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ORIGINAL CONTRIBUTION

Açaí (*Euterpe oleracea* Martius) supplementation in the diet during gestation and lactation attenuates liver steatosis in dams and protects offspring

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Abstract

Purpose Maternal high-fat diet affects offspring and can induce metabolic disorders such as non-alcoholic fatty liver disease (NAFLD). New therapeutic strategies are being investigated as way to prevent or attenuate this condition. The objective of this study was to evaluate the effect of açaí supplementation in the maternal high-fat diet on dams and offspring lipid metabolism.

Methods Female Fisher rats were divided in four groups and fed a control diet (C), a high-fat diet (HF), an açaí supplemented diet (CA) and a high-fat diet supplemented with açaí (HFA) 2 weeks before mating, during gestation and lactation. The effects of açaí were evaluated in the male offspring after birth (P1) and weaning (P21).

Results HFA reduced relative liver weight, fat and cholesterol liver content in dams and improved liver steatosis as confirmed by histological analyses. HFA increased serum cholesterol and expression of *Srebpf1* and *Fasn* genes. In offspring, HFA decreased relative liver weight, and serum cholesterol only in P21. An increase in the *Sirt1, Srebpf1* and *Fasn* genes expression was observed in P21.

Conclusions These results suggest that açaí supplementation may attenuate NAFLD in dams and protect offspring from the detrimental effects of lipid excess from a maternal high-fat diet.

Keywords Açaí · *Euterpe oleracea* Martius · High-fat maternal diet · Metabolic programming · Non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by accumulation of triglycerides in hepatocytes. This disease encompasses a spectrum of conditions ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH) characterized by the presence of inflammation, which can progress to cirrhosis or hepatic carcinoma [1]. The prevalence of NAFLD in children and adolescents has evidenced the role of maternal nutrition during critical periods of fetal development [2]. Metabolic programing is a process by which maternal lifestyle (including diet) promotes modifications in the uterus environment or milk composition that can trigger several changes in the sequence of events, in the gestational or lactation periods, leading to metabolic disorders in the offspring [3]. The molecular mechanisms and pathways involved are not well understood but some studies have pointed epigenetic changes as having a pivot role in the process [4]. This is a current and extremely relevant concept due to the pandemic of metabolic diseases, such as diabetes, obesity and systemic arterial hypertension [5] that might be partially explained by metabolic programming. High-fat maternal diet has been widely used in the literature to induce NAFLD in experimental animal models and the consumption of such diet reflects the current world scenario in which excessive lipid intake may contribute to rise of liver diseases in the population [6, 7]. Over the last decade, considerable

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progress has been made in understanding how the excess of lipid intake via maternal diet alters metabolic pathways in the uterus, predisposing the fetus to accumulation of fat in the liver, and consequently the development of NAFLD in adult life [8].

Sirtuins, a family of proteins dependent on intracellular levels of NAD+, stood out because of their important role in energy metabolism [9]. Sirtuin 1 (SIRT1) has been extensively studied due to its involvement in several metabolic processes: it deacetylates the sterol regulatory element-binding protein (SREBPs) promoting inhibition of its activity [10]. SREBPs are transcription factors and three different isoforms, SREBP-1a, SREBP-1c and SREBP-2, are present in mammalian cells. SREBPs directly activate the expression of more than thirty genes related to the synthesis and uptake of cholesterol, fatty acids, triglycerides and phospholipids, in addition to increase the expression of genes involved in the generation of NADPH, a necessary cofactor used in anabolic reactions such as lipid metabolism [11]. In general, SREBP-1 regulates transcription of lipogenic genes, ranging from genes involved in fatty acid biosynthesis to gene regulation of the enzyme fatty acid synthase (FASN). Studies evaluating the effect of maternal diet have shown that the excess of lipids can reduce the expression and activity of SIRT1 in the liver of mothers and offspring, causing alterations in liver metabolism and promoting fat accumulation [12, 13].

Under normal physiological conditions, fat accumulates in adipose tissue and not in the liver; however, lipid accumulation in the liver can occur when there are alterations between mobilization and lipid oxidation. Studies have shown that excess of fatty acids may promote mitochondrial dysfunction and reduce oxidative capacity of mitochondria in mothers and their offspring [14]. The lipid influx, in addition to compromised oxidative capacity of the mitochondria, can result in accumulation of partially oxidized lipid products and generation of additional reactive oxygen species (ROS), which can overwhelm cell defenses leading to oxidative stress [15]. In this sense, mitochondrial uncoupling protein 2 (UCP2) has emerged as a potential regulator of hepatic steatosis. UCP2 allows the transfer of anions from the inner mitochondrial membrane to the cytosol and the return transfer of protons from the outer to the inner membrane [16]. It is, therefore, possible that UCP2 is capable of attenuating hepatic steatosis through the control of ROS production [17].

Together with studies that seek to better understand changes that occur in the womb and precede development of metabolic disorders, the search for new therapeutic targets and the introduction of foods with a potential beneficial effect on metabolism have emerged in the scientific field. The most common compound studied is resveratrol, a polyphenol naturally found in purple grapes and widely accepted as chemoprotective agent [18]. In models of hepatic steatosis induced by high-fat maternal diet, administration of resveratrol was shown to efficiently reduce plasma and hepatic storage of triglycerides, both studies through SIRT1 upregulation in offspring [10, 19–21]. Moreover, other polyphenols such as flavonoids, flavonols, anthocyanidins, flavonones, and isoflavones have been studied as potential agents for the prevention and treatment of NAFLD [21]. Therefore, the activation of SIRT1 by polyphenols would be beneficial for the prevention and treatment of NAFLD.

Açaí (*Euterpe oleracea* Martius), an Amazon fruit, with a high content of phenolic compounds of the class of anthocyanins, mainly cyanidin-3-rutinoside, cyanidin-3-glycoside, cyanidin-3-sambubioside, peonidin-3-glycoside and peonidin-3-rutinoside [22] has been the subject of research seeking to evaluate its potential beneficial effect on health. Recent work evaluating the effect of açaí on NAFLD pathology demonstrated a hepatoprotective action of this fruit by modulating the expression of genes involved in adiponectin signaling, lipogenesis and oxidation of fatty acids [23, 24]. However, little is known about the effect of açaí on the molecular mechanisms involving hepatic and lipid metabolism in NAFLD induced by high-fat maternal diet and its effect on offspring. Our hypothesis is that, due to its high content of polyphenols, açaí supplementation in dams' diet 2 weeks before mating and during gestation and lactation protects them and their offspring against NAFLD induced by high-fat diet. The aim of this study was, therefore, to evaluate pathways involved in the development of NAFLD in rats, which may be modified by supplementing a high-fat diet with 2% of açaí pulp during gestation and lactation. Moreover, the effect of such intervention was studied in postnatal and post-weaning offspring.

Materials and methods

Açaí pulp

Pasteurized frozen açaí pulp without colorants or preservatives was obtained in a single lot (07/2016) from Icefruit Comércio de Alimentos Ltda (Tatui, São Paulo, Brazil). Chemical analysis of the pulp showed moisture content of 90%, 3.9-g lipids, 2.3-g total carbohydrate, 0.9-g protein, 2.3-g insoluble fiber and 0.4-g soluble fiber per 100 g of pulp.

