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Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity

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ABSTRACT

The bioactive compounds of freeze-dried jaboticaba peel (FJP), serum lipids and the antioxidant status of rats fed high-fat diets containing different doses of FJP were evaluated. Obesity was induced and three groups of the obese animals received high-fat diet with 1%, 2% and 4% FJP added (J1, J2 and J4, respectively). The HPLC–DAD analyses of the polyphenols in the FJP showed four compounds: gallic acid, cyanidin 3-glucoside, ellagic acid, and quercetin. The serum saturated fatty acids were reduced in J1 and J4-fed animals. The antioxidant defenses of plasma were increased in the J2 and J4-fed animals. All the FJP diets prevented lipid peroxidation in the liver and increased its antioxidant defenses. Lipid peroxidation in brain decreased with an increasing FJP content in the diet. The antioxidant status of the kidneys of J2 and J4-fed animals increased. Thus, FJP could be an alternative to minimize the high-fat diet-induced oxidative stress and circulating saturated lipids.

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1. Introduction

Overweight and obesity have become major public health problems, and the prevalence of related chronic diseases is increasing throughout the world (WHO, 2012). Obesity may be a state of chronic oxidative stress, which consists in an imbalance between overproduction of reactive oxygen species or ROS (e.g. hydrogen peroxide, peroxyl, superoxide and hydroxyl radicals) and circulating antioxidants. Oxidative stress is a possible mechanism underlying the development of diabetes and cardiovascular diseases (Vincent, Innes, & Vincent, 2007).

An excess of ROS, over-produced in obesity, can harm proteins, lipids, nucleic acids and cause cell damage and even death. In addition, obesity impairs the enzyme antioxidant system, with depletion of the superoxide dismutase (SOD),

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catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) activities, and also impairs the non-enzymatic oxidative system (reduced thiol content or GSH, vitamins and minerals) (Noeman, Hamooda, & Baalash, 2011; Vincent et al., 2007).

Dietary nutrients and specific foods, rich in polyphenols (e.g. anthocyanins, flavonols), could play an important role in the prevention and control of complications arising from oxidative stress, increasing the circulation of antioxidant compounds (Leite et al., 2011; Lenquiste, Batista, Marineli, Dragano, & Marostica, 2012), since they are capable of neutralizing reactive species, due to their favorable number and position of hydroxyls (Mazza, Kay, Cottrell, & Holub, 2002; Prior, 2003; Vanzo, Vrhovsek, Tramer, Mattivi, & Passamonti, 2011). These compounds are also correlated to the reduction of serum and liver lipids and decreased gene expression of lipogenic enzymes (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

Myrciaria jaboticaba (Vell.) Berg., popularly known as jaboticaba, is a Brazilian berry with a purple peel. Freeze-dried jaboticaba peel (FJP) showed considerable polyphenol and anthocyanin contents, with important *in vivo* antioxidant properties (Costa, Garcia-Diaz, Jimenez, & Silva, 2013; Leite et al., 2011). Moreover, recent studies have reported that jaboticaba peel contains high contents of ellagic acid, tannins (Abe, Lajolo, & Genovese, 2012) and some volatile compounds (Plagemann, Krings, Berger, & Maróstica Júnior, 2012) that could contribute to its antioxidant power. In addition to this bioactive compounds, it was discovered a new depside named jaboticabin in the *Myrciaria cauliflora* specie (Reynertson et al., 2006; Wu et al., 2013b). This compounds is related to cancer cells antiproliferative and antioxidant effects (Reynertson et al., 2006).

The *M. jaboticaba* fruit has been little explored from a scientific point of view, especially in relation to its chemical constituents and *in vivo* effects, and the present study aimed to investigate the effects of jaboticaba on oxidative stress indicators. The authors hypothesized that the ingestion of different FJP concentrations could protect the tissues against oxidative stress and change the lipid profile in serum in obese rats, due to a peculiar content of polyphenols.

2. Materials and methods

2.1. Jaboticaba peel processing

Jaboticaba fruits (*M. jaboticaba* (Vell.) Berg.) were bought at the local market in Campinas, São Paulo State, Brazil. The fruits were washed, manually peeled and frozen at −18 °C. The peels were dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil) at 30 °C, 300 μm Hg for 95 h, and the freeze-dried jaboticaba peel product (FJP powder) stored at −80 °C.

2.2. Freeze-dried jaboticaba peels (FJP) extracts and analyses

The FJP powder was weighed (1 g) and extracted with 20 mL of 80% methanol (MeOH) at 37 °C for 3 h in a shaking water bath. After a centrifugation (2000*g*, 10 min), it was filled to 25 mL and filtered (0.45 μm) before analyses.

An hydrolysis of the glycoside flavonoids was performed in the MeOH extract in order to facilitate the identification and the quantification of the compounds by the HPLC method (Port's, Chisté, Godoy, & Prado, 2013). For hydrolysis, 13 mL of 1 g L^{-1} BHT (2,6-di-tert-butyl-4-methylphenol) in HPLC grade methanol, 12 mL ultrapure water and 10 mL 6 mol L^{-1} HCl were added to 15 mL MeOH extract, which was allowed to react in water bath at 90 °C for 30 min, with refrigerated reflux condenser.

The total phenolic content was determined by the Folin–Ciocalteu method, adapted from (Swain & Hillis, 1959). The absorbance was measured at 725 nm using a spectrophotometer (Sinergy HT, Biotek, Winooski, VT, USA) and the results were expressed in gallic acid equivalents (GAE mg g^{-1} FJP).

The total anthocyanins were also quantified in the methanol extract according to the pH-differential method described by Wrolstad (1993). The absorbance was then calculated using Eq. (1):

$$A = [(A_{510\ nm} - A_{700\ nm})pH = 1.0] - [(A_{510\ nm} - A_{700\ nm})pH = 4.5] \quad (1)$$

The anthocyanin content was calculated as cyanidin 3-glucoside using Eq. (2):

$$C = A \cdot MW \cdot DF \div \xi \cdot 1 \quad (2)$$

where C = cyanidin 3-glucoside (mg 100 g^{-1}); MW = molecular weight (449.2); DF = dilution factor; and ξ = molar absorptivity (26,900 mol L^{-1}), 1 = pathlength (cm).

The ORAC (oxygen radical absorbance capacity) (Davalos, Gomez-Cordoves, & Bartolome, 2004) test was carried out adding 20 μL of samples extract or standard solutions, 120 μL of fluorescein diluted in phosphate buffer (pH 7.4), and 60 μL of AAPH (2,2′-azobis (2-methylpropionamidine) dihydrochloride) to black microplates, in the dark. Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard and the microplate reader (Synergy HT, Biotek) with fluorescent filters: excitation wavelength, 485 nm; emission wavelength, 520 nm. ORAC values were expressed in μmol trolox equivalent (μmol TE) per gram of FJP by using the standard curves (2.5–80.0 μmol TE L^{-1}) for each assay. The linearity between the net area under the curve and the concentration was checked for the samples and the fluorescence readings were used to the appropriate calculations.

