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Short-Term Consumption of a Commercial Honey-Sweetened açai (*Euterpe oleracea*) Beverage Modulates Cytokine Expression and Oxidative Stress in the Visceral White Adipose Tissue of Rats Differently from the Glucose- Sweetened açai Beverage

Renata Silvério[1*], Rodrigo X. das Neves[1], Fernando de O. Rosa[1], Michele J. ALves[1], Rodolfo G. Camargo[1], Anelisa F. A. Magalhães[1], Érico C. Caperuto[2], Marília Seelaender[1]

[1]Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil

[2]Human Movement Laboratory, São Judas Tadeu University, São Paulo, Brazil

[*]**Corresponding author:** Renata Silvério, Cancer Metabolism Research Group, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil. Tel: +551130917225; Email: resfloripa@yahoo.com.br

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Abstract

Scope: Most of commercial açai (*Euterpe oleracea*) beverages present a high glucose content, which could interfere with the potential beneficial effects of this berry.

Methods and Results: In the first study, we examined the effects of a commercial glucose-sweetened açai beverage upon lipid metabolism and adipokine plasma levels. In a second study, we studied the effects of a honey-sweetened açai beverage upon lipid profile, as well as oxidative status and cytokine expression. Rats were supplemented with both beverages for 6 weeks. The consumption of a glucose-sweetened açai beverage induced an increase in body weight gain and augmented visceral white adipose tissue mass, as well as plasma and liver triacylglycerol content. The consumption of the honey-sweetened açai beverage resulted in a reduction in TNF-alpha and an increase in IL-10 content, as well as reduced oxidative stress markers in the visceral white adipose tissue.

Conclusion: These data suggest an effect of the short-term consumption of a honey-sweetened açai beverage in preventing conditions characterized by oxidative stress and inflammation. On the other hand, short-term consumption of an açai beverage containing a high glucose concentration, as that present in most commercially available beverages, leads to alteration of body composition, lipid and carbohydrate metabolism.

Keywords: Açai; *Euterpe oleracea*; Inflammation; Oxidative Stress; White Adipose Tissue

Abbreviations

Açai-H : Commercial Sugar-Sweetened Acai Beverage Group

Açai-S : Commercial Honey-Sweetened Acai Beverage Group

MDA : Molondialdehyde

MTP : Microsomal Triglyceride Transfer Protein

NFκB : Nuclear Factor Kappa B

TNF-alpha : Tumor Necrosis Factor Alpha

SREBP-2 : Sterol Regulatory Element Binding Transcription Factor

Introduction

Oxidative stress and inflammation are key features in a

number of chronic diseases, most notably in those with metabolic alterations [1-3]. Epidemiological studies have identified fruits and vegetables as the key components of dietary patterns that reduce the risk for the development of chronic diseases, including cardiovascular disease, insulin resistance and type II diabetes and the incidence of many tumors [4-6].

Euterpe oleracea Martius is a large palm tree found in South America, especially in the Amazon. Its fruit, commonly known as açai, is a round, black-purple berry [7] and its pulp is traditionally consumed in Brazil. Açai has gained international attention as a functional food owing to its high content of polyphenols and potential health benefits [8].

Açai beneficial effects are related mainly to secondary metabolites such as flavonoids, including anthocyanins and proanthocyanidins, which provide antioxidant activity [9-11]. Several studies showed that açai consumption slows the progression of oxidative stress [12-16] DA SILVA 2017 POULOSE 2017 EL MORSY 2015 and presents anti-inflammatory effect [9,14]. NORATTO2011.DASILVA2017POULOSE2017ELMORSY2015.

Although the açai compounds show positive health effects, most of the commercial beverages containing this berry also present high sugar content. The consumption of sugar-sweetened beverages has been associated with obesity and weight gain [15], impaired glucose and lipid metabolism and promotion of inflammation [16]. Therefore, the high glucose concentration in the açai juices could inhibit the described health effects of this fruit.

Based on this data, we investigated the effects of short-term consumption (6 weeks) of two different commercial açai beverages available in Brazil. First, we examined the effects of short-term consumption of a commercial glucose-sweetened açai beverage on plasma lipid profile of rats. After detecting deleterious metabolic effects, we also studied the effects of short-term consumption of a commercial honey-sweetened açaí beverage in lipid profile, as well as oxidative status and cytokine expression in the visceral white adipose tissue, liver and muscle.

