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The hypocholesterolemic activity of açaí (*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

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ABSTRACT

Previous studies have demonstrated that the ingestion of açaí pulp can improve serum lipid profile in various animal models; therefore, we hypothesized that açaí pulp (*Euterpe oleracea* Mart.) may modulate the expression of the genes involved in cholesterol homeostasis in the liver and increase fecal excretion, thus reducing serum cholesterol. To test this hypothesis, we analyzed the expression of 7α-hydroxylase and ATP-binding cassette, subfamily G transporters (ABCG5 and ABCG8), which are genes involved with the secretion of cholesterol in the rat. We also evaluated the expression of sterol regulatory element–binding protein 2, 3-hydroxy-3-methylglutaryl CoA reductase, low-density lipoprotein receptor (LDL-R), and apolipoprotein B100, which are involved in cholesterol biosynthesis. Female Fischer rats were divided into 4 groups: the C group, which was fed a standard AIN-93 M diet; the CA group, which was fed a standard diet supplemented with 2% açaí pulp; the H group, which was fed a hypercholesterolemic diet (25% soy oil and 1% cholesterol); and the HA group, which was fed a hypercholesterolemic diet supplemented with 2% açaí pulp. At the end of the experimental period, the rats were euthanized, and their blood and livers were collected. The HA group exhibited a significant decrease in serum total cholesterol, low-density lipoprotein cholesterol, and atherogenic index and also had increased high-density lipoprotein cholesterol and cholesterol excretion in feces compared with the H group. In addition, the expression of the LDL-R, ABCG5, and ABCG8 genes was significantly increased by the presence of açaí pulp. These results suggest that açaí pulp promotes a

Abbreviations: ABCGs, ATP-binding cassette subfamily G transporters; ANOVA, analysis of variance; ApoB100, apolipoprotein B100; C, control group; CA, control supplemented with açaí group; CYP7A1, 7α-hydroxylase; H, hypercholesterolemic group; HA, hypercholesterolemic supplemented with açaí group; HDL, High-density lipoprotein; HMG CoA-R, 3-hydroxy-3-methylglutaryl CoA reductase; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor; OAC, Association of Official Analytical Chemists; PCR, polymerase chain reaction; SREBPs, sterol regulatory element–binding proteins; TC, total cholesterol; VLDL, very low-density lipoprotein.

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hypocholesterolemic effect in a rat model of dietary-induced hypercholesterolemia through an increase in the expression of ATP-binding cassette, subfamily G transporters, and LDL-R genes.

1. Introduction

The açaí is the fruit of the *Euterpe oleracea* Martius tree, a species that is currently among the most economically significant palm species in the Brazilian Amazon region. This fruit has become one of the main products of the Amazon estuary and is exported to other regions of the world [1]. The açaí is a rounded fruit and weighs approximately 2 g. Only 17% (pulp with peel) of the fruit is edible because the seed comprises the remaining inedible portion. The color of the mature fruit is purple to nearly black. Açaí gained popularity in North America after being promoted as a "Superfood for Age-Defying Beauty" [2]. It contains approximately 13% protein, 48% lipids, and 1.5% total sugar. It is rich in phenolic compounds (mainly anthocyanins), monounsaturated and polyunsaturated fatty acids, phytosterols (eg, β-sitosterol), and dietary fiber, in addition to being a good source of potassium, magnesium, calcium, phosphorus, sodium, and vitamins E and B_1 [3-5].

There are studies reporting on the antioxidant and anti-inflammatory activities of açaí because it presents high antioxidant capacity in vitro [6,7], antioxidant potential in vivo [8-11], anti-inflammatory properties [12,13], and proapoptotic and antiproliferative activities against HL-60 leukemia cancer cells [14].

