



Applied nutritional investigation

Açaí (*Euterpe oleracea* Mart.) pulp dietary intake improves cellular antioxidant enzymes and biomarkers of serum in healthy women



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ABSTRACT

Objectives: The aim of the present study was to evaluate the effect of açaí pulp (*Euterpe oleracea* Martius) intake on the prevention of oxidative damage by measuring the activity of antioxidant enzymes and biomarkers of protein oxidation in women.

Methods: A nutritional intervention study was conducted with thirty-five healthy women who were asked to consume 200 g/d of açaí pulp for 4 wk. Blood samples were collected, and blood pressure and anthropometric parameters were measured before and after the experimental period. Antioxidant enzymes, superoxide dismutase, catalase, glutathione, production of reactive oxygen species, and total antioxidant capacity were evaluated in polymorphonuclear cells. Serum concentration of protein carbonyl and sulfhydryl groups were also determined.

Results: The açaí intake increased catalase activity, total antioxidant capacity, and reduced the production of reactive oxygen species. Furthermore, it reduced serum concentration of protein carbonyl and increased total serum sulfhydryl groups.

Conclusions: These results show the antioxidant benefit of dietary açaí for the healthy women included in the present study, and may increase understanding of the beneficial health properties of this fruit.

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Introduction

Açaí is a fruit of the native Amazon palm tree *Euterpe oleracea* Martius. The fruit, considered one of the most important fruits of

the Amazon estuary, is widely consumed in Northern Brazil [1]. In the past 10 y, sales of açaí and related products, such as tablets, capsules, juice, and instant drink powders, have increased in Brazil and abroad, including the United States, Japan, and Europe

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[2,3]. The growing interest in açai is mainly due to its health benefits [4–6]. The antioxidant effect is attributable especially to its polyphenolic fraction rich in anthocyanins, mainly cyanidin 3-glucoside and cyanidin 3-rutinoside, as well as monomeric catechin and epicatechin or oligomeric procyanidins [7,8].

Polyphenols are capable of donating hydrogen atoms, which break oxidation chains and chelate transition metal ions, inhibiting the formation of free radicals [9]. Additionally, they may upregulate antioxidant enzymes by activating the NRF2/KEAP1 pathway [10]. In a normal state, when NRF2 interacts with KEAP1, the product is rapidly degraded by proteasomes. Polyphenols promote KEAP1/NRF2 dissociation [11], allowing NRF2 to translocate to the nucleus and heterodimerize with the small MAF protein. The heterodimer then binds to antioxidant-responsive elements within the regulatory regions of multiple antioxidant genes, inducing robust expression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [12]. These enzymes are reactive oxygen species (ROS) scavengers; SOD converts almost all of the superoxide anions produced in the cell to hydrogen peroxide, which is further reduced to water by CAT or GPx [13–15].

High intracellular levels of ROS are often defined as oxidative stress [16]. In addition to this classic definition, oxidative stress is synonymous with a disruption of the redox signaling control [17]. Most clinical studies of the oxidative metabolism rely on the measurement of oxidative damage in biomolecules and biomarkers, such as oxidant and antioxidant molecules [18,19]. For example, protein carbonyl and sulfhydryl groups are considered biomarkers of protein oxidative damage [20]. Sulfhydryl groups represent all protein thiol groups that can form disulfide bonds when oxidized, decreasing in number as oxidative stress increases [21,22].

Some *in vitro* and animal studies have reported the strong antioxidant effect of açai [23–26], but few studies have been conducted in human beings. The kinetics of anthocyanin absorption and a significant increase in the total antioxidant capacity (TAC) of plasma were demonstrated in healthy individuals after a single dose of açai juice [27]. To the best of our knowledge, the effect of habitual dietary açai intake on the activity of antioxidant enzymes and biomarkers of oxidation in subjects has not yet been clarified. Hence, we hypothesized that the phytochemical composition of açai prevents oxidative damage and improves the antioxidant and prooxidant status of healthy humans.

In this study, we aimed to assess the effect of açai daily intake on ROS production, TAC, the activity of SOD, CAT, and GPx on polymorphonuclear (PMN) cells and serum protein carbonyl and sulfhydryl groups of healthy women.

