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ORIGINAL CONTRIBUTION

Ilex paraguariensis (yerba mate) improves endocrine and metabolic disorders in obese rats primed by early weaning

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Abstract

Purpose We showed that early weaned rats developed obesity, hyperleptinemia, leptin and insulin resistance at adulthood. Here, we studied the potential beneficial effects of *Ilex paraguariensis* aqueous solution upon body composition, glycemia, lipid and hormonal profiles, leptin signaling and NPY content.

Methods To induce early weaning, lactating rats' teats were blocked with a bandage to interrupt lactation during the last 3 days (EW group), while control offspring had free access to milk throughout lactation (C group). In postnatal day (PN) 150, EW offspring were subdivided into: EW and EW+ mate groups treated, respectively, with water or yerba mate aqueous solution (1 g/kg BW/day, gavage) during 30 days. C offspring received water for gavage. In PN180, offspring were killed.

Results EW+ mate group presented lower body weight (−10 %), adipose mass (retroperitoneal:−40 % and epididymal:−44 %), total body fat (−43 %), subcutaneous fat (−46 %), visceral adipocyte area (−21 %), triglyceridemia (−31 %) and hypothalamic NPY content (−37 %) compared to EW group. However, hyperglycemia and lower HDL-c levels observed in EW group were not reverted with mate treatment. Although the hyperleptinemia, lower hypothalamic JAK2 and pSTAT3 content of EW group were not corrected by mate treatment, the hyperphagia and higher hypothalamic SOCS-3 content were normalized in EW+ mate group, indicating that the central leptin resistance could be restored.

Conclusion Thus, the therapy with yerba mate solution was capable to reverse abdominal obesity, leptin resistance and hypertriglyceridemia, suggesting an important role of this bioactive component in the management of obesity in this programming model.

Keywords Obesity · Programming · Fat mass · Leptin · *Ilex paraguariensis*

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Introduction

The protective effect of exclusive breastfeeding in the prevention of obesity is receiving considerable interest. In the United States, breastfeeding is included in the recommendations to reduce the prevalence of childhood obesity [1].

Epidemiological, clinical and experimental studies show a relationship between nutritional changes during critical periods of life, as pregnancy and/or lactation, and development of chronic diseases in adult life, such as obesity, type 2 diabetes, dyslipidemia and cardiovascular diseases. This biological phenomenon is named programming [2, 3] or developmental plasticity [4]. Indeed, the association between early weaning and development of obesity has

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also been demonstrated in experimental models. The suppression of lactation through maternal treatment with bromocriptine (a prolactin inhibitor) during the last 3 days of lactation programs the offspring for obesity, hyperleptinemia, resistance to the anorexigenic action of leptin [5], insulin resistance and dyslipidemia in adulthood [6] as well as central hypothyroidism [7]. Recently, in another model of early weaning, without pharmacological treatment or maternal separation, the lactation interruption was made using a breast banding [8], and the adult progeny showed higher adiposity, hypertriglyceridemia and insulin resistance. In addition, these animals also presented lower hypothalamic Janus tyrosine kinase 2 (JAK2), phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and higher suppressor of cytokine signaling 3 (SOCS3) levels, indicating central leptin resistance.

Recently, Yerba mate (*Ilex paraguariensis)* has been studied due to its potential beneficial effects in the treatment of obesity. Yerba mate is a native plant from the subtropical regions and one of the most consumed in South America. Its cultivation is done in Brazil, Argentina, Uruguay and Paraguay. Yerba mate leaves are used to prepare different beverages, such as chimarrão (green dried leaves prepared with hot water in a vessel called cuia), tererê (green dried leaves prepared with cold water in the same vessel) and mate tea (roasted leaves prepared with hot water or used to produce soft drinks). These beverages have various biological compounds, such as flavonoids (quercetin and rutin) and phenolic acids (chlorogenic and caffeic acids), caffeine and saponins [9, 10].

Several studies have demonstrated the beneficial effects of yerba mate. In rodents with a model of diet-induced obesity (DIO), it was showed not only an antioxidant activity and a protective effect against DNA damage [11], but also antiobesity effects by increasing the expression of thermogenic genes, such as uncoupling protein on brown adipose tissues or visceral white adipose tissue [12–14], and by reducing lipogenesis [15]. These changes were associated with an improvement in glucose tolerance and lipid profile [13, 15], type 2 DM and dyslipidemic patients also present them, and an antioxidant effect was also observed [16]. Oliveira et al. [17] showed an improve of glucose tolerance in Wistar rats using 1 g/kg/peso of yerba mate, and Miranda et al. [18] showed that regular ingestion of mate tea increased the resistance of DNA irrespective of the dose ingested (0,5, 1 or 2 g/kg weight).

The pathogenesis of obesity is different if it is programmed or diet induced. Then, here we intend to test the effect of yerba mate in the obesity management, analyzing two parameters that were not observed before, such as hypothalamic leptin signaling pathway and neuropeptide-Y (NPY) content. Thus, in the present study, we investigate the effects of *Ilex paraguariensis* aqueous solution (yerba mate) during 30 days of therapy upon metabolic and endocrine dysfunctions previously observed in obese rats primed by a non-pharmacological model of early weaning.