Materials and Methods

Animals

Male adult Wistar rats obtained from the Institute of Biomedical Sciences, University of São Paulo, were maintained in metabolic cages, in a 12 h light: 12 h dark cycle, and under controlled temperature conditions (22 ± 2ºC). Animals were acclimated to their environment for 1 week before the beginning of the experiment. The Ethical Committee for Animal Research from the University of São Paulo approved all the adopted procedures, which were carried out in accordance with the ethical principles stated by the Brazilian College of Animal Experimentation - Protocol n. 041/2005.

Experimental Design

Two studies were carried out in different moments. In both studies animals were randomly divided into 2 groups, a control group and an açai group. Control group received water and food (NuvilabCR1-Nuvital, Curitiba, Paraná, Brazil) *ad libitum*. Açai group received commercial açai beverage and food ad libitum (these animals had no acess to water).

Study 1: In the first study, animals received a commercial sugar-sweetened açai beverage (Açai-S), containing 40 % acai pulp, 15 % glucose, citric acid and water.

Study 2: Animals received a commercial honey-sweetened açai beverage (Açai-H), containing 70 % açai pulp, 18 % honey, 10 % acerola (*Malpighia emarginata*), 2 % lemon (*Citrus limon*), 0.1 % powdered guarana seeds (*Paullini acupana*) and water.

Açai beverages were always offered in dark bottles to mantain the sensory characteristics and to prevent oxidative processes. Animals were weighed 3 times per week, and their food and liquid intake was recorded daily. After six weeks in each treatment, animals were sacrificed by decaptation after 12 h fasting. Then, the weight of visceral white adipose tissue depots (epidydimal, retroperitoneal and mesenteric), liver and gastrocnemius muscles were measured. Blood and tissues were collected and immediately stored at - 80ºC until the experiments were carried out.

Plasma Measurements and Liver Lipid Content Assessment

Blood plasma was isolated by centrifugation at 3,000 x g for 15 min and stored at - 80ºC. Total cholesterol, HDL-cholesterol, triacylglycerol and glucose were quantified using commercial colorimetric kits (Labtest®, Brazil). Adiponectin and leptin plasma levels were determined by ELISA (Invitrogen, USA) and radioimmunoassay (LincoReasearch Inc., USA), respectively. Liver triacylglycerol content was assessed with the method described by Folch et al. [17].

Cytokines Protein Content Assessment

After euthanasia, the tissues (liver, gastrocnemius and visceral white adipose tissue depots) were rapidly removed and frozen. These tissues (0.1 - 0.3 g) were homogenized in a RIPA buffer (0.625% Nonidet P-40, 0.625% sodium deoxycholate, 6.25 mM sodium phosphate, and 1 mM ethylene-diamine tetra acetic acid at pH 7.4) containing 10 µg/ml of a protease inhibitor cocktail (Sigma-Aldrich, USA). Homogenates were centrifuged at 12,000 × g for 10 min at 4˚C, the supernatant was saved, and the protein concentration was determined using a BCA protein assay reagent (Thermo Scientific, USA). Quantitative assessment of TNF-alpha

(CRC3013), IL-6 (CRC0063) and IL-10 (CRC0103) proteins content was carried out by ELISA (Invitrogen, USA).

Thiobarbituric Acid Reactive Species (TBARS) Determination

As an index of lipid peroxidation in the tissues, we measured the formation of TBARS during an acid-heating reaction [18]. Briefly, tissue samples were mixed with 8.1% tricholoacetic acid (2.5 M, pH 3.4; Sigma, USA) and 0.8% thiobarbituric acid (Sigma, USA). The tubes were covered with aluminium foil and kept in a dry bath for 30 min, followed by centrifugation at 3,000 rpm for 10 min at 4ºC. The absorbance of the supernatant was read at 532 nm with Malondialdehyde (MDA) as an external standard. Data are reported as mmol of MDA/mg of protein.