Materials and methods

Study design and subjects

This prospective nutritional intervention study recruited healthy women through advertisements at the Federal University of Ouro Preto (UFOP) website, local radio, and folders distributed in the city of Ouro Preto. The inclusion criteria were: healthy women aged 18–35 y with a body mass index (BMI) of 18.5 to 29.9 kg/m². The exclusion criteria were: illiteracy, body weight change >10% in the past 2 mo, smoking, presence of inflammatory or chronic disease, presence of eating disorders, use of nutritional supplements, being an elite athlete, chronic medication use (except for contraceptives), pregnancy, lactation, and physical disabilities.

One hundred women were recruited, 42 met the inclusion criteria, and 35 completed the study. Seven women did not complete the protocol because they failed to eat açai daily or missed one of the appointments.

All participants signed an informed consent form. This study complied with the guidelines provided by the Declaration of Helsinki and resolution 196/96 of

the Brazilian Health Council. All procedures were approved by the Human Research Ethics Committee of the UFOP-CAAE 0062.0.238.000- 10.

Experimental design

The study was conducted from April to December 2013 at the outpatient Clinical Nutrition Clinic at the School of Nutrition of the UFOP. The intervention lasted 4 wk, each participant meeting with the researchers once a week to receive the açai pulp enough for intake in the following week and to verify the participants' adherence to the protocol. The first meeting included instructing the participants about the experimental period, collecting a fasting blood sample and blood pressure, anthropometric measurements, level of physical activity, and diet. The participants were asked to eat 200 g of açai pulp per day and to maintain their habitual diet and level of physical activity throughout the period. At the end of the 4 wk, lifestyle and anthropometric data, blood pressure, and fasting blood sample were collected again.

Açai pulp

A single lot of pasteurized, frozen açai pulp without colorants or preservatives to ensure homogeneity was bought at a local supermarket (IceFruit, Auckland, New Zealand). The centesimal analysis [28] showed that the pulp had a moisture content of 90% and a dry content of 10%. Each 10 g of dry weight contained 4.7 g of lipids, 1.1 g of protein, 1.5 g of carbohydrates, 2.7 g of fibers, and 52.7 kcal. The product was kept at –80°C. Total polyphenol content was determined by the Folin-Ciocalteu method [29]. Different concentrations of gallic acid (Sigma-Aldrich, Saint Louis, MO, USA) were used to construct a standard curve for quantifying total polyphenols, and the values were expressed in mg of gallic acid equivalent (GAE) in 100 g of açai pulp. The açai pulp had 131 mg GAE/100 g.

The antioxidant activity of açai pulp was determined by the 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), which evaluates the ability of a substance to sequester the free radical DPPH [30]. The pulp had an EC50 of 512 mg/mL, while the EC50 of trolox standard antioxidant was 214 mg/mL.

Dietary intake and level of physical activity

The researchers administered a previously validated food frequency questionnaire [31] containing 86 food items, including leaf vegetables, legumes, meats, dairy products, snacks and other processed meats and sausages, bread and similar items, grains and starches, fruits, and juices. Dietary data were collected before and after the experimental period to check the food intake pattern of the participants' habitual diet. Dietary nutrient intakes were calculated by the software *Avanutri & Nutrição* (Tres Rios, Brazil).

A self-reported physical activity questionnaire was applied at the baseline and endpoint. The total amount of habitual physical activity performed in 24 h of a typical workday, including activities performed at work, leisure, and sports was calculated and the metabolic equivalent of task (MET) was estimated [32].

Anthropometric and blood pressure measurements

Weight was measured by the digital scale (Welmy, Campinas, Brazil), with the participants wearing light clothing and barefoot. BMI and waist circumference were measured as recommended by the World Health Organization [33]. Percentage of body fat was determined by horizontal tetrapolar bioimpedance (Biodynamics, Seattle, WA, USA).

Systolic and diastolic blood pressures were given by taking the mean of three alternate measurements using the oscillometric device OMRON 795 CP (Omron Healthcare, Kyoto, Japan).

