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Consumption of phenolic-rich jabuticaba (*Myrciaria jaboticaba*) powder ameliorates obesity-related disorders in mice

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Abstract

Accumulating evidence indicates that dietary phenolic compounds can prevent obesity-related disorders. We investigated whether the consumption of polyphenol-rich jabuticaba peel and seed powder (JPSP) could ameliorate the progression of diet-induced obesity in mice. Male mice were fed a control diet or a high-fat (HF) diet for 9 weeks. After this period, mice were fed control, HF or HF diets supplemented with 5 % (HF-J5), 10 % (HF-J10) or 15 % (HF-J15) of JPSP, for 4 additional weeks. Supplementation with JPSP not only attenuated HF-induced weight gain and fat accumulation but also ameliorated the pro-inflammatory response associated with obesity, as evidenced by the absence of mast cells in the visceral depot accompanied by lower IL-6 and TNF-α at the tissue and circulating levels. JPSP-supplemented mice also exhibited smaller-sized adipocytes, reduced levels of leptin and higher levels of adiponectin, concomitant with improved glucose metabolism and insulin sensitivity. The magnitude of the observed effects was dependent on JPSP concentration with HF-J10- and HF-J15-fed mice showing metabolic profiles similar to control. This study reveals that the consumption of JPSP protects against the dysfunction of the adipose tissue and metabolic disturbances in obese mice. Thus, these findings indicate the therapeutic potential of the phenolic-rich JPSP in preventing obesity-related disorders.

Key words: **Adipose tissue: Inflammation: Obesity: Polyphenols**

Adipose tissue is a highly plastic organ and a pivotal player in the pathogenesis of obesity[1]. Besides its role in storing and releasing energy, adipose tissue integrates metabolic, endocrine and immunological functions[2,3]. The maintenance of the structural and functional integrity of this tissue depends on the interplay of adipocytes and their precursors, endothelial cells, fibroblasts and immune cells[4]. Importantly, obesity triggers the recruitment and infiltration of different immune cell types, which provokes a pro-inflammatory response[4]. Unresolved tissue inflammation in turn leads to a systemic low-grade inflammatory state[5,6], which compromises the production of adipokines and insulin sensitivity[7,8]. Thus, both adipose tissue inflammation and dysfunction have become attractive targets to prevent the development and progression of obesity-related disorders.

The consumption of bioactive compounds from fruits has been associated with a lower risk of developing chronic non-communicable diseases[9]. Observational evidence indicates that the intake of phenolic compounds may prevent obesity-related complications[10]. These compounds present several beneficial properties, such as anti-inflammatory and antioxidant, and show potential in improving lipid profile and insulin resistance[11–14].

Abbreviations: HF, high-fat; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP; HF-J5, high-fat with 5 % of JPSP; JPSP, jabuticaba peel and seed powder; vWAT, visceral white adipose tissue.

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Jabuticaba (*Myrciaria jaboticaba*) is a fruit native to the Brazilian Atlantic forest with high content of phenolic compounds[15,16], mainly anthocyanins, ellagic and gallic acid derivatives[17]. Importantly, the majority of the bioactive phenolic compounds are concentrated in the peel and seed of this fruit, fractions that are not usually consumed[18,19]. Whether the consumption of the phenolic mixture from this fruit could alter the pathogenesis of obesity remains unclear. Recently, Inada *et al.*[20] developed a jabuticaba peel and seed powder (JPSP) that preserves the integrity of its compounds resulting in a mixture mainly enriched in anthocyanins and ellagitannins. This phenolic composition profile draws attention for the potential metabolic effects of JPSP on obesity and related disorders. Therefore, this study aimed to evaluate the dose–response effects of JPSP consumption on the development of diet-induced disturbances in obese mice.

Materials and methods

Jabuticaba peel and seed powder

Jabuticaba fruits (*Myrciaria jabuticaba*, cv. Sabará) were purchased at Rio de Janeiro's agricultural trading centre. Fruits were processed as previously described[20], by depulping the fruit, freeze-drying and grounding. Proximate composition (moisture, carbohydrate, protein, lipid, ash and dietary fibre) and phenolic compounds profile of JPSP were previously reported[15,20] and are listed in Table 1.

Mouse study design

Eighty male C57BL/6 mice (three-month-old) were used in this study. Animals were housed in the Biotery of the Department of Pharmacology and Psychobiology, Rio de Janeiro State University at a temperature of 23 (SD 2)°C, relative humidity of 60 (SD 10)%, and under a standard lighting regime of 12-h light–12-h dark cycle, with lights on from 07.00 to 19.00 hours. All the animal procedures were approved by the Ethics Committee for the Care and Use of Experimental Animals of the Rio de Janeiro State University (CEUA No. 51/2016) and were carried out following the National Institutes of Health guide for the care and use of Laboratory animals, 8th edition[21].

