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ORIGINAL CONTRIBUTION

Obesity-related inflammatory modulation by juçara berry (*Euterpe edulis* Mart.) supplementation in Brazilian adults: a double-blind randomized controlled trial

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Abstract

Purpose Obesity is an inflammatory-related disease, which recruits immune system cells triggering to imbalanced production of cytokines. Obesity management and treatment using foods bioactive compounds have gained clinical and scientific relevance. Juçara (*Euterpe edulis* Mart.) fruit is rich in fibers, unsaturated lipids and, anthocyanins showing potential health benefits. Thus, we investigated the effect of juçara pulp intake on inflammatory status of monocytes from obese individuals.

Methods It is a placebo-controlled, randomized double-blind trial. Twenty-seven obese participants (BMI between 30.0 and 39.9 kg/m^2) of both genders from 31 to 59-year-old, divided into two groups: 5 g juçara freeze-dried pulp or 5 g of placebo for 6 weeks. Before and after supplementation, blood samples were collected and monocytes obtained and stimulated with lipopolysaccharides. After 24 h of incubation, the cells and supernatants were analyzed.

Results Post-treatment, juçara reduced TLR4, and IL-6 mRNA compared to placebo. Juçara also increased IL-10 mRNA in post-treatment. The protein expression of TLR4 pathway post-treatment, MYD88 expression reduced in juçara group compared to placebo. The juçara post-treatment reduced pIKKα/β compared to the placebo. Ob-R protein levels were higher in the juçara group post-treatment compared to pre-treatment. IL-6, TNF-α, and MCP-1 production by monocytes were reduced by juçara in post-treatment compared to pre-treatment levels. The supplementation increased IL-10 in juçara group with LPS compared to pre-treatment and versus juçara group without LPS.

Conclusion These results demonstrated a proinflammatory state at the beginning, which was improved by juçara pulp consumption. Our results suggest juçara pulp as a potential tool against the proinflammatory status of obesity.

Keywords Obesity · Monocytes · Inflammation · Juçara · TLR4 pathway

Introduction

Obesity chronic subclinical inflammation is a widely accepted concept and a vast research field nowadays. Indeed, one important trigger of the proinflammatory status in obesity is the recruitment of monocyte cells and the activation of macrophages.

Monocytes are precursor cells of the innate immune system circulating in peripheral bloodstream, when infiltrated in adipose or muscular tissues it differentiates into macrophages, which plays a key role in inflammatory processes [1]. Differentiation of macrophages can occur in two phenotypic subtypes known as classic M1 or alternative M2. The M1 macrophages have classical proinflammatory characteristics, commonly found in adipose tissue of obese humans and animal models triggering the

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proinflammatory cellular response. On the other hand, M2 macrophages are present in the adipose tissue of lean individuals and participate in the maintenance of insulin sensitivity of adipocytes, as well as the production of anti-inflammatory cytokines [2–4].

A common mechanism activated in macrophages is the inflammatory process mediated by toll-like receptors (TLRs) [5, 6]. Specifically, TLR4 receptors have been demonstrating they play critical roles in the pathogenesis of inflammatory noninfectious diseases, such as cardiovascular diseases, insulin resistance, obesity and metabolic syndrome [7–9].

The search for obesity prevention tools, able to modulate the inflammatory status through superfoods, has been widely explored. In this sense, juçara (*Euterpe edulis* Mart.) is a berry from South America rainforest rich in anthocyanins, fibers and monounsaturated fatty acids [10–12]. Despite being a recently well-known food, different usage of the fruit has been explored in the literature [13]. Indeed several experimental animal model studies have already demonstrated its potential anti-inflammatory and anti-obesity effects [14–19]. However, the effect of juçara intake in human obesity subclinical inflammation has not been well explored. Therefore, the purpose of this study was to investigate the effect of juçara pulp intake on the inflammatory status of monocytes isolated from peripheral blood cells from obese individuals.

Materials and methods

Experimental design

This was a placebo-controlled randomized double-blind study. The randomization was carried out with software assistance (http://www.random.org), as a blind spot for the study right after the participant's agreement and initial evaluations. Subsequently, the subjects were double-blinded randomized into the named: placebo group or juçara group. The experimental design is shown in Fig. 1.

The exclusion criteria adopted were: not fitting in the stipulated BMI range (BMI $\leq 30.0 \geq 39.9$ kg/m^2); infectious diseases and/or severe chronic diseases; usage of medication that interferes in the inflammatory cascade, lipid metabolism, and food consumption; alcohol and drug abuse; pregnancy and menopause.

The study procedures were in accordance with the guidelines set in the International Declaration of Helsinki and approved by the Ethics Committee of the Universidade Federal de São Paulo (CEP-UNIFESP No. 0319/2017) and registered in Plataforma Brasil database. The written informed consent was obtained from all volunteers.

Participants description

This study enrolled twenty-seven obese volunteers (inclusion criteria of BMI $\geq 30.0 \leq 39.9$ kg/m^2) of both genders, aged 31–59 [20]. Anthropometric descriptive data of weight,

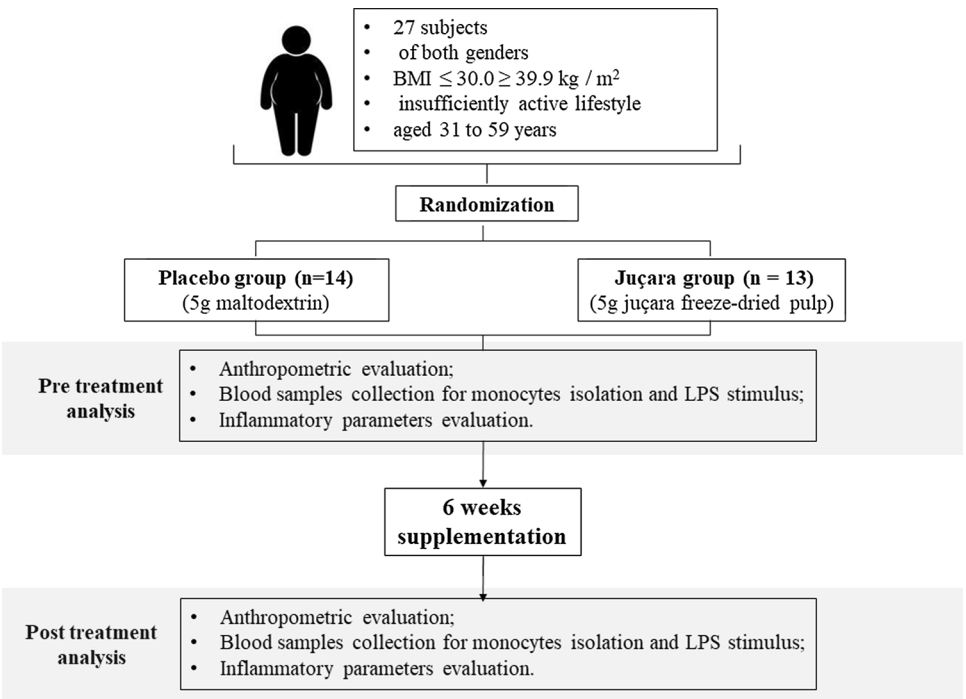

Fig. 1 Fluxogram describing the recruitment of volunteers and the experimental design carried out on this study

height and waist circumference were obtained at pre-treatment and post-treatment of 6-week juçara supplementation. Body mass index (BMI) was calculated and classified as recommended by the World Health Organization. The waist-to-height ratio (W/H) was also calculated considering a 0.5 cut-off point [21]. The sample anthropometric characteristics are described in Table 1.