Polyphenol content of açaí pulp was determined using Folin–Ciocalteu reagent as described previously [25]. A standard curve was constructed using different concentrations of gallic acid for quantifying total polyphenols and values were expressed in mg of gallic acid equivalent (GAE) in 100 g of açaí pulp. The açaí pulp used in this study presented 549.5 mg GAE/100 g. The content of anthocyanins was also measured as reported by Giust and Wrolstad [26]. The assay consists of the pH differential method and the values were expressed as cyanidin-3-glucoside equivalents, mg/L of pulp. The total anthocyanin of açaí pulp was 6.5 mg/L.

Animals and diets

All procedures used in this study were approved by the Ethics Committee in Animal Research of the Federal University of Ouro Preto (Protocol No. 2015/15). Thirty-two female Fischer rats (90 days of age) were obtained from the Laboratory of Experimental Nutrition at the School of Nutrition of the Federal University of Ouro Preto (Minas Gerais, Brazil). Animals were divided into four groups receiving different diets: control diet (C), high-fat diet (HF, 60% of total calories as fat, been 53% saturated fat, 6% soybean oil and 1% cholesterol), control diet supplemented with açaí pulp (CA, control diet plus 2% of açaí pulp) or high-fat diet supplemented with 2% of açaí pulp (HFA). Control diet and high-fat diet were based on the AIN-93G diet, with some modifications according to previous studies [23, 27–29]. All animals were maintained in a standard environment, 23 °C ± 2 °C, 55% humidity and 12-h light/darkness cycle, with food and water provided ad libitum. Initially, animals were fed with the respective experimental diets for 2 weeks. After one week, we evaluated the food intake. After 2 weeks in the experimental diets, the mating was performed with a male rat together with two females for one week. After the mating period, females were separated and housed in individual cages to allow the natural progression of gestation, while continuing to receive the allocated diet during gestation and lactation. The dams' body weight was measured in the first week, pre-mating week, and in the day of euthanise. At birth, some of the male pups ($n = 7$) were anesthetised under isoflurane and euthanised by decapitation (postpartum offspring, P1); whereas, the rest of the pups were kept, six per dam, to guarantee homogeneous growth of the litters. At weaning, the dams and the remaining offspring male (P21) were euthanised as above. Male pups were chosen as to reflect the higher incidence of NAFLD in male population [30] and seven male pups of each group were randomly selected for all the analyses.

Collection of blood and tissue samples

At the end of the experimental period, dams and P21 ($n = 7$ per group) were anesthetised under isoflurane, after 12-h fasting, and killed by total blood collection from the brachial plexus. Blood samples were collected and centrifuged at 3000 g for 15 min at room temperature. Serum was then removed and stored at − 80 °C for further analyses. Livers from dams, P1 and P21 were collected, washed with cold saline solution and weighed. The small hepatic lobe was submerged in liquid nitrogen and immediately stored at − 80 °C for gene and protein expression analyses.

Blood chemistry

Enzymes activities for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in serum samples using a fixed time kinetic reaction following manufactures' instructions (Labtest, Lagoa Santa, Brazil). The levels of serum triglycerides (TG) and total cholesterol (TC) were determined using a colorimetric assay acquired from Labtest (Lagoa Santa, Brazil) following manufacturer's instructions.

Lipid liver content

Hepatic lipids were extracted from liver tissue using a chloroform/MeOH solution (2:1, v/v), as described by Folch et al. [31]. The content of total lipids in the liver was quantified gravimetrically by evaporation of the solvents and dissolution of the dried lipids in 500 µl of isopropanol. Concentrations of TG and TC were determined colorimetrically using TG and TC assay kits (Labtest, Lagoa Santa, MG, Brazil).

Histological examination

Liver smallest lobe was cut and fixed in 4% formalin-buffered solution. After fixation, the tissues were cleared and processed in decreasing concentrations of alcohol and sealed in paraffin. Through a semi-automatic microtome, the paraffin sections were laminated (4 µm), stained with hematoxylin and eosin (H&E) and photographed at 40× magnification (Leica Application Suite, Germany). Liver histology was examined using 15 images obtained at random from the tissue and classified for the degree of macro-vesicular steatosis. The degree of hepatic steatosis was assessed according to scores defined in previous studies and based on the percentage of hepatocytes that present accumulation of fat, being absent < 5%; mild between 6 and 33%; moderated between 34 and 66%; marked > 66% of affected hepatocytes [32].

Quantitative reverse transcription polymerase chain reaction analysis

Total RNA extraction was performed from 10 to 20 mg of frozen liver tissue using TRI Reagent® Solution (Invitrogen, UK) following the manufacturer's instructions. RNA purification was checked by the ratio A260/A280, utilizing a UV/VIS spectrophotometer (Thermo Spectronic, Helios γ). One hundred ng of RNA was transcribed to cDNA by RT-PCR using Super Script III Reverse Transcriptase (Invitrogen, UK) and random hexamers as primers (Promega, UK). The cDNA product was used as template in the quantitative real-time PCR (qPCR) reaction performed with SYBR Green PCR Master Mix kit (Primer design, UK), as recommended by the manufacturer. Reactions were done in duplicate and each reaction had a negative control with water added instead of template. The sequences of oligonucleotide primers for qPCR are noted in Table 1. mRNA levels were analyzed using comparative Ct method and target gene expression was related to the expression of the house keeping gene, β2 microglobulin.

Western blotting

Frozen liver samples were homogenized in Cell Lysis buffer (Cell Signaling Technology, Inc. Danvers, MA, USA) containing 40-mM Tris–HCl (pH 7.5), 300-mM NaCl, 2-mM Na_2EDTA, 2-mM EGTA, 2% Triton, 5-mM sodium pyrophosphate, 2-mM β-glycerophospate, 2-mM Na_3VO_4, 2 μg/ml leupeptin, a cocktail of protease inhibitors (Sigma, St Louis, MO) and 1-mM PMSF following the manufacturer's instructions. Liver homogenates were centrifuged at 13,000*g* for 15 min at 4 °C and supernatants were aliquoted and stored at − 80 °C. Protein concentration was measured by DC™ protein assay (Bio-Rad, UK) following kit guidelines. Thirty μg of total protein for pulled samples from each experimental group was loaded per lane (pulled samples were run in duplicate per gel), subjected to 9% SDS-PAGE, and transferred to polyvinylidene fluoride (PDVF) membranes (GE Healthcare, USA) by wet transfer at 100 V for 1 h using a Mini Trans-Blot cell system (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked using 4% non-fat dry powered milk dissolved in Tris-buffered saline tween-20 (TBST) for 1 h at room temperature. The primary antibodies for SIRT1 (ab110304), SREBP1 (ab28481) and beta actin (ab8227) (all antibodies obtained from Abcam, Cambridge, UK) were used according to the manufacturer recommended dilutions (1:2000 for SIRT1 and SREBP, 1:10,000 for Actin) and were incubated overnight at 4 °C. The membranes were then washed three times for 5 min with TBST, before incubation for 1 h at room temperature with secondary peroxidase conjugated goat anti-rabbit (ab6721, Abcam, Cambridge, UK) or goat anti-mouse (ab205719, Abcam, Cambridge, UK) diluted at 1:5000 in 4% non-fat dry milk–TBST. Membranes were washed as before, and the bound antibodies were visualized by enhanced chemiluminescence (ECL) SuperSignal® (ThermoScientific, USA) using a peqLab Fusion FX7 system (VilberLourmat). Beta actin levels were used as control and levels of SIRT1 and SREBP1 were related to beta actin levels. *Image J* software was used to calculate band intensity.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6 for Windows (GraphPad Software, San Diego, CA). All data were tested for normality using the Kolmogorov–Smirnov test. Parametric data from the four groups were analyzed by one-way ANOVA followed by Tukey test to detect differences between the groups and expressed as mean ± standard deviation (SD). Non-parametric data (western blotting) or semi-quantitative analyses (histology data) were compared using Wilcoxon and Kruskal–Wallis, respectively. The data were presented as median and range (minimum and maximum values). Data from two groups were compared by unpaired Student's *t*-test. Results were considered statistically significant for p values < 0.05.

