food consumption by the energy value of diets. Feed efficiency (FE) was determined as the ratio between the BM gain in grams and the food consumed in kilojoules per animal, multiplied by 100.

2.3. Blood pressure

Systolic blood pressure (BP) was measured every week in the fourth month of life in conscious mice through the noninvasive method of tail-cuff plethysmography (Letica LE5100, Panlab, Barcelona, Spain). Animals went through a 2-week period of adaptation before the beginning of the measurement of BP.

2.4. Blood and tissue sampling

At 3 months of age, an oral glucose tolerance test was performed with 25% glucose in sterile saline (0.9% NaCl) at a dose of 1 g/kg and was administered by orogastric gavage after a 6-h fasting period. The blood glucose concentration was measured prior to glucose administration at 0, 15, 30, 60 and 120 min after glucose intake. On the day before euthanasia, animals were food deprived for 6 h, then anesthetized (intraperitoneal sodium pentobarbital), and blood samples were obtained by cardiac puncture for further analyses. Fat deposits (retroperitoneal and epididymal fat masses) were carefully dissected from both sides of the animal and measured.

Table 1
Composition of experimental diets, low-protein chow (LP), standard chow (SC) and fish oil-enriched standard chow (FO), prepared according to the AIN-93 (the first number refers to the growth diet, AIN-93G, and the number in parentheses refers to the maintenance diet, AIN-93M, when its composition differed from the growth diet)

Ingredients	Diet (g/kg)		
	LP	SC	FO
Casein	50.0	200.0 (140.0)	200.0 (140.0)
L-Cystine	1.5	3.0 (1.8)	3.0 (1.8)
Cornstarch	680	530 (620)	530 (620)
Sucrose	100.0	100.0	100.0
Soybean oil	70.0	70.0 (40.0)	7.0 (4.0)
Fish oil	–	–	63.0 (36.0)
Fiber	50.0	50.0	50.0
Vitamin mixture	10.0	10.0	10.0
Mineral mixture	35.0	35.0	35.0
Choline bitartrate	2.5	2.5	2.5
Antioxidant	0.014	0.014 (0.008)	0.014 (0.008)
Total (g)	1,000.0	1,000.0	1,000.0
Energy (kJ/g)	16.6	16.6 (15.9)	16.6 (15.9)
Carbohydrate (%)	78	64 (76)	64 (76)
Protein (%)	5	19 (14)	19 (14)
Lipid (%)	17	17 (10)	17 (10)

2.5. Biochemical analyses and hormone concentrations

Serum was obtained by centrifugation (120×g/15 min) at room temperature. Total cholesterol (TC), triacylglycerides (TG) and alanine aminotransferase (ALT) were measured by a kinetic-colorimetric method according to the manufacturer's instructions (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil). Fasting glucose concentrations were determined at euthanasia read by a glucometer (Accu-chek, Roche Diagnostic, Germany). The fasting insulin concentration was measured by a radioimmunoassay (Cat. RI-13K for insulin, intra-assay coefficient of variation was 1.4%). The homeostasis model assessment for insulin resistance index was calculated as: fasting glucose (mmol/L)×fasting insulin (μU/mL)/22.5 [18]. Mice serum analysis of TNF-alpha was performed using commercially available ELISA kits (Human/Mouse TNF-alpha ELISA Ready-SET-go, San Diego, CA, USA).

2.6. Adipocyte morphometry

Adipose tissue was fixed (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2), embedded in Paraplast plus (Sigma-Aldrich Co., St. Louis, MO, USA), sectioned (5 μm of thickness) and stained with hematoxylin–eosin. The sectional area of the adipocytes was measured on digital images acquired at random (50 adipocytes/animal, TIFF format, 36-bit color, 1280×1024 pixels) with an LC Evolution camera and an Olympus BX51 microscope, and analyzed with Image-Pro Plus version 7.0 software (Media Cybernetics, Silver Spring, MD, USA) [19].

