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

BARBOSA, P.O., DE SOUZA, M.O., PAIVA, D.P.D., SILVA, M.E., LIMA, W.G., BERMANO, G. and FREITAS, R.N.

2020

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

Priscila O Barbosa[a], Melina O de Souza[b], Deuziane P D Paiva[b], Marcelo E Silva[a,b], Wanderson G Lima[a], Giovanna Bermano[c], Renata N Freitas[a,b]

a – Nucleus of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto, Minas Gerais, Brazil

b – School of Nutrition, Federal University of Ouro Preto, Minas Gerais, Brazil

c – Centre for Obesity Research and Education, School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, UK

Corresponding author: Renata N Freitas

School of Nutrition, Federal University of Ouro Preto
Morro do Cruzeiro Campus, Bauxita 35400-000
Ouro Preto, MG, Brazil.
Tel.: +55 31 3559 1811; Fax: +55 31 3559 18

E-mail address: renata.freitas@ufop.edu.br

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) two 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

Acknowledgements

The authors are grateful to the Jair Pastor Mota and Laboratory of Experimental Nutrition for technical support and supply of animals, Dr Daniela Pala (UFOP, Brazil), Dr Carla Teixeira Silva (UFOP, Brazil), MSc. Miliane Fagundes (UFOP, Brazil), MSc. Ana Maria Viana (UFOP, Brazil), MSc. Talita Magalhães (UFOP, Brazil), MSc. Raiana Souza e Silva (UFOP, Brazil), Maraisa Porfirio (UFOP, Brazil) and Daniel de Souza Paula (UFOP, Brazil) for helping with the animal handling. We thank Professor Dr Maria Terezinha Bahia (UFOP, Brazil) who provided laboratory reagents to perform biochemistry analysis, and Dr Gemma Barron (RGU, UK) for help with gene expression and western blotting experiments.

Funding Sources

This research was supported by Federal University of Ouro Preto (UFOP, Minas Gerais, Brazil), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG, Minas Gerais, Brazil), Coordenação de

42 Aperfeiçoamento de Pessoal de Nível Superior (CAPES) from Brazilian government (P.O.B. scholarship) and
43 Robert Gordon University (Aberdeen, Scotland, United Kingdom).

44

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by accumulation of triglycerides in hepatocytes. This disease encompasses a spectrum of conditions ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH) characterised 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) promote 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 epigenetics 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 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 mobilisation 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 oxidised 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 [19,20,10,21]. 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 signalling, 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 two 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.

2. Materials and Methods

2.1 Açaí Pulp

Pasteurised 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 100g of pulp.

Polyphenol content of açaí pulp was determined by 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 100g of açaí pulp. The açaí pulp used in this study presented 549.5 mg GAE/100g. The content of anthocyanins was also measured as reported by Giust and Wrolstad (2001) [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.

2.2 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 in 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 two weeks. After one week, we evaluated the food intake. After two 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, in order 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 analysis.

2.3 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-hours fasting, and sacrificed 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.

2.4 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.

2.5 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. (1957) [31]. The content of total lipids in the liver was quantified gravimetrically by evaporation of the solvents and dissolution of the dried lipids in 500µl of isopropanol. Concentrations of TG and TC were determined colorimetrically using TG and TC assay kits (Labtest, Lagoa Santa, MG, Brazil).

2.6 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 40x 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].

2.7 Quantitative reverse transcription polymerase chain reaction analysis

Total RNA extraction was performed from 10-20mg 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 analysed using comparative Ct method and target gene expression was related to the expression of the house keeping gene, β2 microglobulin.

2.8 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 13000 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 were 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 1h 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 1h 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, 1:10000 for Actin) and were incubated overnight at 4°C. The membranes were then washed three times for 5 min with TBST, before incubation for 1h 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.

2.9 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 analysed 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 Student *t*-test unpaired one tail. Results were considered statistically significant for p values < 0.05.

3. Results

3.1 Dams

3.1.1 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%, 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 to food intake, the supplementation with 2% of açaí pulp did not affect the caloric intake of the dams (Table 2).

3.1.2 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).

3.1.3 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).

3.1.4 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 (Figure 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 (Figure 1b).

3.1.5 Effect of dietary intervention on gene expression involved in lipid metabolism

In order to determine the potential metabolic pathways by which açaí could improve hepatic fat accumulation, the expression of genes involved in lipid metabolism was assessed (Figure 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* (3-fold change, p=0.0092) and *Fasn* (4-fold change, p=0.0241) genes when compared to the HF group.

3.1.6 Effect of dietary intervention on protein levels

Western blot analysis did not show significant differences in SIRT1 protein levels (Figure 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 (Figure 3b).

3.2 Offspring

3.2.1 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<=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).

3.2.2 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.

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

3.2.4 Effect of dietary intervention on liver steatosis grade

Through histology of P21 livers (Figure 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, Figure 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.

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

In order 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 (Figure 5a).

