



South American herbal extracts reduce food intake through modulation of gastrointestinal hormones in overweight and obese women



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ABSTRACT

Herbal plants have been assessed for possible action that influence gastrointestinal hormonal secretion, modifying gut motility, food intake and energy balance. The effects of herbal extracts derived from native species of South America on food intake and acylated ghrelin and glucagon-like peptide 1 (GLP-1) concentrations after consuming meals were investigated for the first time in humans. Twenty overweight and obese women were recruited for a randomized, single blind, placebo-controlled, crossover design study separated by a 7-day washout period. Participants received an herbal extract combination containing yerba mate, guarana and damiana ('YGD') or placebo. Energy intake at lunch was reduced significantly in the YGD group (-43.3 ± 13.1 kcal; $P = 0.005$). The AUC for acylated ghrelin after lunch was significantly lower in the YGD group (-1004 ± 690.9 vs. 565.9 ± 286.7 , $P = 0.04$). At 60 and 150 min after breakfast, GLP-1 concentrations were higher in YGD ($P = 0.04$) when compared with the control group. The AUC for GLP-1 after breakfast was significantly higher in the YGD group (1003 ± 370.5 vs. 160.3 ± 221.3 , $P < 0.05$). Supplementation with the herbal extract YGD reduced energy and macronutrient intake by modulating the gut hormones in overweight and obese women.

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1. Introduction

The genesis of obesity is multifactorial and involves genetic, endocrine, neurological, and psychological factors, dietary habits, physical activity and socio-cultural conditions (Dawson et al., 2015). Peptide hormones produced from the gastrointestinal tract in response to nutritional intake, such as ghrelin and glucagon-like peptide-1 (GLP-1), are considered to be major regulators of appetite. These hormones act in the arcuate nucleus in the hypothalamus, which communicates with the brain centers (Parker et al., 2013) that synthesize neuropeptide Y/Agouti-related peptide (NPY/AgRP) or pro-opiomelanocortin (POMC), resulting in the release of orexigenic or anorexigenic neuropeptides, respectively.

Abbreviations: GLP-1, glucagon-like peptide 1; YGD, herbal extract combination containing yerba mate, guarana and damiana; AUC, area under the curve; NPY/AgRP, neuropeptide Y/Agouti-related peptide; POMC, pro-opiomelanocortin; BMI, body mass index; WC, waist circumference; DXA, dual energy X-ray absorptiometry; CVs, coefficients of variation; DPP-IV, dipeptidyl peptidase IV.

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Alterations in the secretion of these neuropeptides may affect feeding behavior and energy expenditure (Marathe, Rayner, Jones, & Horowitz, 2013; Sam, Troke, Tan, & Bewick, 2012).

Ghrelin is an orexigenic gut hormone (Bewick et al., 2009; Inui, 2001) and is found in its highest concentrations in the fasting state (Gibbons et al., 2013). Two isoforms of ghrelin are identified, the active (acylated) and inactive (desacylated). Acylated ghrelin is recognized as the preferred measure as it relates more clearly to functionality. Desacylated ghrelin is not devoid of any activities and may have an anorexigenic activity that is contrary to the orexigenic activity of acylated ghrelin (Costantino, 2012). Higher plasma ghrelin levels have been associated with several disorders in metabolism and body weight, reinforcing the importance of ghrelin in the regulation of metabolic homeostasis (Costantino, 2012; Lawrence, Snape, Baudoin, & Luckman 2002). However, GLP-1 is an anorexigenic peptide produced by L intestinal epithelial cells in response to food intake (Macdonald et al., 2002). The secretion of this hormone stimulates glucose-dependent insulin release (Lorenz, Evers, & Wagner, 2013), reduces blood glucose and inhibits secretion of glucagon, attenuates endogenous glucose production (Willms et al., 1996), delays gastric emptying (Holst,

2013; Schirra et al., 2006) and increases satiety (Meeran et al., 1999; Punjabi, Arnold, Geary, Langhans, & Pacheco-lópez, 2011).

Given the high prevalence of obesity and the important role that gastrointestinal hormones play in food intake control (Bewick, 2012; Pimentel et al., 2012), the use of nutritional components in clinical practice is an attractive dietetic opportunity for the modulation of obesity consequences. Thus, herbal plants have been assessed for possible action on physiological processes that influence gastrointestinal hormonal secretion, modifying gut motility, food intake and energy balance (Andersen & Fogh, 2001).