Results

Dams

Effect of dietary intervention on body weight, tissue weight and food intake

The different experimental groups did not present significant changes in body weight in the initial and pre-gestational period. However, at the end of the study, rats receiving HFA had significantly greater body weight (19%, $p = 0.0128$) than the C group (Table 2). Liver weight was also measured at the end of experiment (Table 2): HF group showed a significant increase in the total organ size compared to C (46%, $p = 0.0007$) and CA (73%, $p < 0.0001$) groups, while HFA presented an increase in relation to CA group (42%,

Table 1 Sequence of oligonucleotides

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Sirt1	CTGTTTCCTGTGGGATACCTGACT	ATCGAACATGGCTTGAGGATCT
Srebf1	CCCAGGGCAGCTCTGTACTCC	AAGCTGTCCCGCAGGTA
Fasn	CTTGGGTGCCGATTACAACC	GCCCTCCCGTACACTCACTC
Ucp2	GGTAAAGGTCCGCTTCCAGG	GCAAGGGAGGTCGTCTGTCA
β2-microglobulin	TGACCGTATCTTTCTGGTG	ATTTGAGGTGGGTGGAACTG

Sirt1 sirtuin 1, *Srebf1* sterol regulatory element-binding protein 1, *Fasn* fatty acid synthase, *Ucp2* uncoupling protein 2

Table 2 Body and liver weight, serum lipid profile, liver function, liver lipid content, and food intake of dams

	C	HF	CA	HFA
Initial body weight (g)	210.1 ± 8.83	210.4 ± 10.48	205.4 ± 8.35	218.9 ± 12.84
Pre-Gestational body weight (g)	215.4 ± 11.16	220.7 ± 13.62	212.1 ± 8.49	226.5 ± 15.06
Final body weight (g)	213.1 ± 20.78	230.9 ± 12.61	237.7 ± 27.63	255.6 ± 29.68$^{\#}$
Liver weight (g)	7 ± 1.17	10.23 ± 2.89$^{\#*}$	5.90 ± 0.52	8.39 ± 0.99*
Relative liver weight	3.33 ± 0.73	4.45 ± 1.03$^{\#*}$	2.53 ± 0.55	3.30 ± 0.34§
Total cholesterol (mmol/l)	2.96 ± 0.74	3.6 ± 1.69	2.23 ± 0.68	5.52 ± 1.66$^{\#*\S}$
Triglyceride (mmol/l)	1.14 ± 0.49	0.93 ± 0.38	0.66 ± 0.17	0.83 ± 0.19
AST (U/l)	15.23 ± 4.81	22.63 ± 3.1$^{\#}$	20.69 ± 3.72	23.11 ± 3.68$^{\#}$
ALT (U/l)	23.13 ± 7.4	62.11 ± 20.88$^{\#,*}$	24.61 ± 7.54	60.54 ± 14.2$^{\#*}$
Liver fat (mg/g)	98.62 ± 37.46	214.9 ± 71.8$^{\#*}$	56.79 ± 18.16	117.5 ± 45.62§
Liver cholesterol (mg/g)	3.75 ± 0.4	28.3 ± 4.16$^{\#*}$	3.36 ± 0.33	17.21 ± 6.67$^{\#*\S}$
Liver triglyceride (mg/g)	19.45 ± 13.36	31.39 ± 4.51*	18.08 ± 2.67	22.37 ± 7.84
Food intake (g/d)	13.82 ± 1.36	9.66 ± 1.15$^{\#*}$	14.73 ± 1.14	10.91 ± 0.67$^{\#*}$
Caloric intake (kj/d)	229.82 ± 22.59	217.85 ± 26.08	240.03 ± 18.06	242.38 ± 14.95

$p < 0.05$: $^{\#}$versus C, *versus CA and §versus HF

C control diet, *HF* high-fat diet, *CA* açaí diet, *HFA* high-fat açaí diet. The results are shown as the mean ± SD ($n = 7$ dams per group). One-way ANOVA followed by a Tukey post hoc test

$p = 0.0088$). However, when evaluating the relative liver weight, a statistically significant reduction (25%, $p = 0.0277$) was observed in the HFA group in comparison to HF. HF group presented an increase in relative liver weight in relation to C (34%, $p = 0.0331$) and CA (76%, $p = 0.0002$) groups. Regarding the food intake, the supplementation with 2% of açaí pulp did not affect the caloric intake of the dams (Table 2).

Effect of dietary intervention on serum lipid profile and hepatic function

Dams fed a HFA presented a significant increase in total cholesterol when compared to C (86%, $p = 0.0049$), HF (53%, $p = 0.0492$) and CA (147%, $p = 0.0004$) groups; whereas, no change was observed in serum triglyceride levels (Table 2).

The activities of AST and ALT were determined in serum as biomarkers of the extent of hepatic damage (Table 2). HF and HFA groups showed a significant increase (48%, $p = 0.008$ and 51%, $p = 0.0045$, respectively) in AST when compared to the C group; whereas, ALT activity was significantly increased in the HF (168%, $p = 0.0001$ versus C group; 152%, $p = 0.0002$ versus CA group) and HFA groups (161%, $p = 0.0002$ versus group C; 146%, $p = 0.0003$ versus CA group).

Effect of dietary intervention on liver lipid content

The content of total fat, cholesterol and triglyceride in the liver was evaluated to assess the extent of NAFLD; the results are presented in Table 2. A significant increase in total fat content was observed in the HF group in relation to C (117%, $p = 0.0006$), CA (278%, $p < 0.0001$) and HFA (82%, $p = 0.004$). Interestingly, CA group showed a decrease in fat liver content even if it did not reach statistical difference compared to C group; whereas HFA did not induce an increase in fat liver content as the HF diet did. Hepatic cholesterol levels were higher in the HF (654%, $p < 0.0001$ versus C; 742%, $p < 0.0001$ versus CA) and HFA (358%, $p < 0.0001$ versus C; 412%, $p < 0.0001$ versus CA), but the HFA group presented lower values in relation to the HF group (36%, $p = 0.0001$). Liver triglyceride content in the HF group was also significantly higher than that observed in the CA group (61%, $p = 0.0268$).