Similarly, the expression of lipid metabolism genes was assessed in the liver from pups after the lactation period (Figure 5b). Expression of *Sirt1* (0.5-fold change, p=0.0168), *Srebf1* (4-fold change, p=0.0274) and *Fasn* (5-fold 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 (Figure 5b).

3.2.6 Effect of dietary intervention on protein expression

SIRT1 and SREBP1 protein expression was analysed in offspring P1 (Figure 6a and 6b) and P21 (Figure 6c and 6d) 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.

4. 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 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 [17,16]. 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 to the offspring, açaí consumption during gestation and lactation was able to reduced serum cholesterol and degree of steatosis in P21, suggesting this fruit can to 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, hypertryglyceridemia 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].

In order to better understand our results, we evaluated if modulation of the lipid biosynthesis or fatty acids β-oxidation was responsible for 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 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 to 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 appear to be post-translational regulation, since no changes in SREBP1 protein expression was 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 to 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 adults 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 hyperlipidaemic rats treated with açaí pulp was 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.

In order 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 paediatric 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 becomes 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 in order to justify the effects of açaí supplementation during gestation and lactation.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Brunt EM (2010) Pathology of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 7 (4):195-203. doi:10.1038/nrgastro.2010.21
2. Stewart MS, Heerwagen MJ, Friedman JE (2013) Developmental programming of pediatric nonalcoholic fatty liver disease: redefining the"first hit". Clin Obstet Gynecol 56 (3):577-590. doi:10.1097/GRF.0b013e3182a09760
3. Li M, Sloboda DM, Vickers MH (2011) Maternal obesity and developmental programming of metabolic disorders in offspring: evidence from animal models. Exp Diabetes Res 2011:592408. doi:10.1155/2011/592408
4. Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel JC, Boileau P, Le Bouc Y, Deal CL, Lillycrop K, Scharfmann R, Sheppard A, Skinner M, Szyf M, Waterland RA, Waxman DJ, Whitelaw E, Ong K, Albertsson-Wikland K (2011) Child health, developmental plasticity, and epigenetic programming. Endocr Rev 32 (2):159-224. doi:10.1210/er.2009-0039
5. Fleming TP, Eckert JJ, Denisenko O (2017) The Role of Maternal Nutrition During the Periconceptional Period and Its Effect on Offspring Phenotype. Adv Exp Med Biol 1014:87-105. doi:10.1007/978-3-319-62414-3_5
6. Williams L, Seki Y, Vuguin PM, Charron MJ (2014) Animal models of in utero exposure to a high fat diet: a review. Biochim Biophys Acta 1842 (3):507-519. doi:10.1016/j.bbadis.2013.07.006
7. Speakman J, Hambly C, Mitchell S, Krol E (2008) The contribution of animal models to the study of obesity. Lab Anim 42 (4):413-432. doi:10.1258/la.2007.006067
8. Hughes AN, Oxford JT (2014) A lipid-rich gestational diet predisposes offspring to nonalcoholic fatty liver disease: a potential sequence of events. Hepat Med 6:15-23. doi:10.2147/HMER.S57500
9. Sacconnay L, Carrupt PA, Nurisso A (2016) Human sirtuins: Structures and flexibility. J Struct Biol 196 (3):534-542. doi:10.1016/j.jsb.2016.10.008
10. Ponugoti B, Kim DH, Xiao Z, Smith Z, Miao J, Zang M, Wu SY, Chiang CM, Veenstra TD, Kemper JK (2010) SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. J Biol Chem 285 (44):33959-33970. doi:10.1074/jbc.M110.122978
11. Eberle D, Hegarty B, Bossard P, Ferre P, Foufelle F (2004) SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86 (11):839-848. doi:10.1016/j.biochi.2004.09.018
12. Suter MA, Chen A, Burdine MS, Choudhury M, Harris RA, Lane RH, Friedman JE, Grove KL, Tackett AJ, Aagaard KM (2012) A maternal high-fat diet modulates fetal SIRT1 histone and protein deacetylase activity in nonhuman primates. FASEB J 26 (12):5106-5114. doi:10.1096/fj.12-212878
13. McCurdy CE, Bishop JM, Williams SM, Grayson BE, Smith MS, Friedman JE, Grove KL (2009) Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. J Clin Invest 119 (2):323-335. doi:10.1172/JCI32661
14. Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC, Bateman AC, Clough GF, Poston L, Hanson MA, McConnell JM, Byrne CD (2009) Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology 50 (6):1796-1808. doi:10.1002/hep.23205
15. Rolo AP, Teodoro JS, Palmeira CM (2012) Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med 52 (1):59-69. doi:10.1016/j.freeradbiomed.2011.10.003
16. Nedergaard J, Cannon B (2003) The 'novel' 'uncoupling' proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions. Exp Physiol 88 (1):65-84
17. Baffy G (2005) Uncoupling protein-2 and non-alcoholic fatty liver disease. Front Biosci 10:2082-2096
18. Pan MH, Lai CS, Tsai ML, Ho CT (2014) Chemoprevention of nonalcoholic fatty liver disease by dietary natural compounds. Mol Nutr Food Res 58 (1):147-171. doi:10.1002/mnfr.201300522
19. Tanaka M, Kita T, Yamasaki S, Kawahara T, Ueno Y, Yamada M, Mukai Y, Sato S, Kurasaki M, Saito T (2017) Maternal resveratrol intake during lactation attenuates hepatic triglyceride and fatty acid synthesis in adult male rat offspring. Biochem Biophys Rep 9:173-179. doi:10.1016/j.bbrep.2016.12.011
20. Tiao MM, Lin YJ, Yu HR, Sheen JM, Lin IC, Lai YJ, Tain YL, Huang LT, Tsai CC (2018) Resveratrol ameliorates maternal and post-weaning high-fat diet-induced nonalcoholic fatty liver disease via renin-angiotensin system. Lipids Health Dis 17 (1):178. doi:10.1186/s12944-018-0824-3
21. Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I (2010) Regulation of SIRT1 in cellular functions: role of polyphenols. Arch Biochem Biophys 501 (1):79-90. doi:10.1016/j.abb.2010.05.003
22. Schauss AG, Wu X, Prior RL, Ou B, Patel D, Huang D, Kababick JP (2006) Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, Euterpe oleraceae mart. (acai). J Agric Food Chem 54 (22):8598-8603. doi:10.1021/jf060976g
23. Guerra JFC, Maciel PS, Abreu ICME, Pereira RR, Silva M, Cardoso LM, Pinheiro-Sant'Ana HM, Lima WG, Silva ME, Pedrosa ML (2015) Dietary açai attenuates hepatic steatosis via adiponectin-mediated effects on lipid metabolism in high-fat diet mice. J Funct Foods 14:192-202. doi:10.1016/j.jff.2015.01.025