Among herbal extracts, Yerba Mate (*Ilex paraguariensis*), Guarana (*Paullinia cupana*) and Damiana (*Turnera diffusa*) have been studied in relation to obesity. Yerba mate (*Ilex paraguariensis*) is a native plant of the subtropical region of South America, and it shows antioxidant activity, most likely due to its high polyphenolic content, caffeine, and saponins (Matsumoto et al., 2009; Mejía, Song, Heck, & Ramírez-Mares, 2010). These characteristics have been shown to produce beneficial anti-obesity effects (Arçari et al., 2011a; Resende et al., 2015) by reducing oxidative and inflammatory stress (Arçari et al., 2011b; Matsumoto et al., 2009; Pimentel et al., 2013), adiposity (Hussein et al., 2011; Kim, Oh, Kim, Chae, & Chae, 2015), adipogenesis, energy intake and promoting satiety by regulating GLP-1 (Hussein et al., 2011). Guarana powder has high levels of caffeine, tannins and saponins, compounds responsible for antioxidant, anti-inflammatory and immunomodulatory activities, increasing thermogenesis and reducing food intake (Portella et al., 2013). Damiana is a plant from arid and semi-arid regions. It has anxiolytic, antioxidant and anti-inflammatory properties (Taha et al., 2012), so it is important for obesity treatment. The number of active compounds found in YGD may result in different mechanisms that alter energy metabolism (Harrold et al., 2013). Although randomized, double-blind, placebo-controlled clinical trials with a combination of these three herb extracts (Yerba Mate, Guarana and Damiana; 'YGD') indicated that supplementation significantly reduced adiposity, food intake and the consumption of foods rich in fat, lessened sensations of hunger and the desire to eat and delayed gastric emptying (Andersen & Fogh, 2001; Harrold et al., 2013), no clinical trial has evaluated the effects of YGD on gastrointestinal hormone concentrations.

It has been commented that there is a superior effect of herbal mix when compared to its herbs separately. This is probably due to a synergistic effect between green tea and other ingredients (Krotkiewski & Janiak, 2008). Thus, we sought to investigate whether the South American herbal extract containing Yerba Mate, Guarana and Damiana (YGD) decreased food intake by modulating acylated ghrelin and GLP-1 concentrations in overweight and obese women.

2. Materials and methods

2.1. Study design

The study comprised an acute randomized single-blinded placebo-controlled crossover trial. This study was registered at the Brazilian Clinical Trials Registry (ReBec) as RBR-99395b. The protocol was approved by the local Ethics Committee (number 408.131). All the participants were fully informed of the risks and discomforts associated with the study before providing their written informed consent. This manuscript is reported according to the CONSORT statement.

2.2. Subjects

Participants were recruited by advertisements. Sedentary women (with BMIs of 25–39.9 kg/m²) were recruited for the study.

We considered sedentary women those who remained seated in the workplace and the domestic environment, and during leisure time (Owen, Healy, Matthews, & Dunstan, 2010). The BMI criterion was used due the relevance of gut peptides on appetite in the context of obesity and weight gain. Exclusion criteria were unavailability for one day/week for the study day; participants with autoimmune, liver or kidney disease; participants with hypothyroidism; participants with gastrointestinal symptoms requiring treatment; participants with a history of bariatric surgery; participants on a food restriction program or who underwent a diet in the last 12 months to lose or control weight; participants currently adhering to a specific food avoidance diet; participants taking medication or nutritional supplements known to affect appetite or weight; participants taking immunosuppressive medication or insulin; participants who are pregnant, are planning to become pregnant or are breastfeeding; participants who have undergone menopause; participants with a history of chronic alcoholism; participants with a history of anaphylaxis to food; participants with food allergies, including caffeine or other compounds present in the supplement or any of the study foods; and participants with a history of intolerance and/or allergy to lactose. A total of 29 potential participants showed interest in the study, but nine women were excluded due to hypothyroidism (n = 03), menopause (n = 02), BMI under 25 kg/m² (n = 02) or no time available for the analysis (n = 02); thus, twenty women entered the study. For the analysis, three women were excluded due to missing or lost data (Fig. 1). Therefore, the women needed to visit the laboratory two days after an overnight fast, with seven days of washout. The washout period was chosen based on a previous study with greater security (Harrold et al., 2013).

2.3. Study day

Participants were instructed not to drink caffeinated and alcoholic beverages and remain sedentary throughout the 24 h prior to the study and during the washout period. The last meal on the previous day was standardized (30 g of lettuce, 75 g of tomato, 40 g of carrot, 8 g of olive oil, 170 g of rice, 51 g of beans, 50 g of grilled chicken, 200 mL of orange juice and 40 g of banana) and contained 595.7 kcal, 63.8% carbohydrate, 15.5% protein and 20.8% fat, to eliminate any interference in the secretion of intestinal hormones overnight. The release of gastrointestinal hormones is more involved with the type of nutrient than the volume of the meal (Erdmann, Lippl, & Schusdziarra, 2003; Shiiya et al., 2002; Tschop, Smiley, & Heiman, 2000).