The dietary intake was assessed at pre-treatment and post-treatment period, through a self-reported 3-day Dietary Record and analyzed with the specific software Avanutri 4.0 Revolution (Avanutri and Nutrition Services and Informatics Inc., Três Rios, RJ, Brazil). The diet patterns did not change among groups or evaluation time, these results were previously published [22].

Juçara supplementation

To proceed with the supplementation, juçara pulp was purchased from the Agroecological Juçara Project—Instituto de Permacultura e Ecovilas da Mata Atlântica (Ubatuba, SP, Brazil). The pulp was previously separated from peel and seed in a depulper equipment and, sequent freeze-dried to produce the pulp powder used for the supplementation protocol. The preference for using the pulp is justified considering that this fraction represents the most nutritive part of the fruit and the main source of bioactive compounds as previously described by Inada et al. [23].

The volunteers received the portioned sachets weekly, containing 5 g of the freeze-dried juçara pulp or 5 g of artificially flavored maltodextrin as a placebo corresponding the allocated group. All the volunteers were instructed to consume one sachet per day in the morning period in the way they preferred during the 6-week period of supplementation.

The chosen dose of 5 g of freeze-dried juçara pulp per day was equivalent to the consumption of 50 g of fresh juçara pulp, which could be consumed as one ice cream ball. The fruit dosage was based on previous studies considering the anthocyanins content of the pulp, as well as on our previous dose–response test to offer a physiological consumption which exerts inflammatory modulation [14, 15, 17, 19]. The dose used was also safe for consumption in agreement to juçara cytotoxicity previously investigated by Felzenszwalb et al. [24]. The juçara (*E. edulis* Mart.) composition was summarized by Santamarina et al. [14] and presented in Table 2.

Blood cells separation

Blood samples (20 mL) were collected from the antecubital vein in tubes with 125 IU of heparin sodium, at pre-treatment and post-treatment period. To obtain peripheral blood mononuclear cells, the separation was done by density using Histopaque 1077 and Histopaque 1119 after 30 min of centrifugation at 400×*g* at room temperature. To isolate monocytes, cell culture was required, after incubation, the monocytes got adhered to the plate, while the lymphocytes

Table 2 Juçara composition adapted from Santamarina et al. [14]

Juçara pulp	Concentration in 100 g of fresh matter		References
	Mean	SEM	
Carbohydrates (g)	28.3	3.5	[10]
Proteins (g)	6.0	0.3	[10]
Lipids (g)	29.2	0.9	[10]
SAT (%)	37.44		[11]
MUFA (%)	38.57		[11]
PUFA (%)	20.08		[11]
Fiber (g)	28.3	0.3	[10]
Ashes (g)	8.8	0.8	[10]
Energetic values (kcal)	400.0	23.9	[10]
Cyanidin 3-rutinoside (mg)	191.0	6.5	[18]
Cyanidin 3-glucoside (mg)	71.4	2.1	[18]
Total anthocyanins (mg)	262.4	8.6	[18]
Total phenolic compounds (mg)	415.1	22.3	[10]

SFA total of dietary saturated fatty acids, *MUFA* total of dietary monounsaturated fatty acids, *PUFA* total of dietary polyunsaturated fatty acids

Table 1 Sample descriptive characteristics

	Placebo ($n=14$)		Juçara ($n=13$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Male/female	06/08		05/08	
Age (years)	45.07 ± 3.42		45.76 ± 2.58	
Stature (m)	1.65 ± 0.03		1.66 ± 0.03	
Body mass (kg)	92.23 ± 3.29	92.91 ± 3.57	96.42 ± 4.85	96.81 ± 5.09
BMI (kg/m^2)	33.82 ± 0.71	34.06 ± 0.81	34.63 ± 1.20	34.76 ± 1.30
WC (cm)	102.70 ± 2.66	101.54 ± 2.38	104.59 ± 2.43	104.83 ± 2.85
W/H	0.62 ± 0.02	0.61 ± 0.01	0.63 ± 0.02	0.63 ± 0.01

WC waist circumference, *W/H* waist/height ratio

were dispersed in the supernatant. Sequent, the cell culture medium containing the lymphocytes was discarded. With the monocytes adhered to the plate, the cells were incubated for 24 h with or without LPS in RPMI-1640 medium supplemented with fetal bovine serum and antibiotics (2.5 mg/mL streptomycin and 2.5 IU/mL penicillin) at 37 °C. The supernatants and monocytes adhered to the plate were collected to carry out the next experimental analyses. The experimental groups' design after randomization and cell culture procedures used to perform the subsequential analyzes were displayed in Table 3 to clarify the symbols presented in results graphs.

RNA extraction and RT-PCR

Total RNA from monocyte cells was extracted with Trizol Reagent® (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. A NanoDrop ND-2000 (NanoDrop Technologies Inc., Wilmington, DE, USA) measured the RNA concentration. The RNA purity was observed by a 260/280 nm ratio, which must range between 1.8 and 2.0. Two micrograms of RNA sample were reverse transcribed using a High-capacity cDNA reverse transcription kit (Thermo Fisher Scientific), and complementary DNA was synthesized. The specific primers sequences used were described in Table 4.

The mRNA levels were quantified by RT-PCR using SYBR green PCR master mix in a QuantStudio™ 7 Flex (Thermo Fisher Scientific). The HPRT (Hypoxanthine phosphoribosyltransferase) gene level was used as housekeeping. Results were obtained using the Sequence Detector software (Thermo Fisher Scientific) and expressed as relative units using the method of $2^{-\Delta\Delta C_t}$ previously described by Livak and Schmittgen [25].

Western blotting

Monocyte cell pellets were eluted and incubated in a specific lysis buffer for 30 min. The samples were centrifuged at 20,800×*g* for 40 min at 4 °C, and the supernatant was collected. The total protein concentration was measured by Bradford reagent (LGC Laboratories, Inc., Cotia, SP, Brazil) and normalized to 50 µg per sample. The 10% SDS polyacrylamide gel was performed to separated protein samples by electrophoresis and transferred to nitrocellulose membranes (Bio-Rad Laboratories Inc., Hercules, CA, USA). The membranes were blocked overnight with 1% bovine serum albumin solution at room temperature. The membranes were incubated overnight with the following primary antibodies: pNF-κBp50 (sc-101744), IL6-Rα (sc-374259), IL-10Rα (sc-365374) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and TLR4 (ab22048), TLR2 (ab108998), MYD88 (ab2064), TRAF6 (ab33915), pIKKα/β (ab195907), TNFR1 (ab19139) Ob-R (ab5593). The β-actin (ab6276; Abcam, Cambridge, UK) was used as housekeeping. The specific horseradish peroxidase-conjugated secondary antibodies were incubated for 1 h at room temperature. Bands were visualized

Table 3 Groups distribution after randomization and cell isolation assay resulting in eight different experimental groups, and the correspondent symbols used in graphs

Evaluation time	Pre-treatment				Post-treatment			
Supplementation	Placebo		Juçara		Placebo		Juçara	
Stimulus	Without LPS	With LPS	Without LPS	With LPS	Without LPS	With LPS	Without LPS	With LPS
Symbols in graph	(−) (−)[a]	(−) (+)	(+) (−)	(+) (+)	(−) (−)	(−) (+)	(+) (−)	(+) (+)