Real time PCR

Total RNA was obtained from aliquots of 100 mg of liver by Trizol® reagent extraction according to the manufacturer's instructions. The primers used were: GLUT-2 [NM_012879.2] (sense TTAGCAACTGGGTCTGCAAT, antisense GGTGTAGTCCTACACTCATG); glycogen synthase 2 [NM_013089.1] (sense GTTTCCTGGGAAGTGACCAA, antisense CCATGTTTGTTCATGGCATC), SREBP-2 [NM_005106.4] (sense GGCCTGACAGGTGAAATCAG, antisense ATAGGGGGCATCAAATAGGC); MTP [NM_000253] (sense AATGACCGGCTGTACAAGCTCAC, antisense CCTTTGAAGATGCTCTTCTCTC); Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) [NM_017008.3] (sense AGACAGCCGCATCTTCTTGT, antisense CTTGCCGTGGGTAGAGTCAT). Quantitative real-time PCR was carried out with an ABI 7300 Real Time PCR Systems (Applied Biosystems) and the mRNA levels were determined by a comparative Ct method.

Statistical Analysis

Data are expressed as means ± s.e.m. Statistical analysis was performed using the Graph Pad Prism statistics software package version 5.0 for Windows. Results were analyzed by Student's t test, followed by Tukey's post-test. The 0.05 probability level was considered to indicate statistical significance.

Results

Study 1

Food and beverage intake

Food intake during the experimental period was decreased in Açai-S compared to Control (15.76 ± 0.04 g /day vs. 22.23 ± 0.88 g / day, respectively; $p < 0.05$). However, supplemented animals consumed 58.88 ± 0.52 mL / day of acai beverage. The beverage intake resulted in a higher total caloric intake by the supplemented rats (103.75 ± 1.77 kcal/day vs. 75.61 ± 2.64 kcal/day; $p< 0.05$).

Body and tissues relative weight gain

The increased caloric intake resulted in higher body weight gain in Açai-S compared to Control (Table 1). The weight of all visceral white adipose tissue depots and liver was increased in Açai-S (Table 1).

	Study 1		Study 2	
	Control	**Açai-S**	**Control**	**Açai-H**
Final body weight (g)	223.00 ± 5.34	243.58 ± 5.37	261.13 ± 3.69	265.34 ± 1.74
Body weight gain (g)	32.93 ± 2.98	51.16 ± 3.03[a]	64.38 ± 7.89	65.78 ± 6.69
Relative body weight gain (%)	17.35 ± 1.56	26.66 ± 1.60[a]	35.01 ± 4.58	34.27 ± 5.14
Tissue absolute weight (g)				
Mesenteric adipose tissue (g)	1.04 ± 0.08	1.56 ± 0.14[a]	1.01 ± 0.09	1.08 ± 0.09
Retroperitoneal adipose tissue (g)	1.46 ± 0.16	2.92 ± 0.33[a]	1.91 ± 0.29	2.05 ± 0.20
Epididymal adipose tissue (g)	1.51 ± 0.17	1.90 ± 0.12[a]	2.15 ± 0.15	2.53 ± 0.18[a]
Liver (g)	6.32 ± 0.17	7.27 ± 0.29[a]	7.13 ± 0.27	7.42 ± 0.21[a]
Gastrocnemius (g)	1.34 ± 0.05	1.41 ± 0.03	1.17 ± 0.18	1.37 ± 0.08[a]
Tissue relative weight (%)				
Mesenteric adipose tissue (%)	0.40 ± 0.06	0.63 ± 0.04[a]	0.63 ± 0.04[a]	0.41 ± 0.03
Retroperitoneal adipose tissue (%)	0.65 ± 0.07	1.18 ± 0.11[a]	0.73 ± 0.08	0.77 ± 0.07
Epididymal adipose tissue (%)	0.78 ± 0.08	1.02 ± 0.06[a]	0.82 ± 0.03	0.95 ± 0.05[a]
Liver (%)	2.83 ± 0.07	2.99 ± 0.11[a]	2.73 ± 0.07	2.79 ± 0.05
Gastrocnemius (g)	0.60 ± 0.01	0.57 ± 0.02	0.45 ± 0.06	0.52 ± 0.03[a]

Data are mean ± s.e.m. n = 8 for control and n=12 for acai. [a]Different from the corresponding control (P<0.05).

