Roasted yerba mate and prolonged swimming: effects on body composition, muscle function, and glucose metabolism in obese mice

Yerba mate tostada y natación prolongada: efectos sobre la composición corporal, la función muscular y el metabolismo de la glucosa en ratones obesos

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Abstract

Introduction: Obesity is associated with impaired glucose homeostasis. Exercise is a primary non-pharmacological intervention, whereas the effects of roasted yerba mate combined with prolonged exercise remain poorly understood.
Objective: To investigate the isolated and combined effects of roasted yerba mate (*Ilex paraguariensis*) and prolonged swimming on body composition, muscle function, and glucose metabolism in obese mice.
Methodology: Forty male Swiss Webster mice received a cafeteria diet for 30 days and were randomly assigned to five groups: control, obese sedentary, obese sedentary with yerba mate, obese swimming, and obese swimming with yerba mate. Yerba mate infusion was administered for 30 days, and swimming was performed for 60 minutes daily, five days per week. Assessments included dual-energy X-ray absorptiometry, the inverted grid test, and fasting glucose and insulin measurements.
Results: The swimming groups showed the lowest body weights, while body fat percentage did not differ among obese groups. Only the combined intervention differed from the obese sedentary group in fat-free mass, fasting glucose, and insulin, with values comparable to controls. All interventions reduced insulin resistance. No intervention improved inverted grid test performance.
Discussion: Reduced insulin resistance was consistent with previous rodent studies of yerba mate, despite the lower dose used. The absence of functional improvements may be related to the aerobic nature of swimming.
Conclusions: Roasted yerba mate combined with prolonged swimming was associated with a more favorable glycemic profile and lower insulin resistance in obese mice. Further studies should investigate muscle quality and the underlying mechanisms.

Keywords

exercise; functional foods; Ilex paraguariensis; insulin resistance; obesity.

Resumen

Introducción: La obesidad se ha asociado con alteraciones glucémicas. El ejercicio ha sido su principal tratamiento no farmacológico, pero su combinación con yerba mate tostada ha sido poco estudiada.
Objetivo: Examinar los efectos aislados y combinados de la yerba mate tostada (Ilex paraguariensis) y la natación prolongada sobre la composición corporal, la función muscular y la glucosa en ratones obesos.
Metodología: Cuarenta ratones Swiss Webster machos recibieron dieta de cafetería durante 30 días y se asignaron aleatoriamente a cinco grupos: control, obeso sedentario, obeso sedentario con yerba mate, obeso con natación y obeso con natación y yerba mate. Durante 30 días recibieron infusión de yerba mate y nadaron 60 minutos diarios, cinco días por semana. Se evaluaron composición corporal (absorciometría de rayos X de doble energía), rejilla invertida, glucosa e insulina en ayunas.
Resultados: Los grupos que nadaron presentaron menor peso corporal; el porcentaje de grasa no difirió entre grupos obesos. Solo la intervención combinada difirió del obeso sedentario en masa libre de grasa, glucosa e insulina, comparables a los controles. Todas las intervenciones redujeron la resistencia a la insulina; ninguna mejoró la rejilla invertida.
Discusión: La reducción de la resistencia a la insulina coincidió con estudios en roedores, aunque se utilizó una dosis menor. La ausencia de mejoras funcionales coincidió con la naturaleza aeróbica de la natación.
Conclusiones: La combinación de yerba mate tostada y natación prolongada se asoció con un perfil glucémico más favorable y menor resistencia a la insulina. Se necesitan estudios sobre calidad muscular y mecanismos.

Palabras clave

alimentos funcionales; ejercicio; *Ilex paraguariensis*; resistencia a la insulina; obesidad.

Introduction

Obesity ranks among the greatest public health challenges of our time and is now understood as a complex chronic disease rather than a mere excess of adipose tissue. The World Health Organization estimates that more than one billion people currently live with obesity, a figure that keeps rising across all age groups. Its high prevalence aside, obesity substantially raises the risk of type 2 diabetes mellitus, cardiovascular disease, metabolic dysfunction-associated steatotic liver disease, several cancers and premature death, consequences that place a heavy economic and social burden on healthcare systems worldwide (Blüher, 2019; GBD 2021 Risk Factors Collaborators, 2024).

Its pathophysiology, however, extends well beyond a simple positive energy balance. Excessive expansion of white adipose tissue drives adipocyte hypertrophy, tissue hypoxia, macrophage infiltration and sustained release of pro-inflammatory cytokines, together establishing a chronic low-grade inflammatory state. Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) and monocyte chemoattractant protein-1 (MCP-1) interfere with insulin signaling by activating inflammatory pathways such as nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK), which eventually leads to systemic insulin resistance and metabolic dysfunction (Hotamisligil, 2017; Reilly & Saltiel, 2017).

Skeletal muscle occupies a central position among the peripheral organs affected by obesity, since it accounts for roughly 80% of insulin stimulated glucose disposal under physiological conditions. Sustained exposure to inflammatory mediators and elevated circulating free fatty acids impairs mitochondrial oxidative capacity, reduces metabolic flexibility, promotes oxidative stress and accelerates muscle protein breakdown. Obesity is therefore frequently accompanied by impaired muscle function, reduced strength and a progressive decline in lean mass, features that converge in what has been termed sarcopenic obesity (Blüher, 2019; GBD 2021 Risk Factors Collaborators, 2024).