Materials and methods

Animal use and experimental procedures were approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/017/2009 and CEUA/057/2011). Wistar rats were kept in a temperature-controlled room (25 ± 1 °C) with artificial light-darkness cycles (lights on 0700 h, lights off 1900 h). Virgin female rats (3-month-old) were caged with male rats at the ratio of 3:1. After mating, each female rat was placed in an individual cage with free access to food and water until delivery. We only used pregnant rats that produced from 10 to 12 pups to avoid the influence of the litter size in the programming effect. At birth, to maximize the lactation performance, litters were adjusted to six male pups per dam. Experiments were performed to minimize the number of rats and the suffering caused by the procedures following the ethical doctrine of the three "R"s—reduction, refinement and replacement.

Experimental model of programming by early weaning (EW)

In the first postnatal (PN) day, 20 lactating rats were randomly separated into the two following groups: EW (early weaning, $n = 10$)—mothers were lightly anesthetized with thiopental (0.06 mg/mL/100 g) and wrapped with a breast bandage (physical barrier) to interrupt the lactation during the last 3 days of the period—and C (control, $n = 10$)—mothers whose pups had standard weaning, that is, with a lactation period of 21 days. EW and C groups received free access to food (standard diet for rodents) and pups had easy access to water bottles. The food pellets were placed directly inside the cage in order to facilitate the consumption by the pups.

After weaning, EW and control offspring had free access to water and to a standard diet, and their body weight and food intake were recorded every week. The cumulative food intake was calculated by:

$$\text{Cumulative food intake} = \text{daily consumption} \times 30\,\text{days}\ (150-180\,\text{days-old}).$$

Oral treatment with the aqueous solution of yerba mate

The yerba mate aqueous solution was prepared of roasted yerba mate leaves obtained from Matte Leão®, Rio Grande do Sul, Brazil (lot A326/06). A sample of this lot was

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previously analyzed by our group [19]. The total phenolic (4.33 ± 0.01 g L^{-1}) content was estimated by using Folin–Ciocalteu method. The chlorogenic acid (610 ± 15 mg L^{-1}), caffeine (508 ± 79 mg L^{-1}), theobromine (99 ± 11 mg L^{-1}), quercetin and rutin (both undetected) contents in the sample were quantified by high-performance liquid chromatography. The analysis of soluble powder yerba mate showed 41.20 ± 80 mg L^{-1} of chlorogenic acid, 21 ± 44 mL L^{-1} of caffein and 8.57 ± 10 mg L^{-1} of teobromin, and concentrations of quercetin and rutin were not determined.

The roasted yerba mate solution was prepared fresh each day by dissolving instant mate tea powder (Leao Jr, Curitiba-PR, Brazil) in distilled water (330 mg/ml) using a homogenizer.

In PN 150, two EW offspring of each litter were randomly subdivided into two groups: EW + Mate (EW + Mate, $n = 10$)—rats received instant yerba mate solution 1 g/kg body weight [12, 17]—and EW + water (EW, $n = 10$)—rats received pure water. The controls offspring ($n = 10$) received pure water. Animals received mate tea or water once a day during 30 days by intragastric gavage to guarantee total ingestion. It is important to note that prolonged administration (during 12 weeks) of repeated doses (2 g/kg weight) of yerba mate extract in rats and rabbits caused no apparent symptoms or signs of toxicity, including no change in behavior in comparison with control animals [20].

In PN 180, animals were killed by quick decapitation, with no prior anesthesia (because anesthesia affects hormone and lipid metabolism), to collect blood, carcass, visceral fat mass (retroperitoneal, epididymal and mesenteric white adipose tissues), brown adipose tissue and hypothalamus. Serum and tissue samples were frozen at −80 °C until analysis.

Body composition evaluation

On the day of killing, visceral fat mass was excised (mesenteric, epididymal and retroperitoneal fat depots) and immediately weighed for the evaluation of central adiposity. Total body fat and protein contents were determined by carcass analysis [21]. Pups were eviscerated; the carcass was weighed, autoclaved for 1 h and homogenized on distilled water (1:1). Homogenates were stored at 4 °C for analysis. The homogenate (3 g) was used to determine fat content gravimetrically. Samples were hydrolyzed on a shaking water-bath at 70 °C for 2 h with 30 % KOH and ethanol. Total fatty acids and non-esterified cholesterol were removed by three successive washing with petroleum ether. After drying, overnight in vacuum, tubes were weighed. Protein content was determined in 1 g of the homogenate. Tubes were centrifuged at $2{,}000 \times g$ for 10 min. Total protein concentrations were determined by the Lowry method [22]. Data are expressed as g of fat/100 g of carcass.

