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Basic nutritional investigation

Diet supplementation with acai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats

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ABSTRACT

Objective: We investigated the antioxidant potential and hypocholesterolemic effects of acai (*Euterpe oleracea* Mart.) pulp ingestion in rats fed a standard or hypercholesterolemic diet.
Methods: Female Fischer rats were fed a standard AIN-93 M diet (control) or a hypercholesterolemic diet that contained 25% soy oil and 1% cholesterol. The test diet was supplemented with 2% acai pulp (dry wt/wt) for control (group CA) and hypercholesterolemic rats (group HA) for 6 wk. At the end of the experimental period, rats were sacrificed and the blood and livers were collected. To evaluate the effect of acai consumption, levels of protein carbonyl and sulfhydryl groups, superoxide dismutase and paraoxonase activities, and lipid profiles of the sera were measured.
Results: Animals that were fed the hypercholesterolemic diet presented increased levels of total and non–high-density lipoprotein cholesterol and decreased levels of high-density lipoprotein cholesterol. Supplementing the diet of this group with acai caused a hypocholesterolemic effect by reducing total and non–high-density lipoprotein cholesterol. Serum levels of carbonyl proteins and total, free, and protein sulfhydryl groups were reduced by acai ingestion in animals receiving the standard or hypercholesterolemic diet. Acai supplementation induced a significant reduction in superoxide dismutase activity only in the hypercholesterolemic rats, indicating an association between diet and acai treatment. Also, acai supplementation increased paraoxonase activity in the CA and HA groups.
Conclusion: These results suggest that the consumption of acai improves antioxidant status and has a hypocholesterolemic effect in an animal model of dietary-induced hypercholesterolemia.

Introduction

The distribution of cholesterol among the various fractions of plasma lipoproteins is an important determinant in atherogenesis and is used as a biological marker for the risk of atherosclerosis. Cholesterol in low-density lipoprotein (LDL) is considered to be atherogenic, whereas cholesterol in high-density lipoprotein (HDL) is associated with a protective mechanism against the onset of atherosclerotic lesions [1]. LDLs damaged by reactive oxygen species are taken up by macrophages, which accumulate in the endothelial wall as lipid-laden foam cells during the initial phases of atherosclerotic fatty streak lesions [2]. Therefore, a reduction of total and LDL cholesterol is a primary step in the prevention of vascular disease. Also, preventing oxidative stress could delay the development of atherosclerosis. There is evidence that high antioxidant levels in the plasma are associated with a lower frequency of coronary disease [3], and that decreased antioxidant levels in the plasma can promote the formation of free radicals and consequently LDL peroxidation [4,5].

Current research has focused on identifying ways to prevent atherosclerosis and other diseases through changes in diet. Diets with high fruit and vegetable content can inhibit the development of cardiovascular diseases. The beneficial effects of these foods have been related to the presence of polyphenols and other nutrients, such as unsaturated fatty acids, fiber, and phytosterols. Besides their antioxidant effects, these foods have the ability to change cholesterol levels, especially cholesterol in LDL, in the circulatory system [6–8].

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Acai (*Euterpe oleracea* Mart.) is currently among the most economically significant palm species in the Brazilian Amazon [9] and has become one of the main products of the Amazon estuary exported to other regions of the world. International growth of the acai trade has been attributed to the acai beverage industry and related products [10], where much attention has been given to its antioxidant capacity and associated potential health benefits [11].

Studies have shown that the acai fruit presents high antioxidant capacity in vitro, especially for superoxide and peroxyl scavenging [11,12]. Recently, it has been shown that the consumption of acai juice or pulp by healthy human volunteers caused a significant increase in their plasma antioxidant capacity, which also indicates that acai fruit has antioxidant potential in vivo [13]. In addition, freeze-dried acai presents anti-inflammatory properties, as demonstrated by its ability to inhibit the activities of cyclo-oxygenase-1 and cyclo-oxygenase-2 in cell culture [12]. Acai extracts induced endothelium-dependent vasodilatation in the rat mesenteric vascular bed [14], presented proapoptotic and antiproliferative activities against HL-60 leukemia cancer cells [15], and inhibited nitric oxide production by the activated macrophage cell line RAW 264.7 due to the reduction of inducible nitric oxide synthase expression [16]. Most of these effects are attributed to the polyphenolic fraction of acai, which is rich in the anthocyanins cyanidin 3-glucoside and cyanidin 3-rutinoside, which can be present as monomeric (catechin and epicatechin) or oligomeric procyanidins [17,18].