Biochemical parameters

Blood samples were collected at baseline and at endpoint after an overnight fast of 12 h by venipuncture of the antecubital region using a vacuum system. Serum glucose, total cholesterol, high-density lipoprotein cholesterol, triacylglycerols, and total protein were measured by the enzymatic colorimetric assays (Labtest Diagnostic, Minas Gerais, Brazil). The concentration of low-density lipoprotein cholesterol was given by the Friedewald formula [34]. Fasting plasma insulin was determined by chemiluminescence immunoassay (Access Immunoassay System, Paraná, Brazil). The homeostasis model assessment of insulin resistance evaluated insulin resistance [35].

PMN cells

PMN cells were isolated from heparinized whole blood using Histopaque-1119 in combination with Histopaque-1077 (Sigma-Aldrich, Saint Louis, MO) as instructed by the manufacturer. The cell viability of each sample was determined by trypan blue exclusion and was always greater than 95%.

Production of reactive oxygen species in PMN cells

The ROS production was evaluated using a chemiluminescence assay amplified by luminol (5–1,4 phthalozinedione), as described by Chaves et al. [36]. To perform the assay, 1×10^6 PMN cells in Hank's pH 7.4 solution, were incubated with 500 μ L of luminol in siliconized tubes. The photons emitted were recorded for 30 min at intervals of 1 min and recorded by a computer adapted at the luminometer Berthold Sirius. The values were expressed in relative light unit per minute (RLU/min).

Total antioxidant capacity in PMN cells

Samples were thawed to room temperature and TAC was measured by quantitative colorimetric (BioAssay Systems, San Diego, CA, USA). TAC was expressed in mM Trolox equivalents.

Antioxidant enzymes measured PMN cells

The commercial kits (BioAssay System, San Diego, CA, USA) determined the activities of the enzymes SOD, CAT, and GPx in PMN cells. The activity of enzymes was expressed in U/L.

Protein carbonyl in serum

The concentration of protein carbonyl was determined as described elsewhere [37]. Briefly, 100 μ L of serum were transferred to a microcentrifuge tube containing 600 μ L of DNPH (2,4-dinitrophenylhydrazine). Then, 600 μ L of trichloroacetic acid 20% were added, and the mixture was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was discarded and 800 μ L of ethanol-ethyl acetate were added. The mixture was vortexed until complete dissolution of the pellet and again centrifuged. At the end, the supernatant was discarded and 900 μ L of guanidine were added. The mixture was centrifuged, the supernatant was removed, transferred to a quartz cuvette, and read in a spectrophotometer at 360 nm. A guanidine-containing cuvette was used to reset the device. The concentration of protein carbonyl was expressed as nmol/mg of protein total.

Sulfhydryl groups in serum

The concentration of total serum sulfhydryl groups before and after the intervention was determined by Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid-DTNB) [38]. Briefly, to a microcentrifuge tube were added 800 μ L of methanol, 150 μ L of Tris-HCl pH 8.2, 50 μ L of DTNB, and 40 μ L of serum. The mixture was centrifuged at 10,000 g for 15 min. The absorbances were read in a spectrophotometer at 412 nm. A blank tube containing DTNB was used to reset the device. The results were expressed in mM/L.

Statistical analyses

The data were tested for normality distribution by the Shapiro-Wilk test and are presented as means and standard error (mean \pm SE). All variables were compared using paired Student's *t* test. $P < 0.05$ was considered statically significant. The statistical analyses were performed by the software PASW 18.0 (SPSS, Chicago, IL).

Table 1

Effect of açai pulp on anthropometric, clinical and biochemical characteristics of women after açai consumption for 4 wk