Table 1: Effects of short-term consumption of a commercial açai beverage in body weight gain and tissue weight of experimental study groups.

Triacylglyerol liver content and plasma measurements

The triacylglycerol liver content was augmented in supplemented rats (Açai-S = 74.34 ± 16.53 vs. Control = 39.66 ± 3.82 mg triacylglycerol /mg of tissue, P<0.05). Similarly, triacylglycerol plasma levels were also higher in Açai-S than in (Table 2). Despite these negative effects, the chronic consumption of a sugar-sweetened commercial açai beverage promoted an increase in HDL-cholesterol plasma levels and there was no difference in total cholesterol and glucose levels (Table 2).

	Study 1		Study 2	
	Control	**Açai-S**	**Control**	**Açai-H**
Glucose (mg/dl)	137.37 ± 9.97	144.25 ± 8.73	100.78 ± 3.23	96.64 ± 7.47
Triacylglycerol (mg/dl)	54.55 ± 2.96	73.06 ± 4.16 [a]	70.65 ± 7.25	75.87 ± 10.74
Total cholesterol (mg/dl)	69.27 ± 3.76	76.10 ± 4.17	82.27 ± 2.49	88.59 ± 3.63
HDL – cholesterol (mg/dl)	27.14 ± 2.65	34.82 ± 1.48 [a]	38.46 ± 3.31	38.34 ± 1.91
Leptin (ng/ml)	2.01 ± 0.15	3.11 ± 0.39 [a]	1.59 ± 0.32	1.45 ± 0.15
Adiponectin (µg/ml)	17.33 ± 0.76	27.78 ± 1.22 [a]	22.42 ± 3.43	18.34 ± 1.39

Data are mean ± s.e.m. n = 8 - 10. [a]Different from the corresponding control (P<0.05).

Table 2: Effects of açai sweetened juice beverage short term chronic consumption on glucose, lipid profile and adipokines plasma levels of experimental study groups.

The leptin plasma levels were augmented in the supplemented animals (Table 2), and positively correlated with body weight gain (r=0.6; p<0.05). Since plasma leptin levels are reported to be related with increases on adipose tissue mass this result was already expected. However, adiponectin plasma levels were surprisingly higher in supplemented rats (by 60%, p<0.05) (Table 2). Moreover, adiponectin plasma concentration was also positively correlated with body weight gain (r=0.7; p<0.05).

Gene expression

The liver plays a central role in glucose homeostasis and lipid metabolism; thereby we assessed the effects of short-term sugar-sweetened açai beverage consumption on the expression of important key genes: GLUT-2, glycogen synthase-2, MTP and SREBP-2. Liver GLUT-2 and glycogen synthase-2 mRNA expression was reduced in Açai-S. Reduced GLUT-2 mRNA content in the liver has been associated with insulin resistance in this tissue [10] and a decrease in glycogen synthase-2 gene expression suggests modulation of glucose homeostasis and possible impairment of hepatic glycogen synthesis. There were no alterations regarding the other studied genes (Figure 1).

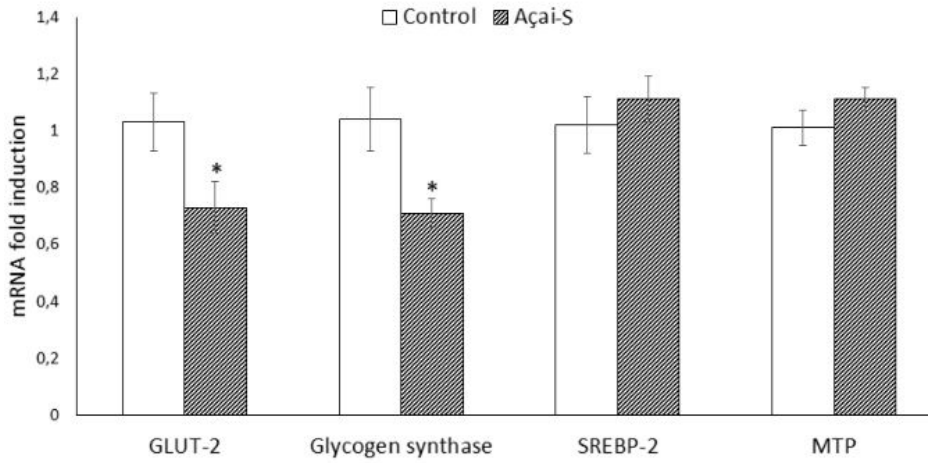

Figure 1: Gene expression of genes related to lipid and glucose metabolism in the liver of animals supplemented with a commercial glucose-sweetened açai beverage and control (study 1). Data are mean ± s.e.m. (n = 8 - 12). * P < 0.05.