2.2.1. Quantification of the phenolic compounds in the FJP by HPLC–DAD

The analysis of the phenolic compounds from the extracts was carried out in a high performance liquid chromatography (HPLC Agilent 1100 Series, Englewood, CO, USA), with manual injection, 20 μL sample loop and ternary pump, coupled to a diode array detector (DAD Agilent G13158). The oven (Agilent 1100) was operated at 25 ± 2 °C, and data was obtained and processed using the software ChemStation (Hewlett Packard, Germany). A reverse phase chromatographic column (C18 Eclips XDB (5 μm × 250 mm × 4.6 mm), Agilent, Englewood, CO, USA) was used. The mobile phase was 1% orthophosphoric acid in water (v v^{-1}) (A) and acetonitrile (B). The elution gradient started at 95:05 (A:B) at 0.7 mL min^{-1}. This condition

was maintained for 5 min and then, the concentration of A was decreased (75:25, A:B), and at 25 min it reached 60:40 (A:B) followed by a linear increase of solvent A to 95% until 35 min.

The detection was done at 210, 254, 280, 300 and 340 nm, which allowed the simultaneous quantification and the tentative identification of the phenolic compounds separated by the HPLC. The comparison parameters were elution time, spectra of absorption and sample fortification. The identification was carried out using the chromatograms obtained at each injection and compared with the absorption spectra and retention time of the standards. Co-chromatography was performed to confirm the identity of the compounds. The concentrations of the identified compounds were calculated from the analytical curves obtained using commercial products under identical chromatographic conditions.

2.3. Rat study

2.3.1. Animals and diets

Thirty weaned male Sprague–Dawley rats were used in this study following all the ethical recommendations, and the protocol (#2226-1) was approved by the UNICAMP Ethics Committee, Brazil. The rats were housed under conditions of constant temperature (22 °C ± 2), relative humidity (60–70%) and a standard dark cycle (19–07 h), and randomized into five groups of 6 animals each: a control group (N or normal), fed a semi-purified diet for 10 weeks (Reeves, Nielsen, & Fahey, 1993); a high-fat control group (C), fed a high-fat diet for 10 weeks; and the FJP groups (J1, J2 and J4) which were fed the high-fat diet for the first 4 weeks (1st experimental phase) and then the high-fat diet plus 1%, 2% and 4% of FJP, respectively, for the following 6 weeks (2nd experimental phase). The doses were based in previous reports (Dragano et al., 2013; Kalt et al., 2008; Leite et al., 2011; Lenquiste et al., 2012). The ingredients used in the normal diet (N) were: casein (15.4%); corn starch (42.66%); maltodextrin (14.17%); sucrose (10.73%); cellulose (5.0%); soybean oil (7.0%; being 58.3% polyunsaturated fatty acids (PUFA), 26.72% monounsaturated fatty acids (MUFA), and 14.99% saturated fatty acids (SFA) (Furlan, Marques, Marineli, & Maróstica Júnior, 2013)); mineral mix (3.5%), vitamin mix (1.0%); L-cystine (0.3%); and choline bitartrate (0.25%) (Reeves et al., 1993). The ingredients used in the control diet (C) were: corn starch (24.98%); maltodextrin (8.29%), sucrose (6.29%); soybean oil (4.0%); and lard (31%; being 18.6% PUFA, 43.66% MUFA, and 37.76% SFA (Furlan et al., 2013). The J1, J2 and J4 diets consisted on C diet plus 1%, 2%, and 4% FJP, respectively; and the amount of cellulose was adjusted to 4.75%, 4.5%, and 4.0% for the same groups. The rats were given free access to water and food. The weight gain and diet consumption were determined weekly.

2.3.2. Diet analyses

Calorie values of diets were determined using Isoperibol Calorimeter 1261 instrument equipped with 1108 oxygen bomb (Parr Instrument Co, Moline, IL, USA).

The total anthocyanin contents were assayed in the FJP diets using the pH-differential method as described previously (Leite-Legatti et al., 2012). The results were expressed as mg cyanidin 3-glucoside 100 g^{-1} diet.

The antioxidant capacity of all the diets was evaluated by the ORAC assay using ethanolic (2:1, v/v) extract of diets, with or without protein precipitation after addition of 0.75 mol L^{-1} metaphosphoric acid and centrifugation at 21,036*g* for 5 min at 4 °C.

2.3.3. Sampling

Blood was obtained from the fasted rats (12 h) by decapitation. The blood samples were collected in tubes containing or lacking ETDA, and centrifuged at 2000*g* for 20 min. Respectively, the plasma and serum was collected, bubbled through nitrogen gas and stored at −80 °C until analyzed. The spleen, liver, kidneys, pancreas and the whole brain were removed, washed, weighted, frozen in liquid nitrogen, and kept at −80 °C. Tissue homogenates (pancreas, kidneys and liver) were prepared in a ratio of about 100 mg wet tissue per 1 mL of 50 mmol L^{-1} phosphate buffer (pH 7.4) or 5% trichloroacetic acid (TCA) solution using a homogenizer (MA102/Mini, Marconi, Piracicaba – SP, Brazil). The homogenates were used in the antioxidant enzyme and GSH assays. The liver and kidneys were also freeze-dried, as also the spleen and whole brain. The organs were manually ground and kept at −80 °C until analyzed for lipid peroxidation and antioxidant capacity.

2.4. Serum FAME from triglycerides and phospholipids

2.4.1. Analytical standards

All reagents used were at least analytical grade. Additionally, the method of choice for the derivatization of the lipids into their respective fatty acid methyl esters (FAME) was base-catalyzed methanolysis with sodium hydroxide as catalyst (NaOMe) (Sigma–Aldrich, Bellefonte, PA, USA). The identity of the analytes was confirmed with the FAME mixture of C8–C25, 37 components and methyl docosahexaenoic acid (DHA) analytical standards (Sigma–Aldrich, Bellefonte, PA, USA).

2.4.2. Chromatographic conditions

For the separation and quantification of the fatty acid methyl esters (FAME) an Agilent HP gas chromatograph model 6890N with a liquid auto-injector was used (Wilmington, DE, USA). This equipment was fitted with a split/splitless injector and FID detector. The separation was performed in a DB-5 (95% poly(dimethylsiloxane)/5% diphenylsiloxane) capillary column (10 m length, 0.10 mm i.d. and 0.10 μm film thickness) (Agilent Technologies, Wilmington, DE, USA). The injector, was operated in splitless mode, and the detection port were held at 250 °C, while the column oven was programmed from 120 °C to 350 °C at a rate of 20 °C min^{-1}. The carrier gas was hydrogen at a constant linear velocity of 77 cm s^{-1}.

