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# Adipose tissue of female Wistar rats respond to *Ilex paraguariensis* treatment after ovariectomy surgery

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ABSTRACT

*Background and aim:* Metabolic disturbances are known for their increasing epidemiological importance. *Ilex paraguariensis* presents a potential option for mitigating lipid metabolism imbalance. However, most of the literature to date has not considered sex bias. This study aimed to evaluate the effect of *Ilex paraguariensis* on the metabolism of different adipose tissue depots in males and females.
*Experimental procedure:* After ovariectomy, female Wistar rats received daily treatment with the extract (1 g/kg) for forty-five days. Biochemical serum parameters and tissue metabolism were evaluated. Oxidation, lipogenesis and lipolysis were evaluated in brown, white visceral, retroperitoneal and gonadal adipose tissues.
*Results and conclusion:* The results showed that treatment with the extract led to a reduced weight gain in ovariectomised females in comparison to control. The triglyceride concentration was decreased in males. Glucose oxidation and lipid synthesis in visceral and retroperitoneal adipose tissues were restored in ovariectomised females after treatment. The response to epinephrine decreased in visceral adipose tissue of control males; however, lipolysis in females did not respond to ovariectomy or treatment. These findings highlight the enormous potential effects of *I. paraguariensis* on lipid metabolism, modulating lipogenic pathways in females and lipolytic pathways in males. Furthermore, the sex approach applied in this study contributes to more effective screening of the effects of *I. paraguariensis* bioactive substances.


## 1. Introduction

Yerba mate (*Ilex paraguariensis,* A. St. Hil., 1822) is a perennial tree belonging to the "holly" family (Aquifoliaceae). It is native to South America and is commonly found in Brazil, Argentina, Paraguay and Uruguay. These population of these countries widely consumes a tea-like beverage called *mate*, which is prepared with the leaves and stems of *Ilex paraguariensis*. In addition to containing compounds with nutritional relevance, mate contains methylxanthines, saponins and polyphenols and these bioactive fractions are relevant for human health research.[1]

Regarding the extract's effects on energy metabolism, several studies have indicated its use as a preventive/therapeutic agent against metabolic syndrome and obesity. As observed in previous studies,[2] insulin levels do not appear to be affected by the extract in male control rats. This was confirmed in the present study, but for the first time in control e ovariecomisated female rats. *Ilex paraguariensis* has been shown to attenuate weight gain, body mass index, waist-hip ratio and the percentage of body fat in humans (men and women were analysed together) after a 12-week treatment.[3] Also in humans, the mate extract was able to modify the

*Abbreviations:* BAT, brown adipose tissue; Epi, epinephrine; KRB, Krebs ringer bicarbonate buffer; WAT, White adipose tissue; WATv, visceral white adipose tissue; WATr, retroperitoneal white adipose tissue; WATg, gonadal white adipose tissue.

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respiratory quotient, indicating an increase in fat oxidation and anti-obesity potential.[4] The extract reduces weight gain in rats after the consumption of a hyperlipidaemic diet, decreases visceral white adipose tissue (WATv) mass, blood insulin, glucose, leptin levels and has been shown to improve the inflammatory profile in liver and muscle.[5,6] The same has been found in C57BL/6J mice with diet-induced obesity, where *Ilex paraguariensis* improves the energetic profile of obese mice, increasing energy expenditure, improving performance in the oral glucose tolerance test, and decreasing blood triglycerides, free fatty acids, leptin, insulin and cholesterol, as well as causing upregulation of thermogenic genes in WAT.[7]

Metabolic syndrome is a critical pathological condition and involves failures in the cardiovascular system, pancreas and liver, which leads to the main causes of death within individuals with this condition: cardiovascular disease, type 2 diabetes mellitus and cirrhosis, among others.[8] World authorities have emphasised their concern about metabolic syndrome because of its significant contribution to the development of metabolic comorbidities. Scientific efforts that favour the prevention and treatment of this disease are quite valid since they can significantly improve the population's quality of life, as well as reducing public health expenditure.

However, most of these studies addressing metabolic syndrome have not yet explored sex bias. Obesity affects both men and women,[9,10] but the distribution of fat depots, energy stock and mobilisation and adipokine secretion show very different patterns between males and females in humans and rodents.[10–12] Circulating sex steroid concentrations alter such factors considerably, leading to androgen and gynoid patterns of adipose tissue.[13] Consequently, the search for new therapeutic agents should involve females/women in study samples, so that basic and clinical research can have better application in the future.

Therefore, faced with the alarming prevalence of metabolic syndrome, the potential use of *I. paraguariensis* extract as a preventive/treatment agent for this condition and the sex dimorphism of adipose tissue, the aim of this study was to evaluate the metabolism of different adipose tissue depots, evaluating male and female Wistar rats separately, after treatment with yerba mate aqueous extract.

## 2. Methods

### 2.1. Animals and experimental design

Fifty-five (N = 55) male and female Wistar rats (8 weeks old) were housed in groups of three per cage, under light/dark cycles of 12 h, at 22 ± 2 °C. All rats were fed with a commercial diet (Nuvilab CR1®) and had access to food and water *ad libitum*. Rats were divided into six groups: male control (C), male treated with mate (CT), female sham (S), female sham treated with mate (ST), female ovariectomy (OV) and female ovariectomy treated with mate (OVT), totalling 8–10 individuals per group. All animal procedures were performed by trained staff to ensure the well-being of the animals.

### 2.2. Ovariectomy protocol

Female rats received Ketamine (90 mg/kg) and Xylazine (10 mg/kg) intraperitoneally. Two midline dorsal skin incisions were made. The ovarian vessels were clamped, and the ovaries removed. Then, the uterine tubes were ligated, and the muscles and skin sutured. Dipyrone (200 mg/kg) was given for analgesia. The sham groups also received anaesthesia and analgesia, and the ovaries were exposed but returned to the abdominal cavity. After recovery, treatment was performed.

### 2.3. Plant materials and aqueous extract preparation

The mate aqueous extract was prepared as follows: 300 mL of filtered water was heated up to 80 °C, then 75 g of commercial yerba mate (Ervateira Barão de Cotegipe, RS, Brazil/Lot 08/17) was added, and left to infuse for 15 min, then filtered and left to cool. Immediately after preparation, the extract was administered by gavage (1 g/kg/day). The preparation was made daily for forty-five days in the late afternoon, due to the nocturnal habits of the animals, for the extract to be administered at the time of awakening.

### 2.4. Euthanasia and tissue procedures

After treatment, animals were weighed and decapitated. The results presented here regarding the females correspond to the dioestrus phase of their cycles. The brown adipose tissue (BAT), visceral adipose tissue (WATv), retroperitoneal adipose tissue (WATr), gonadal adipose tissue (WATg) and serum were collected and processed according to oxidation, lipogenesis and lipolysis protocols. The morphometric parameters were expressed as tissue index (tissue weight/body weight). All euthanasia proceedings followed the NIH publication: Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee (Universidade Federal do Rio Grande do Sul Research System, protocol number: 31500).[14]

### 2.5. Measuring aqueous extract compounds

The same aqueous extract provided to the animals during treatment was used in the following procedures.