Regular physical exercise remains the single most effective non-pharmacological intervention for obesity, improving insulin sensitivity, stimulating mitochondrial biogenesis, increasing fatty acid oxidation and triggering the release of myokines that modulate systemic inflammation. Aerobic training also restores metabolic communication between adipose tissue and skeletal muscle, dampening several of the pathological mechanisms that obesity sets in motion (Hotamisligil, 2017; Reilly & Saltiel, 2017; Saltiel & Olefsky, 2017; Shoelson et al., 2007). Because chronic inflammation drives much of the metabolic dysfunction seen in obesity, interventions able to act simultaneously on inflammatory signaling, oxidative stress and insulin sensitivity have drawn growing scientific interest. Bioactive compounds derived from Ilex paraguariensis fall squarely into this category, given their antioxidant, anti-inflammatory and metabolic regulatory properties (Bracesco et al., 2011; Gambero & Ribeiro, 2015).

Functional foods able to enhance exercise-induced metabolic adaptations have received considerable attention in recent years. Ilex paraguariensis A. St.-Hil., popularly known as yerba mate, stands out among South American botanical species for its rich phytochemical profile and wide range of biological activities, and it is consumed as a beverage throughout much of South America. Its leaves are a notable source of chlorogenic acids, caffeic acid derivatives, dicaffeoylquinic acids, flavonoids, saponins and methylxanthines such as caffeine and theobromine. Together, these compounds display antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic and thermogenic properties that place yerba mate firmly within the category of functional foods (Bracesco et al., 2011; Gambero & Ribeiro, 2015; Heck & de Mejia, 2007).

Both experimental and clinical evidence suggests that the consumption of yerba mate (*Ilex paraguariensis*) may exert beneficial effects on adiposity and glucose metabolism, including reductions in body fat mass and percentage, improvements in insulin sensitivity, and modulation of obesity-associated inflammatory pathways (Arçari et al., 2011; Gambero & Ribeiro, 2015; Kim et al., 2015). Chlorogenic acids seem to act on intestinal glucose absorption, hepatic gluconeogenesis and AMPK signaling, favoring glucose homeostasis and mitochondrial function, while methylxanthines may raise energy expenditure and lipid oxidation. Saponins, for their part, have been linked to improvements in lipid metabolism and inflammatory regulation (Gambero & Ribeiro, 2015; (Heck et al., 2008).

Despite the growing body of evidence regarding the metabolic effects of *Ilex paraguariensis*, much of the available literature addresses yerba mate in general or uses preparations and extracts with limited char-

acterization of their thermal processing, while the specific effects of the roasted form remain less investigated. This distinction is relevant because thermal processing can significantly alter the plant's phytochemical profile and, consequently, its biological properties (Heck & de Mejia, 2007; Riachi et al., 2018).

Thermal processing changes the phytochemical profile of the plant and could therefore alter the biological activity of its bioactive compounds. Previous studies have investigated the association between yerba mate consumption and physical exercise in different experimental models. In healthy mice subjected to chronic swimming, de Souza et al. (2021) evaluated yerba mate preparations and observed changes in lipid mobilization, lactate levels, and irisin concentrations. In healthy humans, studies conducted during submaximal or prolonged exercise demonstrated increased fatty acid oxidation following yerba mate consumption, although these studies were predominantly conducted in physically active individuals without obesity (Alkhatib & Atcheson, 2017). However, evidence remains limited regarding the effects of roasted yerba mate combined with prolonged aerobic exercise in obesity models, particularly with respect to body composition, preservation of lean mass, and glucose homeostasis.

Based on these considerations, we hypothesized that roasted *Ilex paraguariensis* supplementation would potentiate exercise induced metabolic adaptations, improving body composition, preserving lean mass and attenuating obesity-associated insulin resistance. The present study was therefore designed to investigate the isolated and combined effects of roasted yerba mate supplementation and prolonged swimming exercise on body composition, muscle function and glucose metabolism in diet-induced obese mice.

Method

Ethical Approval

All procedures followed the Guide for the Care and Use of Laboratory Animals (National Research Council, USA) and the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA). The protocol was approved by the Animal Ethics Committee of Centro Universitário de Barra Mansa (CEUA–UBM; approval no. 003/2024).

Animals and Experimental Design

Forty male Swiss Webster mice, 60 days old and weighing approximately 40g at the start of the experiment, were obtained from the Animal Facility of Centro Universitário de Barra Mansa (Barra Mansa, Rio de Janeiro, Brazil). They were housed individually in polypropylene cages under controlled conditions (23 ± 1 °C, 55 ± 10% relative humidity, 12 h light/dark cycle with lights on at 07:30 h) and had free access to food and water for the entire experimental period.

Following a seven-day acclimatization period, obesity was induced by a cafeteria diet administered for 30 consecutive days. Animals were then randomly assigned to five groups of eight: standard diet without intervention (CON), obese sedentary (OS), obese sedentary receiving roasted yerba mate (OSYM), obese subjected to swimming exercise (OSW), and obese subjected to swimming exercise combined with roasted yerba mate (OSWYM) for 30 days.