[a]This group was used as control for all the comparative analyses performed

Table 4 Primer sequences used in the performance of RT-PCR

Target genes	Sequences	
	Sense	Antisense
TLR4	5′-GCTCGGTCAGACGGTGATAG-3′	5′-TAGGAACCACCTCCACGCAG-3′
TLR2	5′-TTCTCTCAGGTGACTGCTCGG-3′	5′-AACCCTGTCTTCCTGCCTTCA-3′
IL10Rα	5′-CTCAGACGCTCATGGGACAGA-3′	5′-ACAGGGTCTGGCTACAGTTG-3′
IL-6R	5′-GACTGTGCACTTGCTGGTGGAT-3′	5′-ACTTCCTCACCAAGAGCACAGC-3′
ADIPOR2	5′-AGACACGCGGATCAACTCAC-3′	5′-CTGCACCCCAATCGGTTTTC-3′
Ob-R	5′-ACACCAGAGTGATGCAGGTT-3′	5′-TGCTCAAACGTTTCTGGCTTC-3′
IL-10	5′-CGGCGCTGTCATCGATTTCT-3′	5′-GTTTCTCAAGGGGCTGGGTC-3′
TNF-α	5′-ACTTCCTCACCAAGAGCACAGC-3′	5′-GATGGCAGAGAGGAGGTTGAC-3′
HPRT	5′-CCCTGGCGTCGTGATTAGTG-3′	5′-TCGAGCAAGACGTTCAGTCC-3′

using enhanced chemiluminescence scanned using Alliance 4.7 equipment (Cambridge, UK) after adding ECL reagent (Bio-Rad Laboratories, Inc, Hercules, CA, USA). Scion Image (Scion Image-Release Beta 3b; NIH, Frederick, MD, USA) quantified the band intensity.

Cytokines protein content (IL-6, IL-10, TNF-α, and MCP-1) produced by Monocytes

After 24-h cells incubation, the culture medium was saved, and total protein concentrations determined using NanoDrop ND-2000 (NanoDrop Technologies Inc., Wilmington, DE, USA). Specific ELISA kits (DuoSet ELISA; R&D Systems, Minneapolis, MN, USA) were used to assess the TNF-α, IL-6, IL-10 and MCP-1 protein levels according to the manufacturer datasheet.

Statistical analyses

The sample size was based on G*Power software calculation [26] using the value of effect size as 0.8, power ($1-\beta$ err prob) was set at 95%, and α at 0.05. It was also added a safety margin of 20%, resulting in a sample of 28 volunteers. For the RT-PCR and western blotting analyses, it was performed as a sub-sample calculation following the steps above cited and considering previously reported means and standard errors of the mean [10, 23–28]—this calculation resulted in a sample size of at least four per group for a reliable statistic result.

The data were submitted to the quality tests of Shapiro–Wilk (normality), Levene's (homogeneity) and Grubb's test for outliers. For parametric variables, the analysis of variance (ANOVA) two-way followed by Bonferroni post hoc test was used to compare the groups. For non-parametric variables, we performed the Kruskal–Wallis test. For all comparisons analysis performed, we considered the pre-treatment placebo group without LPS stimulus as the control. All statistical tests were performed in the PASW Statistics 22 software. The results were expressed as the mean ± standard error of the mean (SEM) and the significance level adopted was $p \leq 0.05$.

Results

There was no significant difference (pre versus post-treatment) in the dietary intake of total energy or macronutrients in the treatment and placebo groups, as previously published [22].

Inflammation gene expression in the monocytes from obese adults

At pre-treatment, the placebo group and juçara group demonstrated an increase in TLR4 mRNA caused by the LPS exposure ($p = 0.021$ and $p < 0.001$) compared to the non-stimulated cells. Likewise, under LPS stimulus the TLR4 mRNA pre-treatment was higher in the juçara group versus the placebo group ($p = 0.022$). Post-treatment, the juçara supplementation reduced TLR4 mRNA compared to placebo with or without LPS ($p = 0.016$ and $p = 0.013$ respectively). Therefore, the post-treatment juçara group reduced the TLR4 mRNA under LPS compared to pre-treatment measures ($p < 0.001$) as shown in Fig. 2a. There are no differences in TLR2 mRNA expression (Fig. 2b).

The IL-6 mRNA was increased in pre-treatment juçara group with LPS compared to without LPS group ($p = 0.014$). However, post-treatment analysis demonstrated significant decrease in all the experimental groups compared to pre-treatment measures (placebo $p = 0.003$; placebo with LPS $p < 0.001$; juçara $p < 0.001$; juçara with LPS $p < 0.001$). Post-treatment is noteworthy the reduction of juçara IL-6 mRNA levels compared to the placebo group ($p = 0.024$ and $p < 0.001$) in Fig. 2e. The IL-6R, AdipoR2, and TNF-α mRNA levels did not differ among the experimental groups (Fig. 2c, d, g).

The IL-10 mRNA levels post-treatment in the juçara groups were higher than post-treatment placebo ($p = 0.001$; $p = 0.001$) and pre-treatment juçara groups ($p = 0.011$; $p < 0.001$) in Fig. 2f.

The Ob-R mRNA was higher in the post-treatment juçara with LPS group compared to placebo with LPS group ($p = 0.006$), juçara without LPS ($p = 0.001$), and higher than juçara with LPS pre-treatment ($p < 0.001$) as shown in Fig. 2h.

Inflammation protein expression in the monocytes from obese adults

The data on the inflammatory protein expression of the TLR4/NFκB pathway were evaluated. Despite the fact of no changes in TLR4 and TRAF6 expression, the MYD88 (primary response gene of myeloid differentiation 88) demonstrated to be modulated by the juçara supplementation. In the post-treatment, MYD88 expression reduced in juçara group with LPS, compared to its placebo ($p = 0.021$). In the same way, MYD88 in the placebo group with LPS was higher in post-treatment than baseline measures ($p = 0.044$). The activity of pNFκBp50 was also evaluated and it did not differ among the groups, however, the pIKKα/β was reduced in the post-treatment period in juçara group versus placebo group both without LPS ($p = 0.008$). Furthermore, pIKKα/β