#### 2.5.1. Methylxanthines

An HPLC Shimadzu Prominence 20AT module (Kyoto, Japan) coupled to a photodiode array detector (PDA) SPD-M20A, controlled by LC-Solution Multi-PDA software, was used. A Gemini RP C18 column (Phenomenex, 250 × 4.6 mm i.d.; 5 μm particle size) coupled with a C18 guard column was used as the stationary phase.

Methylxanthines were assayed by HPLC based on a previously validated method employing caffeine and theobromine as external standards.[15] The theobromine (Sigma-Aldrich, St. Louis, MO, USA) and caffeine (Sigma-Aldrich) standards were properly dissolved in methanol: water 30/70 (v/v), at concentrations ranging from 0.48 to 40.0 μg/mL (caffeine) and from 0.495 to 7.005 μg/mL (theobromine). An isocratic system was employed, using methanol/water 30/70 (v/v) as the mobile phase. The flow rate (1.1 mL/min) and temperature (35 ± 1 °C) were kept constant throughout the analysis, which took 10 min. The detection was performed at 280 nm. All samples were properly diluted with methanol/water 30/70 (v/v) seeking the linearity range of standard curves. The total methylxanthine content was determined by the sum of caffeine and theobromine individual concentrations. A representative chromatogram of methylxanthine (caffeine and theobromine) dosage in lot 07/18 of the *Ilex paraguariensis* aqueous extract is shown in Additional file 1.

#### 2.5.2. Total phenols

The total phenols from yerba mate were assayed by the Folin-Ciocalteau method,[16] and the total phenol content was determined by the colorimetric reaction of Folin-Ciocalteau reagent (yellow) to a blue complex when reductive agents were present (phenolic compounds). 0.5 mL of Folin-Ciocalteau reagent was added to 0.5 mL of the sample. Then 0.5 mL of sodium carbonate ($Na_2CO_3$) 10% was added, and after 30 min at room temperature, the measurement was made. Gallic acid (Sigma-Aldrich) was used

as the external standard at concentrations ranging from 2.0 to 6 μg/mL, after dissolution in distilled water. The absorbance was measured in a spectrophotometer (UV-1800 Shimadzu spectrophotometer) at 760 nm wavelength. Values are expressed as gallic acid equivalents.

### 2.6. Serum parameters

Blood was collected in serum prepared tubes, then centrifuged (2150 g/8 min). Total cholesterol, glucose and triglycerides were determined using commercial enzymatic kits (Labtest ®, Minas Gerais, Brazil).

### 2.7. $^{14}C$ glucose incorporation into $^{14}CO_2$

The $^{14}C$ glucose incorporation into $^{14}CO_2$ was performed following a validated method.[17] In brief, tissue samples were sliced and incubated at 37 °C for 60 min in flasks sealed with rubber caps containing KRB (Bicarbonate Krebs–Ringer buffer, pH 7.4), 0.1 μCi [U–$^{14}C$] glucose (55 mCi/mmol, Amersham, Little Chalfont, UK), and glucose 5 mM. The gaseous phase was exchanged with a 5% $CO_2$ and 95% $O_2$ mixture. Small glass wells containing strips of 3 MM-Whatman paper were placed above the level of the incubation medium ($^{14}CO_2$ wells). The assay was stopped by injecting 0.25 mL of trichloroacetic acid solution 50% (v/v) through the rubber caps, and 0.25 mL of NaOH (2.0 M) solution into the $^{14}CO_2$ wells. The flasks were maintained for 12 h at room temperature in order to capture $^{14}CO_2$ in the 3 MM-Whatman paper. The paper contents were transferred to vials containing a liquid scintillation mixture (toluene – Triton X®-100 (2:1, v/v); 2,5-diphenyloxazole (0.4%, v/v) and 2-p-phenylenebis 5-phenyloxazole (0.01% v/v), and radioactivity was measured using a liquid scintillation counter (Tri-Carb 4910 TR liquid scintillation counter, PerkinElmer, MA, USA). Results were expressed as nmol of $^{14}C$ glucose incorporated into $CO_2$ per gram of tissue per hour.

### 2.8. $^{14}C$-glucose incorporation into lipid assay

Samples from the $^{14}C$ glucose incorporation into $^{14}CO_2$ assay were homogenised with chloroform: methanol 2:1 (v/v), followed by lipid extraction performed according to Folch et al. (1957).[18] Next, saline solution (NaCl 0.9%) was added, and tubes were centrifuged for 10 min at 2000g. The chloroform phase was reserved for evaporation, and a scintillation liquid mixture was added. The results were expressed as nmol $^{14}C$ glucose converted to lipid per gram of tissue per hour.

### 2.9. Lipolysis assay

Sample tissues were incubated in KRB (pH 7.4) at 37 °C for 60 min under two different conditions: basal and stimulated. The basal condition corresponded to incubation in KRB, whereas the stimulated (Epi) corresponded to incubation in KRB plus epinephrine (12.5 mM). The samples were assessed through glycerol released into the incubation medium using a UV-method commercial kit (Enzytec™ Fluid Glycerol). Results were expressed as microgram of glycerol released per gram of tissue (μg/g).[19]

### 2.10. Statistical analyses

Results were expressed as the mean and respective standard deviation (SD). Differences among groups were tested by a T-test for male groups (C and CT) and two-way ANOVA for female groups (S, ST, OV and OVT) followed by Tukey post hoc test (Prism® software, 6 edition). Values of $P < 0.05$ were considered significant. Nonparametric data were tested using the Mann-Whitney test for males and Kruskal Wallis (KW) for females, followed by Dunn's post hoc test. Data are expressed by the median and interquartile range (IQR).

Lipolysis data were evaluated by *two-way* ANOVA of repeated measures followed by Sidak's post hoc test, and nonparametric data were analysed by the Friedman test and Dunn's post hoc.

## 3. Results

### 3.1. Aqueous extract compounds

Total polyphenols and methylxanthines (caffeine and theobromine) are the major components present in *Ilex paraguariensis*' extract (Lot 08/17). The extract presented with 5.244 ± 0.124 g% of total phenols (expressed as gallic acid equivalents). Caffeine and theobromine were present at 1.077 ± 0.038 and 0.197 ± 0.007 g%, respectively.

### 3.2. Serum and morphometric parameters

Table 1 shows serum and morphometric data. The extract was able to reduce the triglyceride content in males, and OVT females were determined to have lower levels, as compared to the sham group, by the post hoc test.