Cafeteria Diet-Induced Obesity

Obesity was induced with a cafeteria diet adapted from Eguchi et al., (2008), combining commercial rodent chow, roasted peanuts, milk chocolate and cornstarch biscuits in a 3:2:2:1 ratio. This mixture reached an energy density of approximately 5.11 kcal g^{-1}, against 4.07 kcal g^{-1} for the standard chow, and animals had *ad libitum* access to it for 30 days before the interventions began.

Roasted Ilex paraguariensis

Leaves of *Ilex paraguariensis* were supplied by producers Cláudio Kovaleski and Sílvio Kovaleski at Ervateria Chiru (Chapecó, Santa Catarina, southern Brazil) during November and December 2014. Plants were grown under natural light and the leaves underwent the traditional sapeco drying process; once dried, the material was sealed in airtight plastic bags and stored at −18 °C until use.

The species was botanically identified by Dr. Geraldo Ceni Coelho (Federal University of Fronteira Sul, Chapecó, Brazil), and a voucher specimen was deposited by Dr. Carlos Alberto Bastos de Maria in the Professor Jorge Pedro Pereira Carauta Herbarium, Department of Botany, Federal University of the State of Rio de Janeiro (UNIRIO, Rio de Janeiro, Brazil), under accession code HUNI.

Phytochemical Composition of Roasted Yerba Mate

Dried leaves were roasted in a laboratory chromatographic oven (temperature variation ± 0.1 °C) at 185 °C for 20 min, following the protocol of Riachi et al. (2018), and the roasted material was then used to prepare the infusion administered to the animals throughout the study.

Riachi et al. (2018) had already characterized the phytochemical composition of this same batch of yerba mate under identical roasting conditions. These authors identified phenolic acids, flavonoids and methylxanthines as its main bioactive constituents. The detailed composition is shown in Table 1.

Table 1. Phytochemical composition of roasted *Ilex paraguariensis* infusion used during the experimental protocol.

Phytochemical	Concentration (mg mL^{-1})
Rutin	0.149
3,4- + 3,5-Dicaffeoylquinic acids	0.178
Caffeine	0.183
Syringic acid	0.577
4,5-Dicaffeoylquinic acid	0.650
Ferulic acid	0.832
Chlorogenic acid	0.896
Caffeic acid	0.957

Source: Riachi et al. (2018).

Chlorogenic acid, caffeic acid, ferulic acid, rutin, caffeine and dicaffeoylquinic acids were the major compounds identified, with chlorogenic acids dominating the phenolic fraction and caffeine standing out as the principal methylxanthine.

Preparation of Roasted Yerba Mate Infusion

A fresh infusion was prepared daily by steeping 4 g of roasted *Ilex paraguariensis* leaves in 200 mL of boiling distilled water for 15 min, followed by filtration through Whatman No. 1 filter paper. This preparation corresponded to a nominal concentration of 20 mg/mL of dried yerba mate material. The dosage was initially estimated based on a human intake equivalent to 8 g of dried yerba mate prepared in 500 mL of infusion, corresponding to 16 mg/mL, as adapted from De Souza et al. (2021). Based on a reference human body weight of 70kg, the corresponding volume initially calculated for the experimental animals was 0.3285 mL per animal. Subsequently, the concentration of the infusion was increased to allow the administration volume to be reduced to 0.1 mL per animal, with the aim of minimizing the stress associated with oral gavage and reducing experimental variability, as described by Stutz (2023). Thus, each animal received 0.1mL of the freshly prepared roasted yerba mate infusion by oral gavage once daily at 09:00h for 30 consecutive days, corresponding to approximately 2mg of dried yerba mate material per animal per day based on the nominal preparation concentration. Control animals received an equivalent volume (0.1mL) of distilled water by oral gavage at the same time and under identical handling conditions. The infusion was freshly prepared each day using the same plant material to water ratio, infusion time, water volume, and filtration procedure throughout the experimental period to ensure consistency of the administered preparation.

Swimming Training Protocol

The swimming protocol, adapted from Lima et al. (2013), began with a three-day acclimatization period of 25, 30 and 45 min on consecutive days, after which animals swam for 60 min/day, five days a week, for the remainder of the intervention. Swimming sessions were conducted at 15:00h in glass tanks (40 × 30 × 40cm) holding approximately 18L of water maintained at 31 ± 1°C using thermostatically controlled heaters, with five animals trained simultaneously under the same conditions in each session.

Muscle Function Assessment

Muscle function was assessed with the inverted wire grid test described previously. Each mouse was placed individually on a metal grid (10 × 14 cm) that was then inverted, and the latency to fall was recorded across three consecutive trials separated by 15 min recovery periods; the cumulative latency to fall was the variable used for statistical analysis (Ferraro et al., 2016).

Body Composition

Body composition was evaluated by dual energy X-ray absorptiometry, a well-established, reproducible technique for this purpose in experimental animals. After anesthesia, whole body scans quantified body fat percentage and lean body mass from the differential attenuation of low and high energy X-rays (Nagy & Clair, 2000).

Biochemical Analyses

After an overnight fast, animals were anesthetized with sodium thiopental (100 mg kg^{-1}, intraperitoneally) and blood was collected by cardiac puncture. Serum glucose and insulin were measured with commercially available enzymatic assay kits following the manufacturers' instructions. Insulin resistance and pancreatic β-cell function were estimated using the Homeostatic Model Assessment indexes calculated according to the recommendations of the American Diabetes Association (2010).