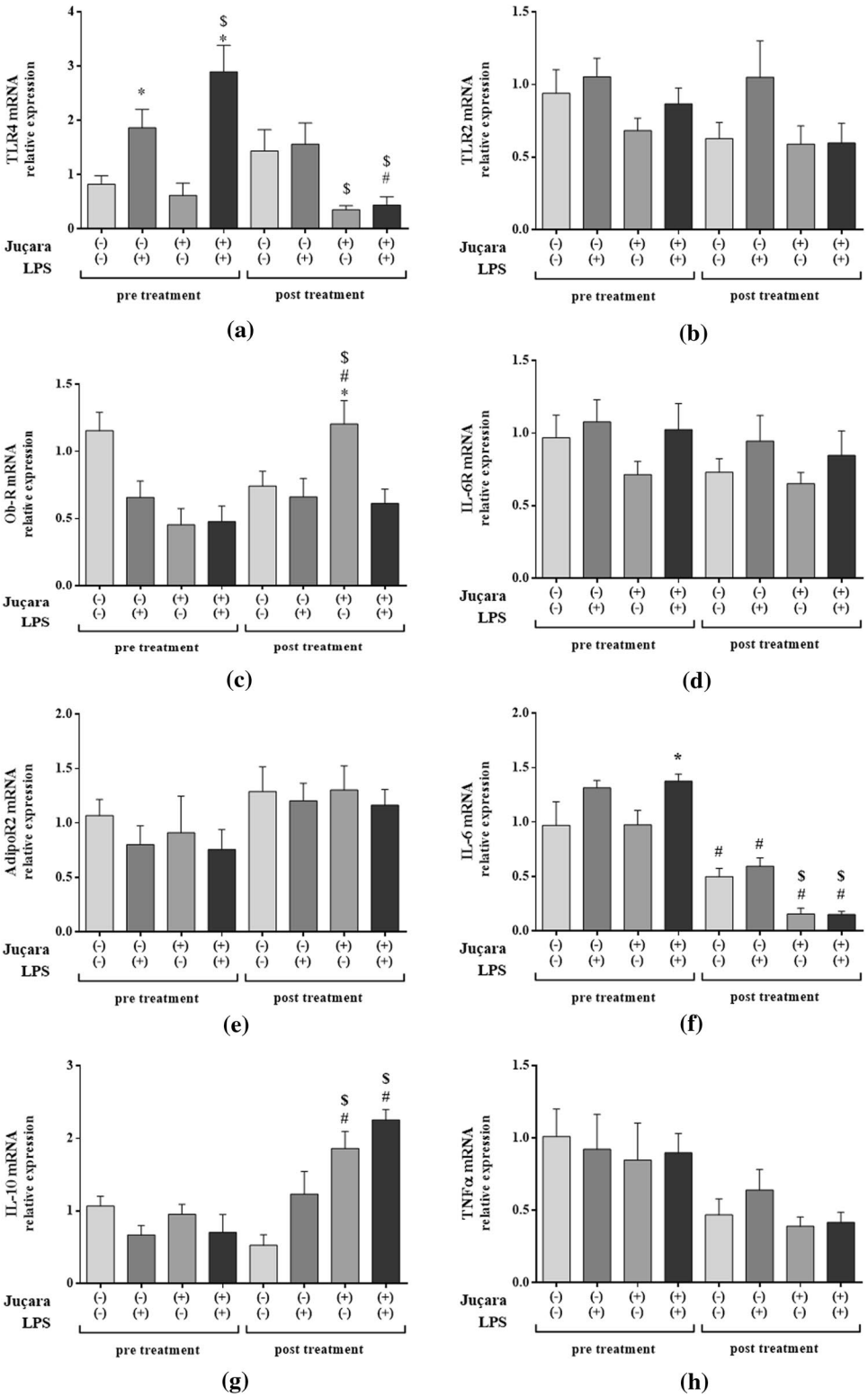

Fig. 2 Monocyte inflammatory markers gene expression between groups before and after supplementation for 6 weeks: **a** TLR4; **b** TLR2; **c** IL-6; **d** AdipoR2; **e** IL-6; **f** IL-10; **g** TNF-α; **h** Ob-R. The housekeeping used for all analyses was HPRT gene expression. The data was normalized by the pre-treatment placebo without LPS group (first bar in the graph) on RT-PCR. The groups description pre-treatment and post-treatment: placebo group without LPS (−) (−); placebo with LPS (−) (+); juçara without LPS (+) (−); juçara with LPS (+) (+). *Without LPS versus with LPS; #baseline versus post-treatment; $placebo versus juçara group ($n = 10$ per group)

in the placebo group without LPS was higher than placebo with LPS ($p = 0.023$; Fig. 3a–e).

The cytokines membrane receptors were also analyzed, in this sense, there are no differences in IL-6Rα, IL-10Rα, TNFR1, and ADIPOR2 did not show differences with placebo either pre or post-treatment period. Although Ob-R levels were higher in juçara group without LPS after treatment compared to baseline expression ($p = 0.037$; Fig. 4a–e).

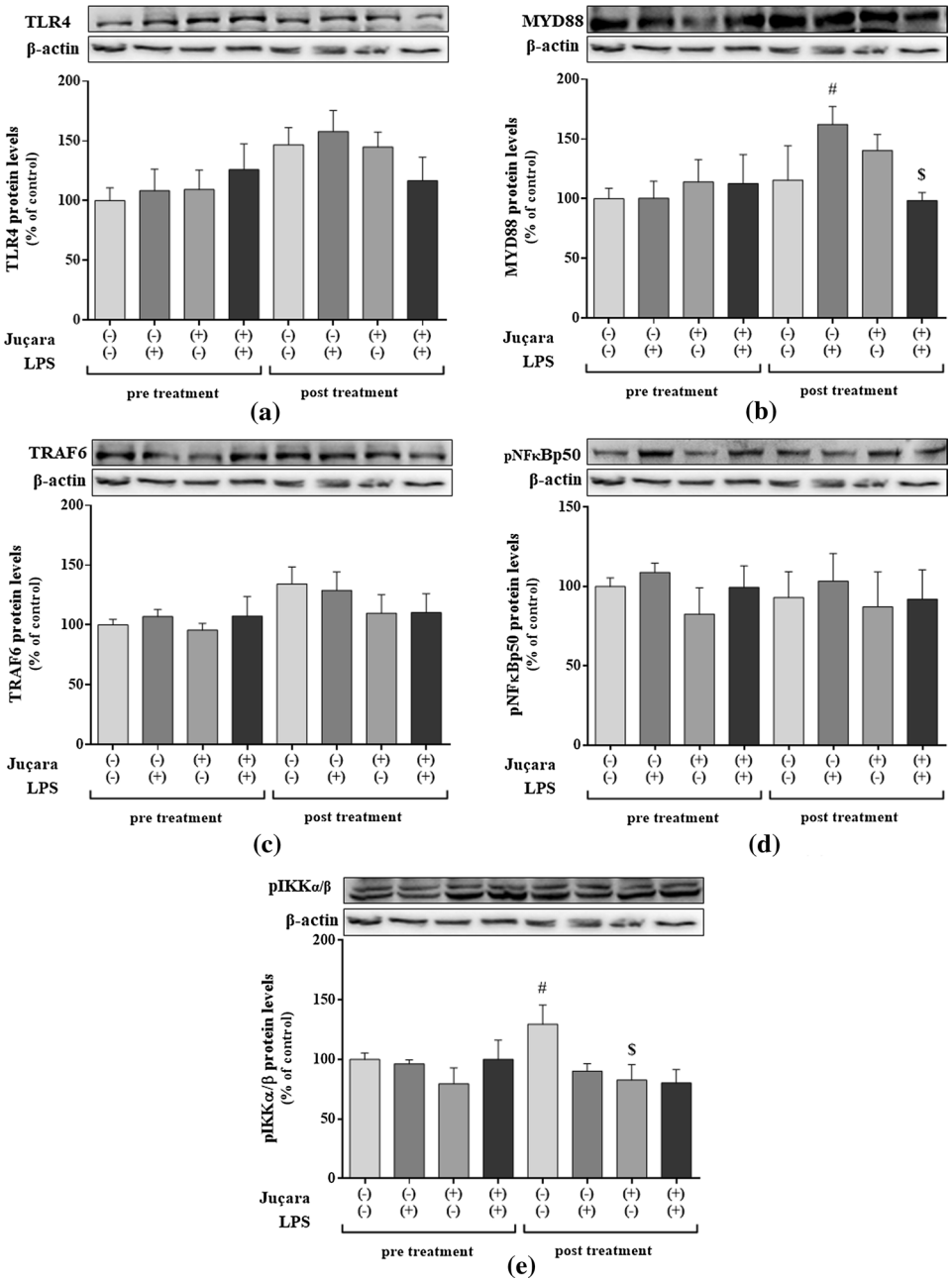

Fig. 3 Monocyte inflammatory markers production levels between groups before and after supplementation for 6 weeks: **a** TLR4; **b** MYD88; **c** TRAF6; **d** pNFκBp50; **e** pIKKα/β. The housekeeping used for all analyses was β-actin protein expression at the same membrane as the target proteins. The data was normalized by the pre-treatment placebo without LPS group (first bar in the graph) on RT-PCR on western blotting. The groups description pre-treatment and post-treatment: placebo group without LPS (−) (−); placebo with LPS (−) (+); juçara without LPS (+) (−); juçara with LPS (+) (+). *Without LPS versus with LPS; #baseline versus post-treatment; $placebo versus juçara group ($n = 10$ per group)

Monocyte cytokines production

The interleukin-6 (IL-6) production by monocytes at pre-treatment was greater in the LPS-stimulated placebo group compared to the non-stimulated placebo group ($p = 0.019$) and post-treatment ($p = 0.034$). Indeed, post treatment there was a reduction of IL-6 levels in the juçara group with LPS compared to the placebo group with LPS ($p = 0.006$) (Fig. 5a).