Regarding the evaluated morphometric parameters, it was observed that the uterus of ovariectomised females presented with a considerable weight decrease. Given that uterine weight maintenance is higher contingent to oestrogen levels, the ovariectomy procedure was successful (Table 1).

The weight gain in males remained unchanged after treatment. However, the mate extract attenuated the weight gain in ovariectomised females. On the other hand, the BAT, WATr and WATg tissue indexes were not different in males or females after treatment. Female groups treated with mate showed a decrease in WATv index according to ANOVA, but no differences were found in the post hoc test. WATg presented with the same pattern as WATv, and only S and OVT groups showed differences in the post hoc test.

### 3.3. Brown adipose tissue metabolism

Fig. 1 shows BAT metabolism. The incorporation of glucose into $CO_2$ or lipids in females did not change with treatment in BAT (Fig. 1D and 1E). Males did not respond significantly to treatment in regard to these parameters (Fig. 1A and 1B). No differences were found regarding $CO_2$ production or lipogenesis.

Intrinsic lipolysis did not change in females (Fig. 1F). Furthermore, the extract was unable to change the response to epinephrine in BAT. Therefore, both males and females demonstrated a response to epinephrine and the lipolysis response remained pronounced (Fig. 1C and 1F).

### 3.4. Visceral adipose tissue metabolism

Fig. 2 shows the WATv metabolism. The incorporation of glucose into $CO_2$ was not different in CT (Fig. 2A). However, OVT females had a significant decrease in this parameter in WATv after 45 days. After treatment with mate extract, these levels returned to the values found in S and ST (Fig. 2D).

The incorporation of glucose into total lipids presented the same response pattern to treatment. Males did not seem to respond. OVT females showed a significant reduction in this parameter, with the extract promoting the restoration of values found in control rats (Fig. 2B and 2E).

No difference in basal lipolysis was observed in either control or

**Table 1**
Morphometric and serum parameters of males, females and ovariectomised females after *Ilex paraguariensis* extract treatment.

| Parameter | Experimental group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | | | |
| | C | CT | T test/Mann- Whitney | S | ST | OV | OVT | ANOVA /Kruskal Wallis | Post hoc |
| **Uterus weight (g)** | – | – | – | 0.446 ± 0.050[a] | 0.513 ± 0.147[a] | 0.116 ± 0.029[b] | 0.109 ± 0.020[b] | Ovariectomy | $P < 0.0001$ |
| **Weight gain (g)** | 42.40 ± 12.03 | 50.45 ± 10.04 | ns | 26.30 ± 7.646[a] | 28.00 ± 5.692[a] | 41.67 ± 12.31[b] | 33.90 ± 7.795[ab] | Ovariectomy | $P = 0.0097$ |
| **BAT index ($10^5$)** | 94.16 ± 4.699 | 85.57 ± 4.362 | ns | 137.7 (121.5/148.2)[a] | 119.1 (108.4/133.7)[ab] | 122.3 (105/143.6)[ab] | 98.79 (86.57/98.79)[b] | – | $P = 0.0045$ |
| **WATv index ($10^4$)** | 64.91 (48.53/76.56) | 50.74 (41.17/60) | ns | 59.89 ± 5.609 | 48.47 ± 6.406 | 65.73 ± 20.24 | 50.21 ± 17.43 | Treatment = 0.0083 | ns |
| **WATr index ($10^4$)** | 119.6 ± 34.82 | 121.4 ± 42.89 | ns | 68.24 (57.4/76.85) | 53.99 (50.64/66.13) | 127.4 (85.66/160.2) | 78.73 (64.8/84.89) | ns | ns |
| **WATg index ($10^4$)** | 105.6 ± 30.07 | 113.1 ± 31.28 | ns | 206.2 ± 53.79[a] | 136.0 ± 24.29[b] | 152.3 ± 58.84[ab] | 127.7 ± 42.58[b] | Treatment | $P = 0.0045$ |
| **Glucose (mg/dL)** | 93.65 ± 14.03 | 87.80 ± 18.10 | ns | 97.224 ± 12.07 | 104.04 ± 23.07 | 111.17 ± 13.37 | 109.72 ± 16.75 | ns | ns |
| **Triglycerides(mg/dL)** | 134.2 ± 50.82 | 82.98 ± 42.02* | $P = 0.028$ | 86.214 ± 29.74[a] | 80.92 ± 34.07[a] | 64.19 ± 26.64[ab] | 44.44 ± 17.36[b] | Ovariectomy | $P = 0.0031$ |
| **Cholesterol (mg/dL)** | 69.65 ± 17.51 | 61.58 ± 16.34 | ns | 77.99 ± 25.87 | 58.04 ± 16.45 | 71.54 ± 19.25 | 66.24 ± 18.18 | ns | ns |
| **Insulin (μUI/mL)** | 2.66 (2.04/5.03) | 2.88(1.87/3.06) | ns | 5.19 ± 1.84 | 3.49 ± 0.75 | 6.21 ± 3.52 | 5.2 ± 2.85 | ns | ns |

Data expressed as mean ± standard deviation or median and 25/75 percentile. Data distribution was evaluated and submitted to T test or Mann-Whitney test for male. For females, two-way ANOVA followed by Tukey post hoc test or Kruskal-Wallis test followed by Dunn's post hoc. 8–10 individuals per group and 3 individuals per group for males insulin levels. (*) indicate difference between groups in males. Different letters indicate statistical difference between groups in females. BAT: brown adipose tissue and WATv: visceral, WATr: retroperitoneal and WATg: gonadal white adipose tissue.

treated males and females (Fig. 2C and 2F). As for the control animals, there was an effect of epinephrine as a lipolysis stimulant in WATv males, but this was not apparent in females. The extract's effect in CT promoted a lack of responsiveness to epinephrine, while in females the response remained the same. That is, the extract promoted a lack of response to epinephrine in males. OV and OVT rats showed no changes (Fig. 2C and 2F).

### 3.5. Retroperitoneal adipose tissue metabolism

Fig. 3 shows WATr metabolism. The treatment response was the same for incorporation of glucose into $CO_2$ and lipids. The incorporation of glucose into $CO_2$ in males did not change after treatment (Fig. 3A and 3B). However, this parameter significantly decreased in the WATr of females 45 days following ovariectomy. After treatment with mate extract, these levels returned to values found in control rats (Fig. 3D and 3E).

The incorporation of glucose into total lipids presented the same response pattern to treatment. Males did not seem to respond (Fig. 3B) while, in females, the extract promoted an increase in these values as compared to the OV group (Fig. 3E).

No difference in basal lipolysis was observed in control or treated males and females (Fig. 3C and 3F). In the presence of epinephrine, this response remained the same in all treated groups (Fig. 3C and 3F).

### 3.6. Gonadal adipose tissue metabolism

Fig. 4 shows WATg metabolism. The incorporation of glucose into $CO_2$ in WATg was not altered either in males or females after treatment. Also, lipid synthesis did not change in control animals. Nevertheless, the extract increased this parameter in WATg of ovariectomised rats. Also, no difference in basal lipolysis or epinephrine responsiveness was observed in control or treated males and females.

