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Trabajo Original

Epidemiología y dietética

A prospective study in women: açaí (*Euterpe oleracea* Martius) dietary intake affects serum p-selectin, leptin, and visfatin levels

*Un estudio prospectivo en mujeres: la ingesta dietética de açaí (*Euterpe oleracea *Martius) afecta a los niveles séricos de p-selectina, leptina y visfatina*

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Abstract

Background: açaí is the fruit of the palm tree *Euterpe oleracea* Martius, which is native to the Amazon region. This fruit has been extensively studied due to its potential effects on human health. Studies have also evaluated the potential effect of açaí on the inflammatory response, but there are still few studies that have assessed this property in humans.

Objective: in this study we aimed to evaluate the effects of 200 g of açaí pulp consumption per day during four weeks on a rich panel of inflammatory biomarkers.

Methods: a prospective nutritional intervention study was conducted on forty apparently healthy women who consumed 200 g of açaí pulp per day for four weeks. A panel of serum inflammatory markers were evaluated before and after the nutritional intervention, namely, cell adhesion molecules (ICAM-1, IVAM-1, P-selectin, MCP-1, and fractalkine), interleukins (IL-1β, IL-6, IL-8, IL-10, and IL-17) and adipokines (adiponectin, leptin, visfatin, and adipsin). The data were analyzed using paired Student's *t*-test to evaluate the effect of the intervention using PASW Statistics, version 18.0, and a p-value of < 0.05 was considered significant.

Results: four weeks of açaí pulp consumption decreased p-selectin, leptin, and visfatin concentrations in the serum of the participating women.

Conclusion: these results show that consumption of açaí pulp was able to modulate important biomarkers of the inflammatory process in apparently healthy women.

Keywords:

Açaí. Adipokines. Cell adhesion molecules. Euterpe oleracea Martius. Healthy women. Interleukin.

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Resumen

Introducción: el açaí es el fruto de la palmera *Euterpe oleracea* Martius, originaria de la región amazónica. Esta fruta ha sido ampliamente estudiada debido a sus posibles efectos sobre la salud humana. Los estudios también han evaluado el efecto potencial del açaí sobre la respuesta inflamatoria, pero todavía hay pocos estudios que hayan evaluado esta propiedad en seres humanos.

Objetivo: en este estudio, nuestro objetivo ha sido evaluar los efectos del consumo de 200 g de pulpa de açaí por día durante cuatro semanas sobre un rico panel de biomarcadores inflamatorios.

Métodos: se ha realizado un estudio prospectivo de intervención nutricional en el que cuarenta mujeres aparentemente sanas han consumido 200 g de pulpa de açaí al día durante cuatro semanas. Se ha evaluado un panel de marcadores inflamatorios séricos antes y después de la intervención nutricional, a saber, moléculas de adhesión celular (ICAM-1, IVAM-1, P-selectina, MCP-1 y fractalquina), interleucinas (IL-1β, IL-6, IL-8, IL-10 e IL-17) y adipocinas (adiponectina, leptina, visfatina y adipsina). Los datos han sido analizados mediante la prueba de la t de Student pareada para evaluar el efecto de la intervención mediante el PASW Statistics, versión 18.0, y todo valor de $p < 0,05$ se consideró significativo.

Resultados: después de cuatro semanas de consumo de pulpa de açaí disminuyeron las concentraciones de p-selectina, leptina y visfatina en el suero de las mujeres participantes.

Conclusión: estos resultados muestran que el consumo de pulpa de açaí ha sido capaz de modular importantes biomarcadores del proceso inflamatorio en mujeres aparentemente sanas.

Palabras clave:

Açaí. Adipocinas. *Euterpe oleracea* Martius. Interleucinas. Moléculas de adhesión celular. Mujeres sanas.

INTRODUCTION

Inflammation is an attempt by the body to protect itself and remove harmful stimuli. However, persistent inflammatory clinical conditions have been related to the pathogenesis of several metabolic disorders (1). One of the molecular types involved in this process is cell adhesion molecules (CAMs) such as ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), and the family of molecular selectins (P-selectin, E-selectin and L-selectin) (2). The association between CAMs and inflammation-related disorders, such as chronic diseases, occurs through an imbalance in steady-state stability, related to the integrity and barrier properties of the vessel walls (3,4). Under inflammatory conditions, there is an increase in the circulating pro-inflammatory monocytes and T lymphocytes that adhere to CAMs through selective binding, resulting in a loss of vascular permeability regulation to macromolecules as well as in atherothrombotic processes and platelet reactivity (5). In addition, CAMs can function as signaling molecules in the activation of intracellular signaling pathways that are critical to maintaining a cellular inflammatory state (6).