After an overnight fast, a flexible venous catheter (Abbotath number 22, Medline Industries, Inc., Illinois, EUA) was placed in the antecubital vein for blood sample collection during the subsequent seven hours. Blood samples were taken at baseline and specific time points, 45, 60, 90, 150, 210 min, after a fixed breakfast containing 200 mL of orange juice, 50 g of bread, 8 g of margarine, 60 g of fresh cheese and 40 g of banana (494.5 kcal, 52.7% carbohydrate, 12.9% protein and 34.5% fat) or a lunch containing salad (18 g of lettuce, 75 g of tomato, 40 g of carrot and 10 g of olive oil), 130 g of rice, 65 g of beans, 55 g of grilled chicken and 200 mL of orange juice (632.0 kcal, 61.7% carbohydrate, 16.9% protein and 21.4% fat). Breakfasts and lunches were prepared in the Dietary Laboratory of Federal University of Goiás and were based on the eating habits of the participants so as not to affect food consumption. The amount of food intake was estimated by subtracting the weight of the leftovers from the weight of the portion determined for each item taken. Subsequently, the data were processed using the program Avanutri, version 3.1.5.

The study occurred over two days, with seven days of washout. On each testing day, the participants received three tablets corresponding to inert placebo containing 100 mg of lactose (Harrold