## 4. Discussion

Considering the aqueous extract compounds, it is important to note that yerba mate exhibits considerable variability in the amounts of the investigated compounds related to the cultivation method, processing, and consumption, thus changing its bioactive properties.[20,21] The results presented here correspond to the biological effect of the aqueous extract of yerba mate containing the concentrations of methylxanthines and polyphenols as mentioned in the results section.

Regarding the serum parameters, the extract was able to reduce the level of triglycerides in males. These data confirm the extract's beneficial effect on the lipid profile, as previously described by other authors in rats.[5,7] However, in these studies the authors showed that such an improvement occurred in control males, highlighting the potential preventive benefit of this extract. To the best of our knowledge, this study represents the first time that a sex approach has been used in rats to study the effect of *Ilex paraguariensis*. However, the effect on the female group remains uncertain, and the potential use of the extract in mitigating energy unbalance in females with decreased sex hormones still needs further investigation.

It is important to note that the ovariectomy surgery protocol presented here did not lead to any significant changes in serum triglycerides and insulin after 45 days. Therefore, the marked changes in energy metabolism of WAT presented below appear to precede the worsening of the lipid profile. These effects on serum parameters presented here still need further study so that they can be extrapolated to humans. Postmenopausal women have a higher

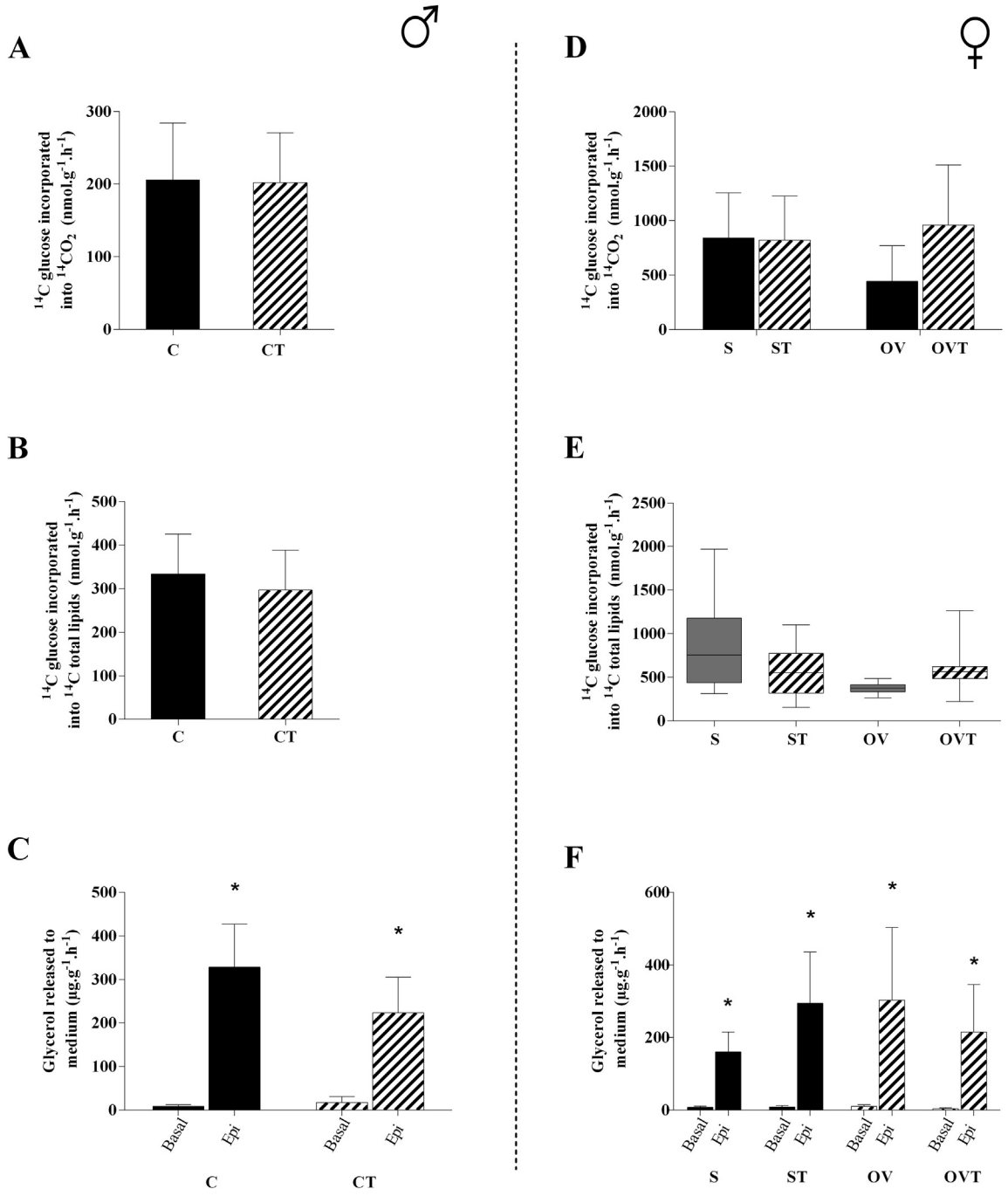


**Fig. 1.** Brown adipose tissue metabolism in males, females, and ovariectomised females submitted to treatment with *Ilex paraguariensis* extract. The metabolism was observed as $CO_2$ (A) and total lipid (B) production from labelled $^{14}$C glucose, and adrenergic responsiveness lipolysis (C) assays. Charts D, E and F present the results of the same assays in females. Data are expressed as the mean and respective standard deviation (SD) or the median and 25/75 percentile. Different letters indicate significant differences; (*) indicates a difference from the respective basal group in lipolysis and (#) indicates difference of basal lipolysis related to other groups. Epi: epinephrine. Values of $p < 0.05$ were considered significant. n = 8–10 in each experimental group. C: male control; CT: male treatment; S: female sham; ST: female sham treatment; OV: ovariectomy; OVT: ovariectomy treatment.

risk of development of cardiovascular and metabolic diseases. Many authors have reported that this is caused by the reduction in circulating oestrogens, which are known to be protective factors in several key metabolic organs, such as the liver and adipose tissue.[13] To the best of our knowledge, no other study has evaluated the effect of yerba mate on oestrogen-deficient females. However, it is necessary investigate if yerba mate extract may mitigate this risk factor in postmenopausal women. In fact, a case-control study with 95 postmenopausal women revealed that consumption of mate extract is associated with a lower occurrence of coronary heart disease, dyslipidaemia and hypertension, confirming the potential use of this extract for this population.[22]