Effect of dietary intervention on liver steatosis grade

To evaluate the effect of the different diets on accumulation of lipids and degree of steatosis in the liver, microscopic analysis was performed. Histological analysis revealed that the HF group had a higher grade of steatosis (moderate and marked), whereas the HFA group had an attenuation of steatosis when compared with HF (Fig. 1a). Scoring of the degree of steatosis confirmed the presence of moderate to marked steatosis in the liver of dams fed a HF diet which was reduced to mild–moderate ($p < 0.01$) by açaí supplementation to HF diet (Fig. 1b).

Effect of dietary intervention on gene expression involved in lipid metabolism

To determine the potential metabolic pathways by which açaí could improve hepatic fat accumulation, the expression of genes involved in lipid metabolism was assessed

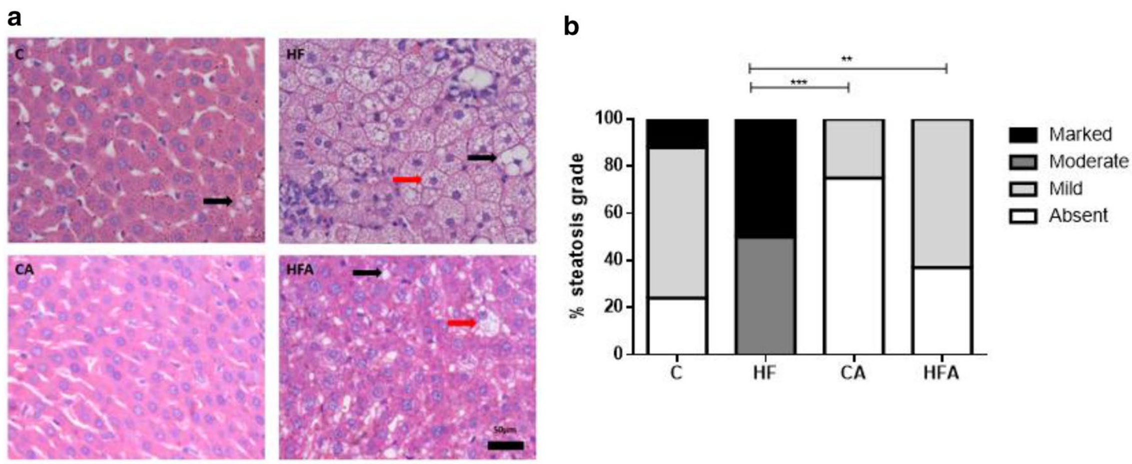

Fig. 1 **a** Representative histological sections of the liver of dams fed with a control diet (C), high-fat diet (HF), açaí diet (CA) and high-fat supplemented with açaí (HFA), stained with hematoxylin and eosin. Black arrow shows macrosteatosis and red arrow shows microsteatosis. The images were photographed at a magnification of 400×. Bar Scale = 50 µm; **b** Grade of hepatic steatosis of dams ($n = 7$ dams per group). Value of $p < 0.05$ was considered statistically significant for the Kruskal–Wallis. **< 0.01, ***< 0.005

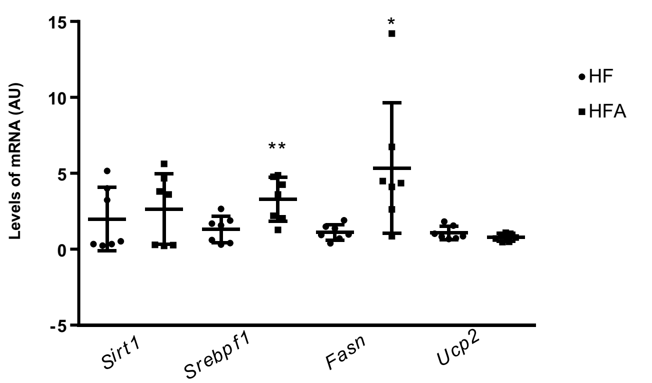

Fig. 2 mRNA abundance for genes related to lipid metabolism in the liver of dams relative to beta-2-microglobulin. *HF* high-fat diet; *HFA* high-fat açaí diet, *Sirt1* sirtuin 1, *Srebf1* sterol regulatory element-binding transcription factor 1, *Fasn* fatty acid synthase, *Ucp2* uncoupling protein 2. The results are shown as the mean ± SD ($n = 7$ dams per group). Analyses by Student's *t* test. *$p < 0.05$; **$p < 0.01$

(Fig. 2). *Sirt1* mRNA abundance was higher in the HFA group compared to HF group, but no statistically significant differences were found. Surprisingly, the HFA group showed an increase in the relative expression of *Srebf1* (threefold change, $p = 0.0092$) and *Fasn* (fourfold change, $p = 0.0241$) genes when compared to the HF group.

Effect of dietary intervention on protein levels

Western blot analysis did not show significant differences in SIRT1 protein levels (Fig. 3a) even if a trend for increased levels in the HFA group could be observed. Although gene expression showed an increase in *Srebpf1* in the liver of the dams fed a HFA diet, protein level did not show statistical difference compared to levels in the HF group (Fig. 3b).

Offspring

Effect of dietary intervention on body and tissue weight

The effect of a high-fat diet supplemented or not with açaí during gestation on offspring was investigated in pups euthanised 1 day after birth (P1). Body weight did not change between groups (Table 3); whereas, when considering the absolute and relative weight of the liver, the pups HFA-P1 showed a decrease of 27% in organ size and 33% in relative weight ($p = 0.0088$ and $p = 0.0126$, respectively; Table 3) compared to HF-P1 group. Similarly, the effect of the different diets during gestation and lactation was assessed in pups culled at the end of the lactation period (P21). An increase in the body weight of pups from HF-P21 (40%, $p = 0.0067$ versus CA-P21) and HFA-P21 (25%, $p = 0.0343$ versus C-P21; 60%, $p < 0.0001$ versus CA-P21) (data shown in Table 3) was observed. The absolute liver weights were also measured at the end of the experiment and pup livers showed an increase from HF-P21 (47%, $p = 0.0002$ versus C-P21; 59%, $p < 0.0001$ versus CA-P21) and HFA-P21 (40%, $p \leq 0.0015$ versus C-P21; 51%, $p = 0.0002$ versus CA-P21). Açaí supplementation reduced the relative weight of the liver by 17% ($p = 0.0263$, HFA-P21 versus HF-P21), whereas feeding a HF diet induced an increase of 35% in relative liver weight (Table 3, $p = 0.0006$, HF-P21 versus C-P21).