One of the primary signaling molecules is nuclear factor-kappa B (NF-kB), which regulates the expression of inflammatory mediators such as interleukins, cytokines, chemokines, and nitric oxide synthase, among others (7). NF-kB is a protein complex formed by two subunits (p65 and p50), and in its inactive form is found in the cytoplasm of cells bound to the Kb inhibitor (IkB) (7). Proinflammatory cytokines, reactive oxygen species (ROS), CAMs, and other signaling molecules may help to activate protein kinases, allowing for the translocation of NF-kB into the nucleus, where it interacts with a DNA promoter region, leading to the transcription of several inflammatory mediators (8). Studies have shown that NF-kB is relatively effective in adipocytes and plays a central role in regulating the release of adipokines, such as adiponectin, leptin, adipsin, and visfatin by adipose tissue, providing a link between inflammation and adipocyte hyperplasia (7,8).

Currently, studies have been conducted to evaluate the effects of dietary antioxidants on the oxidative and inflammatory balance in the body (9-11). A fruit that has these properties and has been extensively studied is the açaí, primarily because of its nutritional and phytochemical composition. Açaí is the fruit of the palm tree *Euterpe oleracea* Martius, and is native to the Amazon region. Interest in açaí has been gaining prominence for more than 10 years since some studies showed the potential health benefits of its consumption, both in vitro and in animal models, primarily correlating those effects to the high concentration of phenolic compounds in this fruit (12-15).

Among the beneficial health effects assigned to açaí, its anti-inflammatory capacity has been described in some studies; however, data concerning humans are still scarce, and there is a lack of data associating the effect of açaí consumption with a complete panel of inflammatory biomarkers in a substantial number of healthy volunteers. Our group has been investigating the potential effect of açaí on oxidative stress, and its association with lipid metabolism, and the preliminary results have led us to investigate the possible effect of the consumption of this fruit on inflammatory markers (16,17). Therefore, the aim of this study was to investigate the effect of daily açaí pulp consumption on ICAM-1, IVAM-1, P-selectin, monocyte chemoattractant protein 1 (MCP-1), fractalkine, interleukins (IL-1β, IL-6, IL-8, IL-10, and IL-17), leptin, visfatin, adipsin, and adiponectin in apparently healthy women.

MATERIALS AND METHODS

STUDY DESIGN AND SUBJECT CHARACTERISTICS

A prospective study on self-controlled nutritional intervention was conducted in apparently healthy women, which consisted of the intake of 200 g of açaí pulp per day in a free-living situation for four consecutive weeks. The participants were recruited through an internet advertisement, and brochures were distributed throughout the town of Ouro Preto, Minas Gerais, Brazil. All the parti-

cipants had to meet the following inclusion criteria: age between 18 and 35 years and body mass index (BMI) between 18.55 and 35 kg/m^2. Exclusion criteria included volunteers presenting more than 10 % changes in body weight within the previous two months; blood pressure > 160/100 mmHg; fasting glycemia > 100 mg/dL; history of dyslipidemia or total cholesterol > 200 mg/dL or triacylglycerols > 150 mg/dL; allergies or food intolerances; engaging in smoking; using nutritional supplements within six months before the study; presence of thyroid or other chronic diseases (cardiovascular, renal, hepatic, or intestinal); presence of infectious or inflammatory diseases; acute illness requiring treatment over the last two months; chronic use of medication, except contraceptives; and being pregnant or lactating.

The volunteers were instructed to maintain their habitual lifestyle, diet and physical activity during the intervention. After enrollment, a total of four (one/week) meetings were held between the researchers and the volunteers. At the first and last meetings, data on each volunteer's anthropometric parameters, body composition, blood pressure, and dietary intake were collected; blood samples were obtained for biochemical analysis, and the results were reported. The baseline data used to characterize the study population are presented in table I. During the first meeting, a sufficient amount of açaí pulp was delivered to last for the following 15 days, and on day 16 the remaining açaí pulp needed for consumption through the end of the study was delivered. In addition, the aim of the weekly meetings was to assist the volunteers and to clarify doubts, in addition to verifying their adherence to the study protocol and checking their intake of 200 g of açaí pulp/day through 24-h dietary recalls. Blood samples from the first and last blood collections were also used to determine a panel of inflammatory markers as described below.