In the case of morphometric parameters, the effect of mate on weight gain has already been described by several authors in animal models of obesity in rats and humans.[3,5,23] Our data demonstrate that treatment did not modify weight gain and adiposity in control males. However, in ovariectomised females, we found original data suggesting a new potential use for the extract: the

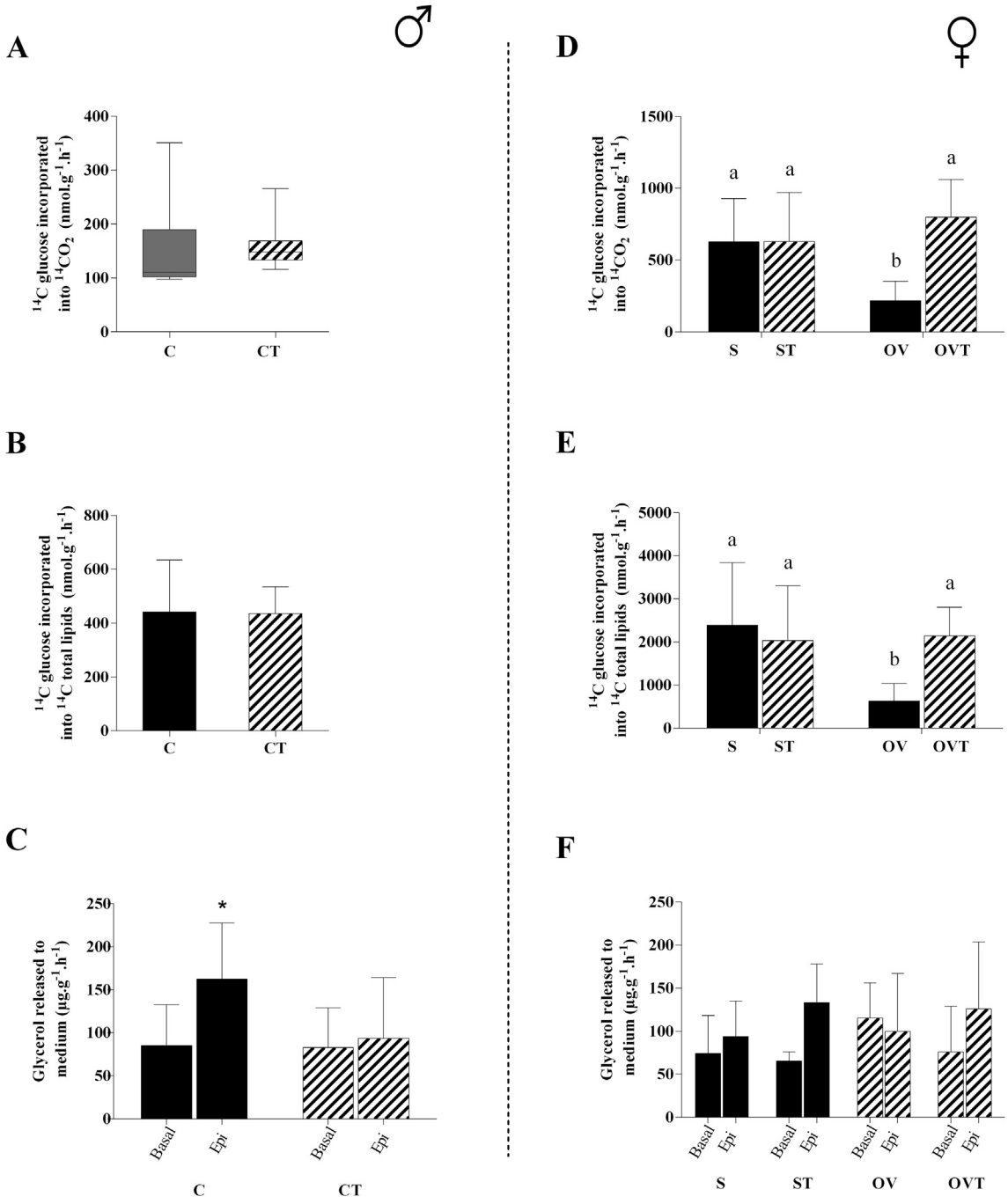


**Fig. 2.** Visceral adipose tissue metabolism in males, females, and ovariectomised females submitted to treatment with *Ilex paraguariensis* extract. The metabolism was observed as $CO_2$ (A) and total lipid (B) production from labelled $^{14}$C glucose, and adrenergic responsiveness lipolysis (C) assays. Charts D, E and F present the results of the same assays in females. Data are expressed as the mean and respective standard deviation (SD) or the median and 25/75 percentile. Different letters indicate significant differences; (*) indicates a difference from the respective basal group in lipolysis and (#) indicates difference of basal lipolysis related to other groups. Epi: epinephrine. Values of $p < 0.05$ were considered significant. n = 8–10 in each experimental group. C: male control; CT: male treatment; S: female sham; ST: female sham treatment; OV: ovariectomy; OVT: ovariectomy treatment.

attenuation of weight gain in oestrogen-deficient female rats. These data should be considered further for investigation of the effect of *I. paraguariensis* on postmenopausal women, who frequently present with changes in adiposity pattern (similar to an androgen pattern) and increased weight gain.

The effect of *Ilex paraguariensis* on morphometric parameters has already been evaluated in humans and the results seem to agree with those observed in the ovariectomised female rats in the present study. Volunteers, men and women, received a standard dose of extract (1 g/day) for 12 weeks. After treatment, there was a significant change in body mass index (BMI), body fat mass and the percentage of body fat. Also, in the same study, the waist-hip ratio decreased in the extract group in relation to the placebo group, without showing adverse effects.[3] Another study, also in humans of both sexes but with higher doses of extract (50 and 100g/day) and in dyspilidaemic volunteers, showed the opposite effect, with decreased levels of triglycerides, but the maintenance of morphometric parameters.[24] This indicates that the investigation of the