Statistical Analysis

Statistical analyses were carried out in GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Normality was checked with the Kolmogorov–Smirnov test; parametric data were compared by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test; non parametric data by the Kruskal–Wallis test followed by Dunn's multiple comparisons test; and correlation analyses (Pearson or Spearman, as appropriate) were performed to examine associations between body weight, fasting glucose, serum insulin, and HOMA indices. Results are reported as mean ± standard deviation (SD), with statistical significance set at $p < 0.05$.

Results

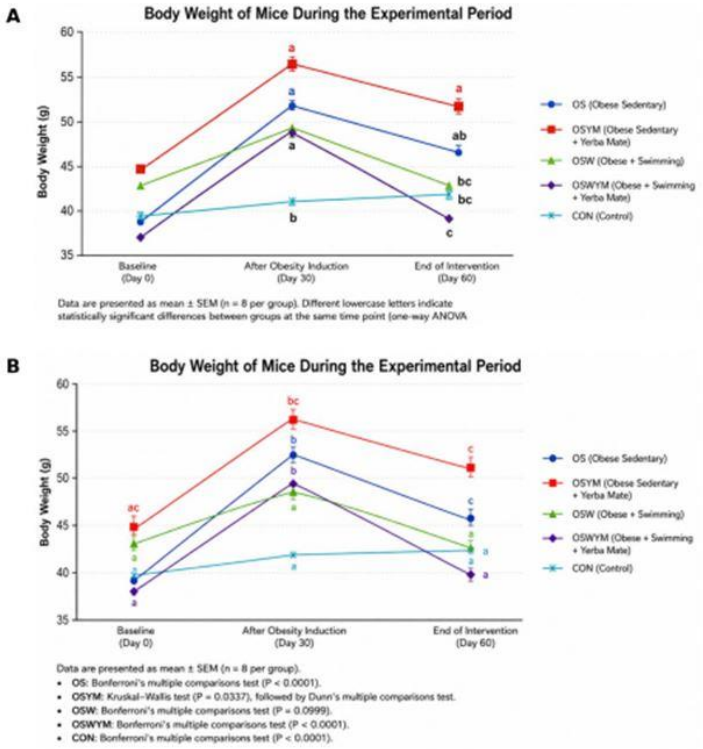
Body Weight

As shown in Figure 1, no statistical differences ($p > 0.05$) were observed among the experimental groups at baseline (Day 0) (OS = 38.40 ± 3.17 g; OSYM = 43.53 ± 7.76 g; OSW = 41.80 ± 4.94 g; OSWYM = 39.83 ± 2.83 g; CON = 40.06 ± 1.27 g). After obesity induction (Day 30) body weight increased significantly ($p < 0.05$) in all obese groups (OS = 52.62 ± 3.86 g; OSYM = 56.02 ± 6.39 g; OSW = 49.5 ± 8.53 g; OSWYM = 50.24 ± 4.39 g) when compared with CON group; and, the mean body weight of these obese groups was statistically similar ($p > 0.05$) among them. The control group maintained the lowest values (CON = 41.36 ± 0.42 g; $p < 0.05$).

After obesity induction period (Day 30), the obese groups showed similar body weight gain among them (OS = 52.62 ± 3.86 g; OSYM = 56.02 ± 6.39 g; OSW = 49.5 ± 8.53 g; OSWYM = 50.24 ± 4.39 g; $p > 0.05$); and, these values are higher than control group (CON = 41.36 ± 0.42 g; $p < 0.05$). These findings indicate that the obesogenic diet effectively promotes body weight gain.

At the end of the intervention (Day 60), the lowest body weight values ($p < 0.05$) were observed in the groups submitted to swimming alone (OSW = 44.07 ± 5.69 g) and swimming combined with yerba mate supplementation (OSWYM = 42.05 ± 2.30 g). Although the OS group showed a numerical reduction in mean body weight from Day 30 (52.62 ± 3.86 g) to Day 60 (47.03 ± 5.08 g), this reduction was not statistically significant ($p > 0.05$). Similarly, the OSYM group did not show a statistically significant reduction in body weight during the intervention (56.02 ± 6.39 g at Day 30 vs. 52.13 ± 4.70 g at Day 60; $p > 0.05$). These body weight evolutions are shown in Figure 1.

Figure 1. Body weight of mice during the experimental period.



Data are presented as mean ± SEM (n = 8 per group). (A) Comparison of body weight among experimental groups at each time point. (B) Body weight changes over the experimental period within each group. Different lowercase letters indicate statistically significant differences between groups at the same time point ($p < 0.05$). OS: obese sedentary; OSYM: obese sedentary + yerba mate; OSW: obese + swimming; OSWYM: obese + swimming + yerba mate; CON: control.

Body Composition and Muscle Function

The CON group showed the lower body fat percentage when compared with obesity group (OS). OSYM, OSW and OSWYM groups were statistically equal OS group ($p > 0.05$). However, OSYM and OSWYM were also equal ($p > 0.05$) CON group. Fat-free mass percentage was higher in CON group when compared with obesity group (OS). All interventions of obese groups were statistically similar ($p > 0.05$) to OS and CON group. The grip strength was available by latency to fall. The CON group showed lower latency to fall when compared with obesity group (OS). All interventions groups were statistically equal ($p > 0.05$) to both CON and OS group, showing a trend toward grip strength gain. The body composition and muscle function data are described in Table 2.