Furthermore, studies have demonstrated that açaí promotes an improvement in the markers of metabolic disease risk. Elevated levels of total and non–high-density lipoprotein (HDL) cholesterol (HDL-C) in the serum and the atherogenic index of rats fed a hypercholesterolemic diet were reduced after diet supplementation with açaí pulp [15]. Supplementation of 2% açaí in food increased the lifespan of sod1 RNAi female flies that were fed a high-fat diet compared with nonsupplemented control flies. Furthermore, açaí administration decreased the transcript level of phosphoenol-pyruvate carboxykinase (Pepck), a key enzyme controlling gluconeogenesis [16]. The long-term administration of açaí seed extract protected C57BL/6J mice fed a high-fat diet that was designed to promote the phenotypic and metabolic characteristics of metabolic syndrome [17]. Açaí juice had atheroprotective effects in hyperlipidemic apolipoprotein E–deficient mice fed a high-fat diet [11] and markedly improved the lipid profile and attenuated atherosclerosis in New Zealand rabbits fed a cholesterol-enriched diet [18]. The cited studies demonstrate that the consumption of açaí improves serum lipid profile and can exert an atheroprotective effect; however, it is not known whether açaí interferes in hepatic cholesterol metabolism.

The liver plays a key role in cholesterol homeostasis because it controls the supply and removal pathways. Cholesterol biosynthesis is partially governed at the transcriptional level by sterol regulatory element–binding protein 2 (SREBP-2) [19]. When cells are deprived of cholesterol, the SREBPs embedded in the membranes of the endoplasmic reticulum are cleaved, enter the nucleus, and bind to the promoters of key genes involved in cholesterol homeostasis. Thus, cleavage activation of SREBP results in increased low-density lipoprotein receptor (LDL-R)–mediated plasma cholesterol uptake and increased cholesterol biosynthesis, in which 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA-R) is a rate-limiting enzyme. Both the LDL-R and HMG CoA-R genes have a sterol regulatory element in their promoter regions and are commonly regulated by SREBP-2 [20-22]. In contrast, the liver eliminates excess cholesterol from the body either by direct secretion into the bile or after its conversion into bile acids via an enzymatic pathway governed by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1) [23,24]. In addition, the ATP-binding cassette, subfamily G transporters (ABCG), ABCG5 and ABCG8, play a significant role in the direct excretion of cholesterol via the bile [25].

In this study, we hypothesized that because of its phytochemical and nutrient components, açaí pulp may modulate the expression of genes involved in cholesterol homeostasis in the liver and increase fecal excretion, thus leading to a reduction of serum cholesterol. Rats fed a diet rich in lipids were used because they develop hypercholesterolemia and liver lipid accumulation [15,26-28]. The present study was undertaken to characterize the effect of açaí pulp on the expression of the genes involved in cholesterol homeostasis in the liver. Owing to their roles in cholesterol biosynthesis, the expression of SREBP-2, HMG CoA-R, LDL-R, and apolipoprotein B100 (ApoB100) was analyzed. To evaluate the proteins involved in the elimination of excess cholesterol from the body, the expression of CYP7A1, ABCG5, and ABCG8 was also investigated. In addition, we investigated the effect of dietary supplementation with açaí pulp on the fecal excretion of cholesterol in rats.

2. Methods and materials

2.1. *Açaí pulp*

Pasteurized açaí (*E oleracea* Martius) pulp was obtained from Icefruit Comercio de Alimentos Ltd. (Tatuí, São Paulo, Brazil). This pulp contained no preservatives or artificial coloring and was pasteurized, vacuum packed, and stored at –18°C. The moisture content was 90%. Each 100 g of dry weight contained 42 g of total fat, 7.0 g of protein, 1.1 g of sugar, and 43 g of fiber, as determined by the Instituto Adolfo Lutz (2008) [29].

2.2. *Animals*

Nine-week-old female Fischer rats weighing approximately 140 g were obtained from the Experimental Nutrition Laboratory of the Federal University of Ouro Preto. The animals were

individually housed in wire-bottomed metabolic cages and maintained in a room with controlled conditions (24°C, 55% humidity, 12-hour light/dark cycles), and food and water were provided ad libitum. The animal experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (no. 2010/23).