In addition to flavonoids, the phytochemical and nutrient component analysis showed that acai pulp has a low sugar content and is not considered a good source of carbohydrates, but is rich in lipids, with high levels of unsaturated fatty acids (oleic and linoleic acids), phytosterols (β-sitosterol) and dietary fiber [18,19]. All these compounds could improve the lipid profile and therefore may have beneficial effects on cardiovascular diseases. There are very few scientific studies evaluating the effects of acai in vivo and therefore more are necessary, because the presence of a compound in food does not ensure its effects.

In the present study, we investigated some functional properties in vivo, especially the antioxidant and hypocholesterolemic effects in control and hypercholesterolemic rats fed acai pulp. These conditions were chosen because they are representative of a normal and a potentially stressing condition such as hypercholesterolemia, in which oxidative stress has been observed [20]. The levels of carbonyl protein and sulfhydryl radicals were determined as biomarkers of protein oxidation. Antioxidative systems were evaluated by the activity of serum superoxide dismutase (SOD). SOD converts superoxide radicals into H_2O_2, and, like catalase and glutathione peroxidase, it is not normally expressed at its maximum capacity but is highly inducible in response to environmental stress by the keap1-Nrf2-ARE pathway (Kelch-like ECH associating protein1-Nuclear-factor erithroid 2-related factor 2-Antioxidant Regulatory Element) [21]. Serum paraoxonase activity was investigated because it is an HDL-associated antioxidant enzyme that prevents and/or inhibits lipoprotein oxidation. It has been reported that paraoxonase (PON) activity is decreased in situations associated with oxidative stress [20]. The results of this study may have a great impact toward a better understanding of the bioactivities of acai in vivo.

Materials and methods

Acai pulp and other reagents

Pasteurized acai (*Euterpe oleracea* Mart.) pulp was obtained from Icefruit Comercio de Alimentos Ltda. (Tatuí, São Paulo, Brazil). The acai pulp was composed of 90% water; and each 100 g of dry weight had 42 g of total fat, 7.0 g of protein, 1.1 g of sugar, and 43 g of fiber, as determined according to the Association of Official Analytical Chemists [22]. The chemical reagents, including 5,5-dithiobis-(2-nitrobenzoic) acid, cysteine, phenylacetate, and paraoxon were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Nine-week-old female Fischer rats weighing approximately 145 g were obtained from our animal facilities (School of Nutrition, Federal University of Ouro Preto, MG, Brazil). Animals were individually housed in wire-bottomed metabolic cages and kept in a room with controlled conditions (24°C, 55% humidity, 12-h light/dark cycles) with food and water provided ad libitum. All experiments were carried out in full accordance with the principles defined by the Colégio Brasileiro de Experimentação Animal [23] and the animal procedures were approved by the ethical committee on research of the Federal University of Ouro Preto.

Diets and experimental design

The rats were divided into four groups, balanced for weight, of eight animals each. The first group served as the control (C) and received a standard AIN-93 diet [24]. The second group (H) received a hypercholesterolemic diet (25% soy oil and 1% cholesterol). The third (CA) and fourth (HA) groups received the same standard and hypercholesterolemic diets, respectively, but supplemented with 2% acai (dry wt/wt). After the pulps were added into the food, they were dried for 3 h at −20°C. The diet composition for each group is presented in Table 1.

Animals received the standard and hypercholesterolemic diets in metabolic cages for 2 wk before the 6-wk experimental period [25]. During the experiment, body weight, food intake, and feces were monitored.

Sample preparation

At the end of the experimental period, fasting rats were anesthetized and sacrificed by total blood collection from the brachial plexus. To determine the levels of serum components, blood samples were collected in 5-mL test tubes and centrifuged. Animal livers were collected and weighed.

Liver function analysis

Serum activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using Labtest (Lagoa Santa, MG, Brazil) kits 79, 53, and 52, respectively.

Protein oxidation analysis

The carbonyl content in serum was determined using the Protein Carbonyl Assay Kit from Cayman Chemical (Ann Arbor, MI, USA).