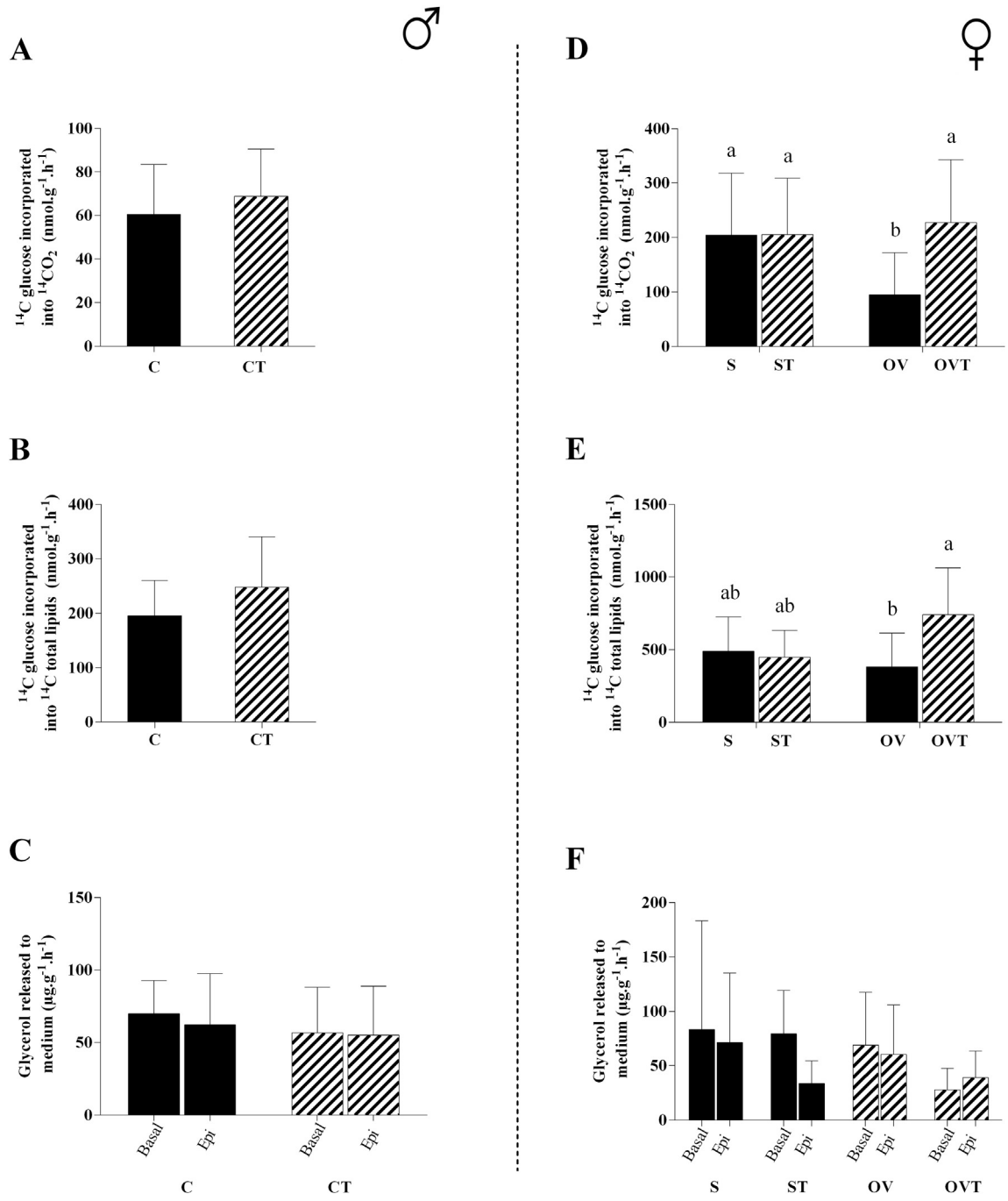


**Fig. 3.** Retroperitoneal adipose tissue metabolism in males, females, and ovariectomised females submitted to treatment with *Ilex paraguariensis* extract. The metabolism was observed as $CO_2$ (A) and total lipid (B) production from labelled $^{14}$C glucose, and adrenergic responsiveness lipolysis (C) assays. Charts D, E and F present the results of the same assays in females. Data are expressed as the mean and respective standard deviation (SD) or the median and 25/75 percentile. Different letters indicate significant differences; (*) indicates a difference from the respective basal group in lipolysis and (#) indicates difference of basal lipolysis related to other groups. Epi: epinephrine. Values of $p < 0.05$ were considered significant. n = 8–10 in each experimental group. C: male control; CT: male treatment; S: female sham; ST: female sham treatment; OV: ovariectomy; OVT: ovariectomy treatment.

effects of *I. paraguariensis* in humans still needs further attention, to determine whether the effect is present only in conditions prior to dyslipidaemia or is maintained in patients with established lipid dysfunction.

Metabolism in brown adipose tissue did not change. Moreover, males and females demonstrated a response to epinephrine and the lipolysis response remained pronounced. BAT has a peculiar metabolism compared to WAT. Besides BAT's increased energy expenditure, essential for its characteristic non-shivering thermogenesis function,[25] it presents high lipogenesis from glucose concomitantly with a high lipolytic rate, which provides a substrate for fatty acids oxidation.[26] Hence, it is highly responsive to adrenergic stimulation via the β3-receptor and, thus, direct innervation of the autonomic nervous system.[10] BAT tissue has a buffering function, using the lipolysis products themselves and free fatty acids from the bloodstream for β-oxidation, decreasing the