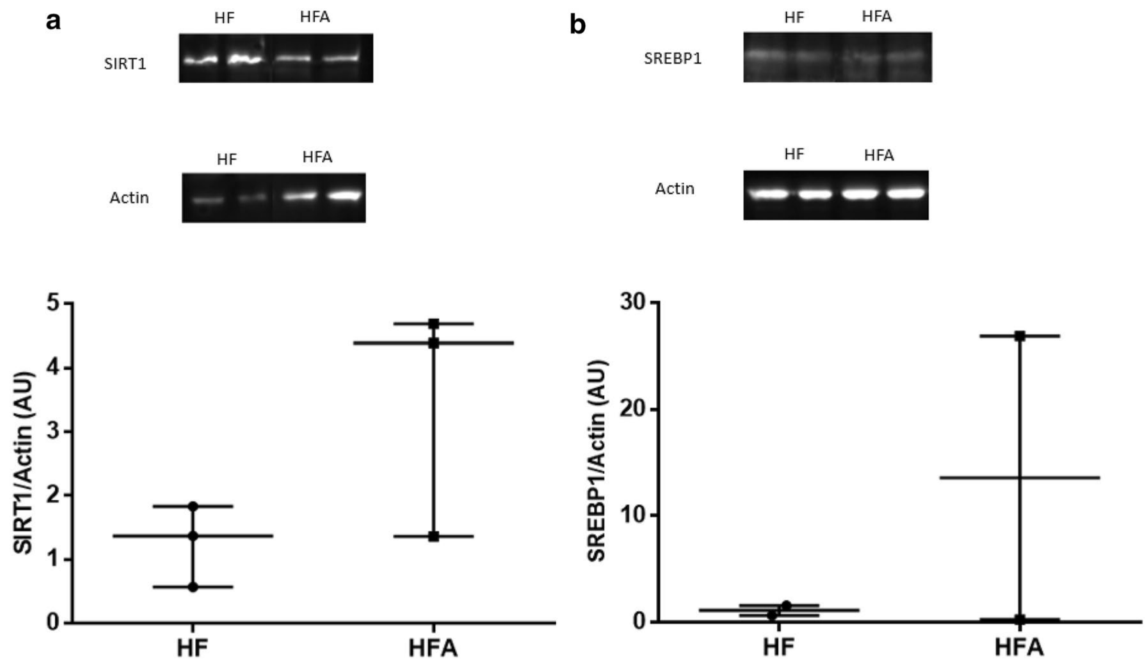

Fig. 3 Western blotting for SIRT1 (**a**) and SREBP1 (**b**) of dams. Graphs represent data from Western blotting quantification. *HF* high-fat diet and *HFA* high-fat supplemented with açaí. Data are shown as median and range (minimum and maximum value) ($n = 7$ dams per group). Value of $p < 0.05$ was considered statistically significant for the Kruskal–Wallis

Table 3 Body and liver weight of offspring P1 and P21

	Pups-P1		Pups-P21			
	HF	HFA	C	HF	CA	HFA
Body weight (g)	5.43 ± 1.07	5.93 ± 0.59	30.49 ± 3	33.67 ± 5.8*	23.96 ± 4.43	38.36 ± 7.46*#
Liver weight (g)	0.296 ± 0.02	0.217 ± 0.06§	1.11 ± 0.14	1.64 ± 0.26#*	1.03 ± 0.21	1.56 ± 0.23#*
Relative liver weight	5.65 ± 1.14	3.75 ± 1.28§	3.67 ± 0.54	4.96 ± 0.89#	4.32 ± 0.38	4.1 ± 0.25§

$p < 0.05$ #versus C, *versus CA and §versus HF

C control diet, *HF* high-fat diet, *CA* açaí diet, *HFA* high-fat açaí diet. Litter size six per dam. The results are shown as the mean ± SD ($n = 7$ pups per group). One-way ANOVA followed by a Tukey post hoc test

Effect of dietary intervention on lipid profile and hepatic function

The effect of the different maternal diets on lipid metabolism was evaluated by measuring serum levels of cholesterol and triglycerides (Table 4). Pups HF-P21 presented, after gestation and lactation, a significant increase in serum cholesterol in relation to C-P21 (58%, $p = 0.0004$) and CA-P21 (48%, $p = 0.0018$) groups, whereas HFA-P21 group induced a significant decrease in cholesterol levels (57%, $p < 0.0001$) when compared to HF-P21 group. No differences were observed for triglyceride concentrations among the different diets.

The activities of AST and ALT enzymes were also determined in the pups' serum after weaning (Table 4) and no difference was found between groups.

Effect of dietary intervention on liver lipid content

To assess the effect of maternal diet on promoting early changes in liver dynamics, lipid metabolism, total content of fat, cholesterol and triglyceride levels were evaluated in the liver of offspring after the lactation period (Table 4). No significant differences were found in liver fat values between groups. HF-21 and HFA-P21, during gestation and lactation, induced an increase in total cholesterol concentration in the liver when compared to C-P21 (144%, $p < 0.0001$ versus HF-P21; 134%, $p < 0.0001$ versus HFA-P21) and CA-P21 (134%, $p < 0.0001$ versus HF-P21; 124%, $p < 0.0001$ versus HFA-P21). Regarding the triglycerides liver content, açaí supplement in control diet was able to prevent the increase in the triglycerides after the lactation period (Table 4). CA-P21 group

Table 4 Body and liver weight, serum lipid profile, liver function and liver lipid content of P21

	C	HF	CA	HFA
Total cholesterol (mmol/l)	4.22 ± 0.51	6.7 ± 1.88#*	4.52 ± 0.77	3.87 ± 0.41§
Triglyceride (mmol/l)	1.2 ± 0.86	0.96 ± 0.66	1.24 ± 0.84	1.24 ± 0.28
AST (U/l)	96.34 ± 10.17	103 ± 17.11	98.22 ± 7.21	94.11 ± 10.43
ALT (U/l)	29.11 ± 5.63	32.26 ± 14.96	23.17 ± 4.36	37.43 ± 9.26
Liver fat (mg/g)	63.89 ± 37.69	88.39 ± 28.63	71.48 ± 7.28	81.89 ± 18.94
Liver cholesterol (mg/g)	4.82 ± 1.35	11.79 ± 3.58#*	5.04 ± 0.45	11.28 ± 2.05#*
Liver triglyceride (mg/g)	22 ± 7.26	26.42 ± 6.40*	14.13 ± 4.84	26.88 ± 7.76*

$p < 0.05$: #versus C, *versus CA and §versus HF

C control diet, *HF* high-fat diet, *CA* açaí diet, *HFA* high-fat açaí diet, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase. The results are shown as the mean ± SD ($n = 7$ pups per group). One-way ANOVA followed by a Tukey post hoc test

presented reduction in liver triglycerides when compared to HF-P21 and HFA-P21 groups (87%, $p = 0.0077$ and 90%, $p = 0.0055$, respectively).

Effect of dietary intervention on liver steatosis grade

Through histology of P21 livers (Fig. 4a), it was possible to observe that HF-P21 had more lipid droplets compared to any other group. In relation to degree of steatosis, HF-P21 group presented a steatosis degree (mild to moderate, Fig. 4b) more pronounced than in CA-P21 and HFA-P21 groups (absent to mild). HFA-P21 group presented a lower degree of steatosis, endorsing the protective effect of açaí in relation to accumulation of hepatic lipids.