Table 1
Composition of experimental diets

	C (g/kg)	CA (g/kg)	H (g/kg)	HA (g/kg)
Casein	140.0	140.0	140.0	140.0
Corn starch	722.5	702.5	502.5	482.5
Soybean oil	40.0	40.0	250.0	250.0
Cholesterol	0.0	0.0	10.0	10.0
Choline	2.5	2.5	2.5	2.5
Mineral mixture*	35.0	35.0	35.0	35.0
Vitamins mixture†	10.0	10.0	10.0	10.0
Cellulose	50.0	50.0	50.0	50.0
Acai pulp	0.0	20.0	0.0	20.0
Energy content (kcal/kg)‡	3810	3812	4820	4822

C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai

* Salt mixture (g/kg of mixture): NaCl, 139.3; KI, 0.79; $MgSO_4 \cdot 7H_2O$, 57.3; $CaCO_3$, 381.4; $MnSO_4 \cdot H_2O$, 4.01; $FeSO_4 \cdot 7H_2O$, 27.0; $ZnSO_4 \cdot 7H_2O$, 0.548; $CuSO_4 \cdot 5H_2O$, 0.477; $CoCl_2 \cdot 6H_2O$, 0.023; KH_2PO_4, 389.0 [22].

† Vitamin mixture (IU or g/kg of mixture): retinol acetate, 2 000 000 IU; cholecalciferol, 200 000 IU; *p*-aminobenzoic acid, 10.00; inositol, 10.00; niacin, 4.00; calcium pantothenate, 4.00; riboflavin, 0.80; thiamin HCl, 0.50; pyridoxine HCl, 0.50; folic acid, 0.20; biotin, 0.04; vitamin B12, 0.003; sucrose, quantity sufficient to 1 kg; choline, 200.0; α-tocopherol, 10 000 IU [22].

‡ Acai pulp energy content: 82 kcal/20 g, using conversion factors: protein 4 kcal/g, fat 9 kcal/g, sugars 4 kcal/g.

Table 2
Food intake, weight gain, and fecal excretion of rats fed a standard diet, a standard diet supplemented with acai, a hypercholesterolemic diet, or a hypercholesterolemic diet supplemented with acai

Variables	Experimental groups				ANOVA (*P*)		
	C	CA	H	HA	Diet	Acai	Acai × diet
Food intake (g/42 d)	710.53 ± 6.56[a,b]	754.32 ± 56.54[a]	676.86 ± 42.77[b]	596.27 ± 73.06[c]	0.0001	0.3610	0.0039
Body weight gain (g/42 d)	26.63 ± 10.88[c]	49.88 ± 5.87[a]	34.38 ± 10.42[b,c]	39.00 ± 7.35[a,b]	0.6220	0.0001	0.0061
Fecal excretion (g/42 d)	10.13 ± 2.01	15.25 ± 1.45	11.83 ± 0.67	14.73 ± 2.46	0.3550	0.0001	0.0877
Food efficiency*	0.04 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.1350	0.0001	0.0784

ANOVA, analysis of variance; C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai
Values are means ± SDs ($n = 8$). Data were tested by two-way ANOVA; when interactions were significant ($P < 0.05$), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($P < 0.05$).
* Food efficiency = (body weight gain) × (food intake)$^{-1}$.

Total and free serum sulfhydryl groups were estimated using Ellman's reagent according to Sedlak and Lindsay [26]. Protein-bound sulfhydryl groups were determined as the difference between the total and free sulfhydryl groups.

Antioxidant enzymes activities

The SOD activities in serum were assayed using Fluka kit 19160 (Steinheim, Germany). This assay uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazoliumchloride to form a red formazan dye that is assayed spectrophotometrically at 505 nm. Inhibition of the production of the chromogen is proportional to the SOD activity present in the sample.

The PON activity in serum (arylesterase activity) was determined according to Beltowski et al. [27]. The enzymatic activity was calculated assuming the molar extinction coefficient of phenylacetate to be 1310 L · mol^{-1} · cm^{-1}. The results were expressed in units per milliliter, where 1 U of arylesterase hydrolyzes 1 μmol of phenylacetate per minute. The enzymatic activity of serum to make paraoxon (PON activity) was calculated assuming the molar extinction coefficient of paraoxon to be 18 290 L · mol^{-1} · cm^{-1}. The results were expressed in units pr milliliter, where 1 U of enzyme hydrolyzes 1 μmol of paraoxon per minute [27].