24. Pereira RR, de Abreu IC, Guerra JF, Lage NN, Lopes JM, Silva M, de Lima WG, Silva ME, Pedrosa ML (2016) Acai (Euterpe oleracea Mart.) Upregulates Paraoxonase 1 Gene Expression and Activity with Concomitant Reduction of Hepatic Steatosis in High-Fat Diet-Fed Rats. Oxid Med Cell Longev 2016:8379105. doi:10.1155/2016/8379105
25. George S, Brat P, Alter P, Amiot MJ (2005) Rapid determination of polyphenols and vitamin C in plant-derived products. J Agric Food Chem 53 (5):1370-1373. doi:10.1021/jf048396b
26. Giusti MM, Wrolstad RE (2001) Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. 00 (1):F1.2.1-F1.2.13. doi:doi:10.1002/0471142913.faf0102s00
27. Reeves PG, Nielsen FH, Fahey GC, Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123 (11):1939-1951. doi:10.1093/jn/123.11.1939
28. Burgueno AL, Cabrerizo R, Gonzales Mansilla N, Sookoian S, Pirola CJ (2013) Maternal high-fat intake during pregnancy programs metabolic-syndrome-related phenotypes through liver mitochondrial DNA copy number and transcriptional activity of liver PPARGC1A. J Nutr Biochem 24 (1):6-13. doi:10.1016/j.jnutbio.2011.12.008
29. Guerra JF, Magalhaes CL, Costa DC, Silva ME, Pedrosa ML (2011) Dietary acai modulates ROS production by neutrophils and gene expression of liver antioxidant enzymes in rats. J Clin Biochem Nutr 49 (3):188-194. doi:10.3164/jcbn.11-02
30. Padilha PC, Rocha HF, Alves N, Peres WAF (2010) Prevalence of nonalcoholic fatty liver disease in obese children and adolescents: a systematic review. Rev Paul Pediatr 28 (4):387-393. doi:10.1590/S0103-05822010000400016
31. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226 (1):497-509
32. Brunt EM (2016) Nonalcoholic Fatty Liver Disease: Pros and Cons of Histologic Systems of Evaluation. Int J Mol Sci 17 (1). doi:10.3390/ijms17010097
33. Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VW, Eriksson JG, Broekman BF (2017) Influence of maternal obesity on the long-term health of offspring. Lancet Diabetes Endocrinol 5 (1):53-64. doi:10.1016/S2213-8587(16)30107-3
34. Desai M, Jellyman JK, Han G, Beall M, Lane RH, Ross MG (2014) Maternal obesity and high-fat diet program offspring metabolic syndrome. Am J Obstet Gynecol 211 (3):237 e231-237 e213. doi:10.1016/j.ajog.2014.03.025
35. Renault KM, Carlsen EM, Norgaard K, Nilas L, Pryds O, Secher NJ, Cortes D, Jensen JE, Olsen SF, Halldorsson TI (2015) Intake of carbohydrates during pregnancy in obese women is associated with fat mass in the newborn offspring. Am J Clin Nutr 102 (6):1475-1481. doi:10.3945/ajcn.115.110551
36. Hachul ACL, Boldarine VT, Neto NIP, Moreno MF, Ribeiro EB, CM OdN, Oyama LM (2018) Maternal consumption of green tea extract during pregnancy and lactation alters offspring's metabolism in rats. PLoS One 13 (7):e0199969. doi:10.1371/journal.pone.0199969
37. Lima NDS, Caria C, Gambero A, Ribeiro ML (2018) The effect of Guarana (Paullinia cupana) on metabolic and inflammatory parameters in adult male mice programmed by maternal obesity. Eur J Nutr. doi:10.1007/s00394-018-1686-1
38. Argentato PP, Morais CA, Santamarina AB, César HC, Estadella D, Rosso VV, Pisani LP (2017) Jussara (Euterpe edulis Mart.) supplementation during pregnancy and lactation modulates UCP-1 and inflammation biomarkers induced by trans-fatty acids in the brown adipose tissue of offspring. Clin Nutr Exp 12:50-65. doi:10.1016/j.yclnex.2016.12.002
39. Mennitti LV, Oyama LM, Santamarina AB, Nascimento OD, Pisani LP (2018) Influence of maternal consumption of different types of fatty acids during pregnancy and lactation on lipid and glucose metabolism of the 21-day-old male offspring in rats. Prostaglandins Leukot Essent Fatty Acids 135:54-62. doi:10.1016/j.plefa.2018.07.001
40. Kemper JK, Choi SE, Kim DH (2013) Sirtuin 1 deacetylase: a key regulator of hepatic lipid metabolism. Vitam Horm 91:385-404. doi:10.1016/B978-0-12-407766-9.00016-X
41. Deng XQ, Chen LL, Li NX (2007) The expression of SIRT1 in nonalcoholic fatty liver disease induced by high-fat diet in rats. Liver Int 27 (5):708-715. doi:10.1111/j.1478-3231.2007.01497.x
42. Nguyen LT, Chen H, Pollock CA, Saad S (2016) Sirtuins-mediators of maternal obesity-induced complications in offspring? FASEB J 30 (4):1383-1390. doi:10.1096/fj.15-280743
43. Borengasser SJ, Kang P, Faske J, Gomez-Acevedo H, Blackburn ML, Badger TM, Shankar K (2014) High fat diet and in utero exposure to maternal obesity disrupts circadian rhythm and leads to metabolic programming of liver in rat offspring. PLoS One 9 (1):e84209. doi:10.1371/journal.pone.0084209