For the chromatographic analysis 75 μL of serum was transferred to a 200 μL conical vial; 50 μL of iso-octane and 100 μL of a 2 mol L^{-1} sodium methoxide, in methanol were added. The vial was vortexed for 2 min and centrifuged at 5422*g*. The organic layer was collected and chromatographed; the injection volume was 3 μL. All analyses were carried out in duplicate.

2.5. Tissues antioxidant defenses analyses

All the absorbance and fluorescence readings for the biochemical analyses were determined in a Synergy HT, Biotek microplate reader (Winooski, VT, USA) with Gen5™ 2.0 data analysis software.

2.5.1. *Lipid peroxidation in the tissues*

2.5.1.1. *TBARS (thiobarbituric acid reactive substances) assay.* The TBARS levels in the plasma, liver, kidneys, spleen and brain were determined using the method described by Ohkawa, Ohishi, and Yagi (1979), with adaptations. The freeze-dried tissues (10 mg mL^{-1}) were sonicated in acetate buffer (pH 3.5) on ice or 100 μL in the case of plasma. The samples were mixed with 8.1% sodium dodecyl sulphate, TBA (2-thiobarbituric acid) powder, 20% acetic acid and 5% sodium hydroxide. After heating at 95 °C for 60 min, the samples were cooled in an ice bath for 10 min and then centrifuged at 10,000*g*, 10 min, 4 °C. The resulting MDA-TBA and related adducts were quantified at 532 nm using a 96-well microplate. A standard curve was prepared using the MDA standard (#10009202, Cayman Chemical Company, Ann Arbor, MI, USA).

2.5.2. *Enzymatic and non-enzymatic endogenous antioxidant systems in the plasma, pancreas, kidneys and liver*

2.5.2.1. *Plasma measurements.* Commercial assay kits from Cayman Chemical Company were used to determine the plasma GSH levels (#703002) and the GPx (#703102), GR (#703202), CAT (#707002) and SOD (#706002) activities.

2.5.2.2. *Superoxide dismutase activity.* One hundred microliters of appropriately diluted samples (phosphate buffer homogenates or PB) were added to a 96-well microplate. One hundred and fifty microliters of a previously prepared solution (0.1 mmol L^{-1} hypoxanthine, 0.07 U xanthine oxidase and 0.6 mmol L^{-1} NTB in phosphate buffer in 1:1:1 proportions) were added just before the readings. The reading was taken at 560 nm and the reaction monitored for 10 min. The SOD activity expressed as U mg^{-1} protein. (Winterbourn, Hawkins, Brian, & Carrell, 1975).

2.5.2.3. *Glutathione peroxidase activity.* GPx activity in the tissues was quantified in PB homogenates by the method described in (Flohe & Gunzler, 1984). The decrease in absorbance was monitored at 365 nm after induction by 0.25 mmol L^{-1} H_2O_2 in the presence of 10 mmol L^{-1} reduced glutathione, 4 mmol L^{-1} NADPH (β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate) and 1 U GR enzyme activity. The results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein.

2.5.2.4. *Glutathione reductase activity.* GR activity was measured in PB homogenates (Carlberg & Mannervik, 1985), following the decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in phosphate buffer. The results were expressed as nmol NADPH consumed min^{-1} - mg^{-1} protein.

2.5.2.5. *Reduced thiol (GSH) contents.* The GSH levels in the tissues were determined in TCA homogenates by Ellman's reaction using DTNB (5′5′-dithio-bis-2-nitrobenzoic acid) (Ellman, 1959). The intensity of the yellow colour was read at 412 nm and GSH was used as the external standard. The GSH concentrations were expressed as nmol GSH μg^{-1} protein. The protein concentrations of all tissue homogenates were determined using the Bradford method (Bradford, 1976).

2.5.3. *Free radical scavenging capacity in the plasma, liver, brain, kidneys and spleen*

2.5.3.1. *Sample preparation.* The plasma samples were treated with ethanol:ultrapure water (2:1) and 0.75 mol L^{-1} metaphosphoric acid (Leite et al., 2011). The freeze-dried tissues were mixed with the same solvents. The samples were centrifuged at 21,036*g* for 5 min at 4 °C and the supernatant removed. These extracts were for the ORAC, TEAC and FRAP assays.

2.5.3.2. *ORAC (hydrophilic oxygen radical absorbance capacity) assay.* The tissues samples extracts were analyzed according to the ORAC assay described above (Section 2.2).

2.5.3.3. *TEAC (trolox equivalent antioxidant capacity) assay.* The tissues TEAC levels were determined based on the method of Rufino et al. (2010) with modifications. The ABTS solution was prepared by mixing 5 mL of 7.0 mmol L^{-1} ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic ac id) diammonium salt) and 88 μL of 145 mmol L^{-1} potassium per sulphate solution, which was allowed to react for 12–16 h at room temperature in the dark. Ethanol (99.5%) was added to the solution until an absorbance of 0.700 ± 0.05

Table 1 – Polyphenols contents and antioxidant activity of freeze-dried jaboticaba peel.[A]

Compounds or antioxidant activity	MeOH extract	Hydrolysated MeOH extract
ORAC (μmol TE g^{-1})	519.14 ± 11.46[a]	608.67 ± 33.07[a]
Total anthocyanins (mg C3G 100 g^{-1})	1737.12 ± 13.60	ND
Total polyphenols (mg GAE g^{-1})	113.80 ± 2.73[a]	115.76 ± 1.34[a]
Gallic acid (mg 100 g^{-1})	4.03 ± 0.07[b]	49.86 ± 0.20[a]
Ellagic acid (mg 100 g^{-1})	348.08 ± 1.75[b]	1545.77 ± 12.56[a]
Quercetin (mg 100 g^{-1})	4.82 ± 0.03[b]	54.96 ± 0.40[a]

C3G = cyanidin 3-glucoside.
GAE = gallic acid equivalents.
Different letters in lines represent statistical difference between extracts ($P < 0.05$).
[A] Data expressed as mean ± SEM. ND = non determined.