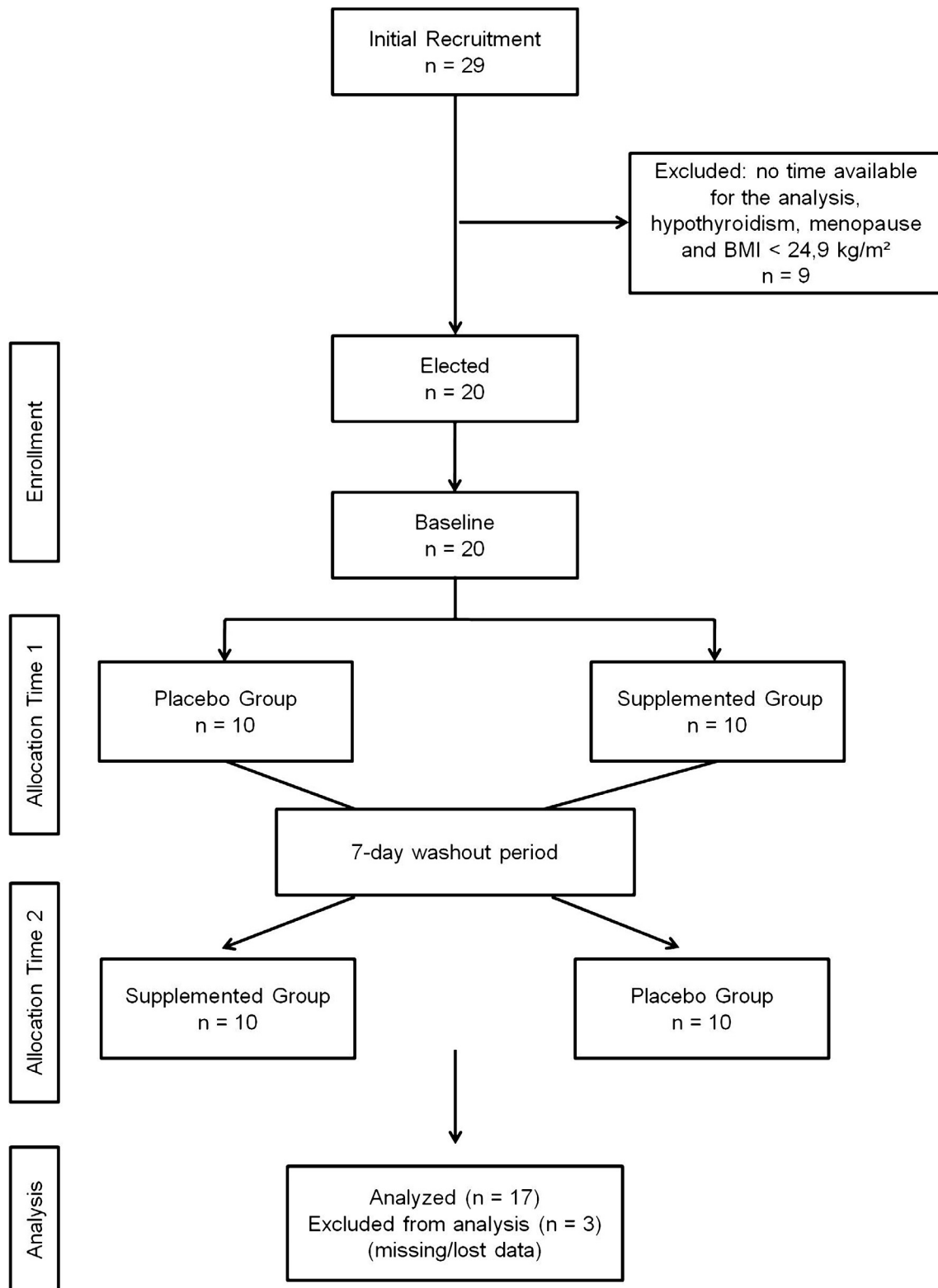


Fig. 1. CONSORT diagram showing the flow of participants through each stage of the randomized crossover trial.

et al., 2013) or three tablets containing Yerba Mate (112 mg), Guarana (95 mg) and Damiana (36 mg) standardized extracts (YGD; Zotrim, Natures Remedies Ltd, Amersham, UK). The YGD and placebo were supplied as individual tablets and packaged in coded containers to ensure the single-blind status of the study. The tablets were consumed with 100 mL of water.

2.4. Identification and quantification of the active components in supplement

The compounds contained in the extract were identified by mass spectrometry. The fragmentation profile of each molecule was used for confirmation. The mass analysis was performed using

electrospray ionization (ESI-MS/MS) LTQXL Thermo Ion Trap. Positive and negative mode ionization were used to investigate the total extract. In summary, 5 mL of sample were injected by directed infusion in N₂ at 10 psi at 10 µL/min flow rate, with a spray voltage of 4.52 kV, a capillary voltage of 17 V and at 280 °C temperature. The flow injection analysis electrospray ionization ion trap mass spectrometry (FIA-ESI-IT-MS/MSn) collision energy was among 20 and 40% (2 eV). The MS/MS spectrum was obtained between 50 and 2000 *m/z*.

The determination of total phenolics content was performed using the Folin-Ciocalteu reagent according to Singleton and Lamuela-Raventós (1999) with some modifications. Briefly, 0.25 mL aliquot of the YGD extract was diluted appropriately and mixed with 0.25 mL of Folin-Ciocalteu reagent and 2 mL of distilled water. Over 3 min at room temperature, 0.25 mL of a saturated sodium carbonate solution was added (Na₂CO₃) and the mixture was placed at 37 °C for 30 min. The absorbance was measured at 750 nm using an Ultrospec Model 2000 UV/visible spectrophotometer. Gallic acid was used as a reference standard, and the results were expressed in percentage (%) of gallic acid equivalents.

The determination of flavonoid content was carried out from the colorimetric method in which occurs the complexation of flavonoids with aluminum chloride according to standard methodology adapted by da Silva, Pezzini, and Soares (2015). Quercetin was used as a standard in aluminum chloride solution.

Caffeine quantification was performed through Waters® Acquity UPLC system (Waters Corp., Milford, MA, USA) consisting of a quaternary pump, autosampler, and mass spectrometer equipped with XevoTqD® source of electro-spray ionization (ESI) and analyzer triple-quadrupole. The source parameters were set as follows: desolvation gas flow 350 L/h, desolvation temperature 120 °C, collision gas flow 1L/h and source temperature 150 °C. Capillary voltage was set to 3.30 V and the cone voltage to 30 V. Caffeine detection was performed using multiple reaction monitoring mode (MRM), the transition 195 > 138 for measurement. Quantification was performed through external standard method, with a caffeine calibration curve with concentrations of 1.0; 1.5; 2.0; 3.0; 3.5 and 5.0 ppm, injected in triplicate in linear regression. It was possible to obtain the value of the correlation coefficient (*r*²) of 0.99%. The value found for the sample was extrapolated from calibration curve to obtain caffeine concentration of YGD extract. All analyzes were performed using MassLynx software.

2.5. Anthropometric and body composition assessments

BMI was calculated as current weight (kg)/height² (m) and classified according to World Health Organization standards (WHO - World Health Organization. Obesity: Preventing, 2004). Waist circumference (WC) was measured with a stretch-resistant anthropometric tape at the shortest perimeter.

Body composition was measured at the baseline of intervention by dual energy X-ray absorptiometry (DXA), using DPX NT densitometry equipment (General Electric Medical Systems Lunar, Madison, EUA). Participants were scanned at the same time of the day (between 7 and 12 am) while barefoot and wearing light clothing. The same operator performed all measurements and calibrations. The performance of the equipment was evaluated by calibration block daily and by spine phantom weekly. The coefficients of variation (CVs) for the DXA tests of muscle and fat mass were 0.75% and 1.03%, respectively.