44. Alberdi G, Rodriguez VM, Macarulla MT, Miranda J, Churruca I, Portillo MP (2013) Hepatic lipid metabolic pathways modified by resveratrol in rats fed an obesogenic diet. Nutrition 29 (3):562-567. doi:10.1016/j.nut.2012.09.011
45. Jump DB, Tripathy S, Depner CM (2013) Fatty acid-regulated transcription factors in the liver. Annu Rev Nutr 33:249-269. doi:10.1146/annurev-nutr-071812-161139
46. Heyman L, Axling U, Blanco N, Sterner O, Holm C, Berger K (2014) Evaluation of Beneficial Metabolic Effects of Berries in High-Fat Fed C57BL/6J Mice J Nutr Metab 2014:12. doi:10.1155/2014/403041
47. Heyman-Linden L, Seki Y, Storm P, Jones HA, Charron MJ, Berger K, Holm C (2016) Berry intake changes hepatic gene expression and DNA methylation patterns associated with high-fat diet. J Nutr Biochem 27:79-95. doi:10.1016/j.jnutbio.2015.08.022
48. de Souza MO, Souza ESL, de Brito Magalhaes CL, de Figueiredo BB, Costa DC, Silva ME, Pedrosa ML (2012) The hypocholesterolemic activity of acai (Euterpe oleracea Mart.) is mediated by the enhanced expression of the ATP-binding cassette, subfamily G transporters 5 and 8 and low-density lipoprotein receptor genes in the rat. Nutr Res 32 (12):976-984. doi:10.1016/j.nutres.2012.10.001
49. Karavia EA, Papachristou DJ, Liopeta K, Triantaphyllidou IE, Dimitrakopoulos O, Kypreos KE (2012) Apolipoprotein A-I modulates processes associated with diet-induced nonalcoholic fatty liver disease in mice. Mol Med 18:901-912. doi:10.2119/molmed.2012.00113
50. Garcia-Heredia A, Kensicki E, Mohney RP, Rull A, Triguero I, Marsillach J, Tormos C, Mackness B, Mackness M, Shih DM, Pedro-Botet J, Joven J, Saez G, Camps J (2013) Paraoxonase-1 deficiency is associated with severe liver steatosis in mice fed a high-fat high-cholesterol diet: a metabolomic approach. J Proteome Res 12 (4):1946-1955. doi:10.1021/pr400050u
51. Fukushima M, Nakano M, Morii Y, Ohashi T, Fujiwara Y, Sonoyama K (2000) Hepatic LDL receptor mRNA in rats is increased by dietary mushroom (Agaricus bisporus) fiber and sugar beet fiber. J Nutr 130 (9):2151-2156. doi:10.1093/jn/130.9.2151
52. Cortez-Pinto H, Machado MV (2009) Uncoupling proteins and non-alcoholic fatty liver disease. J Hepatol 50 (5):857-860. doi:10.1016/j.jhep.2009.02.019
53. Chen YT, Lin YC, Lin JS, Yang NS, Chen MJ (2018) Sugary Kefir Strain Lactobacillus mali APS1 Ameliorated Hepatic Steatosis by Regulation of SIRT-1/Nrf-2 and Gut Microbiota in Rats. Mol Nutr Food Res 62 (8):e1700903. doi:10.1002/mnfr.201700903
54. Bayele HK, Debnam ES, Srai KS (2016) Nrf2 transcriptional derepression from Keap1 by dietary polyphenols. Biochem Biophys Res Commun 469 (3):521-528. doi:10.1016/j.bbrc.2015.11.103
55. Nguyen T, Nioi P, Pickett CB (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 284 (20):13291-13295. doi:10.1074/jbc.R900010200
56. Anderson JW, Baird P, Davis RH, Jr., Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL (2009) Health benefits of dietary fiber. Nutr Rev 67 (4):188-205. doi:10.1111/j.1753-4887.2009.00189.x
57. Garcia-Diez F, Garcia-Mediavilla V, Bayon JE, Gonzalez-Gallego J (1996) Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. J Nutr 126 (7):1766-1771. doi:10.1093/jn/126.7.1766
58. Caz V, Gil-Ramirez A, Largo C, Tabernero M, Santamaria M, Martin-Hernandez R, Marin FR, Reglero G, Soler-Rivas C (2015) Modulation of Cholesterol-Related Gene Expression by Dietary Fiber Fractions from Edible Mushrooms. J Agric Food Chem 63 (33):7371-7380. doi:10.1021/acs.jafc.5b02942
59. Simino LAP, de Fante T, Fontana MF, Borges FO, Torsoni MA, Milanski M, Velloso LA, Torsoni AS (2017) Lipid overload during gestation and lactation can independently alter lipid homeostasis in offspring and promote metabolic impairment after new challenge to high-fat diet. Nutr Metab (Lond) 14:16. doi:10.1186/s12986-017-0168-4
60. Pirola CJ, Gianotti TF, Burgueno AL, Rey-Funes M, Loidl CF, Mallardi P, Martino JS, Castano GO, Sookoian S (2013) Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut 62 (9):1356-1363. doi:10.1136/gutjnl-2012-302962
61. Baselga-Escudero L, Pascual-Serrano A, Ribas-Latre A, Casanova E, Salvado MJ, Arola L, Arola-Arnal A, Blade C (2015) Long-term supplementation with a low dose of proanthocyanidins normalized liver miR-33a and miR-122 levels in high-fat diet-induced obese rats. Nutr Res 35 (4):337-345. doi:10.1016/j.nutres.2015.02.008