Effect of dietary intervention on expression of genes involved in lipid metabolism

To identify some of the potential molecular pathways involved in lipid metabolism and affected by a diet supplemented with açaí during the gestation and lactation process, gene expression was assessed in P1 and P21 offspring, respectively. No statistically significant differences were observed in the gene expression of P1 (Fig. 5a).

Similarly, the expression of lipid metabolism genes was assessed in the liver from pups after the lactation period (Fig. 5b). Expression of *Sirt1* (0.5-fold change, $p = 0.0168$), *Srebf1* (fourfold change, $p = 0.0274$) and *Fasn* (fivefold times, $p = 0.004$) was increased in the HFA-P21 liver when compared to HF-P21. No significant differences were found in *Ucp2* gene expression (Fig. 5b).

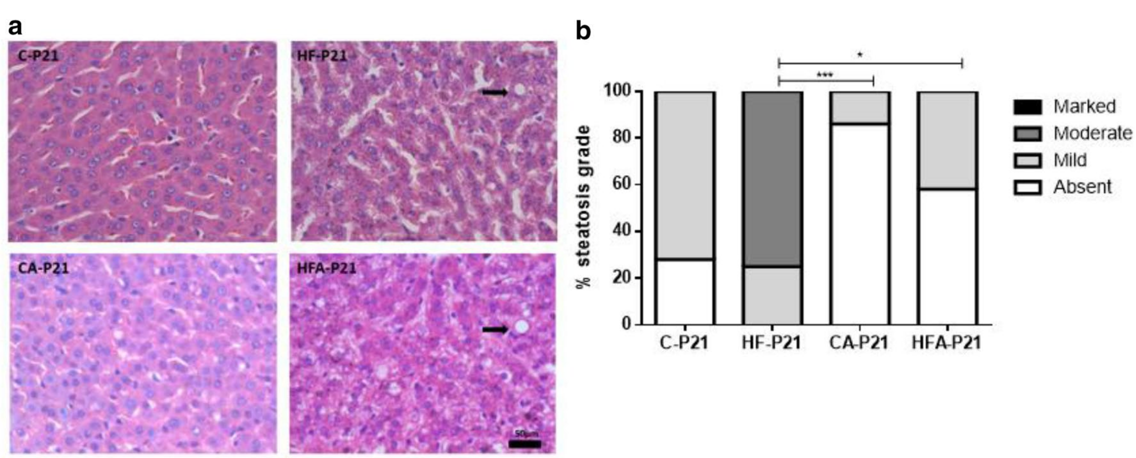

Fig. 4 **a** Representative histological sections of the liver of offspring P21 fed with a control diet (C), high-fat diet (HF), açaí diet (CA) and high-fat supplemented with açaí (HFA), stained with hematoxylin and eosin. Black arrow shows macrosteatosis and red arrow shows microsteatosis. The images were photographed at a magnification of 400×. Bar Scale = 50 µm; **b** Grade of hepatic steatosis of dams ($n = 7$ pups per group). Value of $p < 0.05$ was considered statistically significant for the Kruskal–Wallis. *< 0.05, ***< 0.005

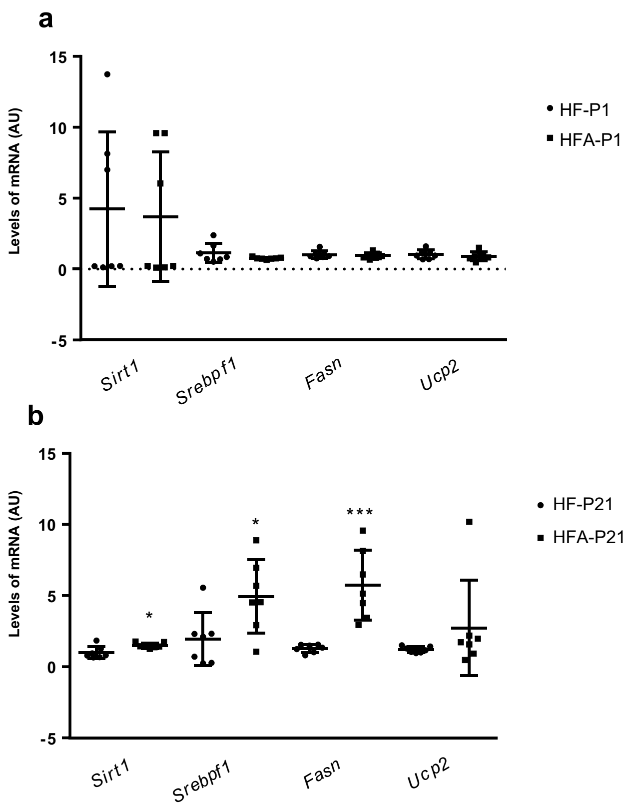

Fig. 5 mRNA abundance for genes related to lipid metabolism in the liver of offspring P1 (**a**) and offspring P21 (**b**) relative to beta-2-microglobulin. *HF* high-fat diet, *HFA* high-fat açaí diet, *Sirt1* sirtuin 1, *Srebf1* sterol regulatory element-binding transcription factor, *Fasn* fatty acid synthase, *Ucp2* uncoupling protein 2. The results are shown as the mean ± SD ($n = 7$ pups per group). Analyses by Student's t test. *$p < 0.05$; ***$p < 0.005$

Effect of dietary intervention on protein expression

SIRT1 and SREBP1 protein expression was analyzed in offspring P1 (Fig. 6a, b) and P21 (Fig. 6c, d) from different maternal diets. No significant differences were observed between the groups. In the same way as it was seen in the dams, the overexpression of *Srebf1* showed no increase in the expression of the respective proteins.

Discussion

In the present study, we evaluated the effects of açaí supplementation in combination with a maternal high-fat diet on lipid and liver metabolism of dams and offspring postnatally or post lactation. Our results revealed that, in dams, the high-fat diet increased absolute liver weight, serum ALT and AST enzyme activity, hepatic total fat content, cholesterol and triglycerides: changes that are consistent with the development of NAFLD. The addition of açaí in the maternal high-fat diet reduced some of the NAFLD characteristics, including relative liver weight and hepatic fat content, in agreement with previous studies conducted with hyperlipidemic and hypercholesterolemic diet in rats and mice which showed açaí to improve hepatic steatosis and reduce the deleterious effects of lipid excess [16, 17]. Although these studies were not performed with rodents during gestation or lactation, the results of our study suggest an important role of açaí also in specific physiological states. Regarding the offspring, açaí consumption during gestation and lactation was able to reduce serum cholesterol and degree of steatosis in P21, suggesting this fruit can modify offspring's lipid metabolism, conferring protective effect to the development of hepatic steatosis.