Table 2. Body composition and muscle function in diet obesity-induced mice 30 days after intervention period.

	OS	OSYM	OSW	OSWYM	CON	P value
Body fat (%)	11.54 ± 0.26^{a}	11.17 ± 0.53^{ab}	11.39 ± 0.31^{a}	10.87 ± 0.42^{ab}	10.33 ± 0.55^{b}	0.0015
Fat-free mass (%)	88.44 ± 0.21^{a}	88.74 ± 0.59^{ab}	88.44 ± 0.76^{a}	89.43 ± 0.53^{bc}	89.86 ± 0.36^{c}	< 0.0001
Latency to fall (seconds)	17.82 ± 4.11^{a}	15.50 ± 7.38^{ab}	10.09 ± 2.90^{ab}	14.02 ± 5.53^{ab}	5.31 ± 1.80^{b}	0.0389

Data are presented as mean ± standard deviation (SD) (n = 8 per group).
Different letters indicate differences statistically significant among groups in the same row.
OS, obese sedentary; OSYM, obese sedentary + roasted yerba mate; OSW, obese + swimming; OSWYM, obese + swimming + roasted yerba mate; CON, control.

Glucose Homeostasis Biochemistry Parameters

Glucose homeostasis was altered after cafeteria diet consumption. All the glucose homeostasis results are described in Table 3.

Table 3. Glucose metabolism in diet obesity-induced mice 30 days after intervention period.

	OS	OSYM	OSW	OSWYM	CON	P value
Glucose (mg/dL)	150.97 ± 12.41a	112.72 ± 5.41ab	107.76 ± 6.17abc	98.69 ± 7.93bc	81.59 ± 5.73c	< 0.0001
Insulin (µUI/mL)	90.42 ± 14.95a	72.92 ± 9.93ac	67.50 ± 12.55ac	47.50 ± 7.36bc	57.08 ± 8.43c	< 0.0001
HOMA-IR	33.69 ± 6.35a	20.33 ± 3.30b	18.07 ± 4.12bd	10.23 ± 1.74c	11.45 ± 1.46cd	< 0.0001
HOMA- β	18.03 ± 2.99a	14.56 ± 1.98abd	13.48 ± 2.51bd	8.48 ± 1.95cd	11.41 ± 1.69d	< 0.0001

Data are presented as mean ± standard deviation (SD) (n = 8 per group).
Different letters indicate differences statistically significant among groups in the same row.
OS, obese sedentary; OSYM, obese sedentary + roasted yerba mate; OSW, obese + swimming; OSWYM, obese + swimming + roasted yerba mate; CON, control.

The OS group presented higher fasting glucose, insulin, HOMA-IR and HOMA-Beta levels than CON group. OSYM and OSW groups showed lower fasting glucose levels than OS, though the difference did not show statistical significance ($p > 0.05$); the OSW group was also equal ($p > 0.05$) CON group, trending towards a significant reduction in fasting blood glucose. However, only OSWYM group (both interventions together) showed the best results in fasting glucose. This group was equal ($p > 0.05$) CON group and showed a normalization of hyperglycemia. The fasting insulin, OSYM and OSW were equal ($p > 0.05$) OS and CON group. OSWYM was only equal CON group and showed lowest insulin concentrations among the obese groups. In the HOMA-IR results, all experimental obese groups (OSYM, OSW and OSWYM) were different from OS group; these groups showed a reduction in HOMA-IR. OSW and OSWYM groups were statistically equal ($p > 0.05$) from CON group. However, the OSWYM group was the standout, as it showed the lowest mean among the obese groups and it was statistically equivalent ($p > 0.05$) only to the CON group. The control group, as expected, kept low HOMA-IR values throughout, reflecting preserved glucose homeostasis. The OSYM group showed higher values to HOMA-β when compared with others interventions obese groups. OSWYM group showed highest and significant ($p < 0.05$) reduction in HOMA-β among obese groups; this group was equal ($p > 0.05$) CON group.

Correlation analysis revealed significant associations between glycemic metabolism, body composition, and functional performance (Table 4). Fasting blood glucose showed a moderate positive correlation with body fat percentage ($r = 0.647$, $p = 0.0001$) and a moderate negative correlation with fat-free mass percentage ($r = -0.647$, $p = 0.0001$), indicating that higher fasting glucose concentrations were associated with greater adiposity and a lower relative proportion of fat-free mass. Similarly, HOMA-IR was positively correlated with body weight ($r = 0.378$, $p = 0.039$) and body fat percentage ($r = 0.483$, $p = 0.0068$), while showing a moderate inverse correlation with fat-free mass percentage ($r = -0.483$, $p = 0.0068$). These findings are consistent with the close relationship between adiposity, altered glucose homeostasis, and reduced insulin sensitivity in obesity.

Table 4. Correlation coefficients between metabolic parameters (insulin, glucose, HOMA-IR, and HOMA-β) with body composition variables, and fall latency.