608 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

609 *Sirt1*: sirtuin 1; *Srebf1*: sterol regulatory element-binding protein 1; *Fasn*: fatty acid synthase; *Ucp2*: uncoupling
610 protein 2
611

612 Table 2: Body and liver weight, serum lipid profile, liver function, liver lipid content, food intake, and food
613 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

614 $p < 0.05$: $^{\#}$ versus C, *versus CA and §versus HF
615 C: control diet; HF: high-fat diet; CA: açaí diet; HFA: high-fat açaí diet. The results are shown as the mean ±
616 SD (n=7 dams per group). One-way ANOVA followed by a Tukey post hoc test.
617

618 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§

619 $p < 0.05$: $^{\#}$ versus C, *versus CA and §versus HF
620 C: control diet; HF: high-fat diet; CA: açaí diet; HFA: high-fat açaí diet. Litter size six per dam. The results are
621 shown as the mean ± SD (n=7 pups per group). One-way ANOVA followed by a Tukey post hoc test.

622

623 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*

624 $p < 0.05$: $^{\#}$ versus C, *versus CA and §versus HF
625 C: control diet; HF: high-fat diet; CA: açaí diet; HFA: high-fat açaí diet; AST: aspartate aminotransferase; ALT:
626 alanine aminotransferase. The results are shown as the mean ± SD (n=7 pups per group). One-way ANOVA
627 followed by a Tukey post hoc test.

628

Legends of figures

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. 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

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. * $p < 0.05$; *** $p < 0.005$

Fig. 6: Western blotting for SIRT1 and SREBP1 of offspring P1 (a and b) and offspring P21 (c and 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