Table 2 – Growth and food intake parameters.[A]

Parameters	Phase	N	C	J1	J2	J4
Weight gain (g)	1st + 2nd	114.80 (102.2–144.8)	176.30 (171.4–191.1)[b]	173.00 (153.9–192.7)	183.40 (142.6–196.6)	191.30 (181.1–210.3)[b]
Food intake (g)	1st + 2nd	650.47 ± 9.71	662.31 ± 17.38	680.52 ± 20.69	687.40 ± 27.44	745.16 ± 36.29
	2nd	386.28 ± 7.03	378.84 ± 9.16	383.80 ± 10.43	399.71 ± 15.18	441.02 ± 22.09[d]
Calorie intake	1st + 2nd	2766 ± 41.31	3864 ± 101.4[b]	3953 ± 120.2[b]	4038 ± 121.5[b]	4263 ± 180.2[b]
	2nd	1642 ± 29.89	2210 ± 53.46[b]	229 ± 60.58[b]	2307 ± 87.64[b]	2503 ± 9687[b,c]
Total TE[B] intake (μmol day^{-1})	2nd	49.61 (46.38–52.48)	22.10 (19.70–23.37)	53.85 (48.12–58.21)	102.30 (88.20–116.2)[e]	218.30 (184.7–236.40)[a,e]
Total TE intake (μmol 100 g^{-1} day^{-1})		25.63 (23.63–26.33)	8.56 (7.66–9.22)	20.68 (18.52–22.28)	38.65 (36.41–40.93)[d]	79.36 (76.03–84.91)[e,g]
NP[C] TE intake (μmol day^{-1})	2nd	35.37 ± 0.64	6.60 ± 0.16[b]	14.36 ± 0.39[b,c]	21.24 ± 0.81[d,e,f]	68.59 ± 3.44[b,e,h,j]
NP TE intake (μmol 100 g^{-1} day^{-1})		17.88 ± 0.64	2.556 ± 0.15[b]	5.469 ± 0.39[b,e]	8.08 ± 0.77[b,e,h]	24.89 ± 2.76[b,e,h,j]

[A] N = normal diet (AIN-93G) group; C = high-fat control diet group; J1 = high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2 = high-fat diet + 2% FJP; and J4 = high-fat diet + 4% FJP. 2nd = last 40 days of experiment.

[B] TE = trolox equivalents.

[C] NP TE = non-protein trolox equivalents. Parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; non-parametric data as in weight gain and total TE intake (Kruskal–Wallis and Dunn tests) were expressed as the median and ranges ($n = 6$).

[a] $P < 0.01$ are compared to the values of the N group.

[b] $P < 0.001$ are compared to the values of the N group.

[c] $P < 0.05$ are compared to the values of the C group.

[d] $P < 0.01$ are compared to the values of the C group.

[e] $P < 0.001$ are compared to the values of the C group.

[f] $P < 0.05$ are compared to the values of J1 group.

[g] $P < 0.01$ are compared to the values of J1 group.

[h] $P < 0.001$ are compared to the values of J1 group.

[i] $P < 0.01$ are compared to the values of the J2 group.

[j] $P < 0.001$ are compared to the values of the J2 group.

Table 3 – Tissue weights in SD rats (% body weight).[A]

Tissues	N	C	J1	J2	J4
Liver	3.88 ± 0.03	5.01 ± 0.04[b]	4.44 ± 0.07	4.62 ± 0.04	5.07 ± 0.05[b]
Kidneys	1.04 (0.87–1.12)	0.72 (0.63–0.75)	0.68 (0.63–0.71)[b]	0.69 (0.62–0.71)[a]	0.69 (0.65–0.75)[a]
Brain	0.96 ± 0.02	0.76 ± 0.02[c]	0.75 ± 0.02[c]	0.76 ± 0.02[c]	0.72 ± 0.02[c]
Pancreas	0.33 (0.30–0.38)	0.28 (0.21–0.31)	0.27 (0.26–0.31)	0.25 (0.24–0.27)[b]	0.27 (0.21–0.30)
Spleen	0.22 ± 0.01	0.18 ± 0.01[c]	0.20 ± 0.01[a]	0.19 ± 0.01[c]	0.19 ± 0.01[b]

[A] N = normal diet (AIN-93G) group; C = high-fat control diet group; J1 = high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2 = high-fat diet + 2% FJP; and J4 = high-fat diet + 4% FJP. Parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; non-parametric data as in kidneys and pancreas ratio (Kruskal–Wallis and Dunn tests) were expressed as the median and ranges ($n = 6$).
[a] $P < 0.05$ compared to the values of the N group.
[b] $P < 0.01$ compared to the values of the N group.
[c] $P < 0.001$ compared to the values of the N group.

was obtained at 734 nm. ABTS solution was added to the sample or trolox standard solutions and allowed to react for 6 min before reading at 734 nm.

2.5.3.4. FRAP (ferric reducing antioxidant power) assay. The ferric reducing ability of the tissues was determined using the FRAP method (Rufino et al., 2010). The FRAP reagent was prepared in the dark with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tris(2-pyridyl)-S-triazine) in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$. The sample or standard solutions, ultrapure water and FRAP reagent were mixed and incubated in a water bath for 30 min at 37 °C. The samples and trolox standard curve were read at 595 nm.

2.6. Statistical analyses

The parametric results were expressed as the means ± standard errors (SEM) and the non-parametric data by the medians and ranges. The statistical analyses of the parametric data were based on a one-way ANOVA followed by a Tukey test. The non-parametric data were submitted to the Kruskal–Wallis and Dunn tests. The limit of significance was set at $P < 0.05$. The statistical analyses were carried out using the GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software.

3. Results

3.1. Chemical composition of FJP

The FJP powder contains compounds of interest, as observed in the Folin–Ciocalteau, total anthocyanins and ORAC (Table 1) methods.

Four polyphenol compounds were identified in the FJP by HPLC–DAD analysis: cyanidin 3-glucoside, gallic acid, free ellagic acid and quercetin (Table 1, Suppl. 1). After hydrolysis,

Table 4 – FAME contents (%) from triglycerides and phospholipids in the serum of experimental groups.