Glucose metabolism biochemical parameters	Body weight (g)	% Body fat	% Fat-free mass	Latency to fall (seconds)
Fasting blood glucose				
R value	0.271	0.647	-0.647	0.635
P value	0.147	0.0001	0.0001	0.0002
Insulin fasting				
R value	0.323	-0.029	0.029	0.461
P value	0.082	0.880	0.880	0.010
HOMA-IR				
R value	0.378	0.483	-0.483	0.619
P value	0.039	0.0068	0.0068	0.0003
HOMA-β				
R value	0.453	0.322	-0.322	0.585
P value	0.0118	0.0829	0.0829	0.0007

Data are expressed as correlation coefficient (r) and P-value.

HOMA-β was positively correlated with body weight (r = 0.453, p = 0.0118), but was not significantly associated with body fat percentage or fat-free mass percentage. The positive association with body weight may reflect changes in pancreatic β-cell secretory activity accompanying alterations in metabolic demand; however, this interpretation should be considered cautiously, since HOMA-β is an indirect estimate of β-cell function and does not provide a direct assessment of pancreatic β-cell activity.

An additional finding was the positive association between latency to fall and fasting insulin (r = 0.461, p = 0.010), fasting blood glucose (r = 0.635, p = 0.0002), HOMA-IR (r = 0.619, p = 0.0003), and HOMA-β (r = 0.585, p = 0.0007). Because greater latency to fall represents better performance in the functional test, these correlations indicate that animals with higher glycemic and insulin-related parameters also tended to remain longer on the apparatus. Although this finding may appear counterintuitive, correlation analysis alone cannot establish the mechanisms underlying this association. The relationship may reflect the combined influence of metabolic status, body composition, and the physiological adaptations induced by the experimental interventions. Therefore, these results should not be interpreted as evidence of a direct beneficial effect of higher glucose or insulin resistance on functional performance.

Taken together, the correlation pattern indicates that greater adiposity was associated with higher fasting glucose and insulin resistance, whereas a greater relative proportion of fat-free mass was associated with lower fasting glucose and HOMA-IR. These findings reinforce the interrelationship between body composition and glucose metabolism in diet-induced obesity. However, given the correlational nature of the analysis, the observed associations should be interpreted as relationships between variables rather than evidence of causal effects.

Discussion

Body Weight

As described previously, no statistical differences (p > 0.05) were observed among the experimental groups at baseline (Day 0), indicating homogeneity of body weight prior to the interventions. After diet-obesity induction (Day 30), the body weight increased significantly (p < 0.05) when compared to the CON group. These findings were described previously in the literature (Eguchi et al., 2008) and confirmed in our results. At the end of experimental period (Day 60), lowest body weight values were observed in swimming (OSW) and swimming plus yerba mate supplementation interventions (OSWYM). Given that OSYM group did not show significant difference (p > 0.05) change in body weight, the results suggest that exercise was the main determinant for weight loss under our experimental conditions. This is consistent with previous evidence that aerobic exercise raises total energy expenditure, enhances fatty acid oxidation and improves metabolic flexibility via AMPK activation and other pathways of mitochondrial energy metabolism, adaptations that together help limit excessive weight gain even under continued exposure to an obesogenic environment (Egan & Zierath, 2013; Hawley et al., 2014).

The absence of a significant effect of yerba mate alone on body weight deserves a cautious reading. Body weight is a relatively blunt marker of metabolic improvement, since it does not distinguish between changes in fat, lean tissue or body water, and several studies have reported that yerba mate supplementation improves metabolic health without necessarily reducing total body weight to a meaningful degree. A lack of significant weight change, therefore, does not rule out biologically relevant metabolic adaptations driven by the bioactive compounds in the infusion (Arçari et al., 2011; Gambero & Ribeiro, 2015). The phytochemical profile of the roasted infusion, rich in chlorogenic acids, dicaffeoylquinic acids, caffeic acid derivatives, rutin and methylxanthines, offers a biologically plausible explanation. The phytochemical profile reported for Ilex paraguariensis, including chlorogenic acids, dicaffeoylquinic acids, flavonoids and methylxanthines, may contribute to the metabolic effects observed in the present study. These compounds have been associated with AMPK activation, increased fatty acid oxidation and improved insulin signaling (Pang et al., 2008; Arçari et al., 2011; Gambero & Ribeiro, 2015). In addition, yerba mate has been shown to increase fatty acid oxidation during submaximal exercise (Alkhatib & Atcheson, 2017), while thermal processing may modify its phenolic composition (Riachi et al., 2018).

Body Composition and Muscle Function

The body composition and muscle function data are described in Table 2. All obese experimental groups (OSYM, OSW and OSWYM) were equal ($p > 0.05$) OS group. However, the OSYM and OSWYM groups showed mean adiposity values closer to the CON group too. Thus, our results show a modest trend for yerba mate alone or associated with swimming to reduce body fat percentage. Some authors also showed modest adiposity reduction following yerba mate supplementation; they also describe the adiposity reduction depend on factors, such as supplementation dose, intervention duration, obesity severity and the phytochemical makeup of the extract used (Dos Santos et al., 2023; Gambero & Ribeiro, 2015). Chlorogenic acids and caffeoyl derivatives have been shown experimentally to modulate adipocyte differentiation, suppress lipogenic pathways and stimulate lipid oxidation through AMPK activation and regulation of peroxisome proliferator-activated receptor gamma (PPAR-γ), improving lipid metabolism even without marked reductions in body weight (Meng et al., 2013; Gambero & Ribeiro, 2015; Kim et al., 2015). Exercise training itself plays a key role in adipose tissue remodeling, increasing lipid utilization, mitochondrial oxidative capacity and metabolic flexibility (Egan & Zierath, 2013; Hawley et al., 2014). No significant reduction in body fat percentage emerged here, but the lower adiposity observed in the combined intervention group may still reflect cumulative metabolic adaptations too small to reach statistical significance under our experimental conditions. This reinforces the idea that metabolic health should not be judged solely by changes in total adiposity, but alongside other outcomes such as insulin sensitivity and body composition (Pedersen & Febbraio, 2012; Li et al., 2025).