Figure 1

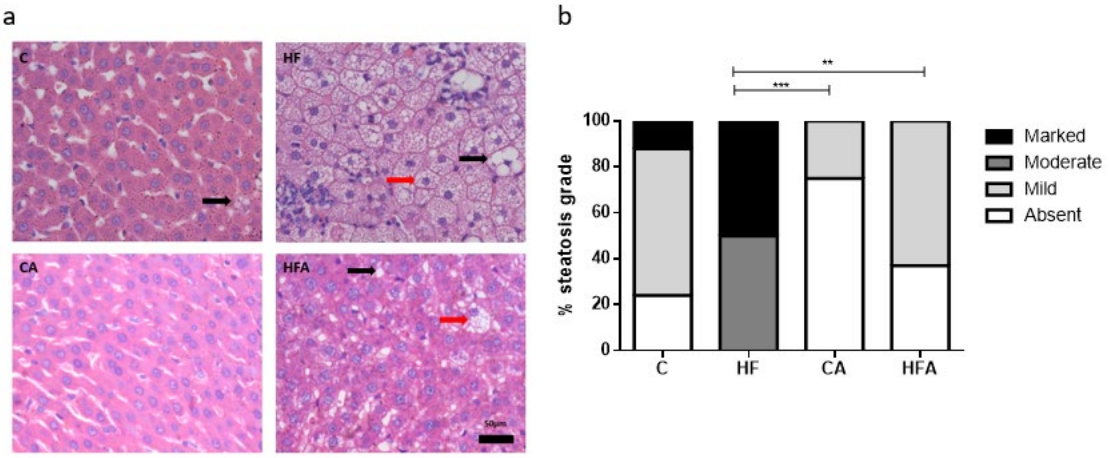

a
C
HF
CA
HFA
50µm
b
**
***
100
80
60
40
20
0
% steatosis grade
C
HF
CA
HFA
Marked
Moderate
Mild
Absent

Figure 2

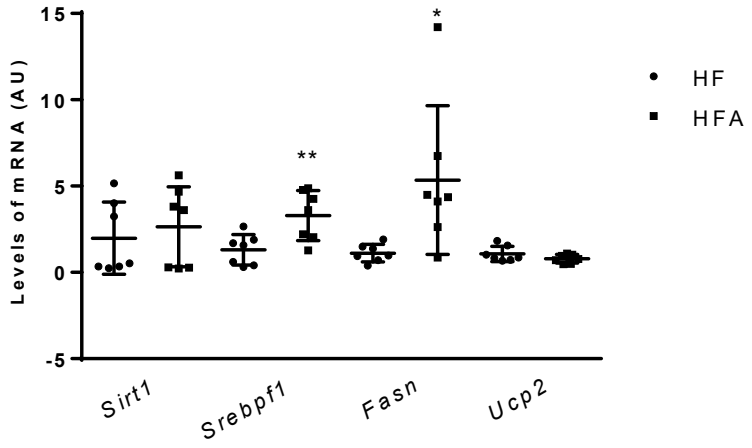


Figure 3

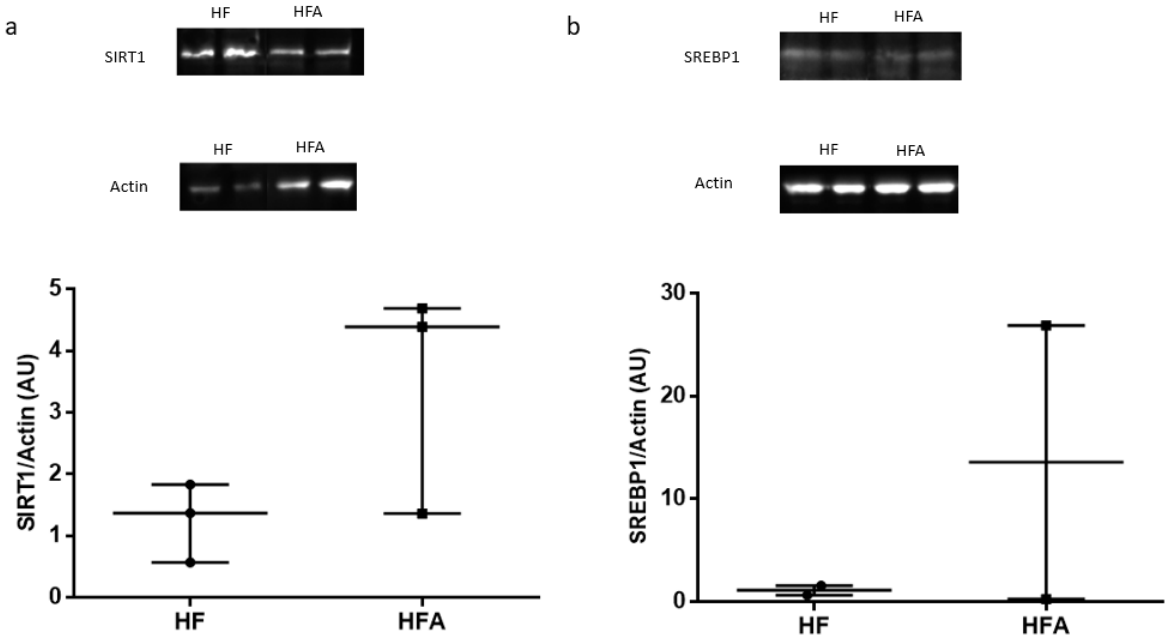

a
HF
HFA
SIRT1
HF
HFA
Actin
SIRT1/Actin (AU)
0
1
2
3
4
5
HF
HFA
b
HF
HFA
SREBP1
HF
HFA
Actin
SREBP1/Actin (AU)
0
10
20
30
HF
HFA