2.6. Blood parameters, fat mass and obesity-associated genotyping

Venous blood sample were collected into EDTA-containing Monovette tubes. These tubes contained a mixture of inhibitors

(dipeptidyl peptidase IV inhibitor [10 µL/mL, EMD Millipore, Massachusetts, EUA, Cat. number DPP4-010] and Pefabloc SC [50 µL/mL, Sigma-Aldrich, St. Louis, USA, CAS number 30827-99-7]) to prevent degradation of the peptides to be measured.

After collection, glucose in venous blood was immediately measured using a portable glucose meter (Accu-check, F. Hoffmann-La Roche Ltd, Switzerland), and other samples were centrifuged for 10 min at 4 °C and 3500 rpm. Samples were immediately pipetted into microtubes and stored at -80 °C until analysis. Acylated ghrelin and GLP-1 were analyzed using a magnetic bead-based multiplex kit (EMD Millipore, catalog. number HMHMAG-34K). The plate reader was a Luminex MagPix (EMD Millipore), and the plate washer was a tecan hydroflex fitted with a magnetic holder. The acylated ghrelin and GLP-1 assay sensitivities were 1.8 and 5.2 pg/mL, respectively. The inter- and intra-assay variabilities were <19% and <11%.

2.7. Statistical analysis

Power calculation was based on the results of a related study (Saghebjo, Hedayati, Fahimi & Ilbeiji, 2013) with the assistance of the G-Power® software (version 3.1.9.2). Assuming a mean difference of 18.2 pg/mL for acylated ghrelin, as well as a standard deviation of 20.65 pg/mL, calculations showed that with an *a* of 0.05 (power = 1 - *b* = 0.80), at least 12 subjects were needed.

Analysis was performed using GraphPad Prism, version 6 for Windows (GraphPad Software, Inc.). Data distributions were evaluated by the Kolmogorov-Smirnov test. Energy intake and grams of macronutrients at breakfast and lunch within each group were compared using a paired samples *t*-test. Because of the large individual variations in the fasting levels of appetite hormones, we computed the change from baseline at each time point for each individual for acylated ghrelin and GLP-1. Differences over time and between conditions (YGD and placebo) were determined using two-factor analysis of variance (ANOVA) with repeated measures. Areas under the curve (AUCs) were calculated using the trapezoidal method. Student's *T*-test was used to determine the differences between the AUCs. All tests were two-sided, and differences were considered significant at *P* < 0.05. Values are expressed as the mean ± SEM.

3. Results

3.1. Participant characteristics

In total, 29 participants were screened, and 20 were recruited to the study; however, three participants were excluded because of missing or lost data (Fig. 1). The mean age and BMI were 32.8 ± 1.6 years and 31.49 ± 0.84 kg/m², respectively. Participants had a body fat between 42 to 55.8% and a waist circumference of approximately 88.0 ± 2.0 cm.

3.2. Chemical characterization of YGD

YGD presented 6.78% of flavonoids (27.44 µg/mL of solution) and 17.45% of total phenolic compounds (196.27 µg/mL of solution). The caffeine content is 1.13% (0.565 µg/mL). The compounds determined using mass spectrometry are presented in Table 1. The qualitative analysis demonstrated the presence of chlorogenic acids glycosylated or not, rutin, vitexin and caffeine.

3.3. Nutrient intake

There was no difference in energy intake at breakfast between groups (*P* = 0.088). However, at lunch, energy intake was signifi-

Table 1
Identification of compounds in herbal extract by FIA-ESI-IT-MS/MSn.

Identification	M.W	m/z	MS/MS
Caffeine	194.19	[M+H] ⁺ 195	MS ² [195] → 138
3-O-(E)-caffeoylquinic acid	354.17	[M-H] ⁻ 353	MS ² [353] → 191
Vitexin	432.38	[M-H] ⁻ 432	MS ² [432] → 415, 397, 379, 367, 351, 337, 313
3,4-di-O-(E)-caffeoylquinic acid	516.09	[M-H] ⁻ 515	MS ² [515] → 353, 335, 191, 179
Vitexin-2''-O-rhamnoside	578.17	[M-H] ⁻ 577	MS ² [577] → 413MS ³ [577] → 293
Rutin	610.10	[M-H] ⁻ 609	MS ² [609] → 301
Diquinic-glucosyl-glucoside	828.16	[M-H] ⁻ 827	MS ² [827] → 664, 707, 502, 383MS ³ [827] → 473, 335, MS ⁴ [827] → 281
Diquinic acid-glucose glucoside + hexose	989.21	[M-H] ⁻ 988	MS ² [988] → 827
Diquinic acid-glucose glucoside + hexose + caffeic acid	1169.25	[M-H] ⁻ 1168	MS ² [1168] → 988
Diquinic acid-glucose glucoside + hexose + caffeic acid + pentose	1301.83	[M-H] ⁻ 1300	MS ² [1300] → 1168

FIA-ESI-IT-MS/MSn: Flow Injection Analysis Electrospray Ionization Ion Trap Mass Spectrometry.