2.7. Liver stereology

The liver was sliced into several minor fragments; some pieces were kept for 48 h at room temperature in the fixative (the same fixative used previously). Random liver fragments obtained from all lobes were embedded in Paraplast plus, sectioned to 5 μm and then stained with hematoxylin–eosin. Several slices were cut per fragment and 10 microscopic fields per animal were analyzed at random (blind analysis) using a video microscope system and a test system composed of 36 test points (P_T) [20]. Briefly, the volume density (Vv) was estimated by point counting fat droplets on hepatic tissue (steatosis): Vv [steatosis, liver]:=P_P [steatosis]/P_T [liver] (P_P is the number of points that hit the structure and P_T is the total test points) [21].

2.8. Western blotting analysis

Liver tissue not used in microscopy had been frozen and homogenized in lysis buffer and supplemented with a protease inhibitor cocktail. The tissue homogenates were centrifuged at 3000×g for 20 min twice at 4°C, and the supernatants were used for Western blotting analysis. Briefly, protein concentrations were determined using the BCA Protein Assay kit (Thermo Scientific). Ten micrograms of protein extract was loaded onto a 10% polyacrylamide gel (Amersham Bio Science) and separated proteins were transferred to a nitrocellulose membrane (Amersham Hybond-P). The membranes were blocked for 1 h 30 min at room temperature with 5% nonfat dry milk in Tris-buffered saline (TBS) (Amersham Bio Science) containing 0.05% Tween-20 (Bio Rad). Then, the membranes were incubated overnight at 4°C with anti-sterol regulatory element binding protein (SREBP-1), anti-PPAR-alpha (rabbit, polyclonal, Santa Cruz Biotechnology) at 1:1000 dilution. Anti-beta actin (mouse, monoclonal, Santa Cruz Biotechnology) at 1:1000 dilution was used as a loading control. After incubation, the membranes were washed with TBS containing 0.05% Tween-20 and then incubated with peroxidase-conjugated secondary antibodies (at 1:8000 dilution for SREBP-1, 1:5000 dilution for PPAR-alpha and 1:5000 dilution for beta-actin). Visualization was performed using the ECL kit according to the manufacturer's protocol (GE Healthcare Bio-Sciences). SREBP-1, PPAR-alpha and beta-actin protein bands were visualized on Amersham Hyperfilm, scanned and quantified by image analysis.

2.9. Data analysis

Data are expressed as mean and standard error of the mean and were analyzed using two-way ANOVA as appropriate, to consider the effects of maternal LP and post-weaning FO. One-way ANOVA, when used, was followed by *post hoc* Tukey test. Differences in the same group at different times were tested with a paired *t* test (Statistica version 8.0, Statsoft, Tulsa, OK, USA). A P value of ≤.05 was considered statistically significant.

3. Results

3.1. Biometry

Maternal LP yielded low birth weight in LP offspring compared to SC offspring. At birth day, the BM of LP and SC mice was 1.54±0.03

Table 2
Data expressed as mean and standard error of the mean for $n=5$ mice per group

Data	Groups			
	SC-SC	SC-FO	LP-SC	LP-FO
Biometry				
BM (g)	45.6±1.4	39.4±1.4	44.4±1.6	35.9±2.5$^{(b)}$
EFM (g)	2.3±0.15	0.9±0.25$^{(a)}$	3.2±0.24	1.4±0.26$^{(b)}$
EFM/BM (%)	5.0±0.3	3.0±0.3$^{(a)}$	6.0±0.4	3.0±0.2$^{(b)}$
RFM (g)	0.7±0.09	0.3±0.08	1.4±0.22$^{(a)}$	0.6±0.15$^{(b)}$
RFM/BM (%)	1.4±0.2	0.6±0.1	2.8±0.4$^{(a)}$	1.3±0.2$^{(b)}$
Biochemistry				
ALT (mg/dl)	23.6±3.1	19.5±4.4	39.2±2.4$^{(a)}$	24.6±2.6$^{(b)}$
Glucose (mmol/L)	9.6±0.2	8.4±0.5	11.4±0.5$^{(a)}$	7.5±0.2$^{(b)}$
HOMA-IR	4.1±0.3	2.0±0.2$^{(a)}$	6.6±0.6$^{(a)}$	3.5±0.3$^{(b)}$
Insulin (μU/ml)	9.6±0.5	5.4±0.5$^{(a)}$	11.2±1.3	9.5±0.6$^{(c)}$
OGTT (auc)	24.3±1.3	20.6±1.2	33.5±3.1$^{(a)}$	21.2±2.1$^{(b)}$
TC (mg/dl)	120.4±2.1	111.4±3.2	149.6±1.7$^{(a)}$	103.0±6.0$^{(b)}$
TG (mg/dl)	51.2±2.0	40.6±2.4$^{(a)}$	47.2±1.3	39.6±1.5$^{(b)}$
TNF-alpha (μU/ml)	65.2±6.7	66.8±3.2	88.1±3.3$^{(a)}$	54.2±3.6$^{(b)}$

