



Juçara (*Euterpe edulis Mart.*) supplementation reduces body weight gain and protects mice from metabolic complications induced by high-fat diet

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Abstract

Purpose To investigate the effects of juçara pulp supplementation on energy homeostasis and metabolic complications in diet-induced obese mice.

Methods Mice received a control (C) or a high-fat diet (H) for 16 weeks and for half the diet was supplemented with 0.5% freeze-dried juçara powder (CJ and HJ groups, respectively). Food intake, body weight, energy expenditure, glucose tolerance, insulin sensitivity, liver steatosis, and uncoupling protein 1 (UCP1) expression in brown adipocytes were analyzed.

Results Juçara pulp supplementation reduced body weight gain in HJ compared to H group but increased body weight in CJ compared to C group. Energy intake was higher in the CJ group than in C, H, and HJ, but similar between H and HJ groups. Despite similar energy intake, juçara pulp supplementation increased energy expenditure in HJ compared to H group. However, UCP-1 expression was not affected by juçara. Also, H mice exhibited a severe diet-induced glucose intolerance and insulin resistance, all of which were significantly improved in HJ mice. In addition, H group had higher fat content in hepatocytes than HJ, whereas no difference was observed between C and CJ.

Conclusions Juçara pulp has a great potential to reduce the metabolic complications associated with obesity.

Keywords Juçara · *Euterpe edulis Mart.* · Anthocyanins · Energy homeostasis · Obesity · Insulin resistance

Introduction

Data from the World Health Organization (WHO) showed that global obesity has nearly tripled since 1975 [1]. In addition, most of the world's population live in countries where overweight and obesity kills more people than underweight

[1]. Worldwide, 39% of adults are overweight and 13% are obese [1]. The prevalence of overweight and obesity has increased dramatically from children to adults and in developed as well as in developing countries [2]. Obesity is strongly linked with chronic diseases such as type 2 diabetes, cardiovascular diseases, dyslipidemia, and non-alcoholic fatty liver disease (NAFLD) [3]. The health and economic burden imposed by obesity and its associated complications clearly demonstrate the need to fight it.

Healthy eating is one of the strategies to counteract some of the detrimental effects of obesity. High consumption of fruits, legumes, and vegetables are highly recommended for diabetic patients [4] and those at increased risk for cardiovascular disease [5] and NAFLD patients [6]. Among other constituents, these foods are a source of polyphenols, the most abundant antioxidant in the diet [7]. Current evidence strongly suggests a positive role for polyphenols in the prevention of

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cardiovascular disease and diabetes [8]. Polyphenols are a large group of compounds characterized by the presence of various hydroxyl groups in aromatic rings. These compounds are divided into two main categories: non-flavonoid and flavonoid compounds [9].

Anthocyanins are pigments of the flavonoid family which are responsible for the red, blue and purple colors of fruits and vegetables [10]. These compounds have high antioxidant capacity and play an important role in modulating the inflammation related to chronic diseases [11]. Experimental studies have shown that anthocyanins have prevented obesity, hyperglycemia, hyperinsulinemia, and liver lipid accumulation in mice fed a high-fat diet [12–14]. The anti-obesogenic effect of anthocyanins involves increased energy expenditure via brown adipose tissue (BAT) UCP-1 expression [15], which is critical for BAT thermogenesis by dissipating energy as heat. BAT activation has been shown to have beneficial metabolic effects including improved insulin sensitivity and reduced hyperglycemia and hyperlipidemia, making this tissue a potential target for the treatment of obesity and type 2 diabetes [16].

The juçara palm (*Euterpe edulis Mart.*) is a species native to the Atlantic Forest in Brazil, and produces spherical purple fruits commonly known as juçara [17]. These fruits contain cis-unsaturated fatty acids, polyunsaturated fatty acids (PUFAs), dietary fiber, and anthocyanins, mainly cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside [18]. Recent studies demonstrated that juçara fruit can improve some of the obesity-associated metabolic complications [19–22]. In this context, the present study aimed to evaluate the effects of juçara pulp supplementation in high-fat diet fed mice on body weight gain and on glucose homeostasis and liver steatosis, two commonly observed metabolic complications associated with obesity.

Methods

Animals and treatment

The experiments were approved by the Institutional Ethics Committee on Animal Use (CEUA n.1750/11). Twelve-week-old male C57Bl/6 mice, obtained from the Center for Development of Animal Models for Medicine and Biology (CEDEME, Federal University of São Paulo), were housed in a temperature-controlled room (22°C) with a 12:12-h light-dark cycle (7:00–19:00h). Mice were randomly distributed into one of four groups receiving for 16 weeks either a control diet (C group), a control diet supplemented with juçara 0.5% freeze-dried powder (CJ group), a high-fat diet (H group), or a high-fat diet supplemented with juçara 0.5% freeze-dried powder (HJ group). The experiment was performed in two series with 4–5 mice/group in each (total $n=8$ –10 mice/group). Body weight was registered once a

week during all the experiment. Energy intake was measured once a month for 48 h, during which mice were kept in individual, transparent cages, close to each other, to obtain individual values. Average daily consumption was determined by subtracting the weight of the food removed from the hoppers after 48 h from the weight of food given, with care taken to account for spillage, divided by the number of days.