Table 2
Energy intake and grams of macronutrients at the test meals during the study.

	Control (n = 17) Mean ± SEM	YGD (n = 17) Mean ± SEM	P value
Energy-Breakfast (kcal)	455.70 ± 12.67	435.25 ± 15.78	0.088
Energy-Lunch (kcal)	548.98 ± 14.37	505.71 ± 17.52	0.005
Carbohydrate-Breakfast (g)	57.73 ± 2.00	56.41 ± 2.35	0.522
Carbohydrate-Lunch (g)	79.66 ± 3.12	70.02 ± 3.82	0.004
Protein-Breakfast (g)	14.92 ± 0.56	13.73 ± 0.76	0.022
Protein-Lunch (g)	24.58 ± 0.42	23.69 ± 0.47	0.039
Lipid-Breakfast (g)	18.34 ± 0.43	17.19 ± 0.67	0.018
Lipid-Lunch (g)	14.67 ± 0.06	14.54 ± 0.07	0.031

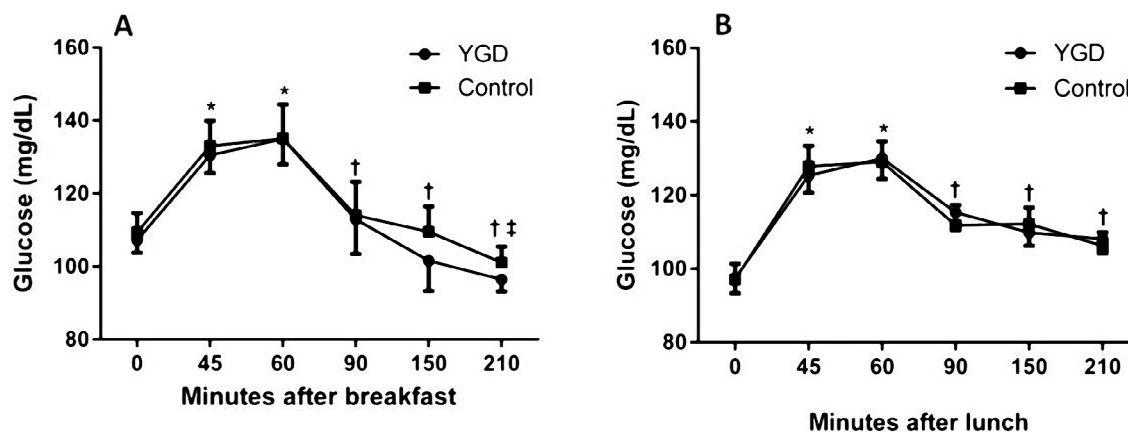
Differences were compared using paired-samples *t*-tests. kcal: calories; g: grams. P values in bold were considered statistically significant.

cantly lower (-43.3 ± 13.1 kcal; $P = 0.005$) in the YGD group compared with the control group, which was accompanied by lower macronutrients consumption ($P < 0.05$) (Table 2).

3.4. Hormone and glucose concentrations

Following breakfast and lunch, glucose concentrations increased after 45 and 60 min ($P < 0.05$) and began to decline after 90 min in both groups (Fig. 2A, B). However, there was a greater reduction in glucose concentrations between 90 and 210 min after breakfast in the YGD group ($P = 0.03$) (Fig. 2A).

The reduction in acylated ghrelin after breakfast was not statistically significant (Fig. 3A, B). However, after lunch, acylated ghrelin concentrations decreased at 60, 150 and 210 min in the YGD group (Fig. 3C). The AUC for acylated ghrelin after lunch was significantly lower in the YGD group than in the control group (-1004 ± 690.9 versus 565.9 ± 286.7 , $P = 0.04$, Fig. 3D).

**Fig. 2.** Glucose concentrations after breakfast (A) or lunch (B) with YGD or placebo supplementation. * $P < 0.05$ vs. 0 min in both groups; † $P < 0.05$ vs. 45 and 60 min in both groups; ‡ $P < 0.05$ vs. 90 min after breakfast only in YGD group.