2.3. Diets and experimental design

The rats were randomly divided into 4 experimental groups of 8 animals each, balanced for weight. The first group served as the control (C) and received a standard AIN-93 M diet [30], the second group (H) received a hypercholesterolemic diet (25% soy oil and 1% cholesterol), the third group (CA) received the same standard diet supplemented with 2% açaí (dry wt/wt), and the fourth group (HA) received the same hypercholesterolemic diet supplemented with 2% açaí (dry wt/wt). The diet composition for each group is presented in Table 1. While in the metabolic cages, for 2 weeks before the 6-week experimental period, the C and CA groups received the standard diet and the H and HA groups received the hypercholesterolemic diet [15]. The food consumption of the animals was measured daily and was corrected for spillage. The feces were collected daily, and the body weight of the animals was recorded weekly.

2.4. Sample preparation

After fasting for 12 hours, blood (0.5 mL) was collected from the retro-orbital sinus into a heparinized capillary tube under light anesthesia with isoflurane (Cristália, Itapira, SP, Brazil). This was collected at the beginning of the experiment and at the end of the second week of adaptation to ensure uniformity in the concentration of total cholesterol (TC) among animals. The sampled blood was centrifuged at 1500 × *g* for 15 minutes, and the plasma was collected and stored at –20°C until TC analysis.

At the end of the experimental period, the rats were fasted for 12 hours, anesthetized with isoflurane (Cristália), and euthanized by total blood collection from the brachial plexus.

To determine the serum component levels, blood samples were collected in 5-mL test tubes and centrifuged at 1500 × *g* for 15 minutes. The animal livers were collected, washed in saline, weighed, immersed in liquid nitrogen, and immediately stored at –80°C for subsequent analysis. The feces were removed from the cecum, dried in a ventilated oven at 60°C, ground, weighed, and stored at –80°C for subsequent analysis.

2.5. Cholesterol analysis in serum and feces

Serum TC was measured with an enzymatic colorimetric Lab Test Kit No. 60-2/100 (Labtest Diagnostic, Lagoa Santa, MG, Brazil), with cholesterol standards as appropriate. After the precipitation of LDL and very low-density lipoprotein (VLDL) with phosphotungstic acid/$MgCl_2$, the HDL-C level in the supernatant was evaluated using a Lab Test Kit No. 13 (Labtest Diagnostic, Lagoa Santa, MG, Brazil). The non–HDL-C level was calculated as the difference between the TC and HDL-C levels [31]. Non–HDL-C represents all potentially atherogenic lipoproteins, that is, LDL and VLDL. The atherogenic index was obtained from the non–HDL-C/HDL-C ratio.

The total fecal fat was extracted with a chloroform/methanol mixture (2:1, vol/vol) (Vetec Química Fina Ltd, Duque de Caxias, RJ, Brazil), according to the method of Folch et al [32]. The total lipid fecal matter was obtained by evaporating the solvents in the extract, and then the TC was measured using a commercial Lab Test Kit No. 60-2/100 (Labtest Diagnostic).

2.6. Quantitative real-time reverse transcriptase polymerase chain reaction assay

The total RNA was isolated from the liver tissue of rats using the RNAgents Total RNA Isolation System (Promega Corporation,

Table 1 – Ingredient composition (in grams per kilogram) and energy content of the diets fed to rats

Nutrients	Standard diet	Standard diet plus açaí	Hypercholesterolemic diet	Hypercholesterolemic diet plus açaí
Casein [a]	140.0	140.0	140.0	140.0
Corn starch	722.5	702.5	502.5	482.5
Soybean oil [b]	40.0	40.0	250.0	250.0
Cholesterol [c]	0.0	0.0	10.0	10.0
Choline	2.5	2.5	2.5	2.5
Mineral mixture [d]	35.0	35.0	35.0	35.0
Vitamin mixture [e]	10.0	10.0	10.0	10.0
Cellulose	50.0	50.0	50.0	50.0
Açaí pulp [f]	0.0	20.0	0.0	20.0
Energy content [g] (kcal/kg)	3810	3812	4820	4822

[a] Isofar (Duque de Caxias, Rio de Janeiro, Brazil), containing 85% protein.
[b] Brasil Foods SA (São Paulo, Brazil).
[c] Vetec Química Fina Ltd (Duque de Caxias, Rio de Janeiro, Brazil).
[d] Mineral mixture as recommended by the AIN-93M rodent diet [30].
[e] Vitamin mixture as recommended by the AIN-93M rodent diet [30].
[f] Açaí pulp energy content: 82 kcal/20 g.
[g] Conversion factors: protein, 4 kcal/g; fat, 9 kcal/g; sugars, 4 kcal/g.