Morphometric analysis of adipocytes

Visceral (epididymal) white adipose tissue was fixed (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2), embedded in paraffin, sectioned (5 µm of thickness) and stained with hematoxylin–eosin. The cross-sectional area of the adipocytes was measured on digital images randomly acquired (TIFF format, 36-bit color, $1{,}360 \times 1{,}024$ pixels) with an Olympus DP71 camera and an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan) and analyzed with the software Image-Pro Plus version 5.0 (Media Cybernetics, Silver Spring, MD, USA). Some of the original color images were converted to gray scale images, for documentation purposes, by using the Adobe Photoshop software. At least 50 adipocytes per animal ($n = 5$) were randomly measured, totaling 250 adipocytes per group.

Lipid profile analysis

Serum levels of total cholesterol, triglycerides (TAG) and high-density lipoprotein (HDL) cholesterol were quantified using Biosystem commercial test kits with an automated A15 spectrophotometer (Biosystems S.A., Barcelona, Spain). Low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol were calculated according to the equation of Friedwald:

$$\text{VLDL-cholesterol} = \text{TAG}/5$$

$$\text{LDL-cholesterol} = (\text{totalcholesterol} - \text{HDL-C-TAG})/5$$

Castelli indexes I and II that correlate with atherogenicity were obtained using the following formulae:

$$\text{Castelli index I} = \text{TAG}/\text{HDL}$$

$$\text{Castelli index II} = \text{LDL}/\text{HDL}.$$

Hormonal determination by radioimmunoassay (RIA)

All measurements were performed in one assay and samples were analyzed in duplicate. Serum leptin was measured by a specific RIA kit (Linco Research, Inc., St Charles, MO, USA) with a range of detection from 0·5 to 50 ng/mL and intra-assay variation was 2.8 %. Serum insulin was determined using an RIA kit (ICN Pharmaceuticals, Inc., Orangeburg, NY, USA), with an assay sensitivity of 0.1 µUI/mL and an intra-assay variation of 4 %. Total corticosterone was measured by RIA kit (Biomedicals, NY, USA) with an assay sensitivity of 50 ng/mL and an intra-assay variation of 6.8 %.

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Insulin sensitivity

Fasting blood glucose was determined using Biosystem commercial test kits with an automated A15 spectrophotometer (Biosystems S.A., Barcelona, Spain). To determine insulin sensitivity, the insulin resistance index (IRI) was calculated: glycemia (mMol/dL) × insulinemia (uU/mL)/22.5.

Western blot analysis

To obtain cell extracts, the hypothalamus was homogenized in ice-cold lysis buffer pH 6.4 (50 mM-HEPES, 1 mM-MgCl2, 10 mM-EDTA and 1 % Triton X-100) containing the following protease inhibitors: aprotinin (10 mg/mL), leupeptin (10 mg/mL), pepstatin (2 mg/mL) and 1 mM-phenylmethylsulfonyl fluoride (Sigma-Aldrich, St Louis, MO, USA). After centrifugation (7,500×g for 5 min), the homogenates were stored at −20 °C. OBR, JAK2, STAT-3, phosphorylated STAT-3 (pSTAT3), SOCS-3 and NPY contents were analyzed by Western blot as described previously [8, 23], using actin as the internal control. Briefly, protein concentrations were determined by the bicinchoninic acid protein assay kit (Thermo Scientific, Rockford, IL, USA). Samples (30 mg of total protein) were separated by 10 % SDS-PAGE according to the molecular weight of each protein and transferred to nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech, Amersham, London, UK). Rainbow standard markers (Amersham Biosciences, Uppsala, Sweden) were run in parallel to estimate molecular weights. The membranes were blocked with 2 % albumin in Tween-Tris-buffered saline (20 mM-Tris–HCl, pH 7·5, 500 mM-NaCl and 0·1 % Tween-20) for 1 h. Specific primary antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) used were anti-OBR, anti-JAK-2, anti-STAT-3, anti-pSTAT-3, anti-SOCS-3, anti-NPY and anti-actin. The membranes were incubated with primary antibodies at 1:500 dilution in Tween-Tris-buffered saline buffer for 1 h, with an appropriate secondary antibody (1:3,000, peroxidase-conjugated IgG; Santa Cruz Biotechnology) for 1 h and then with streptavidin (1:5,000; Zymed, San Francisco, CA, USA) for 1 h. The targeted proteins were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and then exposed to X-ray film. The images were scanned, and the bands were quantified by densitometry using Image J 1.34 s software (Wayne Rasband National Institute of Health, MA, USA).

Statistical analysis

Results are reported as mean ± SEM. The GraphPad Prism 5 was used for statistical analyses and graphics (GraphPad Software, Inc., La Jolla, CA, USA). The experimental data were analyzed by one-way ANOVA and Newman–Keuls multiple comparison tests, except for the protein content measured by Western blot that was analyzed by the non-parametric Kruskal–Wallis and Dunn tests. The significance level was set at $p < 0.05$.

Results

At PN150 (the first day of yerba mate treatment), there was no difference in body weight among groups. However, at PN180 (the last day of treatment), EW presented higher body weight (+9 % vs. C $p < 0.05$), whereas EW + Mate group presented lower body weight (−10 % vc EW, $p < 0.05$), as depicted in Fig. 1a. EW group presented higher cumulative food intake (+21 % vs. C − $p < 0.05$) and yerba mate treatment normalizes this parameter in EW+ Mate group (−18 % vs. EW − $p < 0.05$), as shown in Fig. 1b.