In the signaled cases, $P<.05$ when (a) different from SC-SC, (b) different from LP-SC, (c) different from SC-FO (one-way ANOVA and *post hoc* test of Tukey).
EFM, Epididymal fat mass; auc, area under the curve; RFM, retroperitoneal fat mass.

and 1.86±0.05 g ($P<.0001$), respectively. This difference was maintained at 7 days old (LP=4.22±0.12 g, SC=5.31±0.12 g; $P<.0003$), at 14 days old (LP=8.49±0.23 g, SC=9.28±0.11; $P<.0001$) and at 21 days old (LP=10.12±0.17 g, SC=11.89±0.14 g; $P<.001$). At 4 months of age, the BM was 20% smaller in LP-FO offspring than in LP-SC offspring (Table 2, $P<.01$), without a difference in the naso-anal length of these animals.

No difference was observed in FE among the groups (SC-SC=6.1±2.1 g/kJ; SC-FO=6.6±2.6 g/kJ; LP-SC=5.6±2.0 g/kJ; LP-FO=5.3±3.1 g/kJ). FO offspring had significantly reduced fat pads. The retroperitoneal fat pads and epididymal fat pads of the LP-FO offspring were 60% smaller than the pads of the LP-SC group ($P<.001$). The epididymal fat pads of the SC-FO group were 60% smaller than the pads of the SC-SC group ($P<.006$). The retroperitoneal fat mass and epididymal fat mass/BM ratios were also attenuated in LP-FO offspring than in the LP-SC group (−50%, $P<.001$).

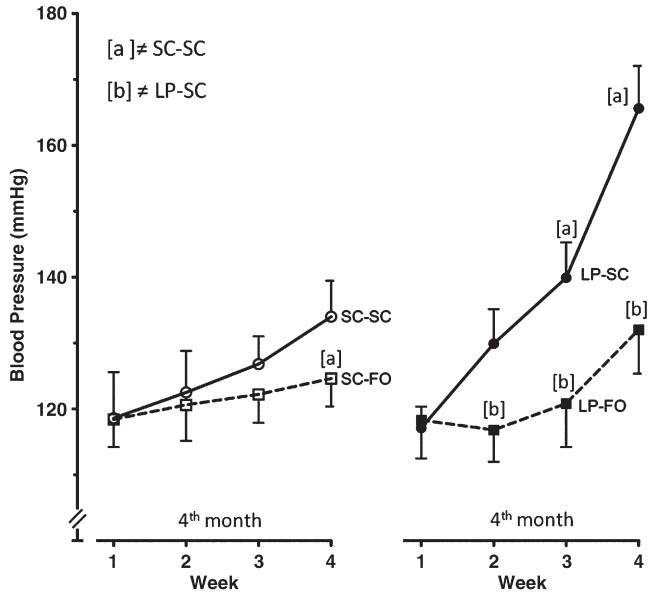

Fig. 1. Systolic blood pressure evolution in the fourth month of life: (○) SC-SC, (□) LP-SC, (●) SC-FO, (■) LP-FO. Values are means with their standard error shown by vertical bars for $n=5$ mice per group. In the signaled cases, $P<.05$ when (a) different from the SC-SC and (b) different from the LP-SC (one-way ANOVA and *post hoc* test of Tukey).