FAMEs	N	C	J1	J2	J4
C16:0	24.97 (24.41–26.34)	21.03 (20.54–24.58)	21.51 (21.15–21.94)	21.44 (19.52–21.96)[a]	20.41 (19.4–21.83)[c]
C18:0	18.82 ± 0.67	22.85 ± 0.91[b]	20.07 ± 0.61	22.85 ± 0.77[b]	20.40 ± 0.61
C18:1-n9	3.69 (3.38–5.638)	4.61 (4.07–5.50)	7.63 (4.33–8.03)[a]	4.91 (0.96–6.30)	4.94 (3.94–5.81)
C18:1-n3	1.49 ± 0.06	0.73 ± 0.06[a]	0.99 ± 0.04	2.49 ± 1.08	0.87 ± 0.02
C18:2-n6	17.95 ± 0.62	13.43 ± 0.63[c]	14.99 ± 0.43[b]	13.52 ± 0.37[c]	14.45 ± 0.48[c]
C20:4-n6	25.41 (24.19–26.26)	28.51 (27.22–30.32)	26.88 (22.82–29.70)	28.77 (5.15–29.54)	30.76 (28.57–34.03)[b]
C22:0	1.36 ± 0.04	1.35 ± 0.03	1.27 ± 0.05	1.30 ± 0.07	1.38 ± 0.07
C22:1-n3	0.20 ± 0.01	0.45 ± 0.02[c]	0.43 ± 0.04[c]	0.41 ± 0.03[c]	0.40 ± 0.03[c]
C22:5-n3	0.30 ± 0.03	0.81 ± 0.1[a]	0.86 ± 0.13[b]	0.87 ± 0.10[b]	0.85 ± 0.11[b]
C22:6-n3	4.74 ± 0.14	4.63 ± 0.12	4.36 ± 0.18	4.44 ± 0.14	4.75 ± 0.35
Non identified	0.31 ± 0.10	0.29 ± 0.08	0.32 ± 0.11	0.33 ± 0.11	0.65 ± 0.14
Total SFA	45.34 ± 0.55	46.04 ± 1.00	42.86 ± 0.73[d]	45.25 ± 0.52	42.24 ± 0.56[a,e,f]
Total MUFA	5.80 ± 0.39	5.90 ± 0.19	8.31 ± 0.60[b,d]	7.34 ± 0.83	6.18 ± 0.25
Total PUFA	48.71 (47.03–49.55)	47.12 (46.05–50.27)	48.10 (42.82–49.05)	47.83 (23.64–48.53)	51.25 (48.02–53.96)
PUFA/SFA	1.05 (1.02–1.13)	1.03 (0.94–1.16)	1.09 (1.027–1.15)	1.04 (0.52–1.11)	1.22 (1.10–1.32)[d,f]
n6:n3	6.61 (5.92–6.97)	6.59 (5.23–6.82)	6.46 (5.51–6.71)	5.23 (2.68–7.21)	6.45 (5.83–9.07)

N = normal diet (AIN-93G) group; C = high-fat control diet group; J1 = high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2 = high-fat diet + 2% FJP; and J4 = high-fat diet + 4% FJP. Parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; non-parametric data as in C16:0, C18:1-n9, C20:4-n6, total PUFA, PUFA/SFA, and n6:n3 (Kruskal–Wallis and Dunn tests) were expressed as the median and ranges ($n = 6$).
[a] $P < 0.05$ are compared to the values of the N group.
[b] $P < 0.01$ are compared to the values of the N group.
[c] $P < 0.001$ are compared to the values of the N group.
[d] $P < 0.05$ are compared to the values of the C group.
[e] $P < 0.01$ are compared to the values of the C group.
[f] $P < 0.05$ is compared to the values of the J2 group.

the content of ellagic acid was significant increased and cyanidin 3-glucoside was supposedly hydrolysated to aglycone cyanidin (Suppl. 1).

3.2. *Weight and intake parameters*

At the beginning of this study, the 5 groups of animals showed similar ($P = 0.58$) body weight (102.6–121.2 g). The animals fed on the high-fat diet showed a 52% increase in weight gain (absolute values) as compared to the standard group. The FJP did not attenuate the weight gain of the rats during the last 40 days of treatment in comparison with C group ($P > 0.05$). The rats fed the C and J4 diets increased their total weight gain ($P < 0.05$) in relation to the N group (Table 2).

There was no statistical difference ($P > 0.05$) in total food intake among the groups, but in the second phase of the experiment, rats from group J4 showed a higher food intake than the C group. The total calorie intake was higher in high-fat-fed animals ($P < 0.001$). However, the same trend of food intake was observed in the second phase of the