As expected (Fig. 2a), EW group had higher retroperitoneal and epididymal adipose tissue (+34 and +39 %,

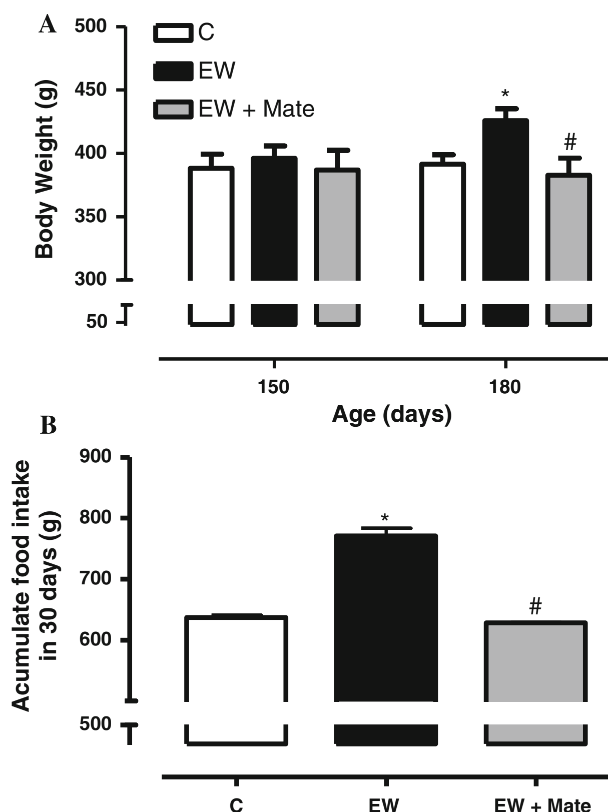

Fig. 1 Body weight and food intake. Body weight at PN 150 and PN 180 (**a**), cumulative food intake (**b**) of adult rats that were normally breastfed for 21 days (**c**), early weaned (EW) or EW that received yerba mate for 30 days (EW + Mate). Values represent mean ± SEM of 12 rats per group. *$p < 0.05$ versus C; $^{\#}p < 0.05$ versus EW

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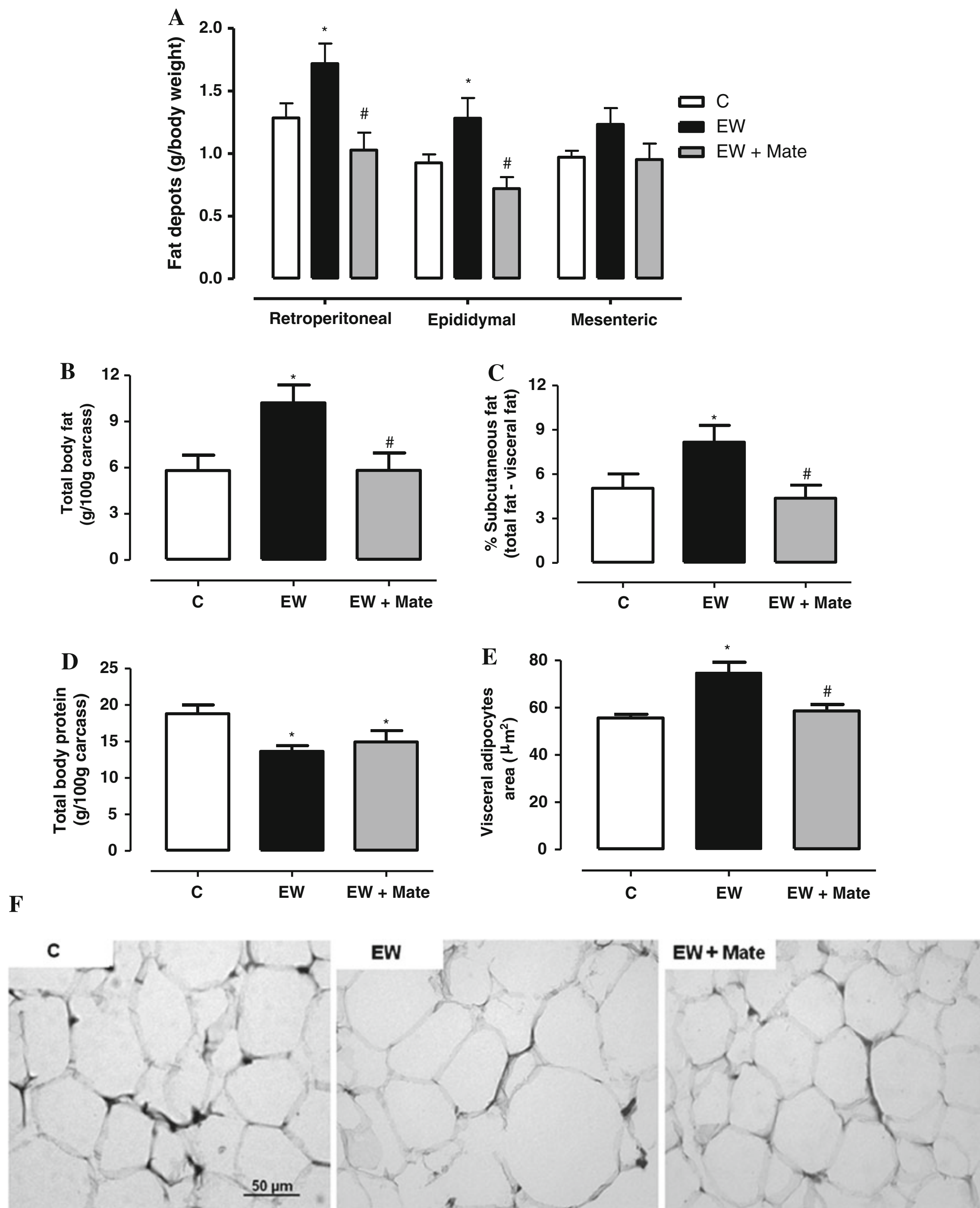