The pre-treatment levels of TNF-α were higher in the juçara group stimulated with LPS compared to the juçara group without LPS ($p = 0.044$). However, in the post-treatment period, there was a reduction of TNF-α in the juçara group with LPS compared to the pre-treatment ($p = 0.049$) (Fig. 5b).

Monocyte chemotactic protein 1 (MCP-1) was increased in the placebo group with LPS compared to the placebo group without LPS at the pre-treatment ($p = 0.001$). Moreover, in the pre-treatment evaluation, the juçara group without LPS had higher levels of MCP-1 compared to the placebo group without LPS ($p = 0.027$). Nevertheless, post-treatment, there was a reduction in the juçara group without LPS compared to the same pre-treatment group

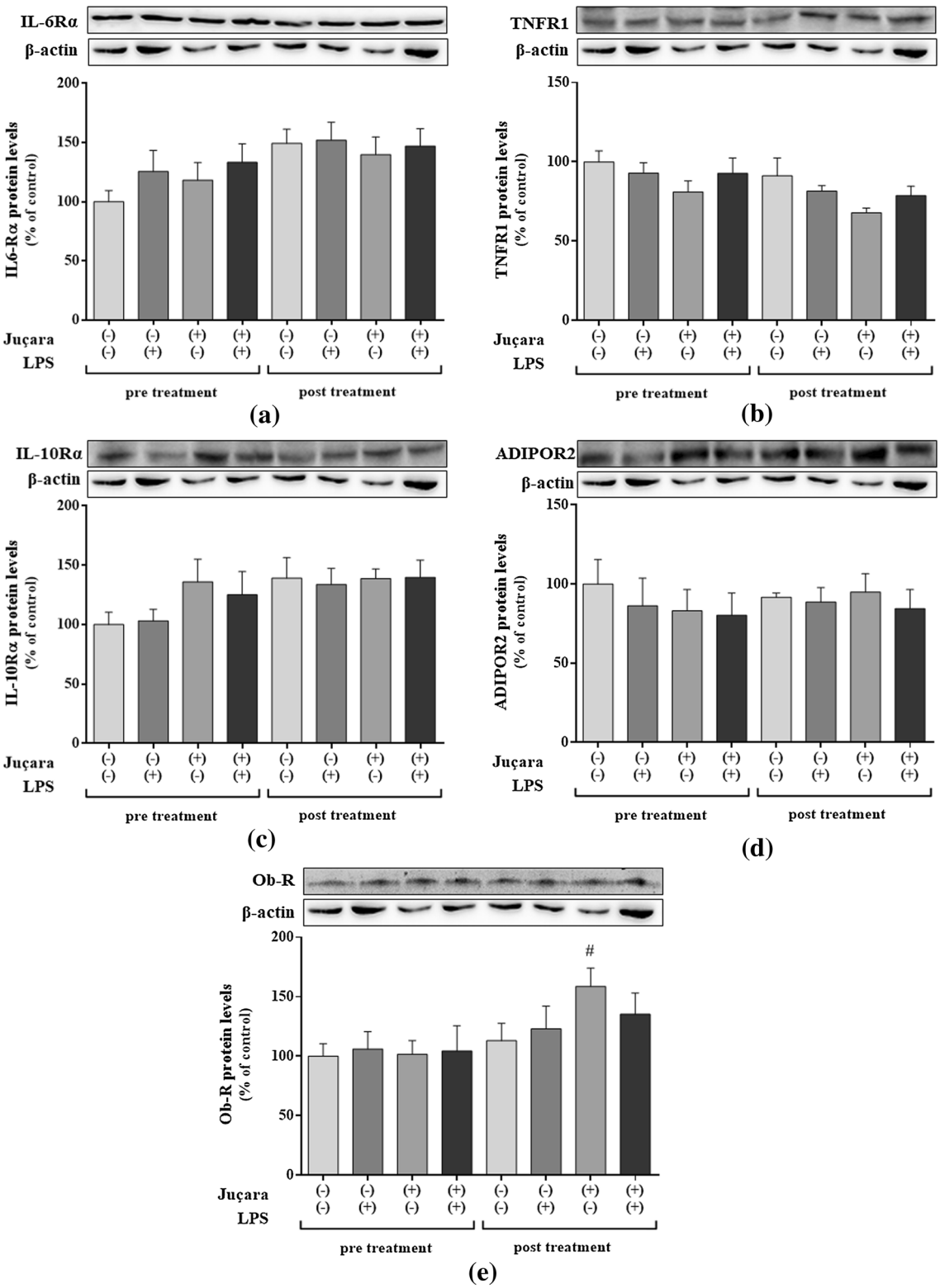

Fig. 4 Monocyte inflammatory markers production levels between groups before and after supplementation for 6 weeks: **a** IL-6Rα; **b** TNFR1; **c** IL-10Rα; **d** ADIPOR2; **e** Ob-R. The housekeeping used for all analyses was the β-actin protein expression at the same membrane as the target proteins. The data was normalized by the pre-treatment placebo without LPS group (first bar in the graph) on western blotting. The groups description pre-treatment and post-treatment: placebo group without LPS (−) (−); placebo with LPS (−) (+); juçara without LPS (+) (−); juçara with LPS (+) (+). *Without LPS versus with LPS; [#]baseline versus post-treatment; [$]placebo versus juçara group ($n = 10$ per group)

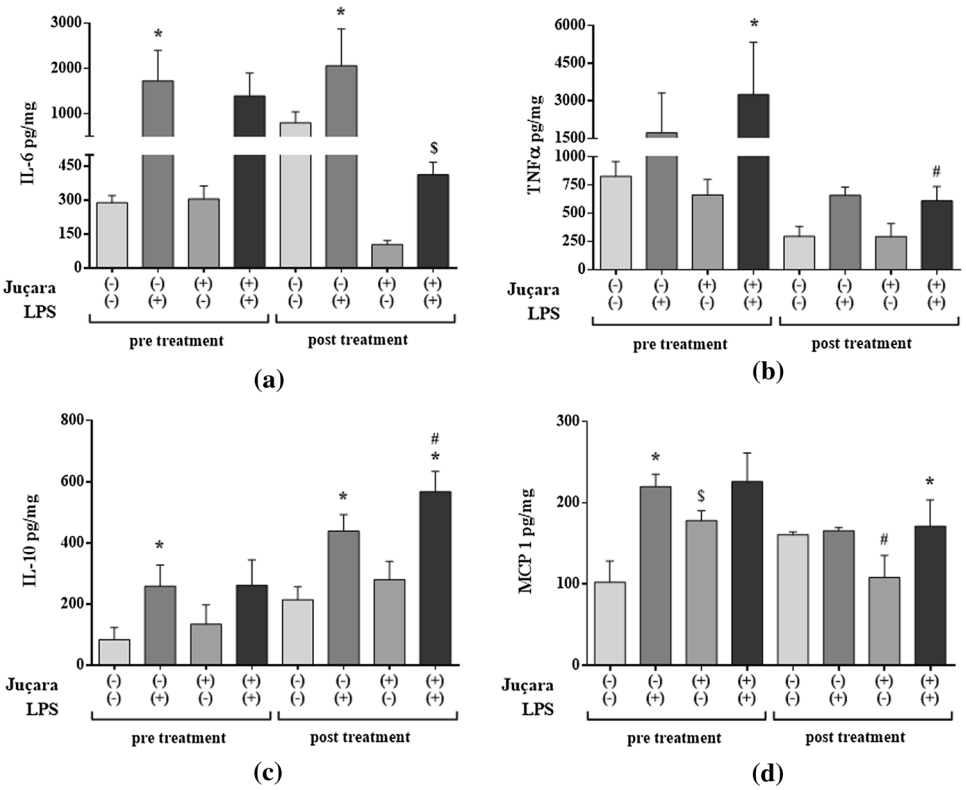

Fig. 5 Monocyte inflammatory markers production levels between groups before and after supplementation for 6 weeks: **a** IL-6; **b** TNF-α; **c** IL-10; **d** MCP-1. The data was normalized by the pre-treatment placebo without LPS group (first bar in the graph). The groups description pre-treatment and post-treatment: placebo group without LPS (−) (−); placebo with LPS (−) (+); juçara without LPS (+) (−); juçara with LPS (+) (+). *Without LPS versus with LPS; #baseline versus post-treatment; $placebo versus juçara group ($n = 10$ per group)

($p = 0.041$) and compared to the juçara group with LPS post-treatment ($p = 0.047$; Fig. 5d).