Triacylglycerol and cholesterol analysis

Serum triacylglycerols and cholesterol were measured enzymatically using Labtest kits 59-4/50 and 60-2/100, respectively. After the precipitation of LDL and VLDL with phosphotungstic acid/$MgCl_2$, HDL cholesterol in the supernatant was determined using Labtest kit 13. Non-HDL cholesterol was calculated as the difference between total and HDL cholesterol. The atherogenic indexes were obtained by the relation between non-HDL cholesterol and HDL cholesterol.

Statistical analysis

Data were expressed as the mean ± standard deviation. All data were analyzed using the Kolmogorov-Smirnov normality test. Data with a normal distribution were analyzed by two-way analysis of variance, and the classification factors were diet and acai and the interactions between diet and acai. Tukey's post hoc test was used to determine the differences among the four groups when a statistical diet-by-acai interaction was observed. Data in which the distributions were not considered normal were subjected to the non-parametric Kruskal-Wallis test. Tests were performed with GraphPad Prism 4.00 for Windows (San Diego, CA, USA). Differences were considered statistically significant at $P \leq 0.05$.

Results

Food intake, weight gain and fecal excretion

We first examined how the addition of acai pulp in the diet affected food intake, weight gain, and fecal excretion. Table 2 shows that the addition of 2% acai pulp did not affect the food intake of animals that were fed the standard diet but significantly lowered the food intake of animals that were fed the hypercholesterolemic diet. Acai supplementation significantly increased the weight gain and fecal production of rats from the control and hypercholesterolemic groups (Table 2). These data show that acai improved the food efficiency index by increasing weight gain without augmenting food intake, as observed in the hypercholesterolemic animals.

Biochemical indicators of liver function

To evaluate whether the hypercholesterolemic diet caused liver damage, liver weight and serum ALT, AST, and ALP activities were measured. The liver weight of the hypercholesterolemic group was greater than the control group (Table 3). The hypercholesterolemic diet also caused an increase in the activities of ALT, AST, and ALP (Table 3). Although ALP activity was significantly lower in the control and hypercholesterolemic rats fed acai pulp, the addition of acai to the diet of hypercholesterolemic rats did not improve their liver function, because the activities of the aminotransferases were not improved compared with rats fed the non-supplemented hypercholesterolemic diet (Table 3).

Table 3
Liver weight and serum AST, ALT, and ALP activities of rat groups fed a standard diet, a standard diet supplemented with acai, a hypercholesterolemic diet, or a hypercholesterolemic diet supplemented with acai

Variables	Experimental groups				ANOVA (*P*)		
	C	CA	H	HA	Diet	Acai	Acai × diet
Liver weight (g)	5.63 ± 0.72	5.30 ± 0.47	9.55 ± 1.26	9.20 ± 1.26	0.0001	0.3440	0.9720
AST (UI)	39.98 ± 4.37	39.80 ± 4.15	59.66 ± 3.98	54.63 ± 4.55	0.0001	0.0957	0.1190
ALT (UI)	29.07 ± 3.54[a]	23.95 ± 0.77[b]	31.36 ± 3.39[a]	33.26 ± 4.80[a]	0.0001	0.1970	0.0076
ALP (U/L)*	35.93 ± 3.39[b]	26.34 ± 6.67[c]	58.00 ± 6.41[a]	50.54 ± 4.90[a]	—	—	—

ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai
Values are means ± SDs ($n = 8$). Data were tested by two-way ANOVA; when interactions were significant ($P < 0.05$), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($P < 0.05$).
* Data subjected to the non-parametric Kruskal-Wallis test; different values are marked with different superscript letters ($P < 0.05$).

Table 4
Levels of carbonyl protein and sulfhydryl groups in serum of rats from groups fed a standard diet, a standard diet supplemented with acai, a hypercholesterolemic diet, or a hypercholesterolemic diet supplemented with acai

Variables	Experimental groups				ANOVA (*P*)		
	C	CA	H	HA	Diet	Acai	Acai × diet
Carbonyl protein (nmol/mg protein)	0.56 ± 0.38	0.21 ± 0.17	0.47 ± 0.23	0.33 ± 0.11	0.8840	0.0200	0.3470
Total SH (μmol/L)	309.55 ± 35.95^{c}	407.64 ± 42.17^{b}	295.96 ± 28.59^{c}	478.41 ± 43.60^{a}	0.0001	0.0420	0.0040
Free SH (μmol/L)	64.89 ± 5.91	113.47 ± 2.83	72.95 ± 7.57	125.90 ± 5.26	0.0001	0.0001	0.2840
Protein SH (μmol/L)	244.66 ± 36.51^{b,c}	294.17 ± 40.57^{b}	223.00 ± 30.54^{c}	352.51 ± 44.62^{a}	0.1880	0.0001	0.0064