Fig. 2 Body composition. Fat depots (**a**), total body fat (**b**), subcutaneous fat depot (**c**), total body protein (**d**) of adult rats that were normally breastfed for 21 days (C), early weaned (EW) or EW that received yerba maté for 30 days (EW + Mate). Values represent mean ± SEM of 12 rats per group. The mean cross-sectional area of epididymal adipocytes (**e**) and photomicrographs of epididymal adipocytes (hematoxylin and eosin staining, same magnification, ×40) (**f**). Values represent mean ± SEM of 5 rats per group. *$p < 0.05$ versus C; #$p < 0.05$ versus EW

respectively, $p < 0.05$). EW+ mate group presented normal retroperitoneal and epididymal adipose tissue (−40 and −44 % vs. EW, respectively, $p < 0.05$). The mesenteric adipose tissue was not different in the three groups. The total body fat (Fig. 2b) and subcutaneous fat (Fig. 2c) were higher in EW group (+76 and +61 % vs. C, respectively, $p < 0.05$) but normal in EW+ Mate group (−43 and −46 % vs. EW, respectively, $p < 0.05$). EW

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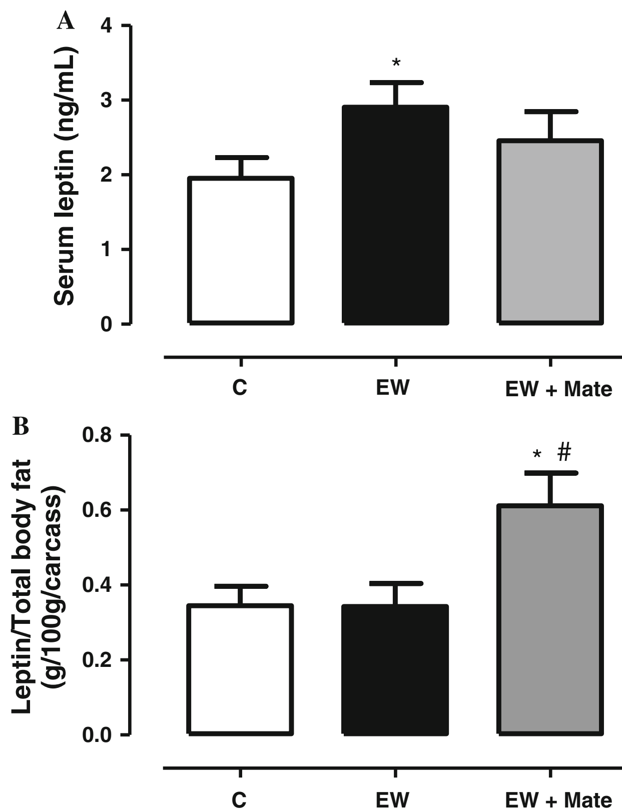

Fig. 3 Leptin levels. Serum leptin levels (**a**) and leptin/total body fat ratio (**b**) of adult rats that were normally breastfed for 21 days (**c**), early weaned (EW) or EW that received yerba mate for 30 days (EW + Mate). Values represent mean ± SEM of 12 rats per group. *$p < 0.05$ versus C; #$p < 0.05$ versus EW

group showed higher visceral adipose tissue area at PN180 (+34 % vs. C, $p < 0.05$), while EW+ Mate showed it normal (−21 % vs. EW, $p < 0.05$) as depicted in Fig. 2e. As expected, EW group presented lower body protein (−27 % vs. C, $p < 0.05$); however, yerba mate treatment for 30 days was not able to reverse this parameter (Fig. 2d).

EW group showed hyperleptinemia (+48 % vs. C, $p < 0.05$), and the yerba mate treatment for 30 days did not reduce significantly the serum leptin level (Fig. 3a). Interestingly, EW+ mate group presented higher leptin/total body fat ratio compared to C and EW group (+77 and +78 %, respectively, $p < 0.05$—Fig. 3b).