Mice were allocated to receive either a standard maintenance diet[22] (*n* 16) or a high-fat diet (*n* 64, HF, 50 % kcal from fat, 23·8 % from lard) for 9 weeks. The HF diet had a higher energy content than the control diet to induce overweight and adipocyte dysfunction. After this feeding period, HF-fed mice were subdivided into four groups (*n* 16/group), receiving HF diet or HF diet supplemented with JPSP in the following concentrations (w/w): 5 % (HF-J5), 10 % (HF-J10) or 15 % (HF-J15) (Fig. 1). Given the JPSP fibre content, diets were formulated with different amounts of cellulose in order to contain similar contents of dietary fibre (Table 2). All groups (including control) underwent dietary intervention for 4 additional weeks. Diet and water were provided *ad libitum* throughout the experiment. Mice body weight was weekly recorded, whereas food intake was monitored every 48 h, taking into account the diet in food compartment and leftovers from the bottom of the cage. Energy intake was estimated using the energy density of the diets, as kJ/g.

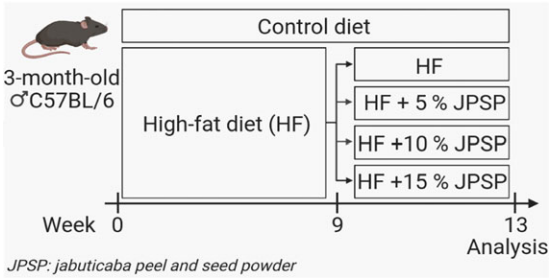

Fig. 1. Experimental design. C57BL/6 male mice were allocated to receive either a control diet (*n* 16) or a high-fat (HF) diet (*n* 64) for 9 weeks. After this period, HF-fed mice were subdivided into four groups (*n* 16 each), receiving HF diet or HF diet supplemented with jabuticaba peel and seed powder (JPSP) for 4 weeks in the following concentrations (w/w): 5 % (HF-J5), 10 % (HF-J10) or 15 % (HF-J15). At the end of 13 weeks, mice were euthanised and blood and adipose tissue were collected for further analyses.

Table 1. Nutritional composition of the jabuticaba peel and seed powder (JPSP)*

	Content
Proximate composition (g/100 g)	
Protein	about 7·5
Ash	about 4·2
Lipid	about 0·5
Carbohydrate	about 51·8
Dietary fibre	about 36·1
Phenolic compounds (mg/100 g)	
Gallic acid	about 50·0
Ellagic acid	about 314·0
Vescalagin	about 740·0
Castalagin	about 898·0
Vescalagin/castalagin isomers	about 543·0
Di-HHDP-glucose isomers	about 240·0
Cyanidin-3-*O*-glucoside	about 904·0
Delphinidin-3-*O*-glucoside	about 70·0
Total phenolic compounds	3759·0

* Proximate composition was determined by official methods, and phenolic compounds were determined by LC-MS previously reported.[15,18]

Table 2. Chemical composition and energy value of the experimental diets

	Experimental groups				
Ingredients	Control	HF	HF-J5	HF-J10	HF-J15
Maize starch (g/kg of diet)	465·7	232·7	245·0	257·2	269·5
Maltodextrin (g/kg of diet)	155·0	115·0	115·0	115·0	115·0
Sucrose (g/kg of diet)	100·0	100·0	100·0	100·0	100·0
Casein (g/kg of diet)	140·0	175·0	175·0	175·0	175·0
Soyabean oil (g/kg of diet)	40·0	40·0	40·0	40·0	40·0
Lard (g/kg of diet)	0·0	238·0	238·0	238·0	238·0
Cellulose (g/kg of diet)	50·0	50·0	37·8	25·5	13·3
Minerals (g/kg of diet)	35·0	35·0	35·0	35·0	35·0
Vitamins (g/kg of diet)	10·0	10·0	10·0	10·0	10·0
Cystine (g/kg of diet)	1·8	1·8	1·8	1·8	1·8
Choline (g/kg of diet)	2·5	2·5	2·5	2·5	2·5
JPSP (g/kg of diet)	0·0	0·0	50·0	100·0	150·0
Total	1000	1000	1050	1100	1150
Energy (kJ/g of diet)	16·7	21·7	21·7	21·7	21·7

HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP; JPSP, jabuticaba peel and seed powder.

Blood and tissue collection

At the end of dietary interventions, mice were fasted for 6 h and euthanised under anaesthesia (thiopental sodium, 70 mg/kg i.p.). Blood samples were collected by cardiac puncture. Plasma was separated by centrifugation at room temperature (120 ***g*** for 20 min) and stored at −20°C until analyses. Subcutaneous (inguinal) and visceral (epididymal and retroperitoneal) fat pads were harvested, weighed and utilised as described below.

Blood and plasma analysis

Blood glucose was measured by using an Accu-Check glucose meter (Roche®). The insulin level was determined by ELISA using the Insulin 125I Ria Kit (RK-400M; MP Biomedicals, LLC). Insulin sensitivity was assessed by using quantitative insulin sensitivity check index (1/log insulin (mU/l) + log glucose (mg/dl))[23]. Leptin, adiponectin and resistin levels were determined by ELISA using commercial kit (RAB0334, Sigma-Aldrich; EK0596 and EK0582, Boster Biological Engineering Co. Ltd, respectively). Plasma levels of TNF-α and IL-6 were determined by ELISA using commercial kits (BMS607-3 and BMS603-2, respectively; Invitrogen).

Adipokines and inflammatory markers of visceral white adipose tissue

Leptin, adiponectin, TNF-α and IL-6 levels in visceral white adipose tissue (vWAT) were evaluated by ELISA using commercial kits (RAB0334, RAB1115, RAB0477 and RAB0309, respectively; Invitrogen) after tissue homogenisation and using the supernatant, as previously described[24]. The results were expressed in ng/mg, pg/mg, pg/mg and pg/mg.