The interleukin-10 (IL-10) levels pre and post-treatment were higher in the placebo group with LPS compared to placebo without LPS ($p = 0.047$; $p = 0.012$). However, there was no difference in IL-10 levels in the basal control group, but post-treatment there was an increase in IL-10 in the juçara group with LPS in relation to the juçara group without LPS ($p = 0.001$) and also compared to the same group in baseline ($p = 0.001$) according to Fig. 5c.

Discussion

This study allows us to suggest the juçara pulp (*E. edulis* Mart.) as a potential inflammatory modulator tool in obesity-related sub-clinical inflammation. It is noteworthy the changes in the TLR4/NFκBp50 pathway in the monocytes of obese individual supplemented with freeze-dried juçara pulp. The peripheral blood mononuclear cells (PBMC), isolated from these obese individuals, reduced the production of inflammatory markers commonly related to obesity and other chronic non-transmissible diseases, even when submitted to a proinflammatory stimulus (LPS) for analysis of the cellular response.

The overcome production and secretion of proinflammatory adipokines in adipose tissue leads to systemic inflammation, and obesity-related metabolic disorders arise [5]. In this process, associated with adipocytes, immune cells such as macrophages and lymphocytes are rich sources of adipokines and contribute to obesity-associated inflammation [5] establishing obesity as chronic low-grade inflammation.

It has been described that the inflammatory process in obesity can be related to TLR4 pathway activation. In fact, the obesity-related lipotoxicity seems to trigger the increased TLR4 signaling transduction, even with recent evidence of no direct binding of fatty acids and this membrane receptor [27].

Despite the fact of no changes in monocytes TLR4 protein expression, the TLR4 mRNA levels demonstrate a notable decrease of gene transcription associated with juçara supplementation. It is well known that genetic information is transmitted from DNA to protein through mRNA molecules. However, several mechanisms are able to modulate the DNA to the protein translation process, causing the remarkable difference in mRNA levels of TLR4, which is not reflected in protein expression. One common explanation for this is the acute LPS exposure (only 1 h), which was enough to trigger cellular mechanism of transcription process increasing mRNA levels, but it is not sufficient for the translation process occurs until the evaluation time. Another possible regulatory mechanism could be attributed to miRNAs generated during the RNA splicing. Indeed, recent studies

demonstrated that numerous different miRNAs blunted protein translation in obesity [28–30]. Hence, we hypothesized that in longer juçara supplementation protocols or more pronounced proinflammatory stimulus could be able to change protein expression, thereby the mRNA levels modifications indicate a modulation of inflammation pathway in intracellular level.

Another indicative of inflammatory pathway modulation can be noticed by the decreased TLR4 signaling transduction by MYD88 in juçara group. The activation of TLR4 through the MYD88-dependent pathway promotes the activation of the MYD88 adapter protein, which in turn recruits and activates IRAK1 and IRAK4 (kinase-associated IL receptor). In consequence, the TRAF6 adaptor protein is activated. The latter forms a complex with enzymes involved in the ubiquitination process and activates TAK1 (kinase 1 activated by transforming growth factor β), which then, activates the complex IKK (IKKα, IKKβ, and NEMO/IKKγ) that triggers decoupling of NFκB by phosphorylation of its inhibitory protein, Iκ-B. Released NFκB migrates to the nucleus by binding to DNA, initiating the gene expression of proteins related to inflammation [31, 32], even with no changes in NFκB expression. Our results of pIKKα/β show less decoupling of the NFκB/IKK protein complex. Consequently, it is possible to suggest that there was a reduced nuclear translocation of this transcription factor after juçara supplementation. This mechanism corroborates our results of proinflammatory markers produced by the monocytes, as well as, the cytokines mRNA levels. Furthermore, polyphenols in a co-cultured cell model of macrophages and adipocytes demonstrated significantly reduced NFκB activation and decreased the release of IL-6 and TNF-α, as well as, suppressed the expression of matrix metalloproteinase (MMP)-2 and MMP-9. The anti-obesity property of polyphenol-rich fruits may be attributed to anti-inflammatory response in adipose tissue associated with infiltrated M2 macrophages [33, 34]. It is possible to explain the results found due to anthocyanins present in juçara or its metabolites by-products which serve as redox buffers suppressing oxidative stress and thereby reducing the inflammatory response by direct ROS scavenging [35–37].

Evaluating the cytokines membrane receptors gene and protein expression did not change after treatment, however, the cytokines per se have undergone major modifications. This result is in agreement with the literature since the modification of membrane receptors is more noticeable in long-term processes and less dynamic than the production of its intra and extra-cellular mediators [30]. The TNF-α is a proinflammatory cytokine produced primarily by macrophages and lymphocytes, and secondarily by muscles and adipose tissue. The decreased TNF-α after the period of juçara supplementation demonstrates a decline of the proinflammatory stimulus. The literature corroborates these results by describing this reduction related to both anthocyanin and MUFA intake, leading to weight loss and reduced risk of cardiovascular diseases [38, 39]. Among many biological activities of TNF-α in intracellular processes, it is included apoptosis, modulating the secretion of adiponectin and IL-6, and accelerate atherogenesis induced by VCAM-1, ICAM-1, MCP-1 [40]. The main function of MCP-1 is to recruit cells of the immune system that participates in the inflammatory process. Higher levels of MCP-1 are related to insulin resistance and the increase of circulating free fatty acids, especially saturated fatty acids, but also linoleic fatty acid (C18: 2n6) [41]. The MCP-1 could promote the recruitment and infiltration of macrophages into adipose tissue increasing its local inflammatory response. Inhibitory effect of polyphenols—such as juçara pulp supplementation—on chemotaxis and release of MCP-1 may contribute to its anti-inflammatory activity. Macrophages cell culture exposed to polyphenols inhibited the migration of macrophages and decreased the expression and release of inflammatory factors from adipocytes through inhibiting NFκB activity and its nuclear translocation [42]. These data corroborate our results since juçara pulp supplementation increased the polyphenols intake, as well as lead to saturated fatty acids reduction and improve the ω-3/ω-6 ratio, contributing to the reduction of the inflammatory parameter MCP-1. On the other hand, IL-10 is characterized as an anti-inflammatory cytokine being associated with IL-6 and TNF-α. In the innate immune system, IL-10 inhibits the activation of macrophages acting as a TNF-α antagonist. Its levels are predominantly reduced in obese individuals, which favors the installation of the proinflammatory process through the activation of NFκB mediated by TLRs and TNFR1 (TNF receptor type 1) [43]. In according to literature, IL-10 production was increased after supplementation in our study, demonstrating that foods rich in phenolic compounds—such as anthocyanins—have influence in the inflammatory pathway [44]. The IL-10 increase especially associated with the LPS exposure after juçara supplementation is a relevant result considering the direct cell-to-cell interactions of biomolecules. This anti-inflammatory behavior of stimulated monocytes indicates that after treatment these cells are more likely to develop the M2 macrophage phenotype on tissue differentiation, hindering the installation of inflammatory status when exposed to LPS [45]. Adipose endocrine function is critical to overall energy balance and homeostasis with adipocyte-derived adipokines playing key roles [5]. One remarkable adipokine is leptin, which is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss. In obesity, the circulating level of the anorexigenic hormone leptin is increased proportionally to adipose tissue mass, whereas it is now established that obese patients are leptin-resistant [46]. Leptin receptors (Ob-R) are encoded by the diabetes gene and belong to the class I cytokine receptor