In the fourth month of life, the LP-SC offspring had the highest BP levels, 10% higher levels than the SC-SC offspring at the third week ($P<.05$) and 23% higher levels at the fourth week ($P<.01$). The FO intake was efficient to lower BP values in LP-FO offspring. In comparison to LP-SC offspring, the BP in LP-FO offspring was reduced by 10% at the second week ($P<.01$), reduced by 14% at the third week ($P<.01$) and reduced by 20% at the fourth week ($P<.0002$). The BP was also smaller in the SC-FO offspring than in the SC-SC offspring at the fourth week ($P<.05$). Moreover, there was interaction between maternal LP and post-weaning FO, showing that FO controlled BP levels ($P<.001$, two-way ANOVA) (Fig. 1).

3.2. Biochemical data

The biochemical data are shown in Table 2. TC concentration was higher in LP-SC compared to SC-SC offspring (+24%, $P<.0003$). FO lowered these values in LP-FO mice in comparison to LP-SC offspring

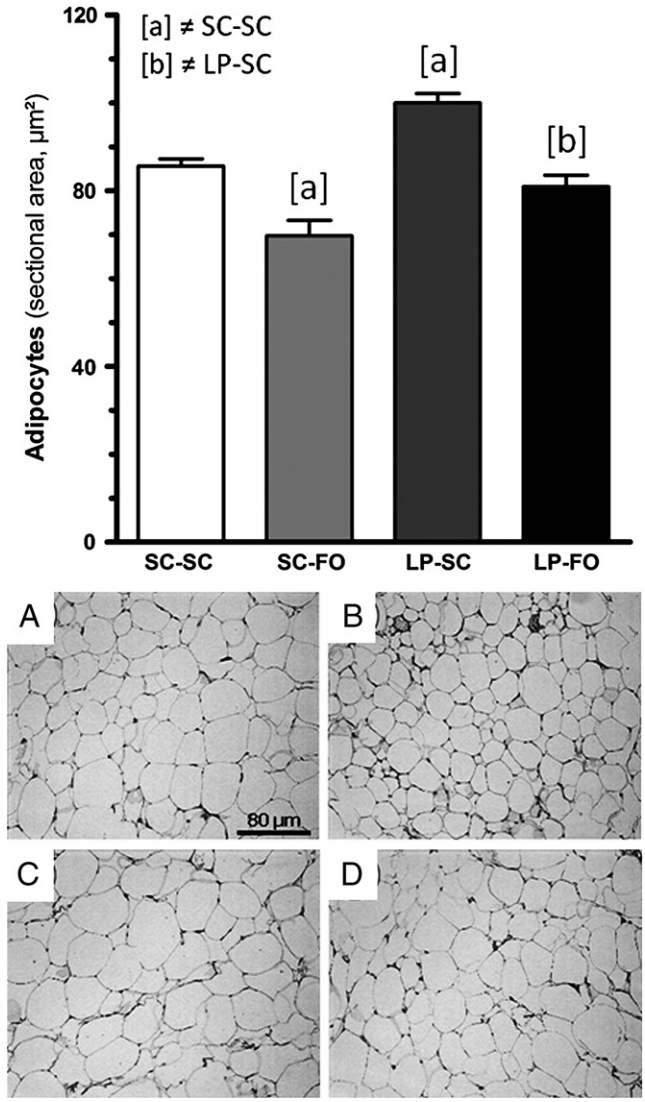

Fig. 2. Mean sectional area of adipocytes. Values are means with their standard error shown by vertical bars for $n=5$ mice per group. In the signaled cases, $P<.05$ when (a) different from the SC-SC and (b) different from the LP-SC (one-way ANOVA and *post hoc* test of Tukey). Photomicrographs of the adipose tissue (hematoxylin and eosin stain, same magnification): (A) SC-SC and (B) SC-FO offspring, with normal adipocytes; (C) LP-SC offspring with adipocyte hypertrophy; and (D) LP-FO offspring, with normal adipocyte size.