Histological analysis

For haematoxylin–eosin staining, fragments of the vWAT fixed in 4 % formaldehyde for 48 h were paraffin-embedded and sectioned (5 µm thick). The average sectional area of adipocytes in vWAT was analysed in ten randomly selected fields of view (20× objective) using the STEPanizer computer-based software. The cross-sectional area of the adipocytes was defined as the ratio of adipocyte volume density:twice the numerical adipocyte density per area. The adipocyte volume density was calculated by the ratio between partial points and total points (using a test frame with sixteen points). The numerical density of adipocyte reflected the number of adipocytes per field of view (excluding the ones that hit the forbidden lines) divided by the area in μm^2 as previously described[25,26]. For the quantification of mast cells, sections were stained with 0·5 % toluidine blue. Images were analysed using Image-Pro Plus version 5.0 software (Media Cybernetics). All images were acquired using an inverted microscope (Olympus) equipped with a digital DP71/BX40 camera.

Quantitative real-time PCR

Total RNA was isolated from vWAT by using a TriZol® reagent (Life Technologies). The cDNA was synthesised with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). TaqMan gene expression assays (Applied Biosystems) were used to detect the following: *Tnfa* (Mm00443258_m1); *Il6* (Mm00446190_m1); *Mcp1* (Mm00441242_m1); *Cxcl9* (Mm00434946_m1) and *Cxcl10* (Mm00445235_m1) mRNA expression. PCR amplification was carried out by using the ABI Prism 7.500 fast (Applied Biosystems) and standard cycling conditions. The expression of each target gene was normalised to the relative expression of *Gapdh* (Mm05724508_g1) mRNA as an internal efficiency control (online Supplementary material). The mRNA fold change was calculated by using the 2(-Delta C(T)) method[27].

Statistical analysis

Results were expressed as mean values and standard deviation. Statistical analyses were performed using GraphPad Prism version 6 software (GraphPad Software Inc.). Comparisons among groups were analysed by one-way ANOVA, followed by Tukey's *post hoc* test for multiple comparisons. Differences were considered significant when $P < 0{\cdot}05$.

Results

Consumption of jabuticaba peel and seed powder prevents high-fat-induced weight gain and adiposity

As expected, mice fed the HF diet for 9 weeks gained significantly more weight than those fed the control diet (Table 3).

Table 3. Food behaviour and body mass variation of male C57BL/6 mice exposed to different concentrations of jabuticaba peel and seed powder (JPSP) (*n* 16 per group)*
(Mean values and standard deviations)

	Experimental groups									
	Control		HF		HF-J5		HF-J10		HF-J15	
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Food intake (g/animal per d)	3·1	0·8	2·9	0·5	3·0	0·7	3·2	0·8	3·1	0·6
Energy (kJ/animal per d)	51·9^{b}	5·8	63·1^{a}	5·3	65·3^{a}	6·0	69·6^{a}	7·9	67·0^{a}	5·9
Consumption of phenolic compounds (mg/animal per d)	–		–		5·7^{c}	0·9	12·0^{b}	1·9	17·5^{a}	2·7
Body mass, week 1 (g)	25·4	0·9	25·3	0·1	25·7	0·1	25·4	0·3	25·1	0·8
Body mass, week 9 (g)	27·0^{b}	2·5	40·1^{a}	4·4	40·0^{a}	4·2	38·8^{a}	5·8	39·5^{a}	5·7
Body mass week 13 (g)	29·5^{c}	1·6	43·8^{a}	0·6	41·2^{a,b}	1·9	40·9^{a,b}	1·8	39·7^{b}	1·3
Weight gain (%)	16·1^{c}	1·9	73·0^{a}	5·4	60·3^{a,b}	5·9	61·0^{a,b}	7·7	58·2^{b}	8·1

HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP.
* Values are expressed as mean values and standard deviation, and different letters indicate a significant difference between the groups (ANOVA followed by Tukey's *post hoc* test, $P < 0.05$).

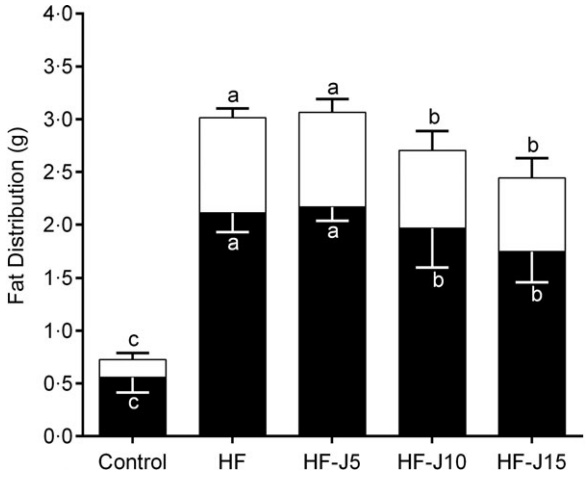

Fig. 2. Body fat distribution. Weight of subcutaneous and visceral fat pads at the end of the experiment (*n* 16 per group). Data are expressed as mean values and standard deviation, and different letters indicate a significant difference between groups, $P < 0{\cdot}05$. JPSP, jabuticaba peel and seed powder; HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP. ▭, Subcutaneous fat pad; ■, visceral fat pad.