Madison, WI, USA), according to the manufacturer's instructions. The concentration and purity of the RNA were estimated spectrophotometrically using the A260/A280 ratio (NanoVue; GE Healthcare, Hertfordshere, UK). Complementary DNA (cDNA) was synthesized from 2 μg of total RNA with random primers using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and following the manufacturer's recommendations. Quantitative real-time polymerase chain reaction (PCR) was performed using a SYBR Green PCR Master Mix reagent (Applied Biosystems) in a final reaction volume of 12 μL. The reaction included 2 μL of cDNA and 0.5 μL of each primer (forward and reverse, 10 μM). The forward and reverse primer sequences for SREBP-2 and CYP7A1 were obtained from published nucleotide sequences [33], as were those for ABCG5 and ABCG8 [34]. The primer sequences for ApoB100 (forward primer 5-AGTAGTGGTGCGTCTTGGATCCA-3′ and reverse primer 5-ACTCTGCAGCAAGCTGTTGAATGT-3′) were derived from the *Rattus norvegicus* genome (National Center for Biotechnology Information GenBank, accession number NM_019287) and were constructed using the Primer-BLAST Program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The forward and reverse primer sequences for LDL-R and HMG CoA-R were obtained from published nucleotide sequences [35], as were those for glyceraldehyde-3-phosphate dehydrogenase [36]. All primers were synthesized by Invitrogen Life Technologies (São Paulo, Brazil). The reactions were performed using an ABI Prism 7000 Sequence Detector (Applied Biosystems) under the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. The specificity of the products obtained was confirmed by analyzing the dissociation curves of the amplified product. As an internal control, the expression of the endogenous glyceraldehyde-3-phosphate dehydrogenase gene was used. The data obtained were analyzed using the comparative cycle threshold method. All analyses were performed in triplicate.

2.7. Statistical analyses

The normality of the data was tested using the Kolmogorov-Smirnov test. Data (Tables 2, 3, and 4) consistent with a normal distribution were subjected to 2-way analysis of variance in which the classification factors were diet (C + CA × H + HA), açaí (CA + HA × C + H), and the interaction between diet and açaí (C × CA × H × HA). The Bonferroni *t* test was used for multiple comparisons among the means. Data that did not fit the normal distribution were analyzed using a Kruskal-Wallis nonparametric test and Dunn posttest. The differences were considered statistically significant when $P < .05$. For the remaining analyses (Fig.), Student unpaired *t* test was used. The results are expressed as means and SDs or as medians and interquartile ranges. The minimum sample size needed to detect a statistically significant difference ($P < .05$) was calculated based on the power of 0.9 (G*Power 3.13, statistical power analyses program; http://www.psycho.uni-deusselforf.de/aap). Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad, San Diego, CA, USA).

3. Results

3.1. Body weight gain, liver weight, fecal excretion, and food intake

We first examined how the addition of açaí pulp in the diet affected body weight gain, liver weight, fecal excretion, and food intake. The data in Table 2 indicate that hypercholesterolemic rats exhibited an increase in weight gain and liver weight. The addition of 2% açaí pulp to the diets did not affect these parameters. The rats of the H group ingested less food and excreted a lower amount of feces compared with the controls. The addition of 2% açaí pulp did not affect the food intake of the animals fed the standard diet, but it significantly increased the intake of the animals fed the hypercholesterolemic diet. The açaí supplementation significantly increased the fecal excretion of the rats in both the control and the hypercholesterolemic groups (Table 2).