Table I. Baseline anthropometric, biochemical, and clinical characteristics of the participating women (n = 40)

Variables	Mean	SD
Weight (kg)	65.5	14.1
BMI (kg/m^2)	24.1	4.4
Waist circumference (cm)	75.0	9.0
Body fat (%)	31.5	5.3
Glucose (mg/dl)	79.1	1.1
Insulin (mIU/ml)	6.4	2.3
HOMA-IR	1.2	0.1
Cholesterol (mg/dl)	190.0	34.0
Triglycerides (mg/dl)	84.0	36.0
Cholesterol-LDL (mg/dl)	108.0	31.0
Cholesterol-HDL (mg/dl)	66.0	14.0
Systolic blood pressure (mmHg)	104.0	11.0
Diastolic blood pressure (mmHg)	72.0	9.0

HOMA-IR: homeostatic model assessment. The results are presented as mean and SD

All the participants gave their informed consent for inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Federal University of Ouro Preto, Minas Gerais, Brazil (project identification CAAE 0062.0.238.000-10).

AÇAÍ PULP

The açaí pulp used in the study was purchased from a local supermarket. The pulp was pasteurized and contained no additives. The required amount was obtained from the same supplier in a single batch (IceFruit® Lot 04/13) to ensure homogeneity of the administered pulp. The pulp was packaged in 100 g units, and the volunteers were instructed to add 200 g of pulp/day (two 100 g packets) to their usual diet.

ADHESION MOLECULES

The participants' plasma concentrations of ICAM-1, IVAM-1, P-selectin, MCP-1, and fractalkine were determined by using multiplex sandwich immunoassay kits (Millipore Corporation, Billerica, MA, USA). The detection sensitivity was 0.019 ng/ml, 0.024 ng/ml, 0.051 ng/ml, 1.9 pg/ml, and 22.7 pg/ml for ICAM-1, IVAM-1, P-selectin, MCP-1, and fractalkine, respectively. The intra- and inter-assay coefficients of variation were 7.9 % and 9.7 % (ICAM-1), 4.5 % and 38 % (IVAM-1), < 20 % and 8.5 % (P-selectin), 6.1 % and 12 % (MCP-1), and 5.3 % and 10.1 % (fractalkine), respectively.

INTERLEUKINS

Plasma IL-1β, IL-6, IL-8, IL-10, and IL-17 concentrations were determined simultaneously by multiplex immunoassay using a commercial MILLIPLEX® MAP (Multiple Analyte Profiling) kit (Millipore Corporation, Billerica, MA, USA) with a sensitivity of 0.8 pg/ml for IL-1β, 0.9 pg/ml for IL-6, 0.4 pg/ml for IL-8, 1.1 pg/ml for IL-10, and 0.7 pg/ml for IL-17.

ADIPOKINES

Adipokine concentrations were determined with multiplex immunoassay kits (Millipore®). The methodology used in the analysis involved MAP and Luminex™ technology, which uses a unique process that internally blends polystyrene microspheres with two different spectral fluorochromes. The sensitivities of the kits for leptin, adiponectin, visfatin, and adipsin were 19 pg/ml, 21 pg/ml, 0.778 ng/ml, and 10 pg/ml, respectively.

DATA ANALYSIS

The sample size was calculated using the expected change in serum cholesterol based on a previous study using 95 % power at

the 5 % level of significance in BioEstat, version 5.9 (18). Thus, 10 volunteers was sufficient for an intervention design, and 40 volunteers completed the protocol of the present study. The statistical analysis was performed using PASW 18.0 for Windows (SPSS, Chicago, IL, USA). A Kolmogorov-Smirnov normality test was performed. All the variables presented a normal distribution, and the data are presented as mean ± standard deviation (SD). Adhesion molecules, interleukins, and adipokines were analyzed using a paired Student's *t*-test to evaluate the effect of the açaí pulp intervention. For all the statistical tests, the significance level was set at 5 %. Statistical analyses were performed with the PASW 18.0 software.

RESULTS

Data on anthropometric, clinical, biochemical, and lifestyle variables (diet and physical activity) were measured before and after the dietary intervention with açaí pulp, and published in a previous study (17). There was no difference in anthropometric, clinical, and biochemical variables after the consumption of açaí pulp by the volunteers. Additionally, as previously described, we checked each volunteer's food intake and physical activity after and before the intervention using questionnaires, and we did not find any significant differences in either total calorie or macronutrient (carbohydrates, fats, and proteins) intake, or in the estimated metabolic equivalents of task (METs) (17). Even though the calculated sample size was 10, we decided to enroll a higher number of women because we were aware of the risk of some losses during the study, both due to the long duration of the study and the need for daily consumption of açaí, which might not have been well accepted.