Accordingly, HF-fed mice displayed higher adiposity in both subcutaneous and visceral depots (Fig. 2). In contrast, HF-induced weight gain was attenuated when HF was supplemented with 15 % JPSP in the 4 final weeks of the dietary intervention. Although only HF-J15-fed mice exhibited lower body mass than HF counterparts ($P < 0{\cdot}001$), mice fed with both HF-J10 and HF-J15 displayed less fat accumulation, which was evidenced by smaller subcutaneous and visceral pads (Fig. 2).

Due to the higher energy content in the HF diet, HF-fed mice displayed a higher energy intake than control counterparts (Table 3). Supplementation of JPSP did not affect food intake (Table 3), suggesting that the lower adiposity observed with HF-J10 and HF-J15 was not explained by changes in energy intake. In turn, the intake of phenolic compounds was 3-fold higher in mice fed with HF-J15 when compared with HF-J5-fed mice due to the nutritional composition of the diet ($P < 0{\cdot}001$) (Table 3).

Jabuticaba peel and seed powder supplementation leads to a healthier expansion of the visceral adipose tissue

Given the lower fat accumulation observed with JPSP supplementation, we examined the effects of JPSP on adipose tissue morphology, composition and functions in the visceral depot. Histological and stereological analyses of adipocytes showed significantly larger adipocytes in HF *v.* control-fed mice, whereas smaller-sized adipocytes were observed in the vWAT from HF-J10 and HF-J15 groups when compared with HF counterparts (Fig. 3a and b). Of note, the adipocyte area from HF-J15 mice was similar to the ones visualised from the control-fed mice.

Staining of vWAT sections with toluidine blue revealed the presence of mast cells in the adipose tissue from HF-fed mice but not in any of the JPSP-supplemented mice neither the control group (Fig. 3c). In line with the presence of mast cells, HF-fed mice had elevated transcriptional levels of inflammatory markers *Tnfa*, *Il6*, *Mcp1*, *Cxcl9* and *Cxcl10* (Fig. 4a–e) concomitant with higher levels of IL-6 and TNF-α in vWAT compared with control (Fig. 5a and b). These mice also displayed higher plasmatic levels of these pro-inflammatory markers, implying that the tissue pro-inflammatory response induced by HF feeding led to a systemic low-grade inflammation (Table 4). In contrast, JPSP-supplemented mice displayed reduced inflammatory markers at both the mRNA and protein levels in the adipose tissue, which was reflected into their lower circulating levels. The magnitude of this reduction was associated with the increase in JPSP supplementation; and the levels of these pro-inflammatory markers in HF-J15 were similar to control-fed mice (Figs. 4 and 5 and Table 4).

Subsequent analysis of adipokines levels showed that HF feeding also promoted an imbalance in their production with increased leptin and decreased adiponectin levels compared with control mice (Fig. 5c and d). This imbalance was detected both at the tissue and systemic levels (Table 4). HF-fed mice also displayed lower plasmatic levels of resistin, indicating that this dietary intervention led to adipose tissue dysfunction (Table 4). Conversely, supplementation with JPSP preserved tissue production of adipokines, with mice fed with HF-J10 and HF-J15 presenting leptin and adiponectin levels similar to that of the control group (Fig. 5c and d). Consistent with tissue levels, circulating leptin decreased with JPSP supplementation, being restored to the control levels by JPSP at a concentration of 10 % and 15 % (HF-J10 and HF-J15 groups), but not 5 % (HF-J5). Moreover, all JPSP-supplemented groups showed resistin plasmatic levels similar to that of the control group, whereas plasma levels of adiponectin detected in JPSP groups were higher when compared with both HF and control groups (Table 4). Taken together, these findings demonstrate that supplementation of HF with JPSP, particularly at 10 % and 15 %, may protect against adipose tissue dysfunction induced by the HF diet.

Supplementation of high-fat diet with jabuticaba peel and seed powder preserves the metabolic health of obese mice

Adipose tissue inflammation and dysfunction are major contributors to the development of metabolic disturbances associated with obesity. Thus, we hypothesised that the healthier state of the adipose tissue from JPSP-supplemented mice may reflect in an improved whole-body glucose metabolism when compared with HF feeding. As expected, fasting blood glucose levels of HF-fed mice were higher compared with control counterparts (Fig. 6a). JPSP supplementation progressively decreased glucose levels, which were restored to the control level for HF-J10 and HF-J15 groups ($P < 0{\cdot}001$). Plasma insulin levels were also elevated with HF feeding (Fig. 6b), and supplementation with different concentrations of JPSP resulted in lower insulin levels, even though still higher when compared with control-fed mice. Consistent with the elevated levels of blood glucose and plasma insulin, HF-fed mice displayed lower insulin sensitivity compared with control mice, which was normalised by the supplementation with 10 % and 15 % of JPSP (Fig. 6c). Collectively, these findings indicate that JPSP supplementation improved