3.2. Cholesterol analysis in serum

To assess the effectiveness of the diet for promoting hypercholesterolemia and to determine the effects of the treatment with açaí, we measured the serum TC of the animals at the beginning of the experiment, after 14 days of adaptation to the control and hypercholesterolemic diets, and at the end of the experimental period (56 days). No difference in the TC levels was found at the beginning of the experiment

Table 2 – Initial body weight, body weight gain, liver weight, fecal excretion, and food intake of rats fed the experimental diets

Measurements	Experimental groups				ANOVA (P value)		
	C	CA	H	HA	Diet	Açaí	Açaí × Diet
Initial body weight initial (g) [a]	139.4 ± 11.9	138.6 ± 11.3	139.3 ± 11.1	138.9 ± 13.7	.9884	.8846	.9653
Body weight gain (g) [a]	55.66 ± 7.12	55.85 ± 7.36	64.81 ± 12.8	66.94 ± 6.59	.0031	.7141	.7588
Liver weight (g) [a]	5.82 ± 0.50	5.60 ± 0.43	8.87 ± 0.62	9.31 ± 0.96	<.0001	.6537	.1685
Fecal excretion (g/d) [a b]	0.58 ± 0.07	0.83 ± 0.11	0.55 ± 0.07	0.69 ± 0.10	.016	<.0001	.1051
Food intake (g/d) [b c]	14.29/13.39-14.61^{A}	13.92/13.57-13.97^{A}	10.62/10.28-10.72^{B}	13.11/12.79-14.13^{A}	–	–	–

[a] Values are expressed as means ± SD (n = 8 per group). The data were tested using 2-way ANOVA ($P < .05$).
[b] Daily per animal (in grams per day).
[c] Data submitted to the nonparametric Kruskal-Wallis test and Dunn posttest; significantly different values are marked with different superscript letters (A-B) ($P < .05$). Values are expressed as the median/interquartile ranges (n = 8 per group).

Table 3 – Initial TC, TC with 14 days of adaptation, and final TC of rats fed the experimental diets

Measurements	Experimental groups				ANOVA (value of P)		
	C	CA	H	HA	Diet	Açaí	Açaí × Diet
Initial TC (mmol/L) [a]	1.68 ± 0.26	1.81 ± 0.25	1.78 ± 0.18	1.74 ± 0.27	.8644	.6091	.3165
TC 14 days (mmol/L) [a]	2.32 ± 0.35	2.48 ± 0.17	3.79 ± 1.66	3.58 ± 2.04	.0107	.9583	.7025
Final TC (mmol/L) [a]	2.20 ± 0.16^{C}	2.24 ± 0.16^{C}	4.24 ± 1.08^{A}	3.25 ± 0.69^{B}	<.0001	.0497	.0353

[a] Values are expressed as means ± SD (n = 8 per group). The data were tested using 2-way ANOVA. The Bonferroni *t* test was used for multiple comparisons among the means. Within a row, significantly different values are marked with different superscript letters (A-C) when a significant interaction was observed ($P < .05$).

(Table 3). At the end of week 2, the TC levels in the hypercholesterolemic rats (H and HA) were 1.6-fold higher than those of the control animals (C and CA). At the end of the experiment, the rats of the H group maintained higher levels of TC than the control rats, and the addition of 2% açaí pulp to the hypercholesterolemic diet (HA group) significantly reduced their TC values (Table 3).

3.3. *Serum lipids, atherogenic index, and cholesterol in the feces*

As expected, the animals fed the hypercholesterolemic diet exhibited increased levels of non–HDL-C, increased fecal cholesterol excretion, decreased levels of HDL-C, and a higher atherogenic index relative to the control group (Table 4). The açaí supplementation significantly increased HDL-C levels and reduced the levels of non–HDL-C in the CA and HA group rats. The addition of 2% açaí pulp to the hypercholesterolemic diet (HA group) promoted a 31% reduction in the atherogenic index and a 44% increase in the fecal cholesterol excretion in comparison with the H group (Table 4).

3.4. *Cholesterol metabolism gene messenger RNA expression*

To investigate the molecular mechanisms involved in the hypocholesterolemic effect of açaí pulp, the expression of the genes involved in cholesterol homeostasis was evaluated by quantitative transcriptase PCR, including SREBP-2, HMG CoA-R, LDL-R, ApoB100, ABCG5, ABCG8, and CYP7A1. As shown in Fig., the H group presented a reduction in the expression of theSREBP-2, HMG CoA-R, and LDL-R genes, relative to the controls. These genes are involved in hepatic cholesterol biosynthesis. The HA group exhibited 1.3- and 2.2-fold increases in the expression of LDL-R and SREBP-2, respectively, relative to the H group. The expression of HMG CoA-R was unaffected by açaí supplementation.