Study 2

Food and beverage intake

Food intake during the experimental period was decreased in Açai-H, when compared with control (14.27 ± 0.37g/day vs. 20.75 ± 1.14g/day, respectively; $p<0.05$). Moreover, supplemented rats consumed 84.24 ± 3.72 mL of açai beverage per day. When total drink and food consumption was analysed, Açai-H showed higher total caloric intake than the rats (107.29 ± 3.20 Kcal/day vs. 70.66 ± 275 Kcal/day, respectively; $p<0.05$).

Body and tissue relative weight gain

Despite the Açai-H augmented caloric intake, both groups showed similar body weight gain (Table 1). Similarly, no differences were found regarding the relative weight of liver, retroperitonial white adipose tissue and mesenteric white adipose tissue, after the experimental period (Table 1). Açai beverage consumption presented solely an effect on the relative weight of the epididymal white adipose tissue and of the gastrocnemius. It is important to note that some supplemented rats showed moderate diarrhea episodes in the first week of supplementation, which was completely abolished along the following week.

Plasma measurements

Table 2 shows the plasma lipid profile, as well as glucose, leptin and adiponectin plasma concentration. There was no difference between the groups regarding all these measurements.

Cytokine protein expression

To assess the possible anti-inflammatory role of the açai beverage we measured cytokine expression in the visceral white adipose tissue (retroperitoneal, epididymal and mesenteric pads), liver and gastrocnemius. The protein content of IL-6 and TNF-alpha, two important inflammatory cytokines, as well as of IL-10, the major anti-inflammatory cytokine, is described in Table 3. Cytokine expression showed depot-specific response inin the white adipose tissue in Açai-H. In the retroperitoneal depot, reduced TNF-alpha expression was observed, resulting in a modified IL10/TNF-alpha ratio. Moreover, IL-10 levels were increased by 56 % in the mesenteric white adipose tissue of Açai-H.

	Control	Açai-H
Retroperitoneal white adipose tissue		
IL-6 (ng.mg of protein^{-1})	918.73 ± 338.63	374.34 ± 89.72
IL-10 (ng.mg of protein^{-1})	156.77 ± 38.84	136.72 ± 9.62
TNF-alpha (pg.mg of protein^{-1})	582.03 ± 104.32	213.84 ± 50.22 [a]
IL-10/TNF-alpha ratio	0.36 ± 0.02	1.10 ± 0.21 [a]
Mesenteric white adipose tissue		
IL-6 (ng.mg of protein^{-1})	26.09 ± 15.80	11.77 ± 1.78
IL-10 (ng.mg of protein^{-1})	18.03 ± 6.37	28.14 ± 2.22 [a]
TNF-alpha (pg.mg of protein^{-1})	108.53 ± 33.57	196.74 ± 54.59
IL-10/TNF-alpha ratio	0.17 ± 0.06	0.28 ± 0.07
Epidydimal white adipose tissue		
IL-6 (ng.mg of protein^{-1})	96.86 ± 33.10	95.24 ± 17.87

IL-10 (ng.mg of protein^{-1})	8.47 ± 2.22	10.57 ± 1.01
TNF-alpha (pg.mg of protein^{-1})	53.51 ± 21.50	18.12 ± 9.58
IL-10/TNF-alpha ratio	0.26 ± 0.12	0.93 ± 0.54
Liver		
IL-6 (ng.mg of protein^{-1})	23.46 ± 1.85	23.44 ± 1.63
IL-10 (ng.mg of protein^{-1})	5.28 ± 0.65	4.56 ± 0.24
TNF-alpha (pg.mg of protein^{-1})	967.55 ± 109.98	930.03 ± 53.93
IL-10/TNF-alpha ratio	0.54 ± 0.02	0.49 ± 0.02
Muscle		
IL-6 (ng.mg of protein^{-1})	0.18 ± 0.03	0.21 ± 0.02
IL-10 (ng.mg of protein^{-1})	0.11 ± 0.01	0.11 ± 0.01
TNF-alpha (pg.mg of protein^{-1})	37.03 ± 3.49	40.26 ± 2.38
IL-10/TNF-alpha ratio	0.31 ± 0.01	0.25 ± 0.02

Data are mean ± s.e.m. n = 8 - 10.*P < 0.05.