The diets were purchased from Rhoster (Rhoster Indústria e Comércio Ltda, Araçoiaba da Serra, SP, Brazil) and prepared according to the Association of Official Analytical Chemists and AIN-93G [23]. The CJ and HJ diets were prepared by adding 5 g of juçara freeze-dried powder in 1 kg of each diet (Table 1). Juçara pulp (*Euterpe edulis Mart.*) was obtained from the agroecological Juçara Project /IPEMA—Institute of Permaculture and Ecovillages of the Atlantic (Ubatuba, SP, Brazil) and then freeze-dried to powder using a lyophilizer. Diets were then stored at -20°C . The composition of the juçara pulp was previously determined [18], and is shown on Table 2. To determine the dose of juçara, we used previous data showing no genotoxic and mutagenic effects of açai in mice, a fruit with characteristics similar to juçara [24]. Also, it has been described that the intake of 100- to 350-mg anthocyanin per day is safe and improves the lipid profile and inflammatory responses in humans [25, 26]. The calculation was applied for the conversion of the rat dose to the adult human dose according to the Food and Drug Administration's Center for Drug Evaluation and Research [27]. The 0.5% dose corresponded to 3.3 mg of anthocyanins/kg/day, which could be obtained

Table 1 Composition of the diets

Ingredient (g/Kg)	Diets	
	C	H
Casein (84% protein) *	202	200
Maize starch	397	115.5
Dextrinized maize starch	130.5	132
Sucrose	100	100
L-cystine	3	3
Fiber	50	50
Soybean oil	70	40
Fat	-	312
Mineral mix (AIN93G) †	35	35
Vitamin mix (AIN93G) †	10	10
Choline chlorhydrate	2.5	2.5
Freeze-dried Juçara powder	-	-
Energy (Kcal/Kg)	3818.7	5230.0

*Values corrected for the protein content in casein. † For detailed composition see [23]. The CJ and HJ diets were prepared by adding 5 g of Juçara in 1 Kg of each diet, which gives a final juçara dose of 0.497%, rounded in the text to the nearest tenth (0.5%). The energy content of the CJ and HJ diets were 3838.7 and 5250.0 Kcal/Kg, respectively

Table 2 Characterization and composition of juçara pulp [18]

Composition	Concentration
Carbohydrates (g/100g) ¹	28.3 ± 3.5
Proteins (g/100g) ¹	6.0 ± 0.3
Lipids (g/100g) ¹	29.2 ± 0.9
Fibers (g/100g) ¹	28.3 ± 0.3
Total phenolic compounds (mg GAE/100 g f.m.)	415.1 ± 22.3
Cyanidin 3-glucoside (mg C3G/100 g f.m.)	47.9 ± 1.5
Cyanidin 3-rutinoside (mg C3G/100 g f.m.)	179.6 ± 5.7
Energetic value (Kcal/100g)	400.0 ± 23.9

¹ Values for dry basis; *f.m.*, fresh matter

by consuming 100 g of fresh juçara pulp or around 10 g of lyophilized juçara per day for an adult of 70 kg [21].

Indirect calorimetry

Indirect calorimetry was analyzed at room temperature (22°C) using an indirect calorimetry system. In short, mice were placed individually in the specifically designed calorimeter chambers (Oxylet System, Panlab-Harvard Apparatus, Barcelona, Spain) with free access to diet and water. They were allowed an acclimation period of 2 h in the chambers before the beginning of the analysis. Oxygen consumption and carbon dioxide production as well as energy expenditure were calculated using the software Metabolism (Panlab-Harvard Apparatus, Barcelona, Spain). Data for energy expenditure were analyzed as areas under the curves (AUC) of energy expenditure in the light and dark cycles using the trapezoidal method [28].

Intraperitoneal glucose tolerance test (ipGTT)

For ipGTT food was withdrawn at 7:00 and a fast blood sample was taken after 8 h from the tip of the tail. Subsequently, each mouse received an intraperitoneal (ip) glucose solution load (2 g/kg body weight), and additional blood samples were collected at 15, 30, 60, and 120 min after injection. Blood glucose during the test was determined by Accu-Check Advantage II (Roche). The AUC was calculated from values of each mouse using the trapezoidal method [28].

Intraperitoneal insulin tolerance test (ipITT)

The intraperitoneal insulin tolerance test was performed in mice fasted for 6 h. Food was withdrawn at 7:00. They were injected ip with 0.5 U/kg body weight of human insulin (Biohulin N, Biobrás, Brazil). Blood samples were collected immediately before and 4, 8, and 12 min after insulin injection for glucose measurement using Accu-Check Advantage II

(Roche). The AUC was calculated from values of each mouse using the trapezoidal method [28].