Maternal high-fat diet affected the health of offspring by promoting changes that may trigger the development of metabolic diseases later in life such as diabetes, insulin resistance, obesity, cardiovascular disease and asthma [33]. Studies have described that excess of maternal nutrition during gestation, in combination with a high-fat postnatal diet, is capable of promoting phenotypic alterations, like increased body weight, hyperinsulinemia, hyperglycemia, hypertriglyceridemia and hypercholesterolemia [34, 35]. In contrast, the introduction of foods such as green tea and guarana can improve serum levels of ALT, cholesterol, triglycerides, HDL and glucose in offspring [36, 37]. In our study, the addition of açaí to the maternal high-fat diet reduced serum levels of total cholesterol in offspring P21 relative to the HF-P21 group. Differently from what was found in the dams, açaí was not able to change the weight and/or fat content in the liver of HF-P21 group. It is possible that the degree of damage caused by the HF diet in the offspring is smaller than in dams and, therefore, the supplementation of açaí in the maternal diet was more effective in mitigating effects at plasma level. In fact, a recent study evaluating the introduction of jussara (a kind of açaí) into a maternal diet enriched with hydrogenated vegetable fat reported a reduction in plasma levels of glucose, total cholesterol and triglycerides in offspring receiving jussara fruit supplementation in a maternal high-fat diet [38]. Another study evaluating the administration of different types of fat (vegetable oil, lard, hydrogenated vegetable oil and fish oil) during gestation and lactation reported that the administration of omega-3 was able to reduce HDL and serum total cholesterol in dams; whereas in the offspring, there was a reduction in the serum and hepatic levels of triglycerides, as well as a decrease in total cholesterol and free fatty acids [39].

To better understand our results, we evaluated if modulation of the lipid biosynthesis or fatty acids β-oxidation was responsible for the improvement of NAFLD in dams and possibly in offspring after lactation and gestation. SIRT1 is an important regulator of lipid metabolism in the liver [40]. Fat-rich diets have been shown to reduce

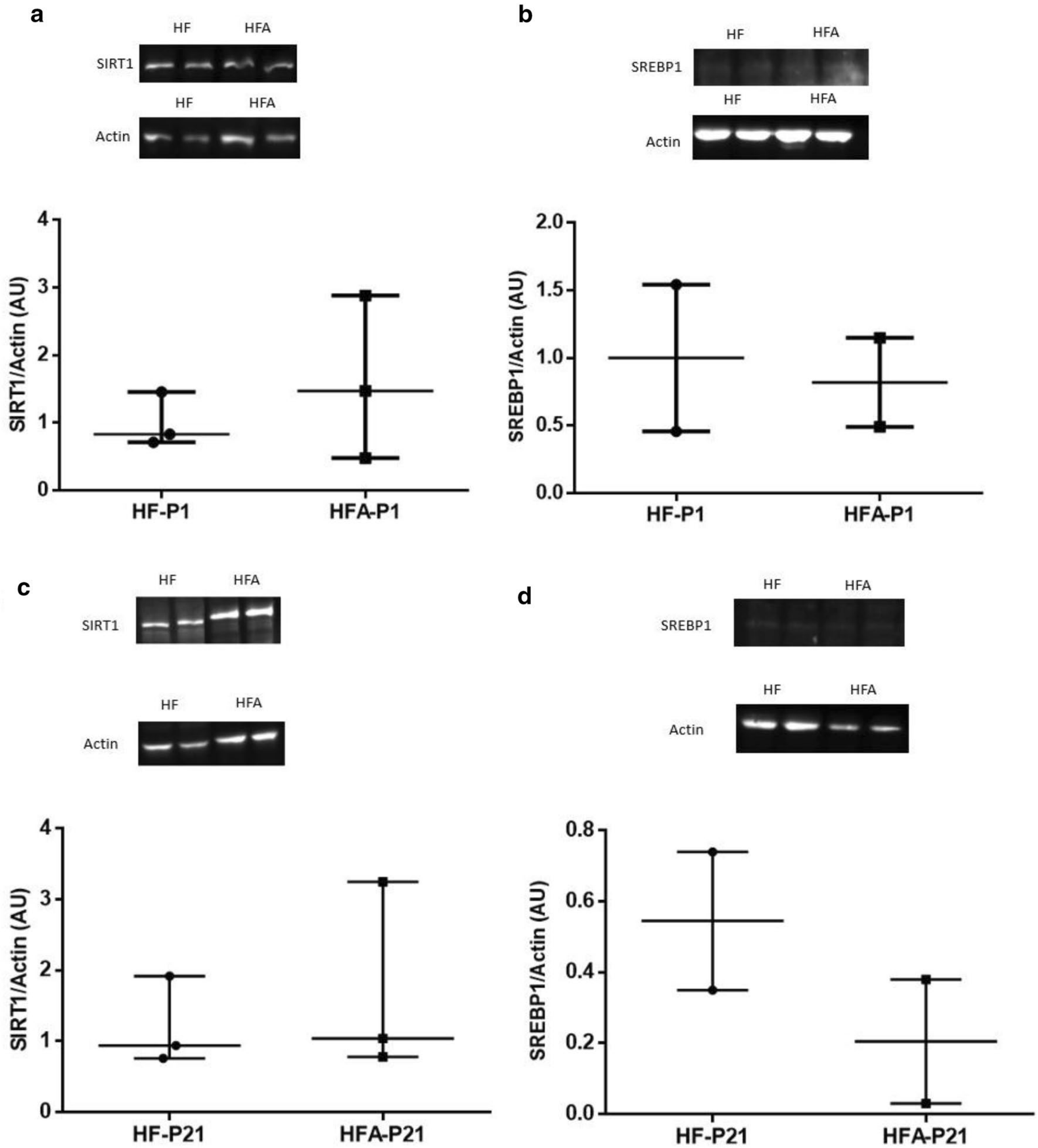

Fig. 6 Western blotting for SIRT1 and SREBP1 of offspring P1 (**a**, **b**) and offspring P21 (**c**, **d**). Graphs represent data from Western blotting quantification. *HF* high-fat diet and *HFA* high-fat supplemented with açaí. The data are shown as median and range (minimum and maximum values) ($n=7$ pups per group). Value of $p<0.05$ was considered statistically significant for the Kruskal–Wallis

the expression of *Sirt1* making the liver more susceptible to fat accumulation [41]. This has also been observed in the liver of animals from a maternal high-fat diet, suggesting that the metabolic programming of NAFLD may be involved in the downregulation of *Sirt1* [42, 43]. In this regard, the use of compounds capable of activating SIRT1 has emerged as an excellent alternative to attenuate fat accumulation in hepatocytes [44]. In the present work, a trend for increased levels of *Sirt1* mRNA expression was observed in dams and P1 group after the addition of açaí to the HF maternal diet, and reached statistical difference in the P21 HFA group (0.5-fold change). However, no changes in protein levels were observed. Açaí is a food that presents high concentration of phenolic compounds, mainly of the class of anthocyanins [22]. We believed that it might be possible to regulate these compounds in the activation of SIRT1 and subsequently in the improvement of NAFLD. It is worth noting that we did not use in this study isolated antioxidant compounds, but the açaí pulp as a food that presents in its composition other dietary compounds that can positively affect the lipid metabolism in the liver through the regulation of other ways.