Parameters	Baseline	Endpoint	<i>P</i>
Body weight (kg)	65.7 \pm 2.4	66.0 \pm 2.3	0.23
Body mass index (kg/m ²)	24.1 \pm 0.7	24.3 \pm 0.7	0.07
Waist circumference (cm)	75.3 \pm 1.6	74.9 \pm 1.6	0.16
Body fat (%)	31.3 \pm 1	31.7 \pm 1	0.10
Systolic blood pressure (mmHg)	104 \pm 2	103 \pm 2	0.48
Diastolic blood pressure (mmHg)	72 \pm 1	72 \pm 1	0.83
Cholesterol total (mg/dL)	182 \pm 35	189 \pm 43	0.33
LDL (mg/dL)	100 \pm 5	104 \pm 6	0.61
HDL (mg/dL)	64 \pm 2	65 \pm 2	0.64
Triacylglycerols (mg/dL)	82 \pm 6	82 \pm 6	0.98
Glucose (mg/dL)	79.6 \pm 1.1	80.3 \pm 1.2	0.59
Insulin (μ IU/mL)	6.2 \pm 0.4	6.6 \pm 0.5	0.36
HOMA-IR	1.2 \pm 0.1	1.3 \pm 0.1	0.32

HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein
Values are means and standard error. *P* value for paired-*t* test at baseline and at endpoint, *n* = 35

Table 2

Estimative by dietary intake and physical activity through questionnaire of women after açai consumption for 4 wk

	Baseline	Endpoint	<i>P</i>
Total energy (kcal)	2102 \pm 108	2110 \pm 134	0.951
Carbohydrate (g)	480 \pm 56	491 \pm 52	0.858
Protein (g)	94 \pm 6	95 \pm 7	0.850
Total lipids (g)	68 \pm 4	68 \pm 5	0.961
Metabolic equivalent of task	40 \pm 2	40.4 \pm 2	0.894

Values are means and standard error. *P* value for paired-*t* test at baseline and at endpoint, *n* = 35

Results

Effect of açai pulp on anthropometric, clinical, and biochemical characteristics

The study included 35 women (24.3 y \pm 8.8). Table 1 shows the anthropometric parameters, the systemic arterial pressure values and blood biochemistry parameters at the baseline and at the endpoint. All evaluated characteristics were not changed by the dietary intervention.

Estimative summary of dietary intake and physical activity

Table 2 shows the daily total energy, macronutrient intake, and quantitative evaluation of physical activity. The total energy intake, carbohydrate, protein, total lipids, and metabolic equivalent of task were unchanged.

Production of reactive oxygen species in PMN cells

Consumption of açai pulp decreased the levels of ROS from 1371.1 \pm 730 RLU/30 min to 289.5 \pm 300 RLU/30 min (Fig. 1).

Total antioxidant capacity in PMN cells

The intervention increased TAC of PMN cells by 104%, from 241.4 \pm 36.01 μ M Trolox equivalents to 493.6 \pm 49.37 μ M Trolox equivalents (Fig. 2).

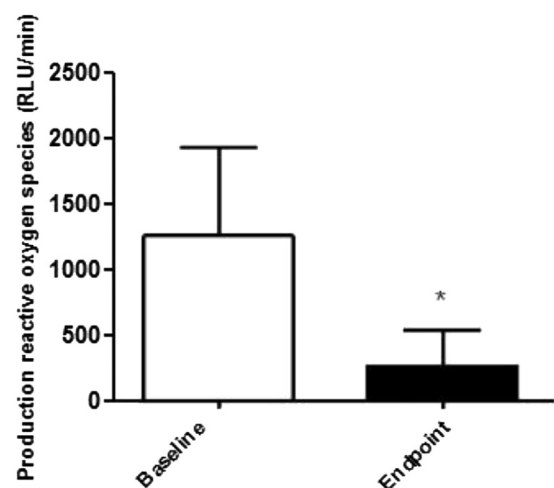


Fig. 1. Effect of açai pulp intake on production of reactive oxygen species of polymorphonuclear cells obtained, *n* = 35. Data are presented as mean and standard error, and were analyzed with paired-*t* test. **P* = 0.004.

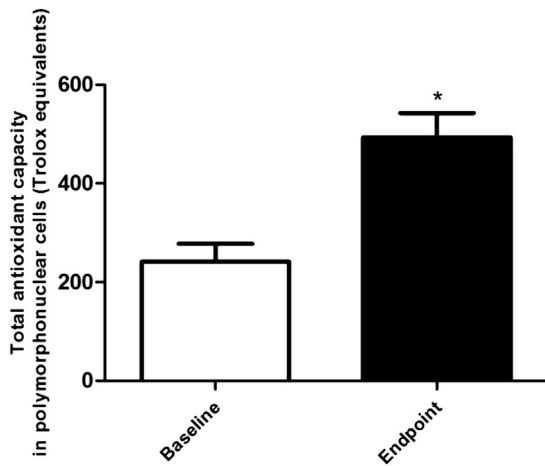


Fig. 2. Effect of açai pulp intake on total antioxidant capacity of polymorphonuclear cells obtained, $n = 35$. Data are presented as mean and standard error, and were analyzed with paired-t test. * $P < 0.001$.