Western blot

The brown adipose tissue was removed and placed in extraction buffer. The total protein content was determined by the Bradford method using the Bio-Rad reagent (Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin (BSA) as the reference. The samples were treated with the LDS Sample Buffer and Reducing Agent (Life Technologies). The proteins (100 µg) were heated for 10 min prior to loading onto the Bolt 4-12% Bis-Tris Plus in a Bolt mini gel tank (Novex, Life technologies, California, USA). Electrotransfer of proteins from the gel to the nitrocellulose membrane was performed for 7 min/2 gels at 20 V for 1 min, 23 V for 4 min, and 25 V for the remainder in an Iblot 2 gel transfer device (Life technologies, California, USA). Nonspecific protein binding to the nitrocellulose membrane was reduced by pre-incubation at 22 °C in blocking buffer containing 1% BSA. The nitrocellulose membranes were incubated overnight at 15 °C with antibodies against uncoupling protein 1 (UCP-1) (ABCAM, Massachusetts, USA). The antibodies were diluted 1:1000 with blocking buffer. The blots were subsequently incubated with a peroxidase-conjugated secondary antibody (ABCAM, Massachusetts, USA) for 1h at 15 °C. Specific bands were detected by chemiluminescence in Alliance 4.7 equipment (Uvitec, Cambridge, United Kingdom). The band intensity was quantified by optical densitometry (Scion Image-Release Beta 3b, National Institutes of Health, Massachusetts). The signals were normalized to β-actin (ABCAM, Massachusetts, USA).

Histopathological analysis

To evaluate the effects of high-fat diet with or without juçara pulp supplementation on liver, histopathological analysis was performed. Liver fragments were collected during euthanasia and fixed in 10% buffered formalin (Merck, Darmstadt, Germany) and embedded in paraplast (Histosec, Merck). Sections of 3–4 µm thick were obtained in microtome (RM 2125RT, Leica, DEU) and stained with hematoxylin and eosin (H.E., Merck). Slides were blindly analyzed by an independent pathologist using a light microscope (Axio Observer D1, Zeiss, USA) at ×200 magnification. To classify the degree of hepatic impairment, steatosis was visually classified into four scores from 0 to 3. The visual score based on histological findings is shown in Table 3.

Statistical analyses

Results are shown as mean ± standard error of the mean (SE). A one- or two-way analysis of variance (ANOVA) with or

Table 3 Score criteria* for hepatopathological changes (including steatosis and inflammation)

Score	Steatosis area (%)	Inflammation
0	0	Preserved cells and histoarchitecture
1	> 0% a 30%	Discrete steatosis
2	> 30% a 50%	Moderate steatosis
3	> 50%	Severe steatosis associated with loss of hepatic lobe structure

*Adapted from [29, 30]

without repeated measures was employed using Statistica software. When an interaction between the factors was detected by ANOVA, the Newman–Keuls *post hoc* test was used. Letters denote significant differences among the groups (different letters indicate significant differences). To evaluate the qualitative variables, referring to the results of the histopathological analysis of the liver, the Kruskal–Wallis non-parametric test was used followed by the Dunn multiple comparison test. An alpha level of $p < 0.05$ was considered statistically significant.

Results

High-fat diet substantially increased body weight and this effect was observed as early as the sixth week. However, juçara supplementation had opposite effects depending on the diet consumed. In control diet fed mice, juçara increased body weight, whereas in high-fat diet fed mice, juçara decreased body weight. This interaction (juçara and diet type) started in the sixth week and continued all over. Body weight of the H group started to be significantly higher than the C group in the 6th week, whereas the difference between HJ and C appeared later (9th week). Similarly, H mice were heavier than CJ from the 7th week on, but this was not observed between CJ and HJ until the 14th week. Consistently, the total body

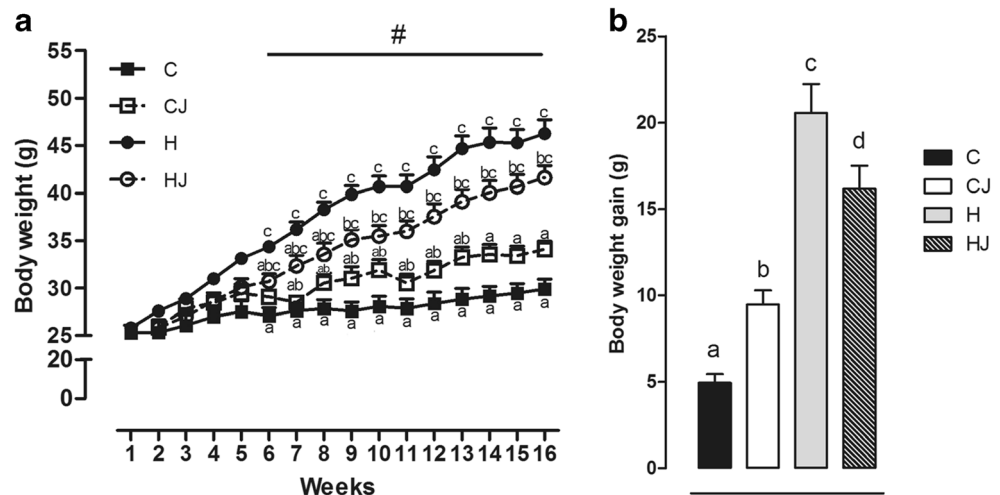
weight gain was affected by high-fat diet with an interaction between diet and juçara. After 16 weeks the body weight gain was higher in CJ compared to C and smaller in HJ compared to H ($C < CJ < HJ < H$) (Fig. 1a and b).

Energy intake was analyzed in the first and every four weeks. High-fat diet increased energy intake in the first week but reduced thereafter, reaching significance for an effect of the diet again at 8th and 12th weeks. On the other hand, juçara increased energy intake from the 4th week on. This effect, however, was evident only in the CJ group, as shown by the interaction between the effects of juçara and diet in weeks 4, 8, and 12. At these time points, the energy intake of CJ was higher than H group, and at weeks 8 and 12, it was also higher than in HJ. Additionally, we used a separate analysis to compare the energy intake only between the H and HJ groups, and no difference was found throughout the experiment (Fig. 2).