The concentrations of the cell adhesion molecules ICAM-1, IVAM-1, P-selectin, MCP1, and fractalkine before and after açaí consumption are presented in table II. The results show a significant reduction (8 %, $p < 0.05$) in P-selectin after açaí pulp consumption. In relation to the other cell adhesion molecules analyzed here, no significant differences were found.

Table III shows the serum concentrations of interleukins as assessed before and after açaí pulp consumption. No significant differences were found for these variables.

We also evaluated the plasma concentrations of four adipokines before and after açaí consumption (Table IV). After the nutritional intervention with açaí pulp for four weeks, there was a reduction in leptin (5 %, $p = 0.006$) and visfatin (44 %, $p = 0.03$).

DISCUSSION

In this study, we evaluated the effect of consuming açaí pulp on a daily basis on a rich panel of inflammatory markers

Table II. Effect of açaí pulp on the plasma adhesion molecules of women before and after açaí consumption (200 g/day) for 4 weeks

Adhesion molecules	Before		After		p
	Mean	SD	Mean	SD	
ICAM-1 (ng/ml)	0.73	0.18	0.74	0.18	0.72
IVAM-1 (ng/ml)	0.55	0.13	0.58	0.12	0.14
P-selectin (ng/ml)	0.48	0.13	0.44	0.12	0.03
MCP-1 (pg/ml)	2.22	0.88	2.23	0.89	0.94
Fractalkine (pg/ml)	36.57	25.60	36.60	21.85	0.10

ICAM: intercellular adhesion molecule; MCP-1: monocyte chemoattractant protein-1; VCAM: vascular cell adhesion molecule. The results are presented as mean and SD; p-values < 0.05 were considered significant for the paired Student's t*-test.*

Table III. Effect of açaí pulp on the plasma interleukins of women before and after açaí consumption (200 g/day) for 4 weeks

Interleukins (pg/ml)	Before		After		p
	Mean	SD	Mean	SD	
IL-10	5.35	6.50	5.70	6.41	0.70
IL-17	0.96	1.10	1.20	1.32	0.39
IL-1 β	3.60	1.57	3.92	1.93	0.36
IL-6	7.39	7.18	7.90	7.70	0.80
IL-8	7.94	6.80	9.28	8.89	0.26

The results are presented as mean and SD; p-values < 0.05 were considered significant for the paired Student's t*-test.*

Table IV. Effect of açaí pulp on the plasma adipokines of women before and after açaí consumption (200 g/day) for 4 weeks

Adipokines (ng/ml)	Before		After		p
	Mean	SD	Mean	SD	
Leptin	0.19	0.04	0.18	0.02	0.006
Adiponectin	20.01	7.90	19.73	8.20	0.737
Adipsin	32.47	6.60	32.37	5.60	0.326
Visfatin	5.21	7.80	2.91	6.90	0.030

The results are presented as mean and SD; p-values < 0.05 were considered significant for the paired Student's t*-test.*

in 40 women, including adhesion molecules (ICAM-1, IVAM-1, P-selectin, MCP-1, and fractalkine), interleukins (IL-10, IL-17, IL-1β, IL-6, and IL-8), and adipokines (leptin, adiponectin, adipsin, and visfatin), for the first time. Our data showed that the intake of 200 g/day of açaí pulp for four weeks decreased serum P-selectin, leptin, and visfatin concentrations. One of the known factors affecting the regulation of inflammatory markers is meal composition, and these results suggest that açaí intake may have a positive effect on the modulation of inflammation in apparently healthy women.

The nutritional and phytochemical components of açaí, such as unsaturated fatty acids, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), dietary fibers and phenolic compounds, have important putative functions and benefits to human health (19). The fatty acid composition of the diet plays a central role in the inflammatory response; some unsaturated fatty acids may have anti-inflammatory effects and improve the balance in the production of cytokines and inflammatory mediators (20). The fatty acid profile of açaí presents oleic (49.72 %), palmitic (25.31 %), and linoleic acids (13.51 %) in the highest proportions (19). The high content of unsaturated fatty acids (> 70 %) present in the açaí lipid fraction might positively affect metabolism and contribute to inflammatory modulation and endothelial dysfunction improvement since dietary unsaturated fatty acids are known to be incorporated into plasma-membrane phospholipids at these modulating cellular signaling events (21). As previously reported, after açaí pulp consumption for four weeks (200 g/day), the volunteers presented an increase by 21 % in MUFA intake and 14 % in PUFA intake (17).