Antioxidant enzyme activities in PMN cells

Antioxidative enzymes status was evaluated by SOD, CAT, and GPx activities in PMN cells at baseline and at endpoint after the experimental period (Fig. 3). CAT activity increased significantly from 0.20 ± 0.02 U/L to 8.30 ± 0.64 U/L (Fig. 3B). The activities of the other enzymes did not change.

Serum protein carbonyl and sulfhydryl groups

In order to evaluate the potential antioxidant effects of açai, we determined the levels of serum protein carbonyl and total sulfhydryl groups at baseline and endpoint (Fig. 4). At endpoint, protein carbonyl had decreased from 1.46 ± 0.05 nmol/mg of protein total to 1.26 ± 0.09 nmol/mg of protein total ($P = 0.027$) and sulfhydryl groups had increased from 370.59 ± 12.21 mM/L to 454.98 ± 13.66 mM/L ($P < 0.001$).

Discussion

In order to evaluate the effects of the antioxidant-rich fruit açai in humans, we conducted a dietary intervention to measure oxidative status in healthy women. The women were asked to maintain their habitual dietary intake and physical activity levels throughout the study period. Our results showed that the daily intake of 200 g of açai pulp for 4 wk improved the antioxidant status. Activity of the antioxidant enzyme CAT and TAC in PMNs cells increased and the production of ROS decreased significantly. Additionally, serum protein carbonyl concentration decreased and the total serum sulfhydryl groups were increased.

Overproduction of ROS can cause oxidative damage to biomolecules and promote the development and progression of many chronic diseases, including atherosclerosis, cancer, and other degenerative diseases [39–41]. Regular intake of fruits, vegetables, and other antioxidant-rich foods is associated with numerous beneficial health effects, reducing the incidence of these diseases [42,43].

In the pathophysiology of various chronic diseases, the production of ROS seems to be directly related to the activation of PMN cells, the principal effector cells of innate immunity [44,45]. The activation of phagocytic cells leads to the production of ROS through oxidative metabolism, so the discovery of new dietary