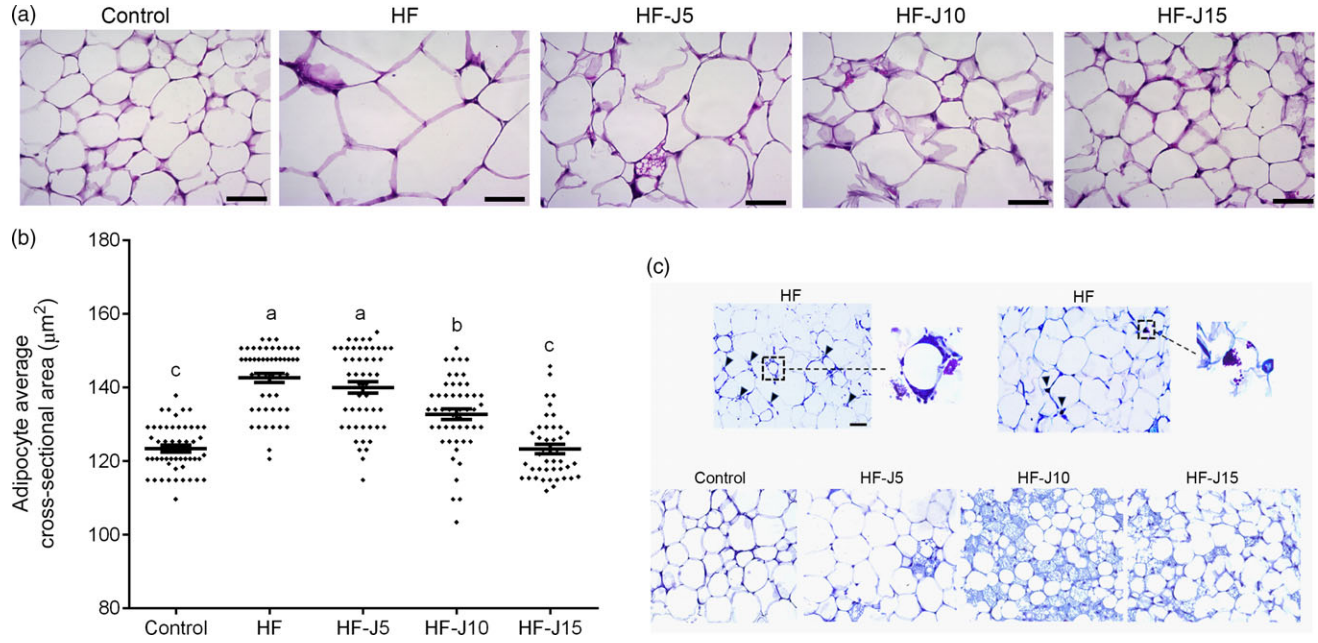

Fig. 3. Effects of jabuticaba peel and seed powder (JPSP) supplementation on cellular and morphological features of visceral adipose tissue. (a) Stereology of visceral white adipose tissue (vWAT). Scale bars: 20 μm. (b) Average cross-sectional area (*n* 10 per group): mice supplemented with high-fat with 10 % of JPSP (HF-J10) and high-fat with 15 % of JPSP (HF-J15) showed smaller adipocyte area compared with HF-fed counterparts and other groups that underwent dietary intervention. Data are expressed as mean values and standard deviation, and different letters indicate a significant difference between the groups, $P < 0{\cdot}05$. (c) Mast cells evidenced in the HF group. Scale bars: 20 μm. Black arrowheads: numerous mast cells distributed in the adipose tissue stroma. Highlighted cells that are in the connective, near the adipocyte, which display purple basophil granules. HF-J5, high-fat with 5 % of JPSP. Scale bars: 20 μm.