Some authors have observed a positive effect of dietary fatty acids, mainly MUFAs and PUFAs, on vascular inflammation markers and leptin. Rallidis et al. (2017) showed that a Mediterranean diet rich in MUFAs decreased P-selectin and E-selectin levels, molecules that facilitate the tethering and rolling of leukocytes along the vascular endothelium (22). Rostami et al. (2017) investigated the association of dietary fatty acids with leptin gene expression in visceral and subcutaneous adipose tissues, and observed a negative association of n-3, n-6, and n-9 fatty acids with visceral leptin gene expression in obese participants (23).

Açaí can be considered a good source of dietary fiber (44.2 % of its dry weight) with a ratio of soluble and insoluble fiber of 1:3 (24). The intake of 200 g/day of açaí pulp contributes approximately 30 % of the daily fiber recommendation (25). Advances in studies involving the role of fibers show that these dietary components also have beneficial effects on inflammatory processes (26). Several studies have shown positive relationships between fiber intake and C-reactive protein, IL-6, IL-8, and TNF-α concentrations (27,28). Although the process by which fibers can modulate inflammation is unclear, the crosstalk of the reduction in the oxidation process has been listed as a possible anti-inflammatory effect of fibers (29,30). In previous studies, we observed that the addition of açaí pulp to the diet is able to decrease oxidative stress biomarkers, which in turn may be related to improvement of the inflammation process (16,17). In addition, the role of fibers on intestinal microbiota and short-chain fatty acid (SCFA) production may also influence the inflammatory process (31). Alqurashi et al. (2017) have shown that, in vitro, açaí is capable of modifying the bacteria that colonize the microbiota as well as SCFA production (32). These molecules, especially butyrate, have important effects on the modulation of inflammatory cells and the release of cytokines (33). Therefore, we may suggest that the improvement observed in the inflammatory profile of these volunteers may also be related to an effect of açaí dietary fiber.

Although the mechanisms are not well elucidated, it is well known in the literature that polyphenols exert a positive effect on inflammatory biomarkers (34). A randomized study evaluated the intake of açaí for 12 weeks in individuals presenting metabolic syndrome, and showed a reduction in IFN-γ concentrations in these subjects (35). The phytochemical composition of açaí is characterized by the presence of five flavonoids of the anthocyanin class, namely, cyanidin-3-rutinoside, cyanidin-3-glycoside, cyanidin-3-sambubioside, peonidin-3-glycoside, and peonidin-3-rutinoside (19). Other phenolic compounds, such as velutin, ferulic acid, epicatechin, p-hydroxybenzoic acid, gallic acid, protocatechuic acid, catechin, ellagic acid, vanillic acid, p-coumaric acid, and lignans, are also found in lower concentrations (36,37). The total polyphenol content of the açaí pulp used in this study was 131 mg of GAE/100 g (16). Thus, the açaí pulp ingested in the study contributed to a total daily consumption

of polyphenols of 262 mg of GAE in the volunteers' diet, which probably contributed to the increase in total antioxidant capacity (TAC) observed in the serum and polymorphonuclear cells of these women, as previously described (16,17). It has been observed that among the flavonoids present in açaí, velutin has the highest anti-inflammatory activity (38,39). Velutin showed the strongest inhibitory effect in terms of NF-kB activation, and exhibited the greatest effects in blocking the degradation of the NF-kB inhibitor, as well as in inhibiting mitogen-activated protein kinase p38 and JNK phosphorylation (39). These are important signaling pathways of inflammation associated with the production of TNF-α and IL-6.

Therefore, in addition to the presence of MUFAs, PUFAs, and fibers, the phenolic compounds found in açaí pulp may be involved in the improvement of the inflammatory profile seen in the participants in this study. We believe that further studies are needed to increase knowledge about other inflammation pathways regulated by the specific compounds of açaí, which was not our objective, and additional studies are also necessary to elucidate the mechanisms involved.

One limitation of this study is that we did not have a control or placebo group, so we cannot exclude the possibility that some changes occurred due to other modifications in lifestyle (such as diet or physical activity) that could interfere with the variables studied. However, as reported before, there was no change in diet or physical activity after açaí pulp intake, which led us to believe that the inclusion of açaí pulp on a daily basis was responsible for the observed changes (17). It is important to highlight that our study aimed to evaluate the effect of açaí as a whole food, as it is consumed by the population. We were interested in testing the addition of açaí pulp on a daily basis as part of a balanced diet, as one of the fruits to be added in the 400 g or 5 servings per day that are recommended by the World Health Organization (WHO).

In conclusion, açaí is a unique fruit that is rich in antioxidants and other bioactive compounds, and a modulatory effect on some inflammatory biomarkers was observed; thus, açaí consumption on a regular basis may benefit the health of women.

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