ANOVA, analysis of variance; C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai; SH, sulfhydryl
Values are means ± SDs ($n = 8$). Data were tested by two-way ANOVA; when interactions were significant ($P < 0.05$), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($P < 0.05$).

Levels of protein carbonyl and sulfhydryl groups

As a biomarker of oxidative stress, serum carbonyl and sulfhydryl groups were measured (Table 4). The addition of acai pulp to the standard and hypercholesterolemic diets significantly decreased the levels of carbonyl proteins. Animals from the CA and HA groups also showed significantly increased levels of total, free, and protein sulfhydryl groups (Table 4).

Activities of antioxidant enzymes

The antioxidant status in serum was also investigated by measuring the SOD, PON-arylesterase, and PON-paraoxonase activities (Table 5). The hypercholesterolemic diet increased SOD activity and decreased the PON-arylesterase and PON-paraoxonase activities (Table 5). The addition of 2% acai pulp increased the activities of PON-arylesterase and PON-paraoxonase in the control group but only increased the activity of PON-arylesterase in the hypercholesterolemic group. Acai supplementation also reduced SOD activity in the hypercholesterolemic group (Table 5).

Serum lipids and atherogenic index

The ability of 2% acai pulp supplementation to modify the serum lipid profile of control and hypercholesterolemic rats was evaluated. As expected, animals fed the hypercholesterolemic diet showed increased levels of total and non-HDL cholesterol, decreased levels of HDL cholesterol, and had a high atherogenic index compared with the control group (Table 6). The elevated levels of total and non-HDL cholesterol in the serum and the atherogenic index of hypercholesterolemic rats were lowered by administering acai pulp (Table 6). Surprisingly, there was a significant decrease in serum triacylglycerol levels of hypercholesterolemic rats compared with control rats (Table 6).

Discussion

Hyperlipidemia and high cholesterol diets are major factors in the development of cardiovascular disease. The search for new compounds and foods that could reduce or regulate cholesterol levels and exert antioxidant action is important for the prevention of vascular diseases. In the present study, we investigated the effects of acai pulp on antioxidant and serum lipid profiles of control and hypercholesterolemic rats.

As expected, our results indicated that the hypercholesterolemic diet caused liver damage and increased oxidative stress and cholesterol levels in rats. Liver injury was characterized by hepatomegaly and increased activities of AST, ALT, and ALP enzymes. The hypercholesterolemic rats also presented a significant increase in SOD activity, which suggests a possible increase in the production of reactive oxygen species, because it has been demonstrated that various antioxidant proteins (including SOD) are induced by oxidative stress [21]. In contrast, the decreased activities of PON-arylesterase and PON-paraoxonase indicated that the present hypercholesterolemic diet impaired the antioxidant capacity, as was observed by others [28]. Moreover, rats fed the hypercholesterolemic diet presented increased levels of total cholesterol and non-HDL cholesterol with reduced HDL cholesterol, providing an appropriate model for dietary hypercholesterolemia. However, rats fed the hypercholesterolemic diet showed a significant reduction in their levels of triacylglycerols. A similar decrease in serum triacylglycerols in animals fed high cholesterol and unsaturated oil was observed by Turbino-Ribeiro et al. [25]. These results could be explained by the high amount of unsaturated oil used in these diets. It is well known that unsaturated fatty acids inhibit important enzymes

Table 5
Serum activity of antioxidant enzymes of rats groups fed a standard diet, a standard diet supplemented with acai, a hypercholesterolemic diet, or a hypercholesterolemic diet supplemented with acai