The obese groups receiving isolated interventions, OSYM and OSW, showed fat-free mass percentages comparable to those of the OS group ($p > 0.05$). In contrast, OSWYM was the only intervention group comparable to CON, suggesting a potential preservation of fat-free mass with the combined intervention. However, because fat-free mass includes muscle, bone, organs, water, and connective tissue, these findings cannot be attributed exclusively to changes in skeletal muscle mass. Previous studies provide biological support for potential effects of Ilex paraguariensis on metabolically active and structural tissues.

Walton et al. (2023) reported increased ATP concentration and mitochondrial efficiency in skeletal muscle following yerba mate supplementation in healthy mice, suggesting an improvement in muscular bioenergetics. More recent studies have also reported effects of yerba mate on bone tissue, including increased osteoblastic and osteocytic cell viability in vitro (Villarreal et al., 2024) and higher cortical and trabecular bone mineral density in postmenopausal women who consumed yerba mate (Brun et al., 2025). Although these findings cannot be directly extrapolated to the present obese mouse model, they support the possibility that the preservation of fat-free mass observed in OSWYM may involve effects on metabolically active and structural tissues beyond adipose tissue. Preserving skeletal muscle matters greatly, since chronic inflammation-obesity induced accelerate protein degradation, impair mitochondrial function and reduce the muscle's regenerative capacity (Egan & Zierath, 2013; Li et al., 2025).

The phytochemical composition of roasted yerba mate offers a plausible mechanistic account. Chlorogenic acids, caffeic acid derivatives, rutin and other phenolic compounds display potent antioxidant and anti-inflammatory activity, reducing oxidative damage and modulating the inflammatory pathways involved in skeletal muscle catabolism (Heck & de Mejia, 2007; Gambero & Ribeiro, 2015). Aerobic exercise, in parallel, stimulates the secretion of myokines such as IL-6, IL-15 and irisin, which promote mitochondrial biogenesis, lipid oxidation and metabolic adaptations within skeletal muscle (Pedersen & Febbraio, 2012; Hawley et al., 2014; Whitham & Febbraio, 2016). Thus, the interventions used in this study may attenuate the inflammation-obesity induced and, therefore, indirectly favor the preservation of lean tissue (Arçari et al., 2011; Gambero & Ribeiro, 2015).

The muscle strength was higher in OS group than CON group. The favorable effects of the combined intervention on body composition and metabolic parameters were not accompanied by proportional increases in grip strength. In the present study, sedentary obese animals (OS and OSYM) exhibited the highest absolute grip strength values, whereas animals subjected to prolonged swimming (OSW and OSWYM) showed lower values. This finding should be interpreted considering differences in body mass among groups, as body size and composition have been associated with absolute grip strength, which may influence the interpretation of muscle performance independently of intrinsic muscle quality (Keevil et al., 2015; Moura-Dos-Santos et al., 2015). Moreover, greater adiposity may coexist with higher lean mass while being associated with poorer muscle quality (Raghupathy et al., 2023).

In the present study, sedentary obese animals were among the heaviest and exhibited the highest absolute grip strength, whereas swimming groups showed lower body mass and lower absolute grip strength. Thus, the greater absolute force observed in obese sedentary animals should not be interpreted as evidence of superior muscle function or quality, since differences in body mass may have contributed to this finding. Because grip strength was not normalized to body mass or lean mass, the present data do not allow definitive conclusions regarding muscle quality or force-generating capacity per unit of muscle tissue (Tomlinson et al., 2016).

The lower grip strength observed after prolonged swimming may also reflect the predominantly aerobic nature of the exercise stimulus, which primarily promotes metabolic, mitochondrial, and oxidative adaptations, whereas greater gains in maximal force and hypertrophy are more strongly associated with resistance exercise and mechanical loading (Hawley et al., 2014; Egan & Zierath, 2013). Therefore, the absence of increased absolute grip strength following roasted Ilex paraguariensis supplementation should not be interpreted as impaired muscle function. Further studies using body-weight-normalized strength and direct measures of skeletal muscle mass and quality are warranted.

The grip strength test measures absolute force, which may be influenced by body mass and therefore does not necessarily reflect intrinsic muscle quality. In support of this interpretation, de Sousa et al. (2021) found that diet-induced obese mice maintained absolute muscle torque but showed lower torque when normalized to body weight, indicating impaired relative muscle performance. Similarly, Shelley et al. (2024) reported reduced soleus muscle power relative to body mass in high-fat diet-fed mice despite preserved absolute isometric force. Thus, greater body mass may increase or preserve absolute force values while masking reductions in relative muscle performance.