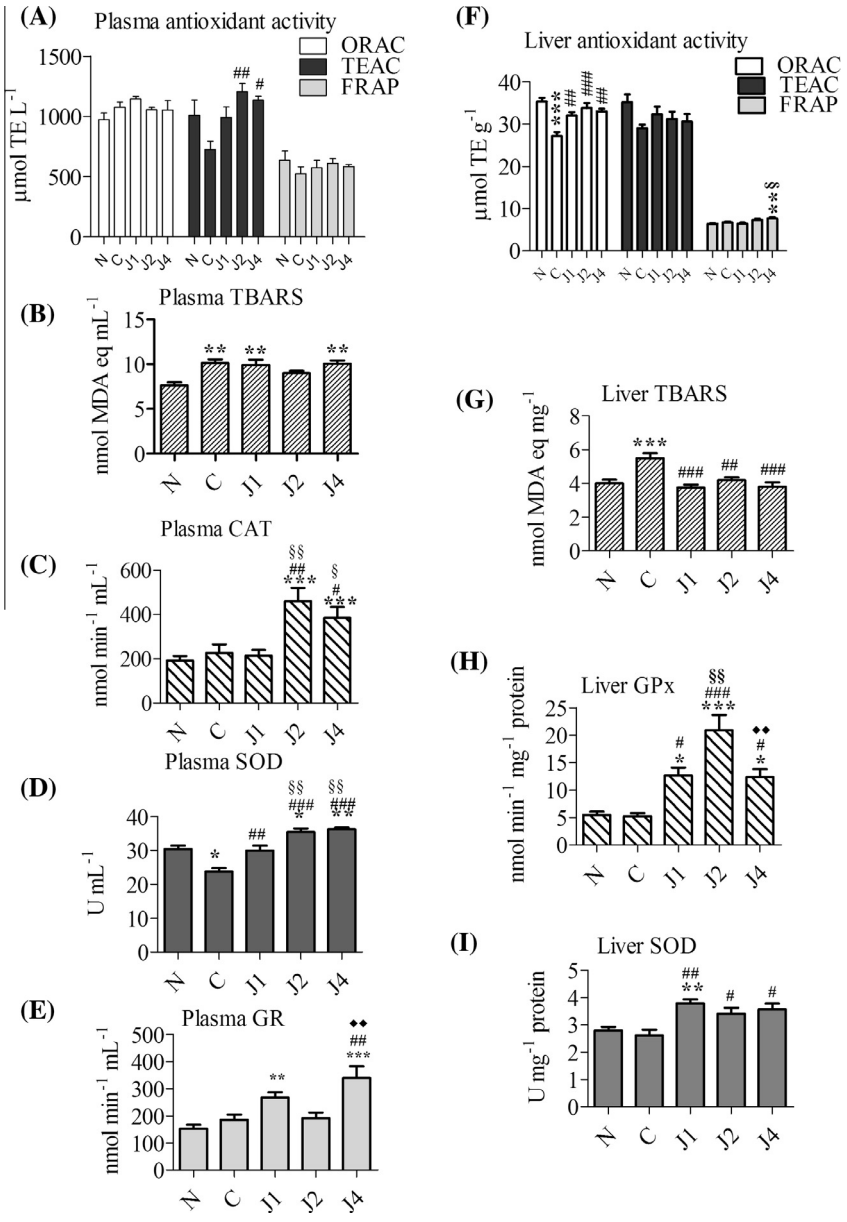

Fig. 1 – Plasma and liver enzymatic, non-enzymatic antioxidant status and lipid peroxidation. (A) and (F) Total antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. (B) and (G) Lipid peroxidation by the TBARS assay. (C) Catalase activity. (D) and (I) Superoxide dismutase activity. (E) Glutathione reductase activity. (H) Glutathione peroxidase activity. N = normal diet (AIN-93G) group; C = high-fat control diet group; J1 = high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2 = high-fat diet + 2% FJP; and J4 = high-fat diet + 4% FJP; eq = equivalents. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data in plasma frap (Kruskal–Wallis and Dunn tests) were expressed as the median and ranges ($n = 6$). *Indicates statistical differences from N; #indicates statistical differences from C; §from J1; and ♦from J2 groups (1 code = $P < 0.05$; 2 codes = $P < 0.01$; and 3 = $P < 0.001$).

experiment to calorie intake by J4. The J4-fed animals showed the highest antioxidant capacity. The animals showed a dependent dose response concerning anthocyanin (1.33, 1.53 and 3.30 mg $100\,g^{-1}\,day^{-1}$, by pH differential method) and polyphenols daily intake (HPLC–DAD). The daily non-protein trolox equivalents consumed by the J4 animals was higher than for all the other groups (Table 2). In addition, the regression analysis showed that the ORAC antioxidant capacity of the FJP diets depended on the total anthocyanin contents ($r^2 = 0.9953$; $r = 0.9686$; $P < 0.001$; $y = -2.0978 + 1.0382x$).

The tissue weights were not altered by the dietary FJP treatment when compared to the C group. The weights of the brains and spleens of the obese rats were lower than those of the healthy rats. The kidneys of the FJP-fed animals were smaller than those of the lean and fat control animals (Table 3).

3.3. *Serum lipids*

The levels of palmitic acid (C16:0) were decreased in the serum for J2 and J4-fed rats; as for stearic acid (C18:0), its content was higher for C and J2 when compared to N group. No alterations on the concentrations of behenic acid (C22:0) were found ($P = 0.612$) among the experimental groups. However, the total SFA were reduced in the serum triglyceride and phospholipid fractions of J1 and J4 animals (6.91% and 8.25%, respectively), when compared with C. These results are consistent with the higher PUFA:SFA ratios in the serum of the J4 group (Table 4).

The animals of N group showed the highest level of linoleic acid (18:2n-6), and the arachidonic acid (C20:4-n6) was increased in J4-fed rats related to N group. However, no differences were found among the high-fat-fed animals for n6 FAME (Table 4 and Suppl. 2).

The animals that received the C diet showed lower levels of serum C18:1n-3 relative to those fed with N diet. The 22:1n-3 and 22:5n-3 FAME were increased in all the high-fat-fed rats (C, J1, J2, J4) and the 22:5n-3 (docosapentaenoic acid) FAME increasing in the FJP-fed animals were more significant ($P < 0.01$). The DHA (C22:6n-3) contents was similar to all experimental groups, as occurred with n6:n3 ratio (Table 4 and Suppl. 2).

The plasma of rats treated with J1 diet had higher concentrations of oleic acid (C18:1n-9) than the N group. These values were the main contributor to the high total content on monounsaturated fatty acids (MUFA) in J1 group in relation to N and C groups (Table 4 and Suppl. 2).

3.4. *Plasma antioxidant status*

The TEAC assay showed that the plasma antioxidant capacity was higher in the J2 and J4 groups as compared to the C group. The FRAP and ORAC assay showed no statistical differences among the groups (Fig. 1A). Although the TBARS levels in high-fat-fed groups were higher than those fed the normal diet, the J2-fed animals showed reduced TBARS levels in absolute values (Fig. 1B). The GSH values were higher in the J1 group (22.17 mmol L^{-1}) in relation to the N group (13.06 mmol L^{-1}), but not as compared to the C-fed animals (17.66 mmol L^{-1}).

The antioxidant enzyme parameters indicated that the FJP intake could promote a protective effect against oxidative stress in the rat plasma. The CAT activity was 2.02 times higher in the J2 plasma and 1.70 times higher in the J4 plasma in relation to the C group (Fig. 1C). The SOD units in the FJP-fed rats were higher in relation to the other groups. The SOD activities in the J1, J2 and J4 plasma was 13.18%, 15.60% and 15.95% higher in comparison to C group (Fig. 1D). The plasma GR increased 1.83 times in the J4 animals when compared to the C group (Fig. 1E). On the other hand, the means for GPx were not statistically different ($P > 0.05$) among the experimental groups, the values ranging from 11.14 ± 1.41 to 14.96 ± 2.29 (nmol $min^{-1}\,mL^{-1}$).

3.5. *Liver antioxidant status*

The obese conditions of the high-fat control group were probably responsible for the high TBARS values and low antioxidant capacity levels in the livers of these animals. Nevertheless, the addition of FJP to the diets improved the antioxidant status according to the ORAC method (Fig. 1F) and ameliorated the lipid peroxidation levels in the rat livers. The TBARS levels were reduced 23.61% in the animals from group J2 and about 31% in the J1 and J4-fed animals (Fig. 1G). A linear trend line showed a significant correlation coefficient ($P < 0.01$) between the liver TBARS and ORAC levels ($r^2 = 0.2492$; $r = -0.4992$; $P = 0.0052$; $y = 41.111 - 2.0886x$).

The diets with added FJP increased the liver GPx and SOD activities (Fig. 1H and I), which corroborates the antioxidant status of the tissue. The addition of 2% FJP to the high-fat diet seemed to be the most efficient dose to increase GPx, since the J2 animals showed a 3.98 times increase as compared to the control group. The GSH contents did not differ ($P > 0.05$) among the experimental groups (values ranging from 41.32 ± 3.88 to 61.50 ± 10.22 nmol μg^{-1} protein). The animals fed the high-fat diets showed the lowest GR activities (14.63 ± 17.19 nmol $min^{-1}\,\mu g^{-1}$ protein) as compared to the N group (23.06 nmol $min^{-1}\,\mu g^{-1}$ protein), but not in the case of the J4 animals (17.98 nmol $min^{-1}\,\mu g^{-1}$ protein).