Concerning leptin signaling pathway, EW group showed lower JAK2 (−30 %, $p < 0.05$—Fig. 4b), pSTAT-3 (−30 %, $p < 0.05$—Fig. 4d) and higher SOCS-3 (+60 %, $p < 0.05$—Fig. 4e) contents. Yerba mate treatment was able to reverse only SOCS3 content (−27 % vs. EW, $p < 0.05$). Moreover, EW group presented higher NPY (+29 % vs. C, $p < 0.05$) that was normalized by mate treatment (−37 % vs. EW, $p < 0.05$) as shown in Fig. 5a.

Table 1 shows lipid profile, fasting glycemia and serum hormone profile of adult rats. EW group presented higher triglycerides, glycemia and IRI (+31, +46 % and twofold increase, $p < 0.05$, respectively), but lower HDL-c (−13 %, $p < 0.05$). Yerba mate treatment for 30 days in EW offspring only normalized serum triglycerides (−35 % vs. EW, $p < 0.05$). Insulin and corticosterone serum levels were not significantly different among groups.

Discussion

Previously, we demonstrated that rats primed by early weaning presented higher visceral and total body fat mass, insulin resistance, hypertriglyceridemia and central leptin resistance at adulthood [8]. Here, for the first time in this model, we evaluated the effects of a 30 days treatment with the *Ilex paraguariensis* aqueous solution (yerba mate) on adiposity, glucose homeostasis, lipid and hormone profiles and hypothalamic signals involved in the central regulation of food intake, which are parameters directly related to obesity and its metabolic disorders. We showed that yerba mate normalized most of the alterations observed in EW model.

Several studies have demonstrated that obese mice chronically treated with yerba mate for 8 weeks [12, 13, 24] and with chlorogenic acid infusion, a main compound of yerba mate, for 3 weeks [25] showed a marked attenuation in weight gain and body adiposity, and restoration of the serum levels of cholesterol, triglycerides, LDL-c and glucose. Hussein et al. [26] showed that the treatment with yerba mate (50 and 100 mg/kg) in mice improved the glucose and lipid metabolism, maybe by the increase in serum GLP-1. In adult rats primed by early weaning, the treatment with yerba mate from PN150 to PN180 days old prevented the development of overweight, higher body adiposity, visceral obesity, higher subcutaneous fat depots and hypertriglyceridemia, evidencing its potential effects on the management of obesity metabolic disorders. However, yerba mate was not able to correct totally the hyperleptinemia, the hyperglycemia and the insulin resistance index of EW offspring, which can be partially due to the treatment period.

In the early weaning model, adult animals are obese and had hyperphagia and central leptin resistance featured by lower hypothalamic JAK2 and pSTAT3 contents, with higher SOCS3 [8]. The treatment with yerba mate during 30 days normalized food intake and hypothalamic SOCS3 content, although it did not change JAK2 and pSTAT3 content. Leptin has other signaling pathways that were not studied here, such as PI3 K that crosstalk with insulin signaling pathway. Also, SOCS3 is able to act as an intracellular feedback for insulin signaling pathway [27]. Thus, despite the unaltered JAK2 and pSTAT3 contents after mate treatment, the normalized SOCS3 with this treatment may normalize the food intake, restoring

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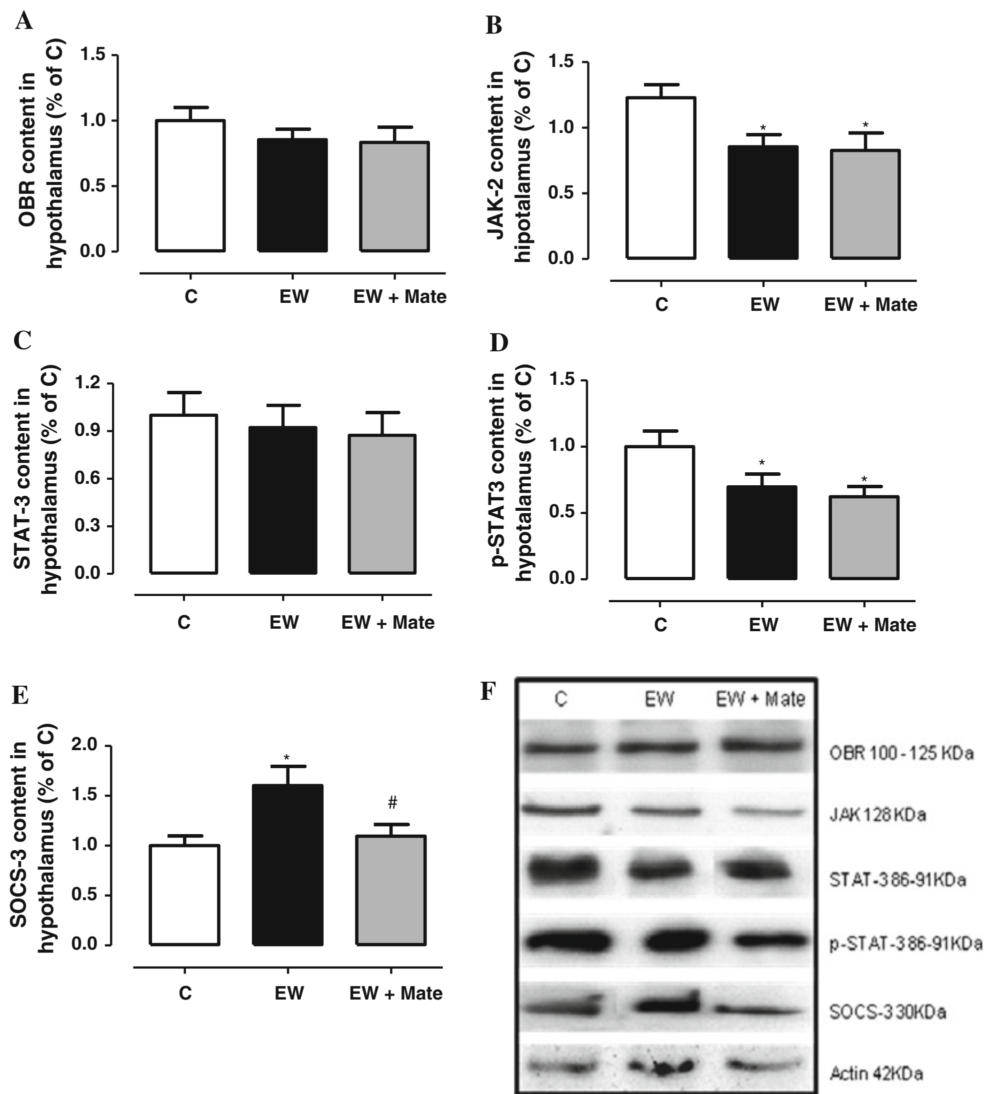