Figure 4

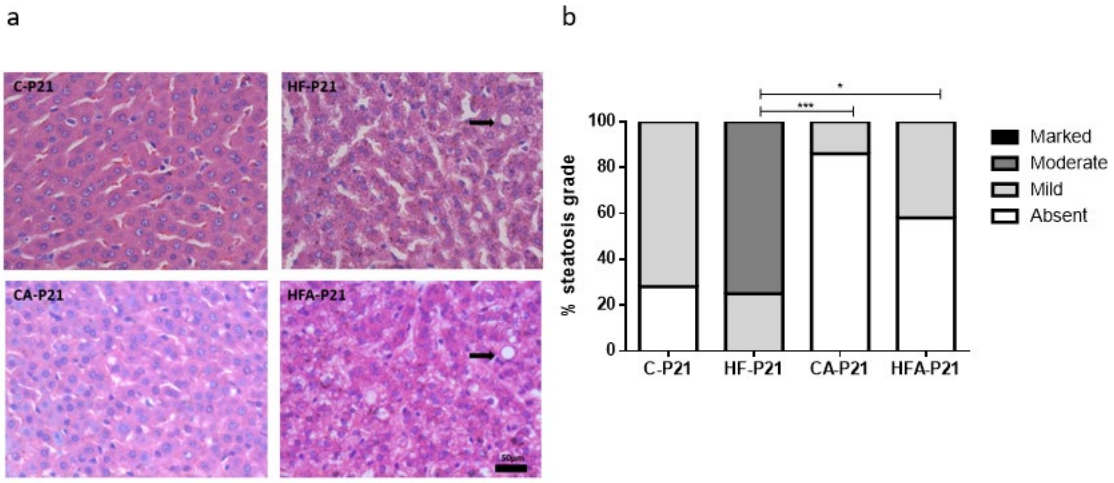

a
b
C-P21
HF-P21
CA-P21
HFA-P21
50µm
% steatosis grade
100
80
60
40
20
0
*
***
C-P21
HF-P21
CA-P21
HFA-P21
Marked
Moderate
Mild
Absent