Table 3: Cytokines (Il-10, TNF-alpha and IL-6) levels in the white adipose tissue, liver and gastrocnemius muscle of control and supplemented rats.

Thiobarbituric acid reactive species (TBARS)

Many studies have shown that the açai fruit presents in vitro [9,19] and in vivo [20,21] antioxidant capacity. Therefore, lipid peroxidation in the visceral white adipose tissue pads, liver and gastrocnemius was assessed (Figure 2). Açai-H had a significant reduction in TBARS formation in the retroperitoneal white adipose tissue and epididymal white adipose tissue of 49.75 % and 44.90 % respectively ($p<0.05$), in relation to the Control.

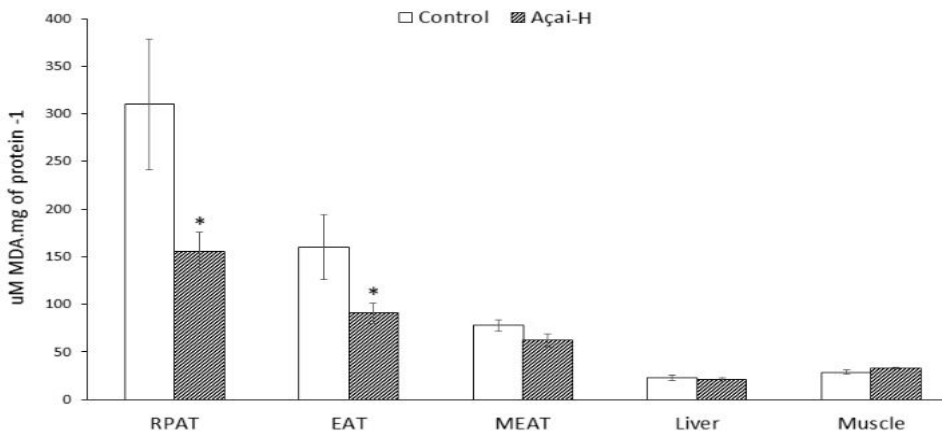

Figure 2: Malondialdehyde (MDA) concentration in the visceral white adipose tissue depots (retroperitoneal, epidydimal and mesenteric), liver and muscle of animals supplemented with a commercial honey-sweetened acai beverage and its control (study 2). Data are mean ± s.e.m. (n = 6 - 10). RPAT: retroperitoneal white adipose tissue, EAT: epididymal white adipose tissue, MEAT: mesenteric white adipose tissue. * P< 0.05.

Discussion

Açai is one of Amazon´s most popular functional foods and widely consumed in the world [22]. Commercial açai beverages claim health benefits due to the antioxidant and anti-inflammatory properties of this fruit. However, our results show that the benefits of consuming these beverages depends on the sweetener used.

We demonstrated that short-term consumption of a honey-sweetened açai beverage is able to modulate cytokine levels in the visceral white adipose tissue, in a depot-specific manner. Results also revealed that this supplementation was able to reduce oxidative stress markers in two visceral white adipose tissue pads.

White adipose tissue is an important endocrine organ being involved in the regulation of many pathological processes [23]. The white adipose tissue is able to secrete a plethora of factors, including cytokines (e.g. TNF-alpha, IL-10, IL-6) and hormones (e.g. leptin and adiponectin), acting locally and distally, with autocrine, paracrine and endocrine actions [24]. Several morpho-functional differences have been reported among intra-abdominal visceral white adipose tissue and peripheral subcutaneous white adipose tissue [25]. Visceral adipose tissue secretessecreares higher amounts of pro-inflammatoryamountshigher of higher amounts of pro-inflammay cytokines, such as TNF- alpha and IL-6, which are associated with many disease conditions (e.g. obesity, metabolic syndrome, diabetes, etc.).