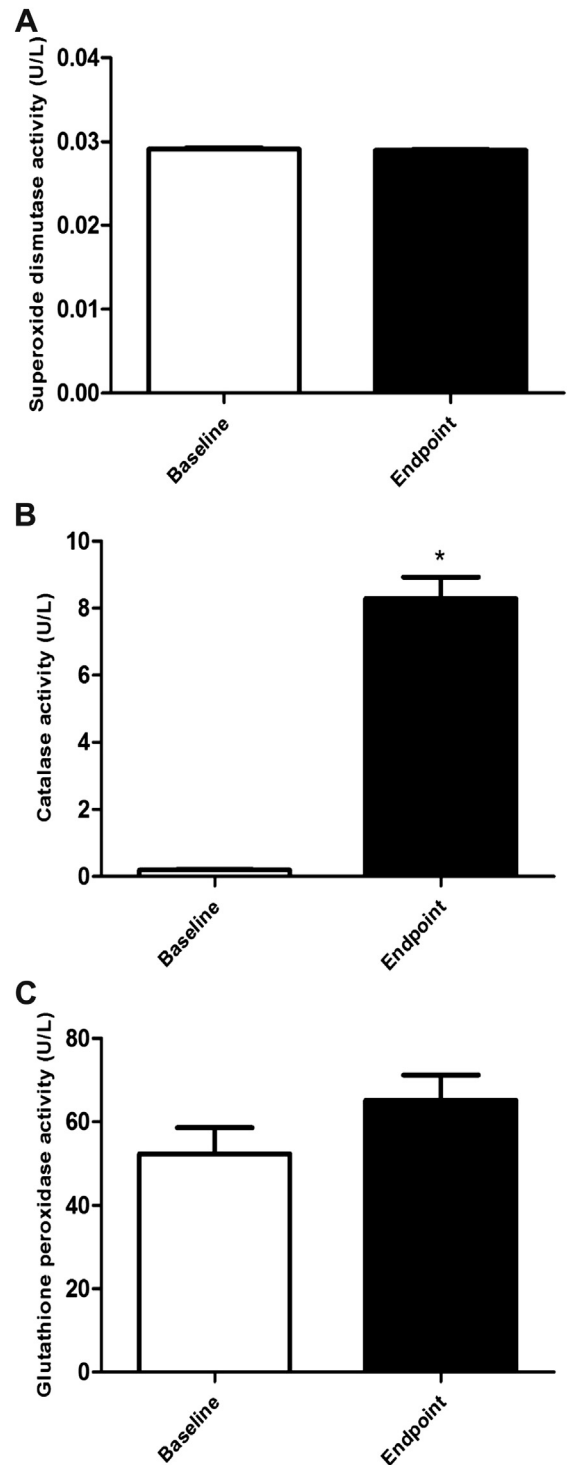


Fig. 3. Effect of açai pulp intake on activities of antioxidant enzymes in polymorphonuclear cells obtained, $n = 35$. (A) Superoxide dismutase; (B) catalase; (C) glutathione peroxidase. Data are presented as mean and standard error, and were analyzed with paired-t test. * $P < 0.001$.

compounds that can modulate this process is of great interest. In this regard, studies have demonstrated that açai inhibits ROS production in isolated neutrophils [5,25,46]. In the same direction, in our in vivo study we found a reduction on ROS production by PMN cells of the women after açai intake. We also found an increased CAT activity and the TAC in the same cells. CAT is

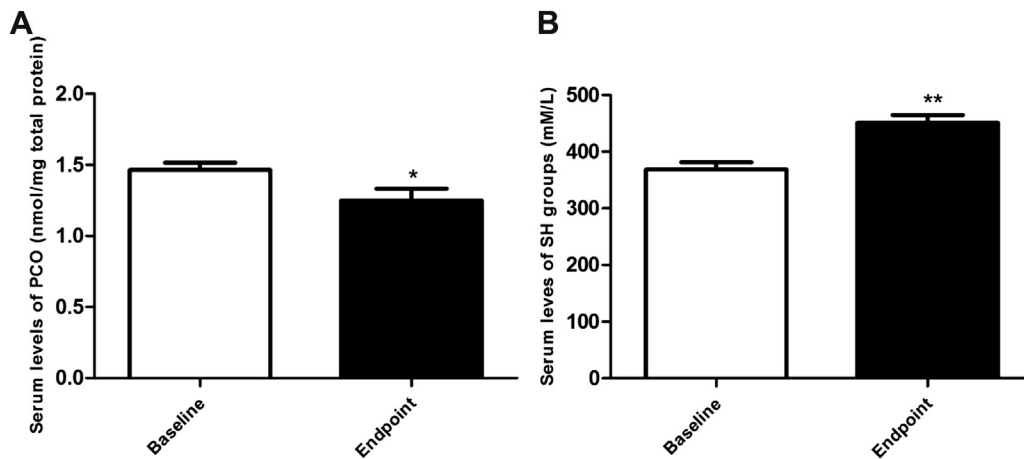


Fig. 4. Effect of açai pulp intake on serum markers of oxidative stress, $n = 35$. (A) PCO, protein carbonyl; (B) SH, total sulfhydryl group. Data are presented as mean and standard error, and were analyzed with paired-t test. * $P = 0.027$, ** $P < 0.001$.

part of the main antioxidant defense of the cell and other studies have found that its activity can be improved by dietary compounds improving the antioxidant systems [47,48]. TAC appears to reflect the capacity of an individual to neutralize free radicals as it is an element of the nonenzymatic part of the system, protecting the body against ROS [49], and we found an increment of 104% of TAC in PMN cells. Previous studies have shown that an acute dose of açai significantly increased TAC in the plasma of healthy subjects [27,50], reinforcing our observation that long-term intake of açai may improve the antioxidant status of healthy women. The dietary intake of wild blueberries, another fruit rich in antioxidants, enhanced the total serum antioxidant status of healthy subjects after the consumption of a high-fat meal [51]. Taken together, these results reinforce the putative effect of dietary açai intake on ROS modulation and antioxidant defense improvements in PMN cells.