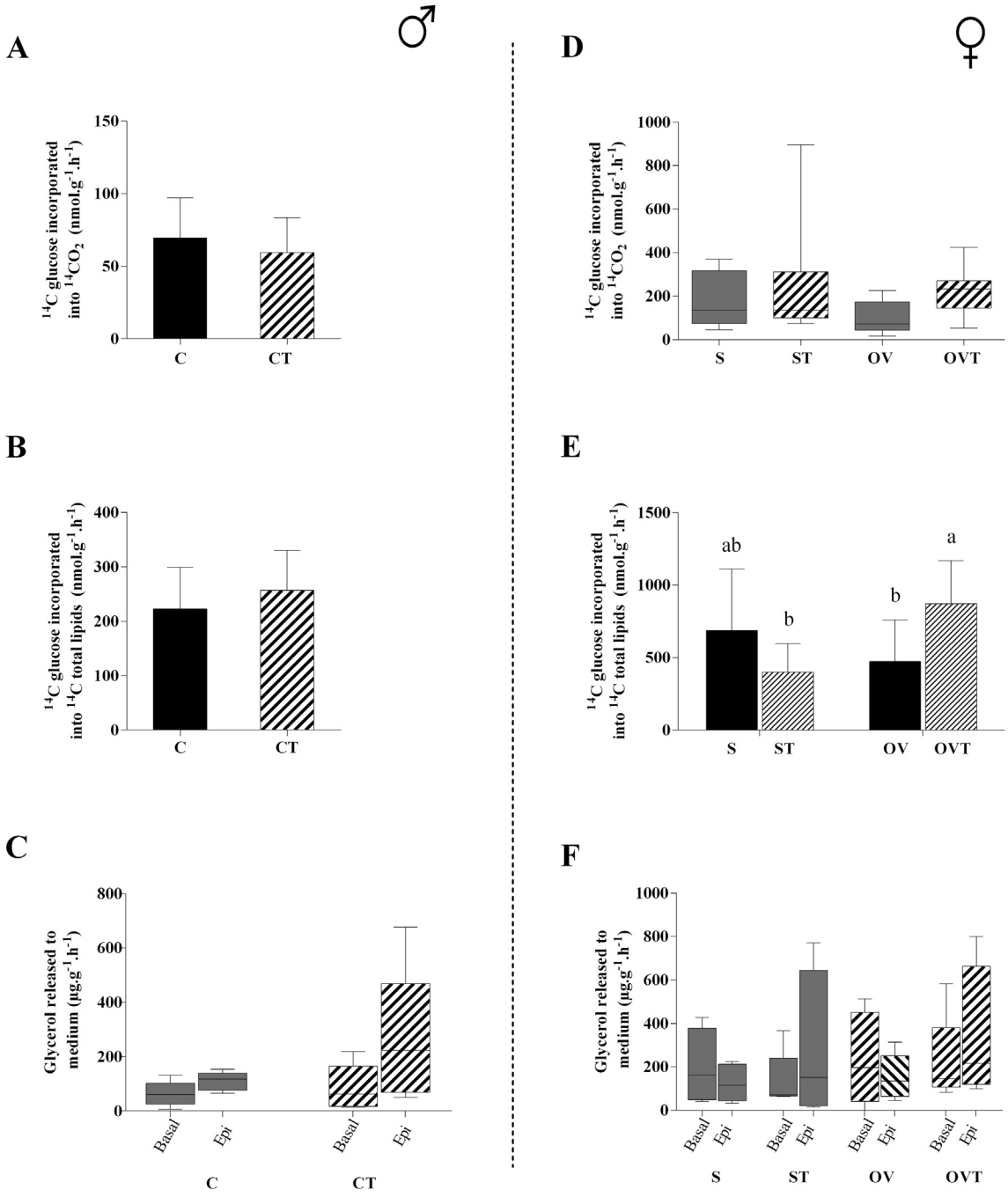


**Fig. 4.** Gonadal adipose tissue metabolism in males, females, and ovariectomised females submitted to treatment with *Ilex paraguariensis* extract. The metabolism was observed as $CO_2$ (A) and total lipid (B) production from labelled $^{14}$C glucose, and adrenergic responsiveness lipolysis (C) assays. Charts D, E and F present the results of the same assays in females. Data are expressed as the mean and respective standard deviation (SD) or the median and 25/75 percentile. Different letters indicate significant differences; (*) indicates a difference from the respective basal group in lipolysis and (#) indicates difference of basal lipolysis related to other groups. Epi: epinephrine. Values of $p < 0.05$ were considered significant. n = 8–10 in each experimental group. C: male control; CT: male treatment; S: female sham; ST: female sham treatment; OV: ovariectomy; OVT: ovariectomy treatment.

circulating levels of lipids.[27] Another critical point to emphasise is the sexual dimorphism of this particular fat depot. Females appear to be more responsive to browning/beigeing via adrenergic and sympathetic stimulation, in addition to having more mitochondria and mitochondrial cristae.[10] Also, women have a higher BAT mass and more energetic activity in this tissue.[28]

Our results indicate the responsiveness to epinephrine in all groups remained present in CT, ST and OVT, showing the tissue responsiveness to epinephrine did not change after treatment. The preservation of epinephrine responsiveness after treatment appears to be beneficial, since the maintenance of the lipolysis/β-oxidation ratio in this tissue is crucial for its capability in regard to buffering lipids from the bloodstream. To the best of our knowledge, the effect of this extract on BAT has never been evaluated by other authors. Unfortunately, according to our results, there was no increase in activity in this tissue; however, the maintenance of responsiveness and the morphometric outcomes indicate that the action of extract components appears to be on the metabolism of

white adipose tissue.

In visceral adipose tissue, OVT females demonstrated a significant change in $CO_2$ production and lipid synthesis in WATv after 45 days. After treatment with mate extract, these parameters returned to the values found in S and ST. The oxidation of glucose to $CO_2$ and its incorporation into lipids depicted the energy activity and lipid storage capacity. In obese C57BL/6J mice, $VO_2$ and glucose tolerance, as metabolites that indicate mitochondrial metabolism and proteins involved in the respiratory chain, were decreased.[29] Additionally, the increase in adipocyte volume itself increases lipolysis.[30] Thus, 45 days after ovariectomy (which led to female weight gain), glucose uptake and use from key tissues, such as muscle and adipose tissue, may have been significantly modified.[31]

Animal studies have shown that mate extract is capable of changing insulin signalling, for example, via the activation of the AKT pathway.[5] Therefore, the extract could restore the glucose metabolic profile, a fact never observed in females. These outcomes are initial; however, they reinforce the potential use of this extract in attenuating metabolic imbalance in postmenopausal women. In humans, the modulation of yerba mate extract still needs to be investigated. A study with male and female non-obese volunteers indicated an increase in total body lipid oxidation with yerba mate extract consumption.[4] Another investigation with postmenopausal women, reported modulation of glycaemic levels after consumption of yerba mate extract.[22] It can hardly be said that the mechanism by which *Ilex paraguariensis* acts on serum glucose levels is only by WAT in an oestrogen-deficient state. However, the impressive modulation of oxidation and lipid synthesis from glucose in WAT contributes to a small elucidation of the broad effects of this extract, which is capable of modulating other key tissues of energy metabolism.[32,33]

Still, regarding WATv, in control animals, the effect of epinephrine as a lipolysis stimulant appeared in the WATv of males, but not females. The extract's effect in CT led to a lack of responsiveness to epinephrine, while in females the response remained the same. That is, the extract promoted a lack of response to epinephrine in males, and OV and OVT rats showed no changes.

As expected, male WATv presented a higher responsiveness to lipolytic stimulation when compared to females. However, the WATv of CT males was less responsive to epinephrine, which emphasises that the extract can decrease the contribution of WATv to lipolysis. In obese individuals, the contribution of WATv is more significant than subcutaneous WAT because it is more sensitive to circulating catecholamines, contributing to the increase of fatty acids in the bloodstream. This leads to a higher risk of ectopic lipid accumulation and, consequently, the development of insulin resistance.[26] Thus, the results indicate that the extract creates a better stock/mobilisation profile, reducing the risk of central obesity.

In females, WATv has a lower lipolysis rate, which characterises a protective stock pattern. Thus, the contribution of this depot to circulating lipids remains lower.[26] In this way, *I. paraguariensis* acts differently in males and females, improving the metabolic profile in males and maintaining the protective pattern in females.

In retroperitoneal adipose tissue metabolism, the incorporation of glucose into $CO_2$ and lipids in males did not change after treatment. However, these parameters significantly decreased in the WATr of females 45 days following ovariectomy. Again, after treatment with mate extract, these levels returned $CO_2$ production to values found in control rats and increased lipid synthesis.

Glucose uptake is significantly altered in WATr and WATv in female rats 45 days after ovariectomy in key tissues, such as the muscle and adipose tissue,[31] potentially leading to the development of progressive insulin resistance. Likewise, in our study, the treatment was able to alter the oxidation and lipogenesis beneficially, indicating an improvement in glucose metabolism.