Short-term consumption of a commercial honey-sweetened açai beverage induced an increase in IL-10 protein content in the mesenteric white adipose tissue, as well as a reduction in TNF-alpha protein levels in the retroperitoneal pad. Moreover, the reduced TNF-alpha expression in the retroperitoneal depot resulted in a modified IL-10/TNF-alpha ratio. Consistent with our observation, Xie et al. [14] showed a reduction on serum levels, gene expression and protein levels of TNF-alpha and IL-6 in resident macrophages from mice fed with açai. Other authors also described that oral administration of an açai stone extract reduced the increase in TNF-alpha expression in the lung of animals exposed to cigarette smoke [26]. Furthermore, a recent research described that açai frozen pulp ingestion prevented increase in IL-1beta, IL-18 and TNF-alpha, reducing the carbon tetrachloride-induced damage in rat brain tissue [27].

TNF-alpha is the most-studied cytokine in white adipose tissue. This cytokine is involved in metabolic, physiological and immunological regulation in this tissue, acting as a mediator of inflammation. On the other hand, IL-10 secreted by adipocytes, white adipose tissue stromal vascular fraction and tissue matrix, inhibits the production of several cytokines, such as TNF-alpha, IL-1beta and IL-6 [28]. IL-10/TNF-alpha ratio has been adopted as an indicator of the inflammatory status and disease-associated morbidity, with lower values associated with poorer prognoses [29].

Açai has been reported to contain many bioactive compounds. Major polyphenolic components in açai pulp include anthocyanins, proanthocyanidins, other flavonoids and lignans [30,31]. Among them, the flavonoids were found to be the major polyphenols. Flavonoids from açai pulp present anti-inflammatory effects at least in part through inhibition of NF-kB activation [32] and by modulating the Toll-Like Receptor-4 (TLR-4) and NF-kB protein expression [33]. NF-kB is a key mediator of inflammation in adipocyte cells and studies have shown a close relationship between TLR-4 and the activation of the NF-κB pathway, which leads to the elevation of pro-inflammatory adipokine genes and protein expression in adipose tissues [34,35].

Flavonoids have been shown, as a group, to exhibit strong antioxidant capacities. The mechanism responsible for the antioxidant activity of flavonoids involves the direct scavenging or quenching of oxygen free radicals or excited oxygen species, as well as the inhibition of oxidative enzymes that generate these reactive oxygen species [36]. We found lower MDA levels in two visceral white adipose tissue pads (retroperitoneal and epididymal) in Açai-H. Studies have shown a similar reduction in MDA content in different tissues of animals treated with anthocyanins or proanthocyanidins- rich fruit extracts [37-39]. Moreover, the açai antioxidant effect was previously shown in the serum and in the liver of ApoE deficient mice [14], in the serum of healthy adults [10] and liver of mice fed with a high-fat diet [8].

The improvement of anti-inflammatory/antioxidant profile of all visceral white adipose tissue depots indicates a protective effect of the short-term honey-sweetened commercial açai beverage consumption against chronic diseases. It is interesting to note that even with the higher epididymal white adipose tissue absolute and relative weight, the Açai-H cytokine profile was not altered. In addition, the MDA content was increased in this tissue.

Adipokines play a role in a wide variety of physiological and pathological process, including immunity and inflammation, in addition to having significant effects on metabolism. Among them, leptin and adiponectin are the most widely investigated. Leptin has a pivotal role in the control of food intake and plasma leptin levels are related with increases on adipose tissue mass. Therefore, an increase in leptin plasma levels only in Açai-S was already expected. Adiponectin is an anti-inflammatory and insulin-sensitising adipokine, playing a central role in glucose and lipid metabolism [40]. Considering that generally adiponectin plasma levels are inversely correlated with body weight, the increased adiponectin plasma level in Açai-S are very intriguing. Studies have reported that chronic consumption of procyanidins or plant sterols, both compounds present in açai, is able to modulate inflammation and oxidative stress by reducing inflammatory markers and

increasing adiponectin plasma levels [41-43]. We hypothesize that this increase in adiponectin plasma levels could be a physiological response to counteract the inflammatory profile induced by the consumption of a glucose-rich beverage. In this respect, a recent study described a similar increase in adiponectin serum levels of rats fed with a high glucose diet [44]. The authors suggest that this effect is due to the pro-inflammatory microenvironment of the adipose tissue of these rats.