3.6. *Brain antioxidant status*

The high-fat diet promoted a decrease in brain antioxidant values according to the ORAC and TEAC assays. Interestingly, the addition of 1% and 4% FJP to the diet reversed the effect caused by the high fat diet. The brain ORAC values were increased in J4 animals and FRAP values were higher in groups J2 and J4 as compared to the C group (Fig. 2A) and brain lipid peroxidation was ameliorated by the FJP diets, exhibiting a dependent dose response pattern (Fig. 2B). In addition, the FRAP results was significantly correlated ($P < 0.001$) with the lipid peroxidation values ($r^2 = 0.4514$; $r = -0.6719$; $P = 0.00005$; $y = 8.4991 - 0.3681x$).

3.7. *Kidney antioxidant status*

As observed in Fig. 2C, the ORAC antioxidant capacity of the kidneys increased in the rats fed the FJP diets in relation to the C group. The TEAC assay also showed an improvement in the antioxidant capacity of the kidneys in the J2 and J4 animals, although FRAP assay showed no significant differences ($P > 0.05$). The values for TBARS in the kidneys were similar

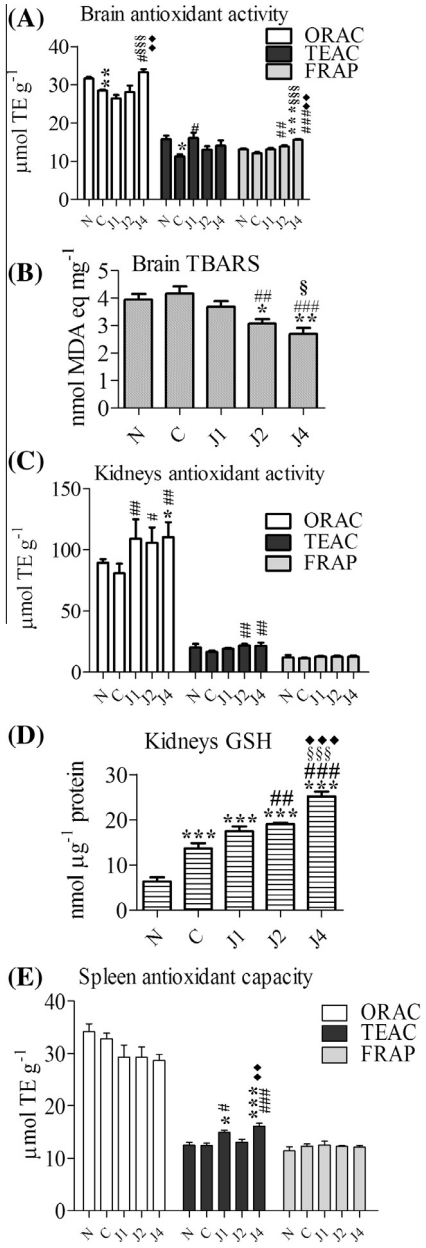

Fig. 2 – Tissue antioxidant capacity and lipid peroxidation. (A) Brain total antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. (B) Brain lipid peroxidation by the TBARS assay. (C) Kidney antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. (D) Kidney GSH. (E) Spleen antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. Results expressed on a dry weight basis. N = normal diet (AIN-93G) group; C = high-fat control diet group; J1 = high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2 = high-fat diet + 2% FJP; and J4 = high-fat diet + 4% FJP; eq = equivalents. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data in kidneys ORAC and TEAC (Kruskal–Wallis and Dunn tests) were expressed as the median and ranges ($n = 6$). *Indicates statistical differences from N; #indicates statistical differences from C; §from J1; and ♦from J2 groups (1 code = $P < 0.05$; 2 codes = $P < 0.01$; and 3 = $P < 0.001$).

among the groups (values ranging from 2.58 to 2.78 nmol MDA equivalents mg^{-1}), except that there was an increase of 24.45% in lipid peroxidation in the kidneys of the C animals (2.90 nmol MDA equivalents mg^{-1}) in comparison to the N-fed ones (2.33 nmol MDA equivalents mg^{-1}).

The GSH values increased in the high-fat-fed animals. Furthermore, groups J2 and J4 showed 39.29% and 84.57% increases in GSH, respectively, as compared to C group (Fig. 2D). The FJP-fed animals showed similar SOD activities as compared to group C, but the J4-fed animals (1.47 U mg^{-1} protein) showed a decrease in activity of this enzyme ($P < 0.05$) when compared to group N (2.20 U mg^{-1} protein).

3.8. *Spleen antioxidant status*

According to the TEAC results, the J4 group showed an increase in antioxidant activity relative to N, C and J2 groups (Fig. 2E), but the ORAC, FRAP and TBARS results did not confirm these findings.

3.9. *Pancreas antioxidant status*

The GSH content of the J4 animals (6.30 nmol μg^{-1}) increased in comparison to J1 (4.27 nmol μg^{-1} protein) and J2 (4.42 nmol μg^{-1} protein), but no differences were found in comparison to group C (5.11 nmol μg^{-1} protein). On the other hand, the SOD units in group J4 (1.97 U mg^{-1} protein) were lower than in groups N (2.60 U mg^{-1} protein) and C (1.75 U mg^{-1} protein).

4. Discussion

The *M. jaboticaba* peel contains high contents of polyphenols, as shown in this study. Corroborating literature results (Abe et al., 2012), the HPLC–DAD analysis showed significant amounts of gallic acid, quercetin and ellagic acid.

The ellagitannins content of jaboticaba peel (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013) probably was responsible for the increased ellagic acid amount in FJP sample, after the acid hydrolysis (Table 1). In addition, as published in previous work (Leite-Legatti et al., 2012), jaboticaba peel contains significant amounts of cyanidin 3-glucoside (1963.57 ± 52.72 mg 100 g^{-1}), a very common compound in deep-purple berries (Prior, 2003). The HPLC–DAD data showed that cyanidin 3-glucoside content of FJP was increased, and the aglycone of cyanidin was supposedly released after acid hydrolysis (Suppl. 1). The polyphenol compounds showed in this study corroborates to the FJP high antiradical activity demonstrated by ORAC assay (Table 1).

The high-fat diets changed the FAME profile from triacylglycerols and phospholipids in serum of the rats. Indeed, the blood FA profile in high-SFA-fed animals is characterized by a higher proportion of SFA and MUFA, as well as by a lower proportion of n-6 PUFA (Tranchida et al., 2012). The activity of Δ6 and Δ9 saturases are also increased in obese conditions (Folsom, Ma, McGovern, & Eckfeldt, 1996). The composition of lipids in serum is correlated with the insulin resistance as shown by studies that demonstrated a positive association between total SFA from plasma phospholipids and cholesterol

ester to type 2 diabetes occurrence (Folsom et al., 1996; Wang et al., 2003). The high percentage of MUFA of J1 animals could indicate an increased activity of Δ9 desaturase, which produces oleic acid by desaturating stearic acid, and is inversely correlated insulin levels (Folsom et al., 1996).