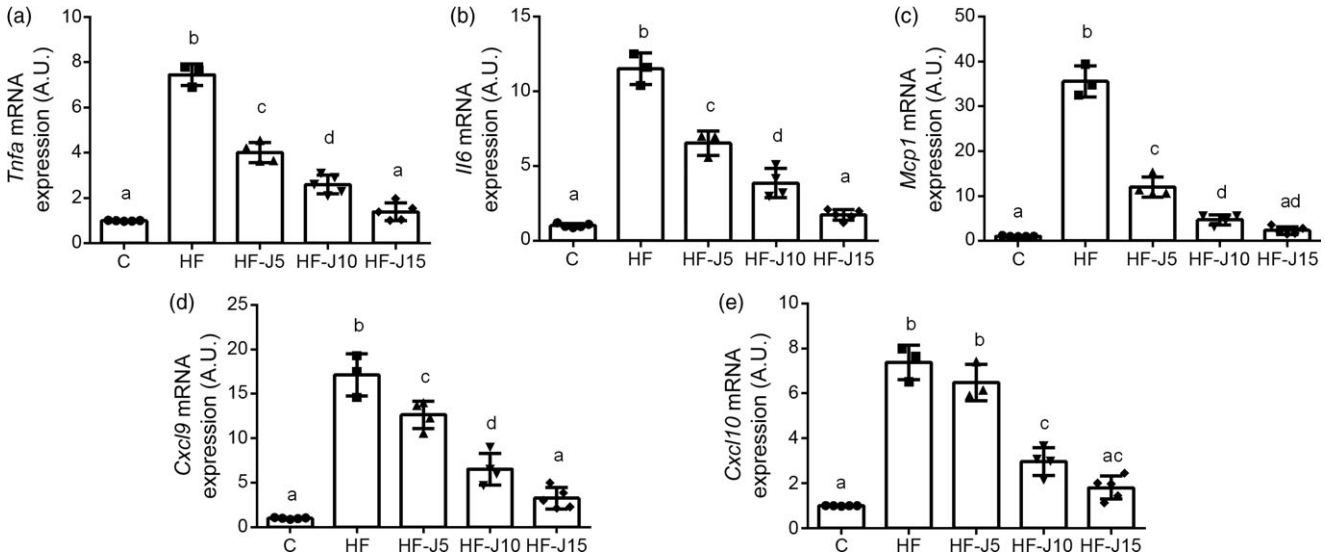

Fig. 4. Transcriptional levels of inflammatory markers. Gene expression of inflammatory markers *Tnfa* (a), *Il6* (b), *Mcp1* (c), *Cxcl9* (d) and *Cxcl10* (e) relative to Gapdh mRNA levels was examined in visceral adipose tissue (*n* 10). Data are expressed as mean values and standard deviation, and different letters indicate a significant difference between the groups, $P < 0{\cdot}05$. JPSP, jabuticaba peel and seed powder; HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP.

glucose metabolism promoting a healthier metabolic profile in obese mice.

Discussion

In the past few years, several researches have reported the beneficial effects of jabuticaba *in vitro*[28], as well as in animal models[29] and, more recently, in humans[30]. In this study, we provided evidence that JPSP prevents the development of obesity complications induced by HF feeding. We have found that the supplementation with JPSP protected obese mice against not only body weight gain and adiposity but also against the development of adipose tissue dysfunction and low-grade inflammatory state, which resulted in improved metabolic profile even under HF feeding conditions (Fig. 7). Thus, our study

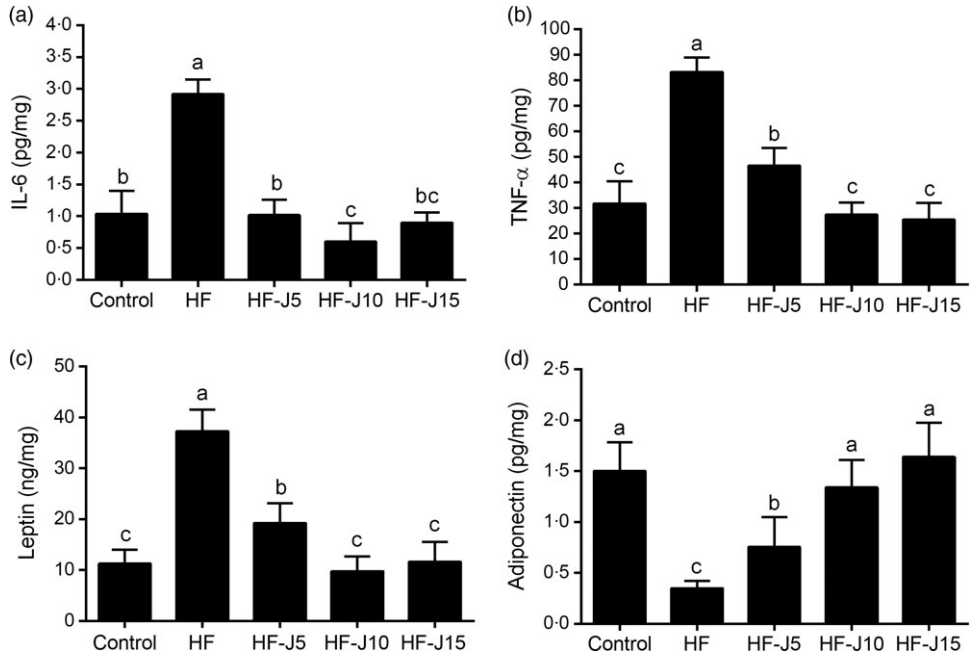

Fig. 5. Tissue levels of adipokines and inflammatory markers. Protein levels of inflammatory markers IL-6 (a) and TNF-α (b) as well as adipokines leptin (c) and adiponectin (d) were assessed in the visceral adipose tissue after supplementation with jabuticaba peel and seed powder (JPSP) (n 10). Data are expressed as mean values and standard deviation, and different letters indicate a significant difference between the groups, $P < 0{\cdot}05$. HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP.

Table 4. Plasma adipokines levels of male C57BL/6 mice exposed to different concentrations of jabuticaba peel and seed powder (JPSP) (n 5)* (Mean values and standard deviations)

	Experimental groups									
	Control		HF		HF-J5		HF-J10		HF-J15	
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leptin (ng/ml)	$6{\cdot}0^{b}$	0·7	$13{\cdot}9^{a}$	3·5	$14{\cdot}8^{a}$	3·4	$6{\cdot}4^{b}$	1·6	$4{\cdot}9^{b}$	1·6
Adiponectin (pg/ml)	$5{\cdot}6^{b}$	1·3	$5{\cdot}48^{b}$	1·4	$7{\cdot}0^{a}$	1·5	$7{\cdot}70^{a}$	0·9	$7{\cdot}4^{a}$	1·9
Resistin (pg/ml)	$1{\cdot}9^{a}$	0·1	$1{\cdot}18^{b}$	0·2	$1{\cdot}8^{a}$	0·18	$1{\cdot}9^{a}$	0·4	$2{\cdot}3^{a}$	0·3
IL-6 (pg/ml)	$15{\cdot}4^{c}$	3·6	$67{\cdot}7^{a}$	5·4	$28{\cdot}9^{b}$	3·8	$15{\cdot}3^{c}$	1·9	$12{\cdot}1^{c}$	4·3
TNF-α (ng/ml)	$3{\cdot}1^{b}$	0·9	$9{\cdot}2^{a}$	1·9	$6{\cdot}0^{a}$	1·0	$4{\cdot}0^{b}$	0·9	$3{\cdot}8^{b}$	0·9

HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP.
* Values are expressed as mean values and standard deviation, and different letters indicate a significant difference between the groups (ANOVA followed by Tukey's *post hoc* test, $P < 0.05$).

unravelled that JPSP has the potential to provide a new therapeutic option to hinder obesity pathogenesis.