Fig. 4 Signalization pathway leptin. Content of OBR (**a**), JAK-2 (**b**), STAT-3 (**c**), pSTAT-3 (**d**), SOCS-3 (**e**) and representative bands (**f**) in hypothalamus of adult rats that were normally breastfed for 21 days (C), early weaned (EW) or EW that received yerba mate for 30 days (EW + Maté). Values represent mean ± SEM of 12 rats per group. $*p < 0.05$ versus C; $^{\#}p < 0.05$ versus EW

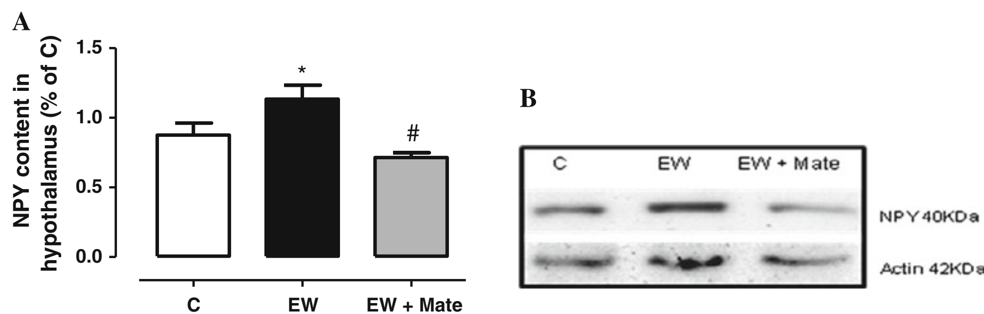

Fig. 5 Hypothalamic NPY. Content of NPY (**a**) in hypothalamus and representative bands (**b**) of adult rats that were normally breastfed for 21 days (C), early weaned (EW) or EW that received yerba mate for 30 days (EW + Mate). Values represent mean ± SEM of 12 rats per group. $*p < 0.05$ versus C; $^{\#}p < 0.05$ versus EW

hypothalamic insulin resistance or acting in the secondary leptin signaling pathway. Also, other signaling proteins should be studied, such as phosphotyrosine phosphatase (PTP)-1B, which can inhibit leptin signaling pathway [28].

NPY is an orexigenic neurotransmitter that is abundant in the brain and negatively regulated by leptin [29]. Adult rats from early weaning model presented higher NPY content in hypothalamus [23] that possibly contributes to

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Table 1 Blood lipid, glucose and hormone levels of adult rats

	180 days old		
	C	EW	EW + Mate
Total cholesterol (mg/dL)	53.9 ± 4.6	60.7 ± 2.9	57.6 ± 3.0
HDL-c (mg/dL)	30.9 ± 1.2	26.8 ± 1.03*	27.1 ± 0.9*
LDL-c (mg/dL)	19.8 ± 3.2	26.3 ± 1.9	23.3 ± 1.5
VLDL-c (mg/dL)	6.5 ± 0.7	8.0 ± 0.9	5.7 ± 0.5
Triglycerides (mg/dL)	30.9 ± 2.9	41.7 ± 4.6*	28.7 ± 2.4#
Castelli index I	1.72 ± 0.10	2.15 ± 0.13*	2.05 ± 0.03*
Castelli index II	0.51 ± 0.09	0.98 ± 0.06*	0.83 ± 0.04*
Glycemia (mg/dL)	119.8 ± 7.0	175.0 ± 16.3*	153.5 ± 14.8
Insulinemia (μUI/mL)	9.3 ± 0.5	10.9 ± 1.0	9.3 ± 0.9
IRI	2.39 ± 0.23	4.80 ± 0.73*	3.91 ± 0.67
Corticosteronemia (ng/mL)	552.3 ± 30.1	698.8 ± 53.3	559.8 ± 59.1