In the YGD group, the GLP-1 concentrations at 45, 60, 90, 150 and 210 min after breakfast increased significantly compared with baseline (Fig. 4A). At 60 and 150 min, differences ($P = 0.04$) were observed in GLP-1 concentrations between the YGD and control groups. Moreover, the AUC for GLP-1 after breakfast was higher in the YGD group compared with the control group (1003 ± 370.5 versus 160.3 ± 221.3 , $P < 0.05$, Fig. 4B). The GLP-1 concentrations after lunch increased at 45 min compared with baseline in only the YGD group ($P < 0.01$, Fig. 4C). The incremental AUC for GLP-1 after lunch did not differ between groups (Fig. 4D).

4. Discussion

Although, previous studies demonstrated that administration of YGD slowed intestinal transit and reduced food and calorie consumption, thus causing weight loss in overweight subjects (Andersen & Fogh, 2001; Harrold et al., 2013), we confirmed that YGD supplementation attenuated energy and macronutrient intake at lunch and increased GLP-1 concentrations after breakfast, followed by decreased acylated ghrelin levels after lunch, indicating an important role of herbal extracts on gastrointestinal hormone modulation and appetite.

The yerba mate chemical composition showed that it is rich in several polyphenols which include caffeic acid, caffeine, caffeoyl derivatives, caffeoylshikimic acid, chlorogenic acid, feruloylquinic acid, kaempferol, quercetin, quinic acid, rutin, and theobromine (Arçari et al., 2009). Among the most prevalent compounds in guarana, it has been highlighted the high concentration of caffeine, theobromine, catechins, and condensed tannins (Ruchel et al., 2016). Phytochemical investigations have revealed that damiana is rich in flavonoids, including acacetin, apigenin, gonzalitosin,

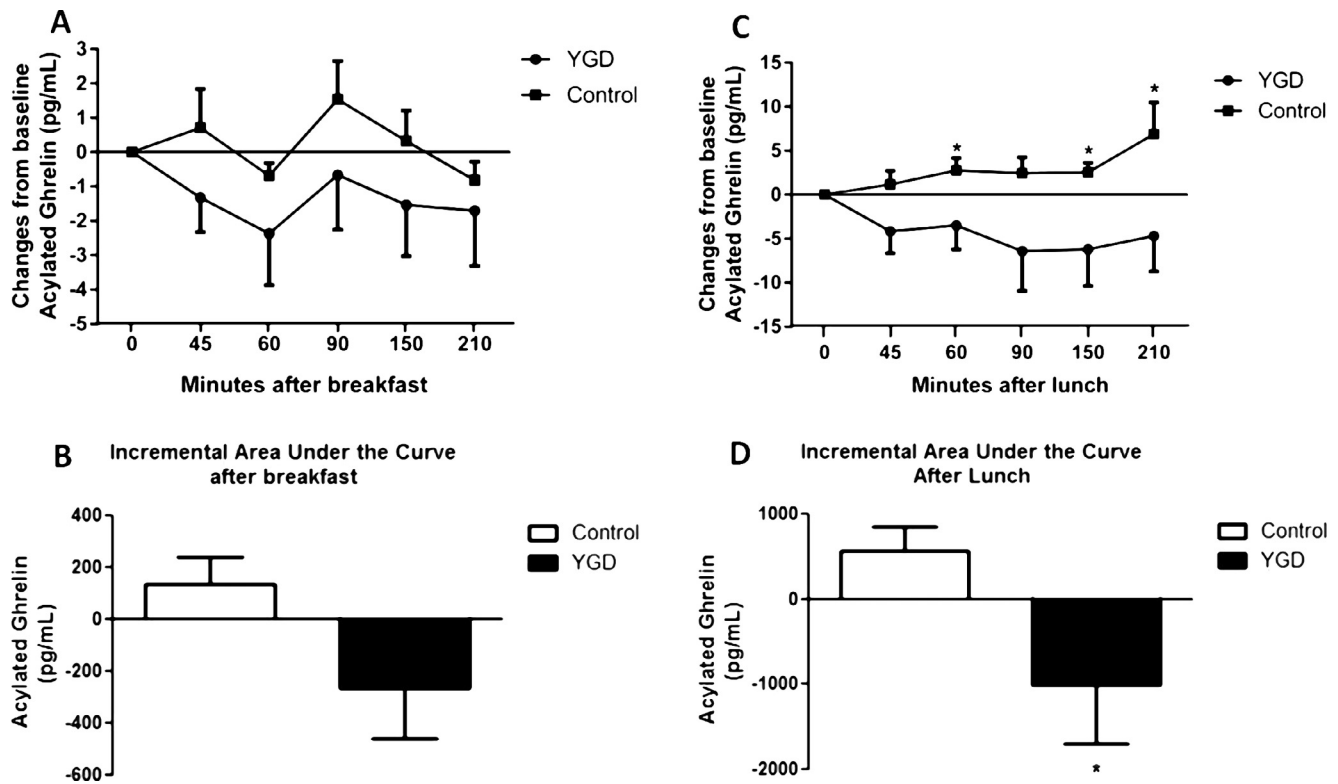


Fig. 3. Postprandial profiles of acylated ghrelin after breakfast (A) and after lunch (C). Incremental area under the curve for acylated ghrelin after breakfast (B) and after lunch (D). * $P < 0.05$ vs. control group.