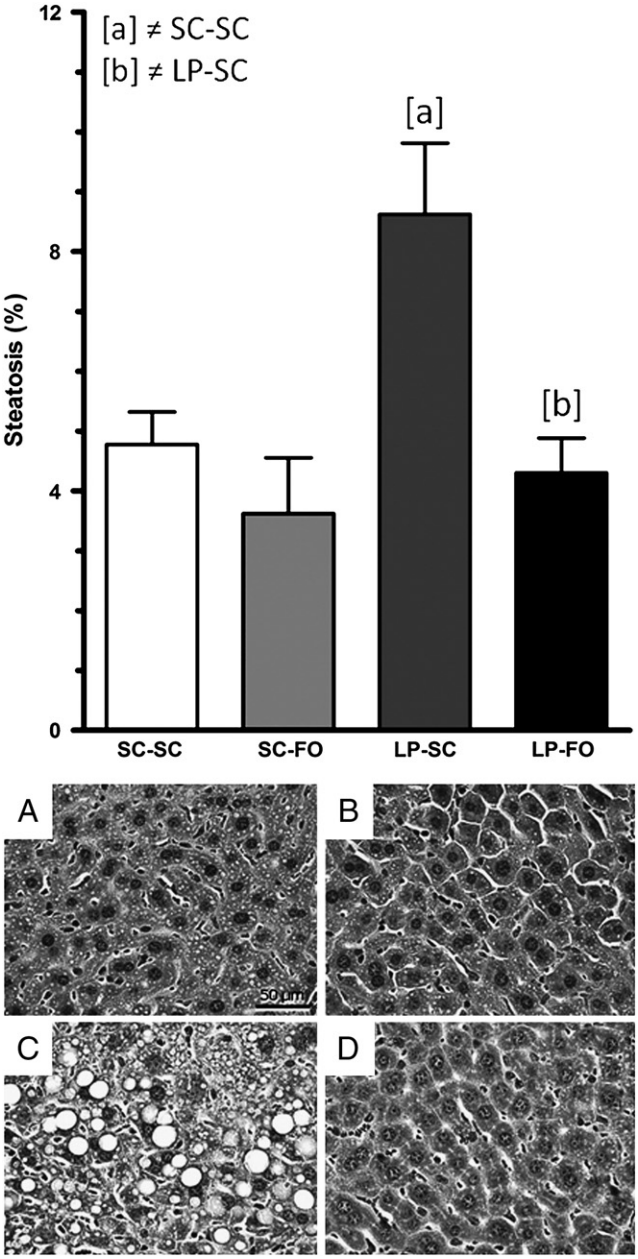

Fig. 3. Volume density of hepatic steatosis. Values are means with their standard error shown by vertical bars for $n=5$ mice per group. In the signaled cases, $P<.05$ when (a) different from the SC-SC and (b) different from the LP-SC (one-way ANOVA and *post hoc* test of Tukey). Photomicrographs of the liver (hematoxylin and eosin stain). The normal appearance in SC-SC and SC-FO offspring is shown in (A) and (B), respectively; macro- and microvesicular steatosis in the LP-SC offspring in (C); and reduced steatosis in LP-FO offspring in (D).

(−31%, $P<.0001$). Interaction in TC was observed between maternal LP and post-weaning FO ($P<.0001$, two-way ANOVA), emphasizing that FO had a beneficial effect on TC levels. Both SC-FO and LP-FO offspring had a significant decrease in TG levels compared to their counterparts, SC-SC *vs.* SC-FO (−21%, $P<.005$) and LP-SC *vs.* LP-FO (−16%, $P<.04$).

Insulin levels were reduced in SC-FO in comparison to SC-SC offspring (−44%, $P<.01$), but in LP-FO offspring insulin level was higher than in SC-FO offspring (+76%, $P<.001$). The LP-SC group had a 20% higher blood glucose level than the SC-SC offspring ($P<.01$), but it was lower in LP-FO than in LP-SC offspring (−34%, $P<.0001$). Interaction was observed between maternal LP and post-weaning FO ($P<.01$, two-way ANOVA), emphasizing that FO had a beneficial effect on glycemia. The OGTT confirmed the beneficial effects of FO on blood glucose. Indeed, the homeostasis model assessment for insulin resistance (HOMA-IR) index was 60% higher in LP-SC than in SC-SC offspring ($P<.004$), but it was 47% smaller in LP-FO than in LP-SC offspring ($P<.004$) and 51% smaller in SC-FO than in SC-SC offspring ($P<.009$).