Values represent mean ± SEM of 12 pups per group. Significant differences between groups: * *vs.* C; # *vs.* EW

HDL-c high-density lipoprotein, *LDL-c* low-density lipoprotein, *VLDL-c* very low-density lipoprotein, *IRI* insulin resistance index

hyperphagia found in these offspring. The treatment with yerba mate improved the content of NPY and may contribute to the correction of hyperphagia in EW offspring, independent of leptin action. Since glucagon-like peptide-1 (GLP-1) inhibits NPY [30], and Hussein et al. [26] showed that yerba mate increases GLP-1, it is possible that the decrease of NPY by mate treatment is dependent of GLP-1.

Thylakoids are membranes isolated from plant chloroplasts. Isolated thylakoids from green leaves leads to retarded fat digestion by adhering to fat globules in the intestine. Thylakoids treatment to apoE-deficient mice fed with high-fat diet presented lower body weight, body fat, free fat acids, triglycerides and serum glucose and higher cholecystokinin (CCK) compared to non-treated animals [31]. In Sprague–Dawley rats at 12 days with high-fat diet, thylakoids are able to reduce food intake, cause slow down of fat digestion, reduce body weight gain and promote satiety by elevated CCK release compared with the control rats [32]. CCK could also act in a similar way that we suggested above for GLP-1. However, we did not find any report about the effects of CCK on NPY neurons. Thus, it is possible that thylakoids found in yerba mate have contributed for the food consumption normalization of EW pups.

It is well known that obesity is associated with an inflammatory profile, and adipose tissue plays an important role in this typical obesity status, since there is an increased production of cytokines in this tissue in obese subjects [33]. It was showed that hypothalamic SOCS-3 can also be induced via the IKKβ-NFκβ pathway during cellular inflammation, and this mechanism can contribute to leptin resistance [27, 34]. Thus, probably the weight loss in adult EW offspring treated with yerba mate improved the inflammatory status that may have contributed to the correction of hypothalamic SOCS-3 contents. Indeed, Arçari et al. [12] showed that treatment with yerba mate during 8 weeks improved the expressions of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in mice fed with high-fat diet.

In the present model, EW pups had an undernutrition during 3 days due to the lack of milk, but after weaning, they quickly recover their body weight without overeating that only started at PN90 [8]. Thus, the recovery in body weight could be explained by a decrease in metabolic rate. In fact, NPY content in hypothalamus was increased in these animals at weaning [23], and this neuropeptide, besides its orexigenic effect, also decreases metabolism, especially decreasing thyrotropin-release hormone [35]. Accordingly, we recently published that EW pups had a decrease in serum thyrotropin and triiodothyronine [36], which could help to explain a decrease in basal metabolic rate. Thus, undernutrition during a critical period of life leads to a sequence of mechanisms that prepare the animal to survive in a poor food supply environment. In this case, the programming strategy is not based only on hyperphagia but maybe more directly through a reduction in metabolic rate.

In the present study, the treatment with yerba mate normalized the adipocytes area of obese EW offspring, corroborating the findings of Kang et al. [15] in DIO mice. The effect of yerba mate upon leptin serum levels is controversial. We and Pimentel et al. [37] showed that yerba mate was not able to change the hyperleptinemia in obese rodents. However, Hussein et al. [38] with a much higher yerba mate concentration showed a serum leptin increase, whereas Pang et al. [14] showed a serum leptin decrease after yerba mate treatment in DIO mice. The contradictory results may be related to different models, species or doses employed in these studies. However, we showed a higher leptin/total body fat ratio in EW+ mate group, which may be associated with a local increase in leptin production and secretion by the adipocyte. Paz-Filho et al. [39] showed that a lower leptin/total body fat mass (measured by bioimpedance) ratio in adult obese subjects with three or more criteria for metabolic syndrome may suggest a leptin deficiency state, which was related to a worse prognosis of metabolic syndrome. Thus, our finding may be suggestive of a protective effect of yerba mate in EW+ Mate.

In the present study, we found that EW offspring had hyperglycemia and insulin resistance, which corroborate our previous study [8], and the treatment with yerba mate did not totally reverse these parameters. Thus, concerning

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the glycemic homeostasis, our findings were not in accordance with previous studies that have shown that *I. paraguariensis* treatment improves glucose tolerance in obese animals [13–15, 24]. Again, maybe these differences can be attributed to dose and the duration of treatment, as well as model of obesity and species studied.

Conclusion

In summary, our study reinforces the idea that obesity can be primed by early weaning and that treatment with yerba mate is able to restore many parameters related to obesity and metabolic syndrome, indicating that this compound may be helpful in preventing and treating some endocrine-metabolic diseases, and for the first time showing the possible hypothalamic mechanism of yerba mate action. Therefore, our study suggests the importance of the future use of yerba mate as therapeutic tool in the obesity management.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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