As body weight gain was decreased in HJ compared to H despite similar energy intake, energy expenditure (Fig. 3a–b) as well as UCP1 protein expression in brown adipose tissue (Fig. 3c) were measured in these groups. Interestingly, juçara increased energy expenditure in the HJ compared to H during the light phase of the cycle. However, UCP1 expression was similar between H and HJ.

Glucose intolerance was present in high-fat diet fed mice (H and HJ) as early as the 8th week. Blood glucose was significantly higher as an effect of the diet at 60 and 120 during the ipGTT

Fig. 1 (a) Body weight and (b) total body weight gain. $n=8-10$ mice per group. Two-way ANOVA with (a) or without (b) repeated measure; # Effect of diet. Different letters indicate significant differences among the groups found in post hoc test performed when an interaction between diet and juçara supplementation was detected. $p < 0.05$. C, control diet-fed group; CJ, control diet fed group supplemented with juçara; H, high-fat diet fed group; HJ, high-fat diet fed group supplemented with juçara



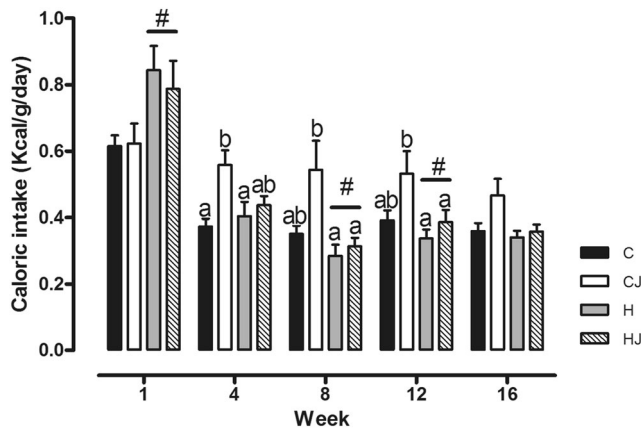


Fig. 2 Energy intake throughout the experiment. $n=8-10$ mice per group. Repeated measure two-way ANOVA; # Effect of diet. Different letters indicate significant differences among the groups found in post hoc test performed when an interaction between diet and juçara supplementation was detected. $p<0.05$. C, control diet-fed group; CJ, control diet-fed group supplemented with juçara; H, high-fat diet-fed group; HJ, high-fat diet fed group supplemented with juçara

(Fig. 4a), resulting in increased AUC in H and HJ compared to C and CJ (Fig. 4b). No effect of juçara was observed in week 8 (Fig. 4a–b). At the end of the study (16 weeks), except for before ip glucose administration (T0), blood glucose was increased by the high-fat diet at all time points during the test but there was also an interaction between diet and juçara at 15, 30, and 120 min. At these points, whereas blood glucose in H was higher than in C (and also than CJ at 30 and 120 min), in the HJ group, the values were intermediate, not differing from the other groups (Fig. 4c). Similarly, there was an effect of the diet and an interaction between diet and juçara for the AUC, which was higher in H compared to HJ, and in both high-fat diet fed groups than in C and CJ groups (Fig. 4d).

At week 8, insulin action was decreased in both groups fed a high-fat diet without any effect of juçara. As shown in Fig. 5a, normalized blood glucose was significantly higher in H and HJ compared to C and CJ mice 4 and 8 min following insulin administration, which resulted in increased area under the blood glucose curve in these groups (Fig. 5b). In week 16, the fat-rich diet worsened insulin sensitivity even further. The normalized blood glucose was higher as an effect of the diet (H and HJ) at 8 and 12 min. However, the severe insulin resistance was partly recovered by juçara supplementation, as the glucose-lowering effect of insulin was more evident during the test in HJ compared to H, reaching statistical significance at min 12. Consequently, the AUC of blood glucose at week 16 was affected by the high-fat diet and there was also an interaction between diet and juçara. The AUC was higher in H than in the other groups (C, CJ, and HJ).

Finally, ectopic fat liver accumulation was evaluated at the end of the study. The H group had higher fat content in hepatocytes than HJ, whereas no difference was observed between C and CJ (Fig. 6 and Table 4).

Discussion

Juçara is a palm tree whose fruit is rich in *cis*-unsaturated fatty acids, PUFAs, and dietary fiber and is a rich source of anthocyanins, compounds known for their health benefits. In view of the potential health-promoting properties of the juçara fruit, we investigated the effect of juçara freeze-dried powder supplementation in mice fed a high-fat diet. We found two important beneficial effects of the fruit. First, juçara decreased