The TNF-alpha level was higher in LP-SC mice compared to the SC-SC offspring (+35%, $P<.02$), and FO significantly lowered the TNF-alpha level as shown by a comparison of LP-FO offspring to LP-SC offspring (−40%, $P<.003$). Maternal LP and post-weaning FO had an additive effect in increasing TNF-alpha levels and an interaction existed between these parameters ($P<.01$, two-way ANOVA). Additionally, LP-SC had higher values of ALT than the SC-SC offspring (+66%, $P<.01$). Conversely, LP-FO showed lower values of ALT in comparison with the LP-SC offspring (−37%, $P<.01$).

3.3. Quantitative microscopic analyses

In the LP-SC group, adipocyte hypertrophy was evident; it was 17% higher than in the SC-SC offspring ($P<.005$). FO significantly lowered adipocyte size by 20% in LP-FO mice compared to the LP-SC offspring ($P<.001$). This result was also observed in SC-FO mice compared to the SC-SC offspring ($P<.002$) (Fig. 2).

There were no significant differences in liver mass among the groups (SC-SC=1.5±0.09 g; SC-FO=1.4±0.05 g; LP-SC=1.6±0.05 g; LP-FO=1.5±0.09 g). However, large accumulation of lipid content in the LP-SC offspring was observed. The LP-SC offspring showed greater hepatic steatosis than the SC-SC offspring (+80%, $P<.02$). LP-FO mice had reduced steatosis in comparison to the LP-SC offspring (−50%, $P<.01$). It is noteworthy that the content of steatosis in LP-FO and SC-SC and SC-FO did not show any difference. We observed lipid vacuoles of various sizes in hepatocytes of the LP-SC offspring (Fig. 3).

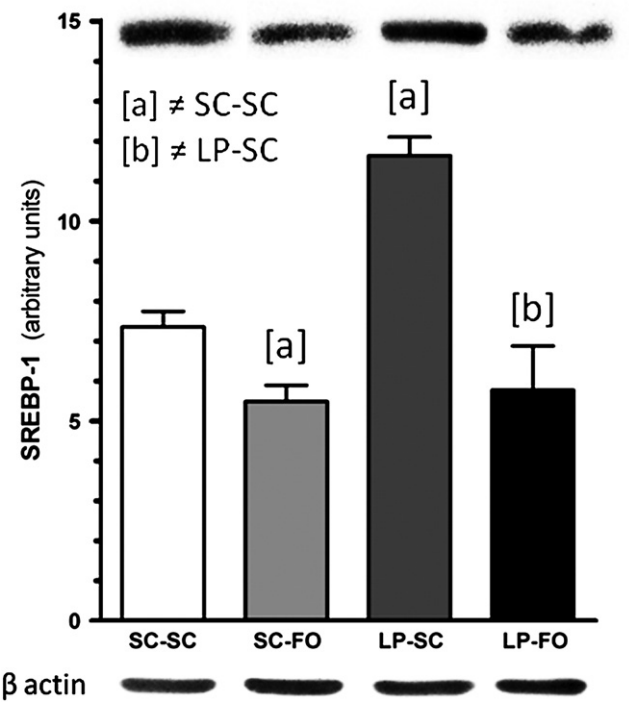

Fig. 4. Hepatic expression of SREBP. In the top, representative Western blots with bands corresponding to groups in the order SC-SC, SC-FO, LP-SC and LP-FO. In the bottom, beta-actin protein bands. Values are means with their standard error shown by vertical bars for $n=5$ mice per group. In the signaled cases, $P<.05$ when (a) different from the SC-SC and (b) different from the LP-SC (one-way ANOVA and *post hoc* test of Tukey).