Consistent with the main function of lipid storage, adipose tissue depots expand under increased nutrient availability reflecting into weight gain[31]. Although displaying similar energy intake, we observed that obese mice supplemented with JPSP had lower weight gain and fat accumulation, which coincided with lower glycaemia and higher insulin sensitivity. Before our work, some preclinical studies in mice have proposed that jabuticaba extracts may protect against weight gain leading to better glucose metabolism[32,33]. Nonetheless, it is worth pointing out that these studies have assessed extracts with different compositions as they have been obtained from the jabuticaba peel, whereas our powder included both the peel and the seed. In addition, previous studies have employed HF diets combined with sucrose and administered the extracts by gavage instead of supplementing the diet. Despite these differences in experimental design, our results corroborate those of earlier reports highlighting the therapeutic potential of jabuticaba, especially their peel and seeds, for obesity treatment.

On this path, other anti-obesity mechanisms may occur by inhibiting the digestive enzymes α-amylase, α-glucosidase and pancreatic lipase[34]. These enzymes catalyse the hydrolysis and absorption of carbohydrates and lipids from the diet. In this way, the inhibition of these enzymes prevents hyperglycaemia and postprandial hypertriacylglycerolaemia[35,36].

As is known, phenolic compounds can inhibit α-amylase, α-glucosidase and lipase through non-specific binding to enzymes[37]. *In vitro* assays showed that proanthocyanidins exhibit potent α-amylase inhibitory activity and moderate α-glucosidase inhibitory activity[38,39] and polyphenols extracted from fruits have previously been reported as inhibitors of lipase

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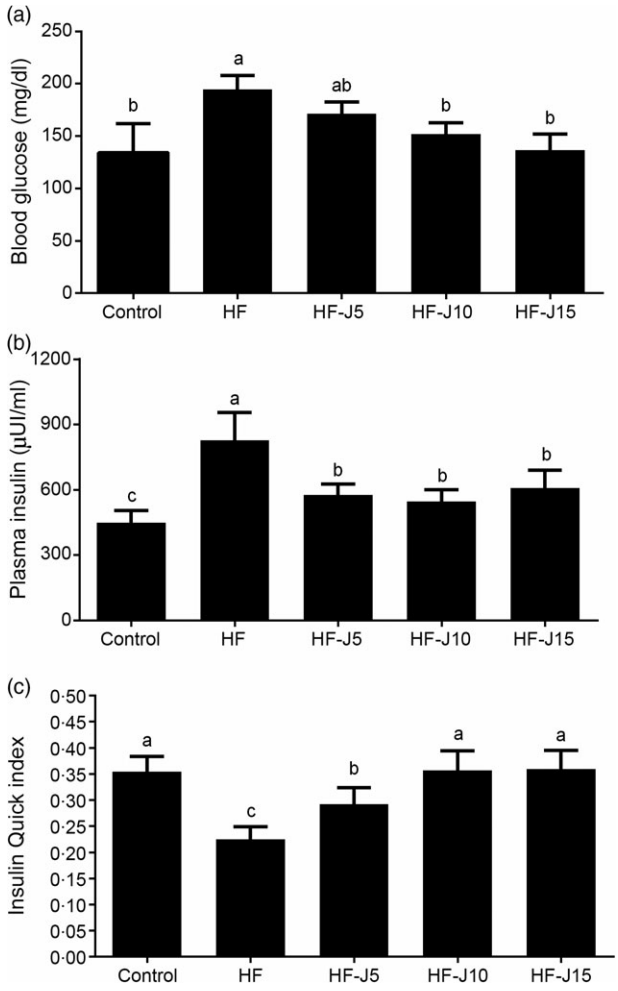

Fig. 6. Concentration of glucose, insulin and insulin Quick index at the end of dietary interventions. (a) Blood glucose (n 10), (b) plasma insulin (n 10) and (c) Insulin Quick index. Data are expressed as mean values and standard deviation , and different letters mean a significant difference between the groups, $P < 0{\cdot}05$. JPSP, jabuticaba peel and seed powder; HF, high-fat; HF-J5, high-fat with 5 % of JPSP; HF-J10, high-fat with 10 % of JPSP; HF-J15, high-fat with 15 % of JPSP.