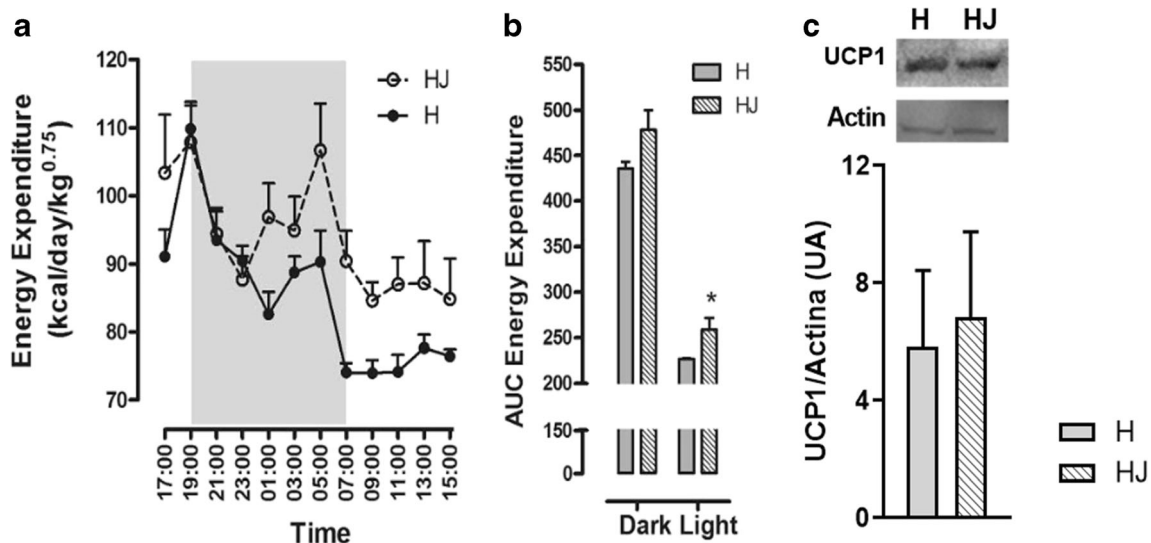
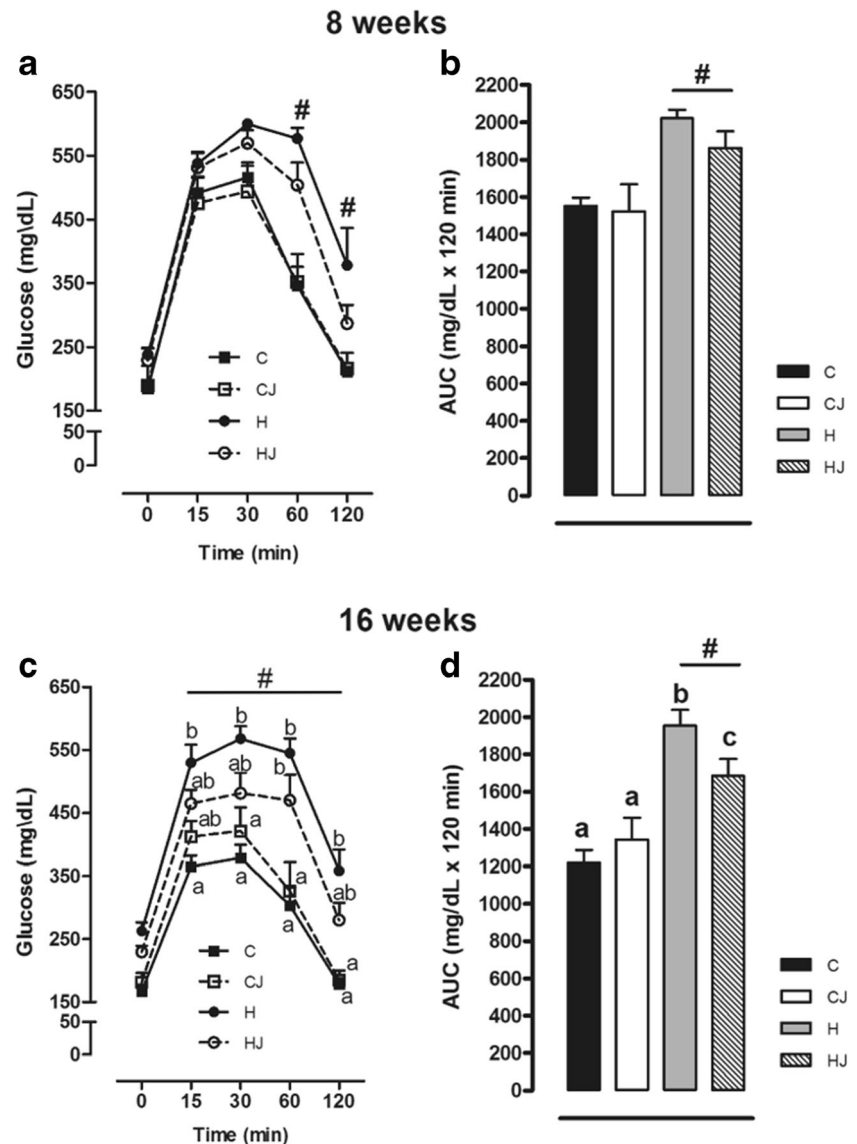


Fig. 3 Energy expenditure (a), AUC of energy expenditure (b), and UCP1 protein expression in brown adipose tissue (c) of mice at the end of the study. $n=4-5$ mice per group. Repeated measure one-way ANOVA

(a), two-way ANOVA (b), and Student’s *t*-test (c), $*p<0.05$. H, high-fat diet fed group; HJ, high-fat diet fed group supplemented with juçara