The H group presented an increase in ApoB100 and a decrease in CYP7A1 expression compared with the C group. The messenger RNA (mRNA) levels for CYP7A1 in the hypercholesterolemic rats that received the açaí supplementation (HA group) did not differ from those in the H group, but the ApoB100 expression was significantly lower in these animals (HA group) than in the H group. The addition of 2% açaí pulp to the hypercholesterolemic diet (HA group) increased the mRNA levels of ABCG5 and ABCG8 compared with the mRNA levels of the genes in the H group (Fig.).

4. Discussion

Owing to the public interest in the prevention and/or treatment of cardiovascular disease and other metabolic diseases, extensive studies have been conducted to identify natural products that can regulate dyslipidemia. Increasing evidence suggests that the various components of açaí contribute to cardioprotection via mechanisms that affect cell membrane receptors, intracellular signaling pathway proteins, and the modulation of gene expression [37-41]. It has been demonstrated that flavonoids regulate the activity of the nuclear receptor regulators of cellular lipid metabolism [42,43].

The present study was designed to investigate the hypocholesterolemic activity of açaí pulp using a rat model of dietary-induced hypercholesterolemia. A 2% açaí pulp dose was chosen because of its relevance to human nutrition. This dosage mimics the addition of a portion of this fruit in food

Table 4 – Serum lipids, atherogenic index, and cholesterol in the feces of rats fed the experimental diets

Measurements	Experimental groups				ANOVA (value of P)		
	C	CA	H	HA	Diet	Açaí	Açaí × Diet
HDL-C (mmol/L) [a]	1.49 ± 0.14	1.66 ± 0.18	0.13 ± 0.01	0.15 ± 0.02	<.0001	.0259	.0638
Non–HDL-C (mmol/L) [a]	0.71 ± 0.20	0.57 ± 0.28	4.11 ± 1.08	3.10 ± 0.71	<.0001	.0232	.0802
Atherogenic index [a b]	0.48 ± 0.16^{C}	0.36 ± 0.21^{c}	31.19 ± 9.13^{A}	21.56 ± 8.26^{B}	<.0001	.0332	.0377
TC feces (mg cholesterol/g feces) [a]	1.61 ± 0.67^{C}	1.10 ± 0.50^{C}	9.14 ± 2.09^{B}	16.16 ± 6.6^{A}	<.0001	.0138	.0051

[a] Values are expressed as means ± SD (n = 8 per group). The data were tested using 2-way ANOVA. The Bonferroni *t* test was used for multiple comparisons among the means. Within a row, significantly different values are marked with different superscript letters (A-C) when a significant interaction was observed ($P < .05$).

[b] Atherogenic index = (TC – HDL-C) × $(HDL\text{-}C)^{-1}$.