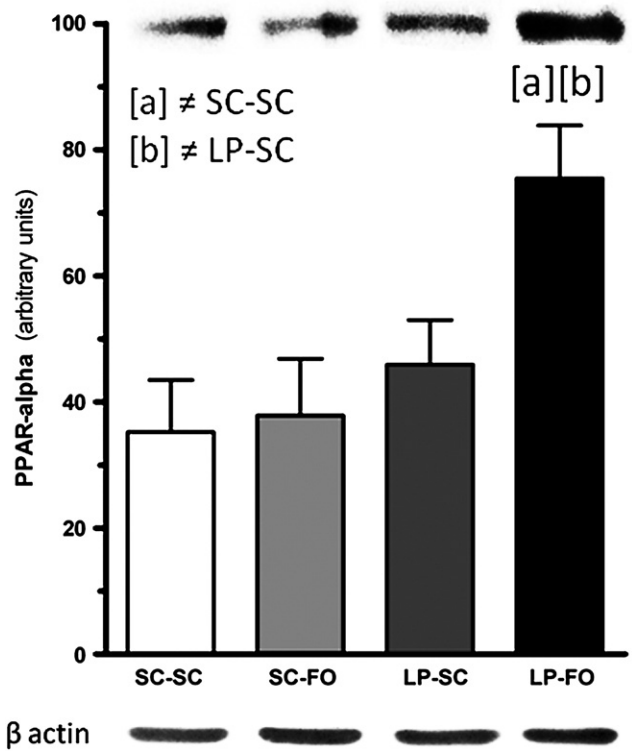

Fig. 5. Hepatic expression of PPAR-alpha. In the top, representative Western blots with bands corresponding to groups in the order SC-SC, SC-FO, LP-SC and LP-FO. In the bottom, beta-actin protein bands. Values are means with their standard error shown by vertical bars for $n=5$ mice per group. In the signaled cases, $P<.05$ when (a) different from the SC-SC and (b) different from the LP-SC (one-way ANOVA and post hoc test of Tukey).

3.4. Western blotting

The hepatic expression of SREBP-1 protein increased in the LP-SC offspring in comparison to SC-SC ($P<.0001$). In contrast, in LP-FO offspring, the expression of SREBP-1 was approximately 50% lower than in the LP-SC offspring ($P<.0001$). In addition, in SC-FO offspring the expression of SREBP-1 was lower than in the SC-SC offspring (Fig. 4).

The hepatic expression of PPAR-alpha protein was more than 60% higher in LP-FO than in LP-SC offspring ($P<.001$). Indeed, the LP-FO offspring showed increased values of hepatic expression of PPAR-alpha protein compared to the SC-FO offspring (+100%, $P<.001$) (Fig. 5).

4. Discussion

Protein restriction during development results in low birth weight and insults to diverse organs as documented in previous studies from our group [22–24]. The effect of early FO administration was evaluated in adult mice, which were subjected to maternal protein restriction during gestation and lactation. LP offspring fed FO had reduced final BM, improved lipid metabolism (reduction in TC and TG levels), reduced fat pad masses and adipocyte size, reduced liver steatosis and reduced BP and TNF-alpha levels.

The changes in BM observed in SC and LP offspring during the suckling period should be physiological. LP offspring remained lighter than the SC offspring throughout all this period agreeing with the literature that considers protein restriction during pregnancy and lactation without catch-up phenomenon [25,26].

Protein restriction of pregnant rodents produced gross adiposity in their mature offspring, which resulted in the increased expression of genes encoding a number of lipogenic enzymes, suggesting that enhanced lipogenesis is one of the factors that lead to visceral adiposity in this animal model [27]. Importantly, we observed that LP offspring that were fed FO just after weaning had lower fat body deposition. The FO was effective in reducing the proportion of fat deposits, although the animals had proportionally eaten the same amount of food as the other groups. Dietary n-3 PUFA induces the genes of the FA oxidation pathway and also reduces body fat deposition in animals and humans. This reduction in body fat occurs by inducing the expression of genes involved in thermogenesis; therefore, total body heat production increases [28]. These effects on thermogenesis could explain, at least in part, why dietary FO reduced BM and fat deposition in the LP-FO offspring.