Variables	Experimental groups				ANOVA (*P*)		
	C	CA	H	HA	Diet	Acai	Acai × diet
SOD activity (inhibition rate, %)	37.38 ± 2.09^{b}	36.60 ± 1.27^{b}	66.95 ± 19.17^{a}	47.22 ± 7.19^{b}	0.0001	0.0208	0.0124
PON-arylesterase activity (U/mL)	37.10 ± 7.60^{b}	65.17 ± 7.45^{a}	16.70 ± 3.24^{c}	27.39 ± 9.09^{b}	0.0001	0.0001	0.0019
PON-paraoxonase activity (U/mL)	0.20 ± 0.05^{b}	0.35 ± 0.06^{a}	0.07 ± 0.01^{c}	0.10 ± 0.03^{c}	0.0001	0.0001	0.0001

ANOVA, analysis of variance; C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai; PON, paraoxonase; SOD, superoxide dismutase
Values are means ± SDs ($n = 8$). Data were tested by two-way ANOVA; when interactions were significant ($P < 0.05$), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($P < 0.05$).

Table 6
Serum lipids and atherogenic index of rats fed a standard diet, a standard diet supplemented with acai, a hypercholesterolemic diet, or a hypercholesterolemic diet supplemented with acai

Variables	Experimental groups				ANOVA (*P*)		
	C	CA	H	HA	Diet	Acai	Acai × diet
Total cholesterol (mmol/L)	2.16 ± 0.21^{c}	2.06 ± 0.23^{c}	8.12 ± 2.32^{a}	5.42 ± 0.98^{b}	0.0001	0.0041	0.0069
Non-HDL cholesterol (mmol/L)	0.52 ± 0.25^{c}	0.29 ± 0.15^{c}	7.84 ± 2.30^{a}	5.12 ± 0.97^{b}	0.0001	0.0024	0.0091
HDL cholesterol (mmol/L)	1.64 ± 0.33	1.78 ± 0.09	0.28 ± 0.04	0.30 ± 0.07	0.0001	0.2220	0.3320
Triacylglycerol (mmol/L)	0.76 ± 0.09^{a}	0.64 ± 0.03^{b}	0.31 ± 0.04^{c}	0.36 ± 0.06^{c}	0.0001	0.1290	0.0005
Atherogenic index*	0.35 ± 0.22^{c}	0.16 ± 0.08^{c}	28.08 ± 7.15^{a}	18.04 ± 5.69^{b}	0.0001	0.0037	0.0050

ANOVA, analysis of variance; C, standard diet; CA, standard diet plus acai; H, hypercholesterolemic diet; HA, hypercholesterolemic plus acai; HDL, high-density lipoprotein

Values are means ± SDs ($n = 8$). Data were tested by two-way ANOVA; when interactions were significant ($P < 0.05$), Tukey's post hoc test was performed to determine the specific differences between mean values. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($P < 0.05$).

* Atherogenic index = (total cholesterol total − HDL cholesterol) × $(\text{HDL cholesterol})^{-1}$.

related to VLDL metabolism, thereby resulting in lower levels of triacylglycerols in the plasma [29].

A complete nutrient analysis of freeze-dried acai fruit pulp/skin powder showed that it is very rich in polyphenols, especially anthocyanins (cyanidin 3-glucoside and cyanidin 3-rutinoside), and other compounds such as unsaturated fatty acids (oleic and linoleic acids), phytosterols (β-sitosterol), and dietary fiber [18,19]. Although there are reports showing that the fruit of acai has high antioxidant abilities in vitro due to the flavonoids [11,12,13,17], very little is known about its effects in vivo.

Acai supplementation significantly reduced food ingestion in the HA group. This finding suggests, as observed by other researchers [30], that polyphenols also play a role in the modulation of appetite. It remains to be explained why this effect was not observed when the standard diet was used. The presence of acai did not alter food ingestion in the CA group but did lower it in the HA group, although better food efficiency was observed in both cases, indicating better nutrient utilization that led to a clear increase in weight gain when the fruit was added to the standard diet. The differences found between the association of the acai-supplemented standard diet and the acai-supplemented hypercholesterolemic diet indicate that other factors of the diet affect the action of the active components of the fruit. It is of interest to further study the physiologic implications of the relation between acai pulp and/or its components and different diets.