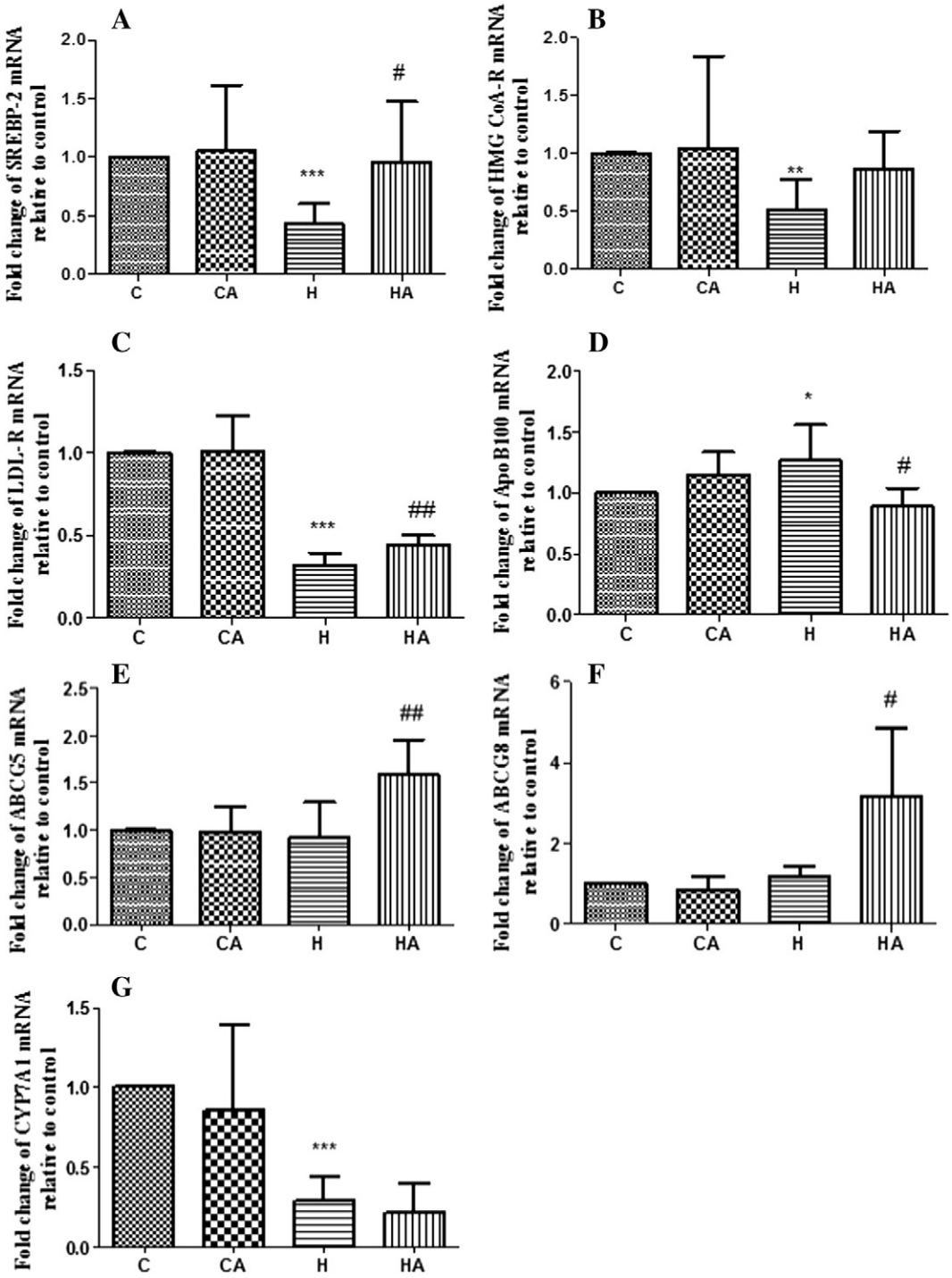

Fig. – Effect of açaí supplementation on the mRNA expression of genes involved in cholesterol metabolism in the rat. Rats were fed the following diets: C, CA, H, and HA groups. SREBP-2 (A), HMG CoA-R (B), LDL-R (C), ApoB100 (D), ABCG5 (E), ABCG8 (F), and CYP7A1 (G). Values are expressed as means ± SD (n = 6 per group). The data were tested using Student unpaired *t* test. $^{*}P < .05$ with respect to the C group; $^{**}P < .01$ with respect to the C group; $^{***}P < .001$ with respect to the C group; $^{\#}P < .05$ with respect to the H group; $^{\#\#}P < .01$ with respect to the H group; $^{\#\#\#}P < .001$ with respect to the H group.

[44] and has demonstrated effects in previous studies [10,15,16]. Corroborating our previous results [15], açaí supplementation improved the lipid profile in the rat. Thus, we focused on characterizing the effects that açaí pulp supplementation in the diet would have on the transcription of the genes involved in cholesterol metabolism and fecal cholesterol excretion.