Fig. 4 Blood glucose (a, c) and AUC of blood glucose curve (b, d) during ipGTT at weeks 8 and 16. $n=8-10$ mice per group. Repeated measure two-way ANOVA (a, c) and two-way ANOVA (b, d); # Effect of diet. Different letters indicate significant differences among the groups found in post hoc test performed when an interaction between diet and juçara supplementation was detected. $p<0.05$. C, control diet-fed group; CJ, control diet-fed group supplemented with juçara; H, high-fat diet-fed group; HJ, high-fat diet fed group supplemented with juçara



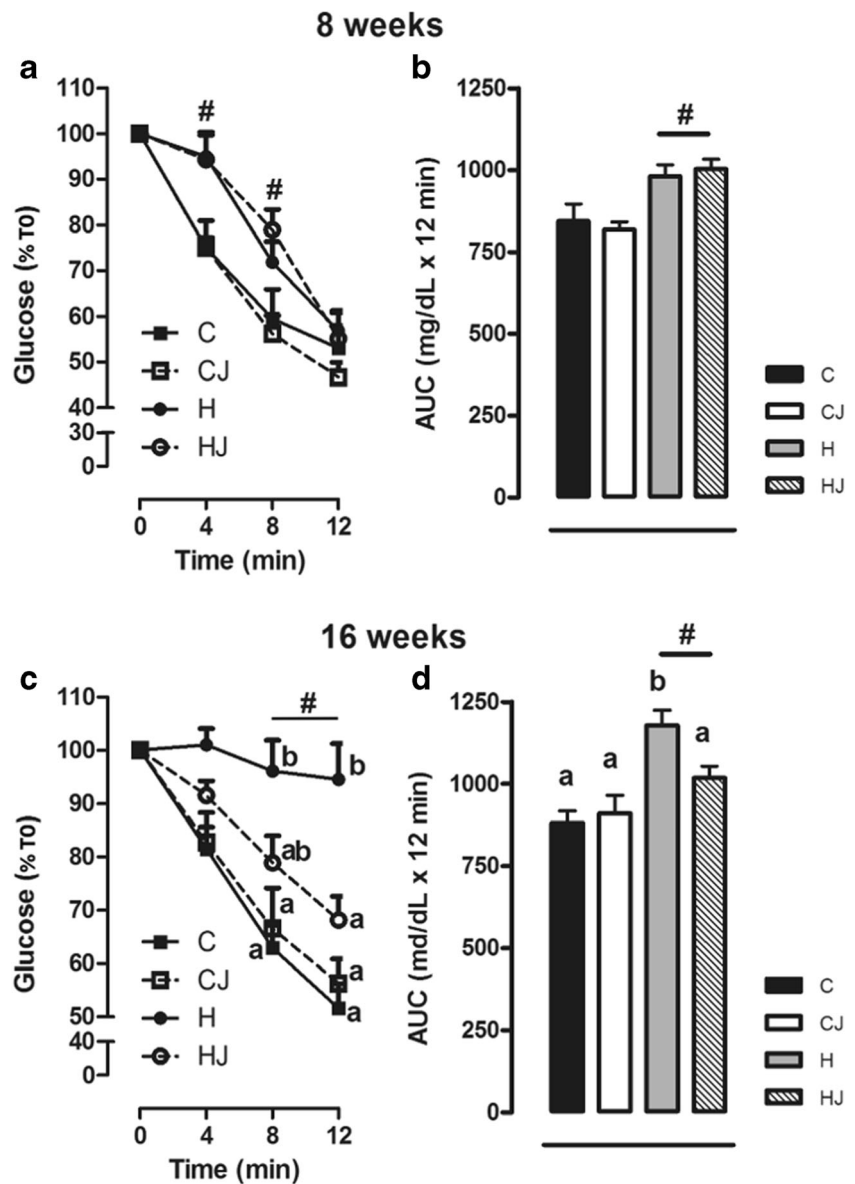
body weight gain and second, it improved substantially the metabolic parameters in diet-induced obese mice.

Several studies have reported the protective effects of anthocyanins-rich compounds against diet-induced obesity in rodents [12, 31]. In humans, increased consumption of most flavonoids, including anthocyanins, is inversely associated with body weight [32]. To understand how juçara protects against diet-induced obesity, energy intake and expenditure were assessed. Whereas juçara supplementation had no effect on energy intake, it increased energy expenditure in mice fed a high-fat diet in the light cycle. As diet-induced thermogenesis is highest during the dark cycle due to feeding [33] and we did not find any difference in 24-h locomotion (data not shown), we suggest that the effects of juçara on energy expenditure could be via basal metabolic rate or nonshivering cold-induced thermogenesis, for which brown adipose tissue has a prominent role.

Hoek-van den Hil et al. (2015) examined different flavonoids, including anthocyanins, and all reduced high-fat diet induced body weight gain but this effect could not be explained by changes on energy intake or energy expenditure [34]. Jia et al. (2020), on the other hand, found that oral administration of cyanidin-3-O-glucoside, an anthocyanin found in juçara, decreased body weight gain and increased energy expenditure and brown adipose tissue UCP-1 protein expression in mice fed a high-fat diet [15]. Juçara supplementation, in a maternal diet enriched with trans-fatty acids, increased UCP-1 expression in brown adipocytes of 21 days old offspring [35]. In our experimental model, however, juçara supplementation did not change brown adipose tissue UCP-1 protein content.