laricitrin, vitexin, luteolin-8-propenoic acid, orientin, orientin-3'-ketone, pinocembrin, and syringetin (Szewczyk & Zidorn, 2014). As expected, our qualitative results confirm the presence of caffeine, vitexin, rutin and several chlorogenic acids.

Among the compounds of YGD formulation, only yerba mate has been associated with gastrointestinal hormone modulation. Hussein et al. (2011) demonstrated that yerba mate may induce anorexic effects by direct induction of satiety and by stimulation of GLP-1 secretion in mice. Regarding the YGD bioactive compounds, methylxanthine alkaloids such as caffeine, polyphenols and saponins are able to increase GLP-1 production and regulate glucose homeostasis (Hussein et al., 2011; Liu et al., 2013) by stimulating insulin secretion, inhibiting food intake and reducing blood glucose concentrations (Liu et al., 2013). Similarly, it was demonstrated in humans, that GLP-1 secretion was significantly increased after the consumption of decaffeinated coffee which is rich in chlorogenic acid, a phenolic acid very abundant in yerba mate (Johnston, Clifford, & Morgan, 2003). Likewise, the methylxanthine alkaloids present in YGD may produce similar results after breakfast, for which an increase in GLP-1 levels and a significant reduction in glucose concentration between 90 and 210 min after YGD consumption were observed.

Associated with these compounds, the flavonoids present in Damiana (Zhao, Dasmahapatra, Khan, & Khan, 2008) can act as GLP-1 receptor agonists (Wooten et al., 2011). The vitexin, one of the flavonoids detected in YGD, was previously described as able to significantly reduce the postprandial blood glucose levels in rats (Choo, Sulong, Man, & Wong, 2012). An acute clinical trial also noted that the flavonoids from other vegetal sources intake increased the plasma GLP-1 concentration, with a concomitant reduction in intestinal glucose absorption (Johnston, Clifford, & Morgan, 2002). Furthermore, Yogisha and contributors (Yogisha & Ravisha, 2010) have suggested that flavonoids are able to inhibit

the enzyme dipeptidyl peptidase IV (DPP-IV) *in vitro*, thus increasing GLP-1 release and improving the diabetes by attenuating blood glucose concentrations (Mulvihill & Drucker, 2014). In the present study, the supplement offered to participants had 6.78% of flavonoids, and the YGD group exhibited higher concentrations of GLP-1 throughout the study, including significantly higher concentrations after breakfast. Flavonoids present in YGD may be acting as GLP-1 agonists. A chemical series of hydroxyl flavonols with the ability to selectively augment calcium signaling in a peptide agonist-specific manner, with effects only on truncated GLP-1 peptides and exendin-4 were identified in the study conducted by Wooten et al. (2011).

Some reasons for lower food intake found in the YGD group compared with the control group can be explained by the fact that GLP-1 also stimulates contraction of the pylorus, resulting in delayed gastric emptying and reduced gastrointestinal transit. Moreover, GLP-1 stimulates the secretion of amyloid peptide and amylin, which reduce gastric emptying by vagal action (Baggio & Drucker, 2014; Rayner, Samsom, Jones, & Horowitz, 2001; Schirra, Wank, Arnold, Goke & Katschinski, 2002). Thus, the reduced energy intake at lunch could be associated with an increase in postprandial GLP-1 concentrations after breakfast. In contrast, ghrelin stimulates gastrointestinal motility through specific ghrelin receptors located on myenteric, vagal and central neurons (Peeters, 2003). Our study indicated that YGD decreased postprandial acylated ghrelin concentrations after lunch and maintained lower acylated ghrelin concentrations after breakfast without significance due to variability in concentrations. Moreover, these beneficial effects on appetite and plasma gut hormones could be associated with the capacity of ghrelin in increasing the GLP-1 release response (Gagnon, Baggio, Drucker, & Brubaker, 2015).

There are several compounds present in YGD that also may act synergistically or directly on satiety and energy intake. Caffeine, for