The liver plays a key role in cholesterol homeostasis, and the conversion of cholesterol to bile acids is a major pathway of cholesterol catabolism. The present study demonstrated

that a hypercholesterolemic diet promoted a reduction in the expression of CYP7A1. These results are in agreement with other studies [45,46]; however, açaí supplementation had no effect on CYP7A1. The activity of CYP7A1 can have a major impact on the overall catabolism of excess cholesterol, but other metabolic processes favor cholesterol elimination from the body, such as cholesterol secretion into the bile via the ABCG5 and ABCG8 transporters [47,48]. The addition of açaí pulp to the hypercholesterolemic diet up-regulated the expression of the ABCG5/G8 transporters. These transporters are expressed, almost exclusively, in the liver and intestine and mandatorily form a functional heterodimer that acts as a transporter for cholesterol efflux [49]. ATP-binding cassette, subfamily G transporters 5 and 8 transgenic mice that were overexpressing the transgene in the liver and the intestine were crossed into the atherosclerotic LDL-$R^{-/-}$ genetic background, and these mice developed significantly less atherosclerosis than the wild-type controls [50]. Yu et al [25] demonstrated that increased expression of ABCG5 and ABCG8 in the liver and small intestine in mice causes profound alterations in cholesterol trafficking, which is characterized by an increase in the biliary cholesterol secretion and a reduction in cholesterol absorption. Diet supplementation with açaí pulp increased the mRNA levels of the ABCG5/G8 transporters in hypercholesterolemic rats. Up-regulation of the transporters is the likely mechanism underlying the decreased concentration of serum cholesterol and increased fecal cholesterol excretion.

The hepatic cholesterol metabolism associated with the concentrations of plasma and intracellular cholesterol is mainly regulated by SREBP-2, a nuclear transcription factor that binds to the membranes of the endoplasmic reticulum. Sterol regulatory element–binding protein-2 is regulated both at the transcriptional level by sterol depletion and at the posttranslational level by a proteolytic cleavage cascade [19]. The hypercholesterolemic rats exhibited a lower expression of SREBP-2, suggesting that a hypercholesterolemic diet would lead to a saturated cholesterol state in hepatocytes and resulting in a down-regulation of the de novo synthesis of cholesterol with a decline in SREBP-2 expression. In addition, the açaí pulp decreased the cholesterol concentration, which, in turn, up-regulated the expression of SREBP-2.

In cells deprived of cholesterol, SREBP-2 binds and activates the promoters of LDL-R and HMG CoA-R genes. Increased hepatic LDL-R expression results in an improved clearance of plasma LDL-C, which has been strongly associated with a decreased risk of the development of cardiovascular disease in humans [51]. Because the LDL-R is also regulated by the intracellular concentrations of cholesterol, the hypercholesterolemic diet and the açaí pulp affected the expression of this receptor in response to SREBP-2 similarly, suggesting a possible mechanism of action of açaí in the reduction of serum non–HDL-C and, therefore, of TC. Similar to the regulation of LDL-R, cholesterol concentrations modulate the expression and activity of HMG CoA-R. The results of other studies indicate that expression of the HMG CoA-R gene in the liver of rats on a high lipid diet is slightly down-regulated compared with that of the control rats, which is similar to the results found in this study [20,52,53].

Apolipoprotein B100 is associated with hepatic-derived non–HDL-C and is incorporated into the nascent lipoprotein particles, along with cholesterol and triglycerides [54]. Owing to the positive effects of açaí in reducing the levels of non–HDL-C and the fact that polyphenols affect apolipoprotein B secretion rates [55,56], we decided to evaluate the gene expression of this apolipoprotein. Açaí supplementation decreases the mRNA levels of ApoB100, suggesting that the reduction in the overall secretion of the VLDL is caused by modifications in the packaging of this lipoprotein.

In conclusion, the present study is the first to study the effect of açaí on cholesterol balance. Our results provide insight into the molecular mechanisms involved in the cholesterol-lowering properties of açaí. However, our study is limited in that only the gene profile was analyzed; thus, it is important to confirm if alterations of genes expression are reflected by protein levels. Based on these results, we accept our hypothesis that açaí pulp exerts a hypocholesterolemic effect by inducing differential gene expression in the rat. The hypocholesterolemic effect observed in the rats fed açaí pulp can primarily be attributed to the enhanced expression of the ABCG5 and ABCG8 transporters. These alterations directly increased the rate of biliary sterol excretion and increased uptake of LDL cholesterol by the liver via the up-regulation of LDL-R.

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