Browning of white adipocytes is known to increase energy expenditure [36] and could be involved in the thermogenic effects of juçara. High-fat diet fed mice treated with cranberry

Fig. 5 Normalized blood glucose (a, c) and AUC (b, d) during ipITT at weeks 8 and 16. *n*=8–10 mice per group. Repeated measure two-way ANOVA (a, c) and two-way ANOVA (b, d); # Effect of diet. *p*<0.05. Different letters indicate significant differences among the groups found in post hoc test performed when an interaction between diet and juçara supplementation was detected. C, control diet-fed group; CJ, control diet fed group supplemented with juçara; H, high-fat diet-fed group; HJ, high fat-diet fed group supplemented with juçara



polyphenolic extract exhibited browning in inguinal and epididymal white fat [37]. Moreover, multiple thermogenic mechanisms beyond UCP-1 have been found recently in thermogenic brown and beige fat, including creatinine and

calcium cycling [36]. Future studies are warranted on these alternative mechanisms to explain how juçara increased energy expenditure.

Energy intake of the mice fed a high-fat diet was higher only in the first week, consistent with other studies [38, 39]. Markers of inflammation, reactive gliosis, and neuron injury have been found in hypothalamus of mice fed a similar high-fat diet as early as the first week, after which they subside but eventually return later as high-fat feeding continues, and may become permanent [39]. Thus, it seems that the period of higher energy intake coincides with the initial hypothalamic inflammatory phase in mice fed a high-fat diet. Interestingly, juçara consistently increased the energy intake of mice fed a control diet, which resulted in increased body weight gain but without evidence of metabolic impairment. As food was offered ad libitum, we speculate that juçara might have

Table 4 Score of hepatic steatoses of mice at the end of the experiment

Groups	n° of mice	0 (absence)	1 (discrete)	2 (moderate)	3 (severe)
C*	5	5	0	0	0
CJ*	5	5	0	0	0
H	5	0	0	0	5
HJ*	5	0	2	2	1

**p*<0.05 when compared with the group that received a high-fat diet. Kruskal-Wallis test followed by Dunn’s similar comparison test

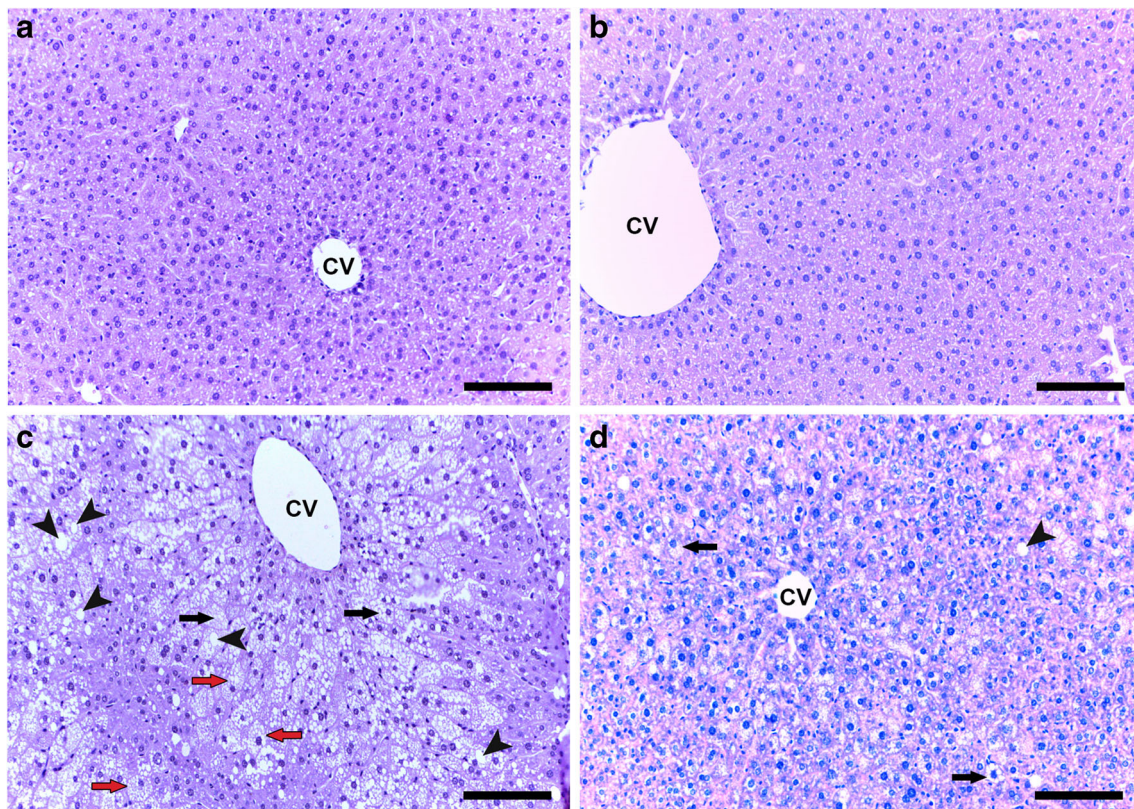


Fig. 6 Representative photomicrographs of mice livers. **a** and **b** Normal hepatic cytoarchitecture (score 0) in the control (**a**) and control supplemented with juçara (**b**) groups. **c** High-fat diet-fed group showing pericentral microvesicular (black arrow) and macrovesicular (arrowhead) steatosis (small and large lipid droplets present in hepatocytes), and

hepatocellular hypertrophy (red arrow) (score 3). **d** Note in the high-fat diet-fed group supplemented with juçara decreased liver injury with reduced microvesicular and macrovesicular steatosis area. CV, central vein of the hepatic lobule. Bar: 50 µm; ×200 magnification

increased the palatability of the diet leading to its higher consumption. Oyama et al. (2016) found not effect of 0.5% or 2% juçara supplementation on body weight in mice fed a hypercaloric and high-fat diet, but at the higher dose, the body weight gain was significantly increased in the control group, which also displayed glucose intolerance [19].