superfamily, which includes receptors for IL-6. The alternative splicing of this gene produces six receptors isoforms with cytoplasmic domains of different length. The Ob-R is necessary for the intracellular signaling in the Janus Kinase (JAK)-STAT pathway. The JAK-2 activation leads to the phosphorylation of the cytoplasmic-residues binding with different transcription factors. The leptin functional receptor is expressed not only in the hypothalamus to regulate energy homeostasis and neuroendocrine function, but also in all cell types of the innate and adaptive immune system [47, 48]. The role of leptin in regulating the immune system of obese subjects remains less well defined.

It has been postulated that leptin resistance might be due to defective leptin transport across the blood–brain barrier. It is also possible that leptin resistance is caused by defects in the downstream mediators of leptin receptor [46]. Hence, Ob-Rb receptor function may be decreased, indicating a state of leptin resistance. In this sense, the increase observed in Ob-R demonstrates an improvement of leptin sensibility after the juçara pulp supplementation. In fact, the literature demonstrates that antioxidant foods can reverse the resistance of leptin, improving the Ob-R function [49–51]. This modification may be related to the epigenetic modulation promoted by juçara previously reported [22], moreover it has been proved that the leptin receptor functionality, as well as the leptin signaling pathway, could be modulated by epigenetic markers. Indeed, it was established that methylation of a proximal region of LEP gene promoter constitutes a determinant of leptin expression in human adult tissues since that DNA methylation has a role in long-term gene silencing and that demethylation is able to increase and reactive leptin expression [52].

Summing up, juçara pulp (*E. edulis* Mart.) is able to exert anti-inflammatory role on obesity-related sub-clinical inflammation, modulating TLR4/NFκBp50 pathway in the monocytes from obese individuals. It is noteworthy the suppression of gene and protein expression of key molecules involved in inflammation such as TLR4 and IL-6. Furthermore, the juçara supplementation could modulate the energy homeostasis from the leptin receptor. In addition, further studies are necessary to investigate the beneficial effects of chronic exposure to juçara pulp supplementation.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical standards The study procedures were in accordance with the guidelines set in the International Declaration of Helsinki and approved by the Ethics Committee of the Universidade Federal de São Paulo (CEP-UNIFESP No. 0319/2017) and registered in Plataforma Brasil database. The written informed consent was obtained from all volunteers.

References

1. Engin AB (2017) Adipocyte-macrophage cross-talk in obesity. In: Engin AB, Engin A (eds) Obesity and lipotoxicity. Springer International Publishing, Cham, pp 327–343
2. Dey A, Allen J, Hankey-Giblin PA (2015) Ontogeny and polarization of macrophages in inflammation: blood monocytes versus tissue macrophages. Front Immunol 6:1–15. https://doi.org/10.3389/fimmu.2014.00683
3. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL (2014) Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? Front Immunol 5:1–6. https://doi.org/10.3389/fimmu.2014.00470
4. Lovren F, Pan Y, Quan A et al (2010) Adiponectin primes human monocytes into alternative anti-inflammatory M2 macrophages. AJP Heart Circ Physiol 299:H656–H663. https://doi.org/10.1152/ajpheart.00115.2010
5. Brikos C, O'Neill LAJ (2008) Signalling of toll-like receptors. Handb Exp Pharmacol 183:21–50. https://doi.org/10.1007/978-3-540-72167-3-2
6. O'Neill LAJ (2008) The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226:10–18. https://doi.org/10.1111/j.1600-065X.2008.00701.x
7. Gribar SC, Anand RJ, Sodhi CP, Hackam DJ (2008) The role of epithelial Toll-like receptor signaling in the pathogenesis of intestinal inflammation. J Leukoc Biol 83:493–498. https://doi.org/10.1189/jlb.0607358
8. Jialal I, Kaur H, Devaraj S (2014) Toll-like receptor status in obesity and metabolic syndrome: a translational perspective. J Clin Endocrinol Metab 99:39–48. https://doi.org/10.1210/jc.2013-3092
9. Kim K-A, Gu W, Lee I-A et al (2012) High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One 7:e47713. https://doi.org/10.1371/journal.pone.0047713
10. Da Silva NA, Rodrigues E, Mercadante AZ, De Rosso VV (2014) Phenolic compounds and carotenoids from four fruits native from the Brazilian Atlantic forest. J Agric Food Chem 62:5072–5084. https://doi.org/10.1021/jf501211p
11. Silva P, Carmo L, Silva G et al (2013) Physical, chemical, and lipid composition of juçara (*Euterpe edulis* Mart.) Pulp. Braz J Food Nutr 24:7–13
12. Sousa De Brito E, De Araújo MCP, Alves RE et al (2007) Anthocyanins present in selected tropical fruits: acerola, jambolão, jussara, and guajiru. J Agric Food Chem 55:9389–9394. https://doi.org/10.1021/jf0715020
13. Menaa F (2015) The berry fruit açai (*Euterpe oleracea* Mart): bringing Health benefits and exotism to the modern table. Adv