Animals that received LP during gestation and/or lactation had altered lipid metabolism as shown by elevated TC and TG levels. These changes in LP offspring may be a consequence of the altered expression of key genes involved in the regulation of fat metabolism and insulin signaling, and the features emerged with aging [29]. Ingestion of enriched FO diet has been associated with increased beta-oxidation and down-regulation of genes encoding for lipogenic enzymes [11]. LP-SC offspring had high glycemia, an impaired OGTT and high HOMA-IR index, and borderline high values of insulinemia, indicating that insulin action on peripheral target tissues resulted in insulin resistance. In contrast, the LP-FO offspring showed a reduction in all these parameters. In response to n-3 PUFA ingestion, fat oxidation increases in skeletal muscle, which is associated with a reduction in TG droplets and an improvement in glucose uptake and glycogen storage in animals other than humans [30]. The hypolipidemic effect of FO decreases the availability of lipid fuel within the skeletal muscle and may, in turn, restore glucose oxidation and help normalize insulin resistance [10].

The increased expression of TNF-alpha has been associated with insulin resistance, obesity, hypertriglyceridemia and glucose intolerance [9,31], suggesting that TNF-alpha interferes with insulin action by altering the catalytic activity of the insulin receptor. In the present study, TNF-alpha levels increased in the LP-SC offspring, but the LP-FO offspring showed a significant decrease in TNF-alpha levels.

Maternal protein restriction programs for chronic diseases in adult life, including hypertension [32]. The mechanism of the appearance of hypertension in offspring from dams fed low-protein diet during gestation is not completely known, but the renin–angiotensin system seems to be involved [33], as well as the impairment of the glomerulogenesis [24]. In the present study, offspring from maternal protein restriction showed high BP levels in the fourth month of life than animals from normal dams. The FO intake efficiently reduces the BP in both SC and LP mice. Experimental evidences have shown that FO reduces BP in animals [14,34,35]. The anti-hypertensive effect of FO has been attributed to altered biosynthesis of eicosanoids [36], suppression of the synthesis and release of TNF-alpha [37], reduction of TG and viscosity [38], and a decrease in intracellular sodium concentrations [39]. In the present study, as mentioned, the LP-FO offspring showed significantly reduced TNF-alpha, TC, TG, insulin and glucose levels; therefore, this reduction may, at least in part, be responsible for the lowering effect of FO on BP.

Adipose tissue metabolism correlates with the size of the adipocytes [40]. LP-SC offspring had bigger adipocytes than the other groups. In contrast, FO administered to LP offspring was able to limit adipocyte hypertrophy. It is well known that n-3 PUFA activates PPARs and that the expression of the adipocyte PPAR-gamma isoform controls the expression of genes involved in adipogenesis as well as in lipid and glucose metabolism [41].

As mentioned, the LP-SC offspring were severely affected by hepatic and elevated levels of ALT and SREBP-1. Fatty liver, caused by chronic hepatocyte accumulation of lipids, can ultimately lead to inflammation and scarring, with the potential to progress to cirrhosis and liver failure [42]. It is well documented that fatty liver is related to the high expression of SREBP-1, which is elevated in response to hyperinsulinemia [43]. Moreover, insulin resistance is a strong driver

of fat accumulation in the liver [44]. Interestingly, FO intake was accompanied by a significant decrease of hepatic lipid accumulation in LP-FO offspring, which is in agreement with previous findings [12]. Additionally, an FO-enriched diet was capable of reducing SREBP-1 protein expression in LP offspring. Alternatively, PPAR-alpha protein expression was higher in LP-FO offspring. Insulin and glucose stimulate lipogenesis by activating the transcription factors SREBP-1 and carbohydrate response element binding protein (ChREBP), respectively [45,46]. The n-3 PUFA inhibits both the expression and nuclear translocation of SREBP-1 and ChREBP, which suppresses lipogenesis [47]. The beneficial effects of FO ingestion during hepatic steatosis may be attributed to the suppression of lipid synthesis in the liver and to the up-regulation of FA oxidation through PPAR-alpha activation [48]. Fish oil, which contains DHA and EPA, is traditionally used as a functional food against metabolic diseases. These beneficial health effects of DHA and EPA are thought to arise from their binding and activation of PPARs [49]. This idea can explain the mechanism involved in the reduction of hepatic steatosis in animals fed FO.

In conclusion, the present findings show compelling evidence that early FO intake by offspring from protein-restricted mothers can revert the negative responses on BP, body adiposity, glucose and lipid metabolisms, and liver structure by adulthood.

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