The characteristic alterations of type 2 diabetes, insulin resistance, and insufficient compensation of insulin secretion, leading to overt hyperglycemia, can be successfully reproduced in high-fat diet fed mice [40]. In our study, glucose intolerance and insulin resistance were present already at week 8 in high-fat diet fed mice, but the effects of juçara could be observed only at a later stage. Other studies have also observed an improvement of hyperglycemia and insulin resistance with anthocyanins or anthocyanins rich fruits [12, 41, 42]. Nevertheless, beyond anthocyanins, juçara fruit is also rich in fibers, whose beneficial effects on glucose homeostasis, insulin resistance, and other metabolic complications are well established [43].

Juçara supplementation (0.5%) in maternal diet rich in trans-fatty acids during gestation and lactation improved glucose levels, body composition, and gut microbiota and reduced low-grade inflammation in the colon of 21-day-old rat

offspring [44]. Similarly, Oyama et al. (2016) found that 0.5% juçara improved glucose tolerance in mice after 10 weeks [19]. However, a higher dose (2%) led to glucose intolerance and increased cholesterol levels in mice fed a control diet and to the loss of the positive effect in the high-fat high-caloric group. These results reinforce the need for more studies to determine the optimal time and dose for an effective and safe use of juçara [19].

The mechanisms linking obesity to insulin resistance and type 2 diabetes mellitus are yet not fully understood but include inflammation [45], oxidative stress [46], and ectopic lipid accumulation [47]. Here we found that juçara protected high-fat diet fed mice from liver steatosis after 16 weeks, a mechanism that could be involved in the improved insulin sensitivity observed in HJ compared to H mice. Similarly, supplementation with cyanidin 3-glucoside prevented the increase in total liver lipids and triglycerides induced by high-fat diet [12]. Even though we did not measure inflammation and oxidative stress, evidence shows that high consumption of anthocyanin-rich foods is associated with lower insulin and inflammation levels in adult females [48]. An inverse association between high anthocyanin intake

and inflammatory biomarkers has also been observed [49]. Additionally, acute consumption of juçara pulp in humans increased the activity of catalase (CAT), an indispensable enzyme in the antioxidant defense [50]. In a recent study, obese participants that received 5g of juçara freeze-dried pulp for 6 weeks showed a reduction in interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α), and monocyte chemoattractant protein-1 (MCP-1) levels compared to placebo [22]. Finally, reduction in oxidative stress markers has been shown in healthy humans supplemented with anthocyanin-rich strawberry [51] and in streptozotocin-induced diabetic rats [41]. Together, these studies highlighted the potential benefits of anthocyanin-rich food in the prevention of metabolic complications associated with obesity, and our results suggest that juçara might be included among them.

Conclusion

In conclusion, juçara pulp supplementation can improve insulin resistance and glucose intolerance besides protecting mice from liver steatosis and diet-induced obesity (as summarized in the graphical abstract). Additional studies are needed to understand its action mechanistically.

Abbreviations AIN, American Institute of Nutrition; ANOVA, analysis of variance; AUC, area under the curve; BSA, bovine serum albumin; C, control diet group; CAT, catalase; CEDEME, Center for Development of Animal Models for Medicine and Biology; CEUA, Institutional Ethics Committee on Animal Use; CJ, control diet group supplemented with juçara 0.5% freeze-dried powder; H, high-fat diet group; HE, hematoxylin and eosin; HJ, high-fat diet group supplemented with juçara 0.5% freeze-dried powder; IL-6, interleukin 6; IPEMA, Institute of Permaculture and Ecovillages of the Atlantic; ipGTT, intraperitoneal glucose tolerance test; ipITT, intraperitoneal insulin tolerance test; MCP-1, monocyte chemoattractant protein-1; NAFLD, non-alcoholic fatty liver disease; PUFAs, polyunsaturated fatty acids; SE, standard error of the mean; TNF- α , tumor necrosis factor alpha; UCP-1, uncoupling protein 1; WHO, World Health Organization

Code availability Not applicable.

Author contribution MB, CAMO and LPP were responsible for the design of the study; MB and CAMO conducted the study; MB, CAMO, TLM, FPC, IDB, DAR, DE, LLM and VVR helped with experimental procedures, data analysis and discussion.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval All procedures were approved by the Institutional Ethics Committee on Animal Use (CEUA n.1750/11) from Federal University of Sao Paulo.

Consent to participate Not applicable.

Consent for publication All authors listed have contributed to the work, read, approved, and agreed to submit the manuscript.

Competing interests The authors declare no competing interests.

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