Food Technol Nutr Sci Open J 1:1–4. https://doi.org/10.17140/AFTNSOJ-1-101
14. Santamarina AB, Jamar G, Mennitti LV et al (2018) The use of juçara (*Euterpe edulis* Mart.) supplementation for suppression of NF-κB pathway in the hypothalamus after high-fat diet in Wistar rats. Molecules (Basel, Switzerland) 23:1–13. https://doi.org/10.3390/molecules23071814
15. Argentato PP, Morais CA, Santamarina AB et al (2017) Jussara (*Euterpe edulis* Mart.) supplementation during pregnancy and lactation modulates UCP-1 and inflammation biomarkers induced by trans-fatty acids in the brown adipose tissue of offspring. Clin Nutr Exp 12:50–65. https://doi.org/10.1016/j.yclnex.2016.12.002
16. Almeida Morais C, Oyama LM, De Oliveira JL et al (2014) Jussara (*Euterpe edulis* Mart.) supplementation during pregnancy and lactation modulates the gene and protein expression of inflammation biomarkers induced by trans-fatty acids in the colon of offspring. Mediat Inflamm 2014:1–11. https://doi.org/10.1155/2014/987927
17. Oyama LM, Silva FP, Carnier J et al (2016) Jucąra pulp supplementation improves glucose tolerance in mice. Diabetol Metab Syndr 8:1–8. https://doi.org/10.1186/s13098-015-0122-4
18. Morais CA, Oyama LM, de Moura Conrado R et al (2015) Polyphenols-rich fruit in maternal diet modulates inflammatory markers and the gut microbiota and improves colonic expression of ZO-1 in offspring. Food Res Int 77:186–193. https://doi.org/10.1016/j.foodres.2015.06.043
19. Jamar G, Santamarina AB, Mennitti LV et al (2018) *Bifidobacterium* spp. reshaping in the gut microbiota by low dose of juçara supplementation and hypothalamic insulin resistance in Wistar rats. J Funct Foods 46:212–219. https://doi.org/10.1016/j.jff.2018.05.002
20. WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation
21. Haun R, José Gondim Pitanga F, Lessa I (2009) Razão Cintura/estatura Comparado a outros indicadores antropométricos de obesidade como preditor de risco coronário elevado. Artigo Original Rev Assoc Med Bras 55:705–711. https://doi.org/10.1590/S0104-42302009000600015
22. Santamarina AB, Jamar G, Mennitti LV et al (2018) Supplementation of juçara berry (*Euterpe edulis* Mart.). Modulates epigenetic markers in monocytes from obese adults: a double-blind randomized trial. Nutrients 10:1899. https://doi.org/10.3390/nu10121899
23. Inada KOP, Oliveira AA, Revorêdo TB et al (2015) Screening of the chemical composition and occurring antioxidants in jabuticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits and their fractions. J Funct Foods 17:422–433. https://doi.org/10.1016/j.jff.2015.06.002
24. Felzenszwalb I, da Costa Marques MR, Mazzei JL, Aiub CAF (2013) Toxicological evaluation of *Euterpe edulis*: a potential superfruit to be considered. Food Chem Toxicol 58:536–544. https://doi.org/10.1016/j.fct.2013.05.029
25. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
26. Faul F, Erdfelder E, Lang A-G, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39, pp. 175–191. https://doi.org/10.3758/BF03193146
27. Lancaster GI, Langley KG, Berglund NA et al (2018) Evidence that TLR4 is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism. Cell Metab 27:1096–1110.e5. https://doi.org/10.1016/j.cmet.2018.03.014
28. Arner P, Kulyté A (2015) MicroRNA regulatory networks in human adipose tissue and obesity. Nat Rev Endocrinol 11:276–288. https://doi.org/10.1038/nrendo.2015.25
29. Nunez Lopez YO, Garufi G, Seyhan AA (2017) Altered levels of circulating cytokines and microRNAs in lean and obese individuals with prediabetes and type 2 diabetes. Mol Biosyst 13:106–121. https://doi.org/10.1039/C6MB00596A
30. Marques-Rocha JL, Samblas M, Milagro FI et al (2015) Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J 29:3595–3611. https://doi.org/10.1096/fj.14-260323
31. Pisani LP, Estadella D, Ribeiro DA (2017) The role of toll like receptors (TLRs) in oral carcinogenesis. Anticancer Res 37:5389–5394. https://doi.org/10.21873/anticanres.11965
32. Estadella D, da Penha Oller do Nascimento CM, Oyama LM et al (2013) Lipotoxicity: effects of dietary saturated and transfatty acids. Mediat Inflamm 2013:1–13. https://doi.org/10.1155/2013/137579
33. Kang L, Heng W, Yuan A et al (2010) Resveratrol modulates adipokine expression and improves insulin sensitivity in adipocytes: relative to inhibition of inflammatory responses. Biochimie 92:789–796. https://doi.org/10.1016/j.biochi.2010.02.024
34. Kang NE, Ha AW, Kim JY, Kim WK (2012) Resveratrol inhibits the protein expression of transcription factors related adipocyte differentiation and the activity of matrix metalloproteinase in mouse fibroblast 3T3–L1 preadipocytes. Nutr Res Pract 6:499–504. https://doi.org/10.4162/nrp.2012.6.6.499
35. Valenti L, Riso P, Mazzocchi A et al (2013) Dietary anthocyanins as nutritional therapy for nonalcoholic fatty liver disease. Oxid Med Cell Longev. https://doi.org/10.1155/2013/145421
36. Hassellund SS, Flaa A, Kjeldsen SE et al (2013) Effects of anthocyanins on cardiovascular risk factors and inflammation in pre-hypertensive men: a double-blind randomized placebo-controlled crossover study. J Hum Hypertens 27:100–106. https://doi.org/10.1038/jhh.2012.4
37. Azzini E, Giacometti J, Russo GL (2017) Antiobesity effects of anthocyanins in preclinical and clinical studies. Oxid Med Cell Longev 1:1. https://doi.org/10.1155/2017/2740364
38. Campión J, Milagro FI, Goyenechea E, Martínez JA (2009) TNF-α promoter methylation as a predictive biomarker for weight-loss response. Obesity 17:1293–1297. https://doi.org/10.1038/oby.2008.679
39. Naranjo MC, Bermudez B, Garcia I et al (2017) Dietary fatty acids on aortic root calcification in mice with metabolic syndrome. Food Funct 8:1468–1474. https://doi.org/10.1039/c7fo00143f
40. Neele AE, Van Den Bossche J, Hoeksema MA, De Winther MPJ (2015) Epigenetic pathways in macrophages emerge as novel targets in atherosclerosis. Eur J Pharmacol 763:79–89. https://doi.org/10.1016/j.ejphar.2015.03.101
41. Boden G (2011) NIH Public Access. Curr Opin Endocrinol Diabetes Obes 18:139–143. https://doi.org/10.1097/MED.0b013e3283444b09.45Obesity
42. Woo HM, Kang JH, Kawada T et al (2007) Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. Life Sci 80:926–931. https://doi.org/10.1016/j.lfs.2006.11.030
43. Lira FS, Rosa JC, Dos Santos RV et al (2011) Visceral fat decreased by long-term interdisciplinary lifestyle therapy correlated positively with interleukin-6 and tumor necrosis factor-α and negatively with adiponectin levels in obese adolescents. Metab Clin Exp 60:359–365. https://doi.org/10.1016/j.metabol.2010.02.017
44. Palacz-Wrobel M, Borkowska P, Paul-Samojedny M et al (2017) Effect of apigenin, kaempferol and resveratrol on the gene expression and protein secretion of tumor necrosis factor alpha (TNF-α)

and interleukin-10 (IL-10) in RAW-264.7 macrophages. Biomed Pharmacother 93:1205–1212. https://doi.org/10.1016/j.biopha.2017.07.054

45. Lovren F, Pan Y, Quan A et al (2010) Adiponectin primes human monocytes into alternative anti-inflammatory M2 macrophages. AJP Heart Circ Physiol 299:H656–H663. https://doi.org/10.1152/ajpheart.00115.2010
46. Klok MD, Jakobsdottir S, Drent ML (2007) The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev 8:21–34. https://doi.org/10.1111/j.1467-789X.2006.00270.x
47. Matarese G, Moschos S, Mantzoros CS (2005) Leptin in immunology. J Immunol 174:3137–3142. https://doi.org/10.4049/jimmunol.174.6.3137
48. Otero M, Lago R, Gomez R et al (2006) Towards a pro-inflammatory and immunomodulatory emerging role of leptin. Rheumatology 45:944–950. https://doi.org/10.1093/rheumatology/kel157
49. Różańska D, Regulska-Ilow B (2018) The significance of anthocyanins in the prevention and treatment of type 2 diabetes. Adv Clin Exp Med 27:135–142. https://doi.org/10.17219/acem/64983
50. da Costa GF, Santos IB, de Bem GF et al (2017) The beneficial effect of anthocyanidin-rich *Vitis vinifera* L. grape skin extract on metabolic changes induced by high-fat diet in mice involves antiinflammatory and antioxidant actions. Phytother Res 31:1621–1632. https://doi.org/10.1002/ptr.5898
51. de Bem GF, Costa CA, Santos IB et al (2018) Antidiabetic effect of *Euterpe oleracea* Mart. (acai) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: a positive interaction. PLoS One 13:e0199207. https://doi.org/10.1371/journal.pone.0199207
52. Crujeiras AB, Carreira MC, Cabia B et al (2015) Leptin resistance in obesity: an epigenetic landscape. Life Sci 140:57–63. https://doi.org/10.1016/j.lfs.2015.05.003