Previous studies with FJP-fed obese animals showed no significant differences in the serum concentration of triglycerides (Batista et al., 2013). However, the plasma insulin levels were decreased in FJP-fed rats (Lenquiste et al., 2012), and, in the present work, the FJP intake reduced serum saturated lipids. SFA are strongly related with inflammation and insulin resistance (Dragano et al., 2013; Tranchida et al., 2012). Thus, the low levels of saturated FAME in serum triglycerides and phospholipids from J1 and J4-fed animals might act as a protection factor against hyperinsulinemia and inflammation. The 4% FJP dose was more effective, since the J4-animals showed the better PUFA:SFA ratio among the experimental group.

Indeed, our previous studies showed that the 4% FJP dose added to high-fat diets increased triglycerides output, decreased hyperinsulinemia, hepatic inflammation and improve insulin sensitive in *Sprague–Dawley* rats and *Swiss* mice (Batista et al., 2013; Dragano et al., 2013; Lenquiste et al., 2012). In addition, the lower serum levels of SFA of J4 animals could indicate a down-regulation of gene expression of lipogenic enzymes, a result cited in a studies using anthocyanin and ellagic acid-rich diets and extracts (Tsuda et al., 2003; Wu, Ma, & Tian, 2013a). Folsom and co-workers have shown that PUFA and SFA from phospholipids are correlated with dietary pattern and endogenous synthesis, respectively, which corroborates this discussion (Folsom et al., 1996).

An investigation reported that a concentration of 13.09 ng mL^{-1} of total anthocyanins was found in human serum 4 h after the consumption of freeze-dried blueberries (1.16 g 100 g^{-1} total anthocyanins) (Mazza et al., 2002). In the present study, a daily consumption of bioactive compounds from FJP diets enhanced the plasma antioxidant status even under 12 h fasting conditions (Tables 1 and 2 and Fig. 1). In agreement with previous studies (Feillet-Coudray et al., 2009), the plasma results showed that the FJP diets did not alter the values obtained for lipid oxidation, GSH or GPx in obese rats. Conversely, the enzymatic analyses (CAT, SOD and GR activities) showed expressive increases in their activities in the plasma of the FJP groups.

The higher plasma antioxidant status found in the J2 and J4 animals could explain previous studies that showed increased cardio protective parameters in obese rats fed a FJP diet (Dragano et al., 2013; Lenquiste et al., 2012). Moreover, 2% FJP was also capable of increasing the antioxidant potential in healthy rats (Leite et al., 2011) showing that it could prevent diseases under normal conditions.

The high liver weights of the J4-fed animals did not seem to impact the antioxidant status of the tissue, possibly because of the higher intake of polyphenols and trolox equivalents that compensated the damage caused by the calorie intake. The oxidative stress parameters of the livers of the FJP-fed animals showed that all FJP doses promoted oxidative protection, especially the J2 diet, as observed in the ORAC, TBARS and GPx results. Based on the results, it can be seen that the liver antioxidant defenses in the rats fed high-fat diets with 1%, 2% and 4% FJP added, were similar to those of normal diet-fed animals. The ORAC data was shown to correlate with the TBARS data, improving the antioxidant defenses of the liver.

In contrast with some studies (Feillet-Coudray et al., 2009; Lee, Choi, & Seo, 2009), hepatic GSH showed no differences among the groups evaluated, possibly because of the use of different methods or the absence of protein precipitation. The liver GPx activity was expressively higher in the FJP-fed animals, which seemed to contribute to an improvement in the endogenous antioxidant defenses. Polyphenols are related to increase antioxidant enzymes expression (SOD and GPx), via activation of transcription factors (such as Nf-E2/Nrf2) (Chuang & McIntosh, 2011), which could explain these findings. SOD and GPx act by removing the superoxide radical and hydrogen peroxide, respectively, and thus prevent the formation of the hydroxyl radical, a potent ROS responsible for much cell damage (Vincent et al., 2007).

Concomitantly, the brain/ body weight ratio was significantly reduced (about 13%) in the high-fat-fed animals (C, J1, J2 and J4 groups), which corroborates other findings in the literature (Jeon et al., 2012). SFA-rich diets is linked with an increased risk of neurodegenerative disease related to diabetes, which could explain these findings (Jeon et al., 2012; Lenquiste et al., 2012; Winocur & Greenwood, 2005). However, the present data suggested a better brain antioxidant defense for animals that received diets with added FJP, based on the results of the ORAC, TEAC, FRAP and TBARS assays. The polyphenol-rich diet, as shown in this study, could protect neurons against inflammation degenerative action of SFA intake by acting in insulin transductions signals in the brain as the IR-AMPK-GSK3β axis and inhibiting NFκB nuclear translocations and TNFα expression (Jeon et al., 2012; Lau, Joseph, McDonald, & Kalt, 2009). Thus, as brain and liver could be the main tissues benefited by the intake of FJP.

The kidneys are responsible for the catabolism of the polyphenols, which are widely distributed in this tissue (Talavera et al., 2005; Vanzo et al., 2011). In fact, the non-enzymatic antioxidant parameters increased in the J1, J2 and J4 groups, but lipid peroxidation was higher and did not alter with the use of the jaboticaba treatment. Kidney lipid peroxidation may be a consequence of increasing ROS production by renal tissue damaged by high intrarenal pressures (Noeman et al., 2011).

In this work, only the TEAC results showed an increase in antioxidant power of the spleen. The present findings also suggested macroscopic spleen atrophy arising from the obesity, which could be allied with changes in its physiological functions, such as immunological cell recycling.

The β-pancreatic cells are very sensitive to oxidative stress, particularly because they are poor in the antioxidant enzymes (Tiedge, Lortz, Drinkgern, & Lenzen, 1997). No significant changes concerning the antioxidant status of the pancreas was observed in FJP-fed animals.

In conclusion, this study indicated that the oxidative stress arising from obesity was minimized in many tissues by the intake of FJP. The diets with added 2% and 4% FJP showed better *in vivo* antioxidant properties, leading to plasma, liver, brain and kidney antioxidant status enhancement, probably due to the presence of polyphenols, such as

anthocyanins, ellagic acid, gallic acid, quercetin derivatives and others, which were identified in the FJP. In addition, the serum PUFA:SFA ratio of FAME were increased in obese rats that received diets containing 4% FJP. Thus, the regular consumption of jaboticaba peel could increase the circulation of polyphenols in the body, which could possibly improve antioxidant defenses and lipidemia in obese animals, protecting their cells against oxidative stress damage and obesity-related diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2013.11.011.

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