Figure 5

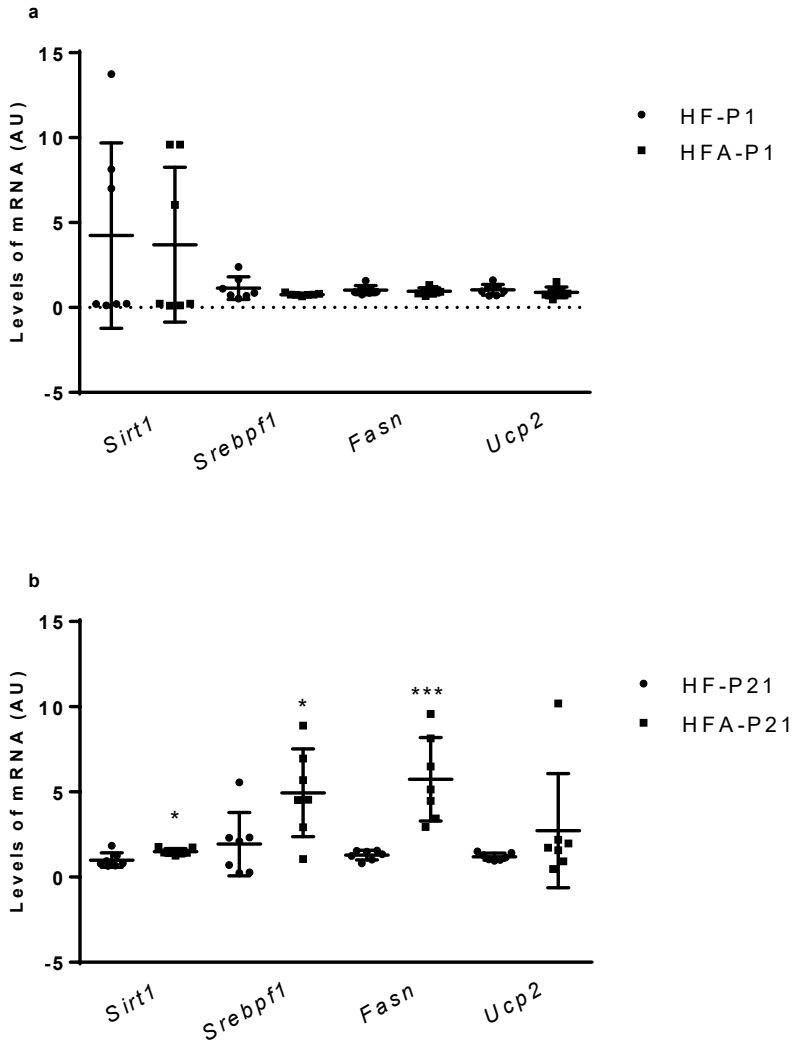
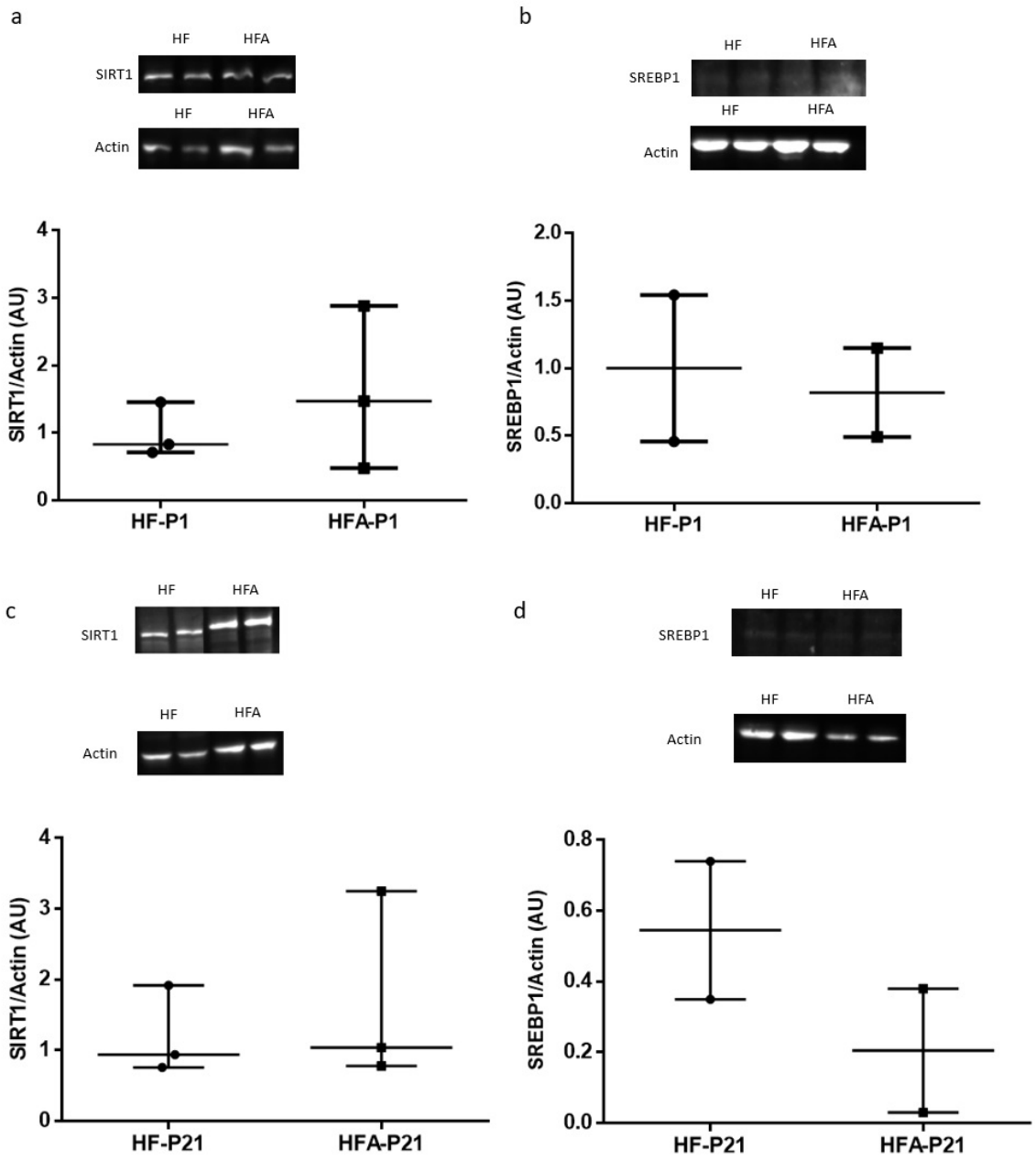

a
Levels of mRNA (AU)
15
10
5
0
-5
Sirt1
Srebpf1
Fasn
Ucp2
HF-P1
HFA-P1
b
Levels of mRNA (AU)
15
10
5
0
-5
*
*
***
Sirt1
Srebpf1
Fasn
Ucp2
HF-P21
HFA-P21

Figure 6


a
HF
HFA
SIRT1
HF
HFA
Actin
SIRT1/Actin (AU)
0
1
2
3
4
HF-P1
HFA-P1
b
HF
HFA
SREBP1
HF
HFA
Actin
SREBP1/Actin (AU)
0.0
0.5
1.0
1.5
2.0
HF-P1
HFA-P1
c
HF
HFA
SIRT1
HF
HFA
Actin
SIRT1/Actin (AU)
0
1
2
3
4
HF-P21
HFA-P21
d
HF
HFA
SREBP1
HF
HFA
Actin
SREBP1/Actin (AU)
0.0
0.2
0.4
0.6
0.8
HF-P21
HFA-P21