Pathways associated with lipid metabolism are dependent on the expression and activation of SREBP1 and key enzymes of lipid biosynthesis such as FAS and dietary components like PUFA and MUFA fatty acids have been shown to regulate the expression of *Srebf1* and lipogenic genes, reducing the accumulation of hepatic fat [45]. Therefore, the high proportion of unsaturated fatty acids (> 70%) present in the lipid fraction of açaí, besides the presence of phenolic compounds, may affect positively lipid metabolism in the liver [22]. In this study, dams and HFA-P21 groups showed an increase in the *Srebf1* and *Fasn* mRNA compared to the HF group. Although the results show higher levels of *Srebf1* mRNA in the HFA group, there appears to be post-translational regulation, since no changes in SREBP1 protein expression were observed in dams and P21. Such results reflect the complexity of lipid metabolism regulation by dietary components. As an example, a study carried out in mice to investigate the effect of different fruits, including açaí, on obesity and metabolic disorders, showed that the groups of animals receiving a high-fat diet supplemented with açaí presented higher glucose and fasting insulin levels compared to groups that received other fruits [46]. In addition, açaí-fed animals showed increased regulation of genes associated with lipid and cholesterol biosynthesis, such as *Cidea*, *Cidec* and *Anxa2* [47]. In general, the results showed an exacerbation of fatty liver disease by açaí. However, it is important to note that the amount of açaí used in that study was 20%, different from our study that evaluated the effect of supplementation with 2% açaí pulp. Moreover, in other study, açaí has been shown to have beneficial effects on cholesterol concentration by increasing its elimination by bile via modulation of gene expression for *Abcg5* and *Abcg8* carriers, as well as up-regulation of the *Srebf2* mRNA [48]. This intriguing observation raises another question about how açaí is able to improve the liver fat accumulation. The current study does not provide data to directly answer this question, but other pathways can be altered. It is possible that the presence of fibers in the açaí can increase the excretion of cholesterol and consequently influence on the lipid metabolism, as observed in previous studies with adult rats [48]. In addition, modifications in oxidative metabolism may contribute to the improvement of hepatic lipid accumulation found in this study. Pereira et al., showed that hyperlipidemic rats treated with açaí pulp were able to prevent the oxidation of LDL and to increase the expression of PON1 and ApoA-I, important molecules related oxidative stress and lipid metabolism [24, 49, 50]. However, this study it was not conducted in a specific state such as gestation and lactation. Other hand, unsaturated fatty acids may provide an increase in the expression and activity of the LDL receptors in the liver [51]. PUFAs found in high amounts in açaí can act as potent activators of the peroxisome proliferator-activated receptor family (PPARs) that regulate other genes involved in lipid metabolism.

To verify the ability of açaí to increase lipid oxidation and thus improve lipid accumulation, levels of *Ucp2* mRNA were assessed. No differences were found in the liver of dams and P1. Regarding the offspring P21, although the *Ucp2* gene expression was 166% higher, no statistically significant difference was observed. In view of the role of UCP2 in reducing ROS and promoting efficient mitochondrial oxidation, an increase in *Ucp2* expression could suggest an increase in the beta oxidation of fatty acids in P21. In fact, a study evaluating the effect of açaí aqueous extract on hepatic steatosis in adults mice showed an increase in carnitine-palmitoyl transferase (CPT-I), a key enzyme in the entry of fatty acids to β-oxidation [23]. In addition, uncoupling proteins also carry the transport of fatty acid anions and lipoperoxide anions through the inner mitochondrial membrane [17]. This mechanism can be interpreted as a way to relieve the matrix of lipids excess. Therefore, UCP2 could also act in the protection of the liver against hepatocellular lipotoxicity [52]. One hypothesis is that the presence of bioactive compounds in açaí confers a beneficial effect in the fat liver accumulation through the reduction of oxidative stress, since açaí is rich in polyphenols and anthocyanins, and regulation of the production of ROS alleviates accumulation of fat droplets in the liver, as observed in our study with an improvement in fat liver content and grade of steatosis. A study by Chen et al. (2018), using sugar kefir, demonstrated a reduction in lipid peroxidation levels and increased the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes [53]. The mechanisms involve the activation of NRF2, an important regulator of oxidative stress and the production of ROS [54, 55]. However, future studies involving redox metabolism need to be performed.

Açaí has also a high fiber content (30%), of which more than 20% is of the soluble type [22]. Fibers are known to promote a lower intestinal absorption of cholesterol from the diet and, consequently, increase the release of this sterol through chylomicrons [56]. Dietary fiber has been shown to be responsible for the increased biliary excretion in rats, thereby reducing serum cholesterol and blocking the enterohepatic circulation preventing reuse of bile acids by the liver [57]. In addition, dietary fibers seem to act indirectly in the expression of genes involved in the metabolism of hepatic cholesterol through secondary signals generated by metabolites produced in the intestine during fermentation [58]; however, this mechanism has not yet been fully elucidated. It is possible that the antioxidant effect of açaí can act directly on the pathways of oxidative stress, neutralizing free radicals and softening the damage caused by excess of lipids. It is important to remember that the açaí used in this study is a whole fruit. It is difficult to define which compound is responsible for the improvements observed in dams and offspring: a synergism between the different macro and micronutrients, as well as phytochemicals present in açaí may be responsible.

Currently, due to the increase in NAFLD in the pediatric population and the high prevalence of maternal obesity, several studies have emerged to understand how the maternal high-fat diet is able to "programming" the fetal liver and predispose the organism to early metabolic disorders. Nevertheless, studies that report the effects of combining a high-fat diet and foods or bioactive compounds into the development of NAFLD through molecular pathways are still limited. Epigenetic studies become important in metabolic programming models, since post-translation modifications in mRNA as repression or degradation may be occurring via microRNAs [59]. In addition, the increase or reduction of methylation in gene promoting regions is also related to regulation in gene expression [60]. Recent work had reported alterations in epigenetic mechanisms and possible regulation through bioactive compounds [61]. Furthermore, it is known that NAFLD is a complex disease that involves, besides lipid metabolism, changes in the insulin cascade, which were not evaluated in this study. The acetylated/deacetylated fractions of the SREBP1 transcription factor were not evaluated, which could promote a more accurate response in relation to the increase in *Sirt1* expression and its effect on SREBP1. We have observed an improvement in the liver lipids of the HFA dams. We do not believe that this effect was in detriment of the offspring once the total and relative liver weights (HFA-P1 and HFA-P21), as well as the total serum cholesterol were reduced in HFA-P21. High-fat diet promotes changes in lipid metabolism involving the crosstalk between liver and adipose tissue and this could explain the alterations in the dams lipid liver metabolism; however, one of the limitations of the study is the lack of data on the adipose tissue of dams and offspring. Although modifications in adipose tissue have not been evaluated, our work contains valuable data on açaí supplementation during specific physiological periods, such as gestation and lactation. Future studies could be conducted to evaluate the effect of açaí on lipid metabolism of adipose tissue.

In summary, the introduction of açaí to the maternal high-fat diet was able to exert a beneficial effect on the lipid metabolism of the dams, reducing the accumulation of hepatic fat, liver levels of total cholesterol and degree of steatosis. Açaí effects were observed in the offspring at serum level, suggesting that the hepatic damage caused by the high-fat maternal diet in offspring could be delayed with the introduction of foods rich in bioactive compounds and, therefore, have beneficial effects on health. More studies are needed to better understand the mechanisms involved to justify the effects of açaí supplementation during gestation and lactation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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