In WATr, no difference in basal lipolysis was observed in control or treated males and females. In the presence of epinephrine, this response remained the same in all treated groups. As already pointed out, male WAT presents higher responsiveness to lipolytic stimulation in comparison to females. However, WATr of males and females showed no responsiveness to epinephrine. The lower contribution of this depot to circulating lipids characterises a stock pattern,[26] and this beneficial phenotype was maintained after treatment. Additional studies should be conducted to verify the extrapolating sexual dimorphism from rodents to humans, since they present differences.[12,13]

Again, the results indicate that the extract interferes with the metabolism of white adipose tissue only through the synthesis pathways in OVT females, increasing lipid stock in adipose tissue. Cell studies show a decrease in adipocyte differentiation, lipid accumulation and inflammation. Studies with rodents have revealed that mate extract modulates adipogenic, antioxidant, inflammatory and insulin signalling.[34] Taken together, the outcomes reported here agree with the overall result of an improvement in WAT energy metabolism, and specifically reveal that the use of glucose by this tissue is significantly altered in relation to oxidation and lipogenesis from glucose.

The gonadal adipose tissue metabolism presents a high response plasticity to different treatments; however, it should be noted that this depot is quite important in rodents, but this is not necessarily true in humans.[12]

Increased lipid synthesis in the WATg of ovariectomised females initially indicated a beneficial response to treatment related to increased buffering of glucose to stock, but extrapolation to humans is not straightforward, considering its higher metabolic importance to rodents in comparison to humans.

We believe, the potential use of *Ilex paraguariensis* has never been explored in an animal model of ovariectomy. Even with indicating the energy imbalance benefits in postmenopausal women,[22] this issue needs further investigation. Nonetheless, the outcomes shown here, specifically the use of glucose substrate and lipolysis, are original, contributing to the elucidation of metabolic pathways in which this extract can beneficially modulate the adipose tissue of oestrogen-deficient females.

Regarding the mechanisms potentially involved in the outcomes presented here, it is necessary to emphasise that *Ilex paraguariensis* acts directly on adipose tissue and indirectly on key tissues in energy metabolism, such as the liver and skeletal muscle. Considering its direct action on white adipose tissue, animal model studies with high fat diet-induced obesity showed an increase in the expression of peroxisome proliferator-activated gamma receptor (PPARγ) and HMG-CoA reductase genes and an increase in phosphorylated AMPK in gonadal WAT.[35] Another study, in the same model, showed that mate extract modulated several obesity-related genes in WAT and decreased TNF-α, IL-6 and leptin, in addition to restoring PPARγ and adiponectin expression. This same study evaluated BAT, which showed an increase in PGC-1α and UCP-1 after treatment.[36]

Other key tissues in energy metabolism may also contribute to improving WAT metabolism. *Ilex paraguariensis* extract was shown to attenuate hepatic metabolic imbalance in obese rats, decreasing the IKK/phosphorylated AKT ratio and NF-κB levels.[32] Also in obese mice, it led to an improved response to insulin administration and restoration of hepatic and muscular insulin substrate receptors. The authors also reported a decrease in TNFα, IL-6 and iNOS, but emphasised that they could not confirm whether these levels were the cause or effect of the observed weight loss.[37]

It is not possible to confidently state which of the bioactive fractions of the extract is responsible for the effects observed here.

However, some studies have already evaluated the isolated action of these compounds. Polyphenols have already been shown to promote modulation of carbohydrate metabolism through their action on microbiota and absorption of secondary phenolic metabolites, but also largely via the inhibition of carbohydrate absorption by the gastrointestinal tract. Thus, this fraction may explain the attenuation of imbalances in glucose homeostasis, as well as in the prevention of these same events in healthy individuals.[38]

Studies have been also paid attention to the stimulatory properties of methylxanthines, one of bioactive fractions of the *Ilex paraguariensis* extract..[39,40] The specific effect of yerba mate's methylxanthine-rich fraction was also evaluated, and it was demonstrated to improve the lipid profile, reduce lipogenesis and increase lipolysis in adipose tissue, in addition to reducing abdominal adiposity in Wistar rats submitted to a high-fat diet.[41]

Another group of compounds present in *Ilex paraguariensis* extract is the saponins, also known as matesaponins.[42,43] These molecules have been demonstrated to have anti-inflammatory and hypocholesterolaemic effects, in addition to improving the potential for glycaemic control.[44,45] However, further studies, with a focus on health applications, are needed to elucidate the action of matesaponins. One of the few studies focusing on matesaponins described that the saponin-rich fraction derived from yerba mate has important properties in regard to lipid metabolism, and is capable of increasing the faecal excretion of fat, even though there was an increase in lipogenesis in adipose tissue in rats.[41]

In fact, more studies are needed to elucidate the anti-obesity pathways through which *Ilex paraguariensis* may be acting. Still, the evaluation of these effects must be interpreted with caution, since although a particular bioactive compound can lead to such outcomes, the effects can also result from the combined effect of phenols, xanthines and saponins together.

The consumption of *Ilex paraguariensis* is widespread in South American countries, and the extract is already popularly known for its stimulating and anti-obesity effects.[46] In this regard, studies on humans have been previously performed and have shown possible beneficial effects.[3,47,48] However, the mechanisms and dose required for such effects to emerge have yet to be explored. Its use is feasible in obesity prevention during the menopausal period, or as a therapeutic adjunct in previously obese women. However, the specific contribution it makes towards reducing energy imbalance requires further investigation, either as a potential agent alone or combined with low doses of antidiabetic drugs.

## 5. Conclusions

In this study, we evaluated the potential of *Ilex paraguariensis* extract as a preventive/therapeutic agent for energy imbalance. This effect was confirmed and rats treated with the extract presented with a beneficial response; mate was observed to promote a stock phenotype in both males and females, and modulated lipogenic pathways in females and lipolytic pathways in males. Considering the current alarming rise of lipid metabolism unbalances worldwide, and their health implications, we have contributed with original results, showing the beneficial effects of the mate extract in females after ovariectomy. In this regard, this extract shows excellent potential to be used, not only for men, but also for women, and in particular postmenopausal women, as a beneficial modulator of WAT metabolism.

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## Author contributions

All authors contributed to the study conception and design. Débora Santos Rocha coordinated all the steps of this work participating in all described activities. Maiza von Dentz and Jéssica Maschio participated in the animal's treatment and tissue collection. Jorge Felipe Argenta Model and Matheus Vieira Lima participated in the ovariectomy surgeries, animal's treatment, and tissue collection. Renata Ohlweiler and Samir Khal de Souza participated in subsequent processing of the samples destined to the lipogenesis, lipolysis, and oxidation techniques. Elaine Sarapio and Éverton Lopes Vogt participated in tissue collection and initial sample processing. Mairique Waszczuk, Simony Martiny, and Valquíria Linck Bassani evaluated the aqueous extract and performed the evaluations of polyphenols and methylxanthines. Luiz Carlos Kucharski coordinated all above-described work. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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