To assess the effects of acai pulp supplementation on the antioxidant status in vivo, the levels of carbonyl proteins and sulfhydryl groups and the activity of two antioxidant defensive enzymes were measured. Protein carbonyls are normally used as a biomarker for protein damage caused by oxidized amino acid residues in stress conditions [31]. Other important markers of oxidative stress are thiol groups in the plasma that can function as physiologic free radical scavengers [32]. In the present study, the addition of acai pulp to the standard and hypercholesterolemic diets caused a significant improvement in the antioxidant capacity by decreasing the level of carbonyl protein and thiobarbituric acid-reactive substances in the control animals (data not shown) and increasing the sulfhydryl groups in the sera of control and hypercholesterolemic animals. This is in agreement with the increased plasma antioxidant capacity observed in healthy human volunteers consuming acai pulp and juice [13]. Similar results were observed in tissue culture and in animals treated with other polyphenols [33,34].

As for the effect of acai pulp on the antioxidant enzymes, the activities of SOD and PON were analyzed. The increased SOD activity observed in the animals consuming the hypercholesterolemic diet could be the result of counteracting the excess superoxide anions that formed as a response to hypercholesterolemic stress [35,36]. We observed that acai supplementation in the hypercholesterolemic diet caused a significant reduction of SOD activity compared with the control rats, which suggests that the stress reduction caused by the acai diminished the need for protective responses. Conversely, when PON activity was assessed, we observed that acai pulp supplementation in the control and hypercholesterolemic animals increased PON activity. Reports on the effect that polyphenols have on SOD activity have been contradictory, causing no effect or increased activity [37,38], whereas PON activity has always been found to increase [39,40]. These inconsistent results on the activity of antioxidant enzymes could be explained by the different stress conditions used, the enzymes being analyzed, and the source of the dietary compounds. Nevertheless, we showed that the intake of acai pulp had a positive effect on the antioxidant enzyme activities of SOD and PON, suggesting that the flavonoids in acai pulp could function to reduce the stressful environment caused by the hypercholesterolemic diet. In this scenario, flavonoids act by reducing reactive oxygen species production by neutralizing them or by chelating metal ions [41,42]. Moreover, there is a growing body of evidence showing that polyphenols may also function by altering the activities of metabolizing enzymes and at the transcriptional regulation level by modulating gene expression and nuclear receptors [43].

Concerning the effect of acai pulp on the serum lipid profile, this is the first study, to our knowledge, addressing the potential benefits of acai intake in the cholesterol profile. We observed that the addition of acai pulp in the hypercholesterolemic diet had a hypocholesterolemic effect by reducing cholesterol levels (total and non-HDL), which could be explained by a lower food intake and higher fecal excretion. Nevertheless, the exact mechanisms behind this hypocholesterolemic effect should be examined because various components of the fruit might cause this effect. Polyphenol-rich foods and beverages, such as red wine [44], green tea [45] or apples [46], have been associated with reduced intestinal absorption of dietary lipids by interfering with their emulsification, digestion, and micellar solubilization [47]. In addition to the polyphenols, other compounds may be involved in explaining how acai pulp modulates the lipid profile, because it is significantly rich in unsaturated fatty acids, dietary fiber, and phytosterols. The ingestion of polyunsaturated (such as linolenic and linoleic acids) and monounsaturated (such as oleic acid) fatty acids is associated with improved lipid profiles by decreasing total and LDL cholesterol plasma levels [48,49]. Nevertheless, in the present experimental model, the hypocholesterolemic effect of acai may not be mediated by the fatty

acid content, because the H and HA diets were already fully loaded with soybean oil. Dietary fiber induces a notable effect on plasma lipids and lipoproteins specifically by reducing the amount of bile acids undergoing enterohepatic recirculation, which results in higher levels of LDL cholesterol being removed from the plasma for bile synthesis [50]. Phytosterols are hydrophobic molecules that are structurally related to cholesterol and naturally occur in vegetable oils. Plant sterols reduce the absorption of dietary and biliary cholesterol from the intestinal tract by 30–50%. The presence of increased quantities of plant sterols in the gut lowers the micellar solubility of cholesterol, thus lowering the amount of cholesterol available for absorption [51]. In addition, it has been reported that the regular consumption of foods containing phytosterols reduces total cholesterol and LDL cholesterol levels without modifying triacylglycerol and HDL cholesterol concentrations [52]. Taking all these considerations together, it is reasonable to assume that, in addition to the polyphenols in acai pulp, other nutrient fractions may account for the hypocholesterolemic effects observed in our experiments.

Conclusion

Our results may have a substantial impact in understanding the bioactivities of acai pulp in vivo and reveal that this pulp has antioxidant action and promotes an improvement in the cholesterol status of diet-induced hypercholesterolemic rats.

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