The preservation of fat-free mass observed in the OSWYM group should not necessarily be interpreted as increased skeletal muscle mass, since fat-free mass also includes bone, organs, water, and connective tissue. Moreover, muscle quantity alone may not reflect muscle quality, which is influenced by the relationship between muscle mass, strength, and body composition (Barahona-Fuentes et al., 2024; Galindo-Perdomo et al., 2025). In obesity, greater adiposity may coexist with higher lean mass but poorer muscle quality (Boncella et al., 2025). Therefore, further studies using direct measures of skeletal muscle mass and quality are needed to confirm whether the observed preservation of fat-free mass reflects specific muscular adaptations. Improvements in metabolic health, it seems, do not necessarily translate into immediate gains in muscle function, particularly when the exercise stimulus is predominantly aerobic (Egan & Zierath, 2013; Hawley et al., 2014; Pedersen & Febbraio, 2012). Swimming involves sustained aerobic activity with comparatively low mechanical loading relative to resistance exercise, so the physiological adaptations it induces relate mainly to oxidative metabolism, mitochondrial function and cardiovascular capacity rather than to maximal force production (Egan & Zierath, 2013; Hawley et al., 2014). Prolonged endurance exercise predominantly induces metabolic and oxidative adaptations in skeletal muscle, whereas substantial increases in muscle strength and hypertrophy are more strongly associated with resistance exercise and mechanical loading (Hawley et al., 2014; Coffey & Hawley, 2007). In mice, chronic swimming combined with yerba mate consumption has been associated with alterations in lipid mobilization and metabolic markers, although muscle hypertrophy and strength have not been directly assessed (de Souza et al., 2021).

Glucose Metabolism

Among the most relevant metabolic findings in our study was the improvement in glucose homeostasis following the combined intervention. The OSWYM group showed lowest fasting glucose and insulin, HOMA-IR and HOMA-β and equal ($p > 0.05$) CON group; while OS group kept the highest glucose homeostasis biochemistry parameters at the end of experimental period.

Hyperglycemia obesity-associated stems mainly from impaired insulin signaling secondary to chronic inflammation, ectopic lipid accumulation and mitochondrial dysfunction in skeletal muscle and liver (Hotamisligil, 2017; Reilly & Saltiel, 2017). Persistent activation of inflammatory pathways reduces insulin receptor signaling, lowering peripheral glucose uptake and contributing to a progressive deterioration of glucose homeostasis (Arçari et al., 2011; Hotamisligil, 2017).

Interventions that simultaneously improve insulin sensitivity and reduce systemic inflammation would therefore be expected to favor glycemic control (Gambero & Ribeiro, 2015). Exercise is recognized as one of the most effective non-pharmacological strategies for improving glucose metabolism, stimulating

GLUT4 translocation independently of insulin, enhancing mitochondrial oxidative capacity and increasing glucose utilization by skeletal muscle (Egan & Zierath, 2013; Hawley et al., 2014). Furthermore, chlorogenic acid and other phenolic compounds presents in roasted yerba mate have previously been linked to inhibition of hepatic glucose production, delayed intestinal glucose absorption, AMPK activation and improved insulin sensitivity (Gambero & Ribeiro, 2015; Stutz et al., 2023). Thus, these molecular mechanisms can explain our results.

As skeletal muscle is the main site of insulin-mediated glucose disposal, the glucose metabolism improves showed in OSWYM group can be related a possible maintaining lean tissue decribed in free-fat mass percentage results in the same group. This hypothesis is justificated for the close relationship between skeletal muscle integrity and glucose homeostasis (Egan & Zierath, 2013; Hawley et al., 2014; Li et al., 2025).

The marked reduction HOMA-IR in OSWYM group indicates that the combined intervention effectively attenuated obesity-induced insulin resistance (Arçari et al., 2011; Gambero & Ribeiro, 2015; Hawley et al., 2014). Insulin resistance is a hallmark of obesity that develops from chronic low-grade inflammation, ectopic lipid deposition and impaired intracellular insulin signaling. Activation of inflammatory mediators, particularly TNF-α, IL-6 and NF-κB, promotes serine phosphorylation of insulin receptor substrate proteins, reducing insulin receptor signaling and glucose uptake by skeletal muscle (Hotamisligil, 2017; Saltiel & Olefsky, 2017).

Assessment of pancreatic β-cell function also showed that the combined intervention lowered HOMA-β in obese animals. Since elevated HOMA-β in obesity generally reflects compensatory hyperinsulinemia secondary to peripheral insulin resistance, the reduction seen in the OSWYM group fits with a decreased pancreatic secretory demand rather than impaired β-cell function (Arçari et al., 2011; Saltiel & Olefsky, 2017).

As obesity progresses, pancreatic β-cells initially compensate for reduced insulin sensitivity by secreting more insulin. However, the prolonged hyperinsulinemia eventually contributes to β-cell exhaustion and a higher risk of type 2 diabetes (Hotamisligil, 2017; Saltiel & Olefsky, 2017). Pancreatic morphology and molecular pathways were not evaluated in this study, but our results support the hypothesis that roasted yerba mate may indirectly help preserve β-cell function.

Conclusions

The combination of prolonged swimming and supplementation with roasted yerba mate promoted a weight loss, a decrease in body fat percentage, an increase in fat-free mass percentage, and favorable adaptations in glucose metabolism in diet-induced obese mice. Thus, our results reinforce that the bioactive compounds of roasted yerba mate appear to potentiate the physiological adaptations that exercise induces, particularly in the body composition and glucose metabolism improving.

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