In addition to evaluating how a compound modulates antioxidant enzymes, biomarkers of oxidative damage can be used to assess individual oxidative (antioxidant/prooxidant) status. Products of protein oxidation are commonly used for this purpose because free radicals also attack proteins, which are abundant in the body [52]. We found that serum protein carbonyl had decreased after açai intake. The accumulation of protein carbonyl has been observed in many diseases, including Alzheimer's disease, diabetes, and others [53]. Moreover, the levels of protein carbonyl of young individuals with features of nonalcoholic fatty liver disease decreased significantly after 4 wk of blueberry juice treatment [54].

Açai intake also effectively modulated serum protein thiol levels. Sulfhydryl groups contribute to 50% of the total antioxidant capacity of healthy subjects [55], and polyphenol-rich beverages have been found to increase the number of sulfhydryl groups [56,57]. Taken together, lower protein carbonyl and higher sulfhydryl group levels after açai experimental period confirm that açai prevents oxidative damage. The same was observed in hypercholesterolemic rats fed a diet with 2% açai pulp [58].

Del Pozo-Insfran et al. [7] estimated the antioxidant capacity of açai pulp to be 48.6 μmol Trolox equivalents/mL. Compared with other antioxidant-rich fruits studied by Silva et al. [59], açai has 4.8, 6.1, and 7.5 times the antioxidant capacity of blackberries, blueberries, and strawberries, respectively. The antioxidant effect of açai has been attributed to its phytochemical

composition comprising hydroxybenzoic acids and flavan-3-ols, along with cyanidin 3-*O*-rutinoside and cyanidin 3-*O*-glucoside as the predominant anthocyanins [8]. Since anthocyanins are the least bioavailable polyphenols [60], studies of the antioxidant effect of açai in humans are necessary before to include them in the antioxidant food list.

Besides its antioxidant effect, an antiinflammatory and a hypocholesterolemic effect of the açai pulp has been shown [5,58], and the pulp presents a high content of polyunsaturated fatty acids, phytosterols, and fiber [8]. An extensive nutritional analysis of the freeze-dried pulp did not reveal a remarkable content of other antioxidant compounds [8]. We believe that the benefits of the fruit are probably due to a synergistic effect of its phytochemical and nutrient composition.

Expectedly, açai intake did not affect the anthropometric parameters because, according to the lifestyle questionnaires, the total energy intake, macronutrients, or level of physical activity were unchanged. The biochemical parameters and blood pressure also did not change, contrary to Udani et al. [61], who reported better glucose, insulin, and total cholesterol levels in their pilot study. However, in this study, subjects were advised to avoid foods containing nitrates, such as bacon and hot dogs, which could lead to the results observed in the glucose, insulin and cholesterol concentrations. On the other hand, Kardum et al. [62] reported that intake of chokeberry, a fruit source of polyphenol-rich juice, for 12 wk did not affect their volunteers' systemic arterial pressure values or anthropometric and blood biochemistry parameters.

One limitation of our study was determining whether or not the participants maintained their habitual diet and/or levels of physical activity during the intervention. However, these data were collected by trained interviewers, so measurement errors were most likely avoided. We wanted the study to have minimal impact on the participants' lives and to assess the effect of the fruit as it is usually consumed, not as tablets or supplements. We believe that this is the strength of our study, in addition to the high number of subjects, the use of several biomarkers of oxidative status, and other risk variables associated.

Conclusion

Our results indicate that dietary açai intake modulates the antioxidant/prooxidant status of healthy women. The

antioxidant effects of açai may stem from the neutralization of free radicals, preventing their attack on other molecules, and/or from the modulation of enzymes involved in oxidative stress. These results pave the way for better understanding the effects of the daily dietary intake of açai in humans. Future studies will be needed to determine how much of this potential “functional food” is necessary to maintain health and prevent chronic diseases.

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