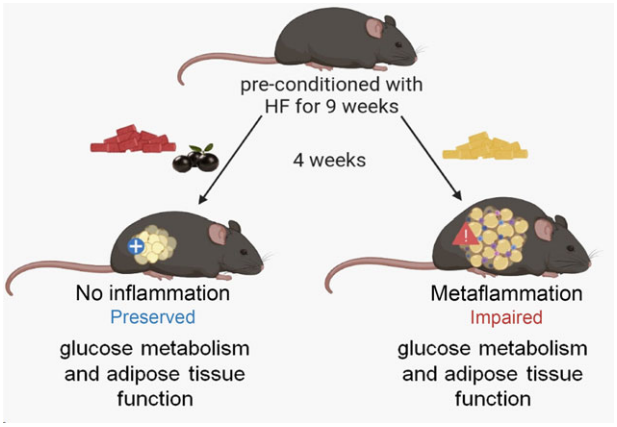

Fig. 7. Scheme depicting effects of jabuticaba peel and seed powder (JPSP) consumption counteracting the development of obesity-related complications. Besides preventing weight gain and adiposity, supplementation with JPSP suppressed the inflammatory response and retained adipose tissue (AT) functions under high-fat (HF) feeding favouring a healthier metabolic profile when compared with HF-fed mice.

activity[40,41]. Likewise, the results of most human intervention studies support the hypothesis that anthocyanins can positively affect obesity markers[42].

Besides its role of energy storage, adipose tissue also plays a critical function in whole-body homoeostasis by secreting adipokines, such as leptin and adiponectin, which influence distinctly energy homoeostasis, glucose and lipid metabolism, cardiovascular function and immune response[43]. Leptin, which is primarily secreted by fully differentiated adipocytes, has a regulatory role in the intake and expenditure of energy by controlling appetite and glucose metabolism[44]. Concomitant with greater fat accumulation, obese individuals generally show elevated circulating levels of leptin paralleled with lower leptin efficacy as a result of leptin resistance[45]. Unlike leptin, adiponectin is down-regulated in obesity and its circulating levels are inversely correlated with body fat. This adipokine improves energy metabolism and fatty acid oxidation, promoting insulin sensitivity and improving glucose tolerance[43]. Thus, it is well recognised that an imbalance in the production and levels of these adipokines underpins the development of metabolic complications associated with obesity. In this regard, it is worth noting that supplementation with 15 % JPSP resulted in tissue levels of both leptin and adiponectin similar to mice fed a control diet, which may explain the improved glucose metabolism observed in the supplemented mice.

Interestingly, connections between preserved production of adipokines and glucose metabolism have been previously observed upon treatment with jabuticaba extracts[29], which reinforces the idea that by preserving adipose tissue functions, supplementation with JPSP favourably affects the metabolic profile of obese rodents. Moreover, as no effect on adipokine levels has been described with the extract from jabuticaba peel only[46], it is plausible that the preservation of adipose tissue functions depends on either composition or concentration of phenolic compounds, probably related to the seeds phenolic profile, rich in ellagic acid and ellagitannins.

Several *in vitro* and *in vivo* studies reported that these phenolic compounds possess antihyperglycaemic properties[47–51]. Given that these phenolic compounds comprised 73 % of the total phenolic content in JPSP, it is tempting to speculate that they mediate the antihyperglycaemic effect observed in our study. Further investigation deciphering the contribution of individual and combined JPSP phenolic compounds will be valuable to understand the glycaemic effects as well as the preservation of adipose tissue functions achieved with JPSP supplementation.

The role of inflammation in the development of obesity complications is solidly established, with overnutrition inducing adipose expression of inflammatory cytokines and chemokines including TNF-α, IL-6, MCP-1, CXCL9 and CXCL10, which are secreted by activated monocytes/macrophages as well as other cells including adipocytes[52].

In line with this hypothesis, it has become clear in recent years that these mast cells are also pivotal players in this process[53,54]. Thus, the absence of these cells in the adipose tissue of supplemented mice could explain, at least in part, their reduced levels of inflammatory markers. However, it is also

conceivable that the absence of an inflammatory response with JPSP may reflect the preserved adipose tissue function of these mice. In line with this hypothesis, it has been reported that adiponectin supports macrophage polarisation to an anti-inflammatory profile (M2) and its decline with increasing obesity may conversely favour the inflammatory phenotype (M1) of macrophages in the adipose tissue[55].

Supporting our findings, a recent *in vitro* study showed decreased leptin expression and secretion and increased transcriptional and protein levels of adiponectin when adipocytes were treated with elderberry fruit (*Sambucus nigra*), which were associated with an anti-inflammatory status[56]. However, given that previous studies reported that ellagic acid reduces gene expression levels of TNF-α and IL-6, and chemokine C-C motif ligand-2 secretion in LPS-stimulated macrophages and adipocytes[57,58], it is also possible that supplementation with JPSP diminished the inflammatory markers through direct mechanisms. Regardless the underlying mechanisms, our findings strongly indicate that by suppressing local and systemic inflammation, JPSP supplementation favours a healthier metabolic profile in obese mouse.

Conclusion

Overall, our results provided evidence that dietary incorporation of JPSP, rich in phenolic compounds, prevented obesity complications and led to a healthier metabolic profile. Despite major advances in understanding obesity pathogenesis, treatment and prevention of its progression continue to pose an important clinical challenge. Our findings support the perspective that supplementation with JPSP has therapeutic potential and may offer a new option to counteract the development of obesity complications induced by HF feeding. Clinical trials are still needed to confirm the efficacy of this approach.

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P. T., E. S. and J. D. designed the research; P. T, E. S., F. M. and K. I. conducted the experiment and analysed the data; P. T., M. R., D. P., M. M., V. S. M. and J. D. wrote the paper.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521001136

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