Diets rich in sucrose have been strongly associated with an increased prevalence of obesity, type 2 diabetes and cardiovascular risk factors. High sucrose feeding is able to induce steatosis, hepatic insulin resistance and hypertriglyceridemia [44,45]. In this study, the short-term consumption of a glucose-sweetened açai beverage resulted in high liver and plasma triacylglycerol content. Despite these negative effects, Açai-S animals showed increase in HDL-cholesterol plasma levels. Similarly, an increase in HDL-cholesterol in apolipoprotein deficient mice fed with açai was previously described [14]. On the other hand, the consumption of the honey-sweetened açai beverage failed to show any improvement on plasma lipid profile. Some studies reported açai hypocholesterolemic effect in pathological conditions [7,14,20]. Thus, it could be possible that the beneficial effect of açai is more pronounced when some alteration in plasma lipid profile is present.

The liver is the primary organ responsible for glycogen and lipid metabolism. Biosynthesis of glycogen and lipids is the primary means by which the body stores excess nutrients. Under normal conditions, glycogen is the primary storage form of excess energy. Glycogen production is regulated primarily via enzymes such as glycogen synthase [46]. The reduction in the glycogen synthase and GLUT-2 gene expression observed in Açai-S indicates impaired liver glucose metabolism in these animals. Enhanced lipogenesis and decreased glycogen synthesis are hallmarks of hepatic insulin-resistance, which might subsequently lead to the development of type 2 diabetes mellitus [47]. Adipose tissue physiology is an important contributor to the regulation of insulin resistance and fatty liver disease [46]. Many of the interactions among adipose tissue, insulin resistance, and hepatic steatosis are orchestrated by adipokines. Adiponectin expression was associated with the up-regulation of insulin-sensitizing genes in the liver (i.e., GLUT-2 and PPARγ) in an obesity model of insulin resistance [48]. Therefore, we can hypothesize that the increase in adiponectin plasma levels could be a physiological response to counteract not only the inflammatory response, but, as well the disruption in liver glucose metabolism induced by the consumption of a glucose-rich beverage.

It is important to note that commercial beverages used in our study contained other bioactive compounds besides açai. Antioxidant effects have been described for acerola (*Malpighia emarginata*) [49,50], lime (*Citrus limon*) [51], guarana (*Paullinia cupana*) [52] and honey [53]. Interestingly, a recent study showed that the intake of acerola juice decreased the level of inflammatory proteins in the adipose tissue of obese rats [54]. The antioxidant effect of acerola is attributed to the high vitamin C level, as well as to the polyphenols content in this fruit [49]. In addition, guarana also exhibits an important stimulant property because of its high caffeine content [55]. Açai-H animals had a higher caloric intake, but the weight gain during the experimental period was similar to control animals. We can speculate that the presence of guarana in the beverage could be responsible for such intriguing effect. Guarana has a high caffeine content, which varies from 3% to 6% in the dried seeds [56-60]. Numerous studies described the beneficial effect of caffeine on energy expenditure [61-65], therefore, it can potentially be considered as a body-weight regulator.

In summary, the short-term consumption of a honey-sweetened açai beverage was able to modulate cytokine levels and reduce oxidative stress markers in the visceral white adipose tissue, in a depot-specific manner. These data suggest a protective effect of the short-term consumption of a honey-sweetened açai beverage against conditions characterized by oxidative stress and inflammation. Moreover, the short-term consumption of an açai beverage containing a high glucose concentration, as that present in most commercially available beverages, leads to alteration of body composition, lipid and carbohydrate metabolism. Nevertheless, the consumption of this beverage interestingly induced an enhancement in adiponectin plasma levels, which could represent a compensatory response aimed at controlling the metabolic disruption induced by supplementation.

Author Contributions

R.X.N., F.O.R., M.J.A. and R.G.C. carried out all animal studies; R.S., A.F.A.M. and E.C. designed the study; R.S. and M.S. have written the manuscript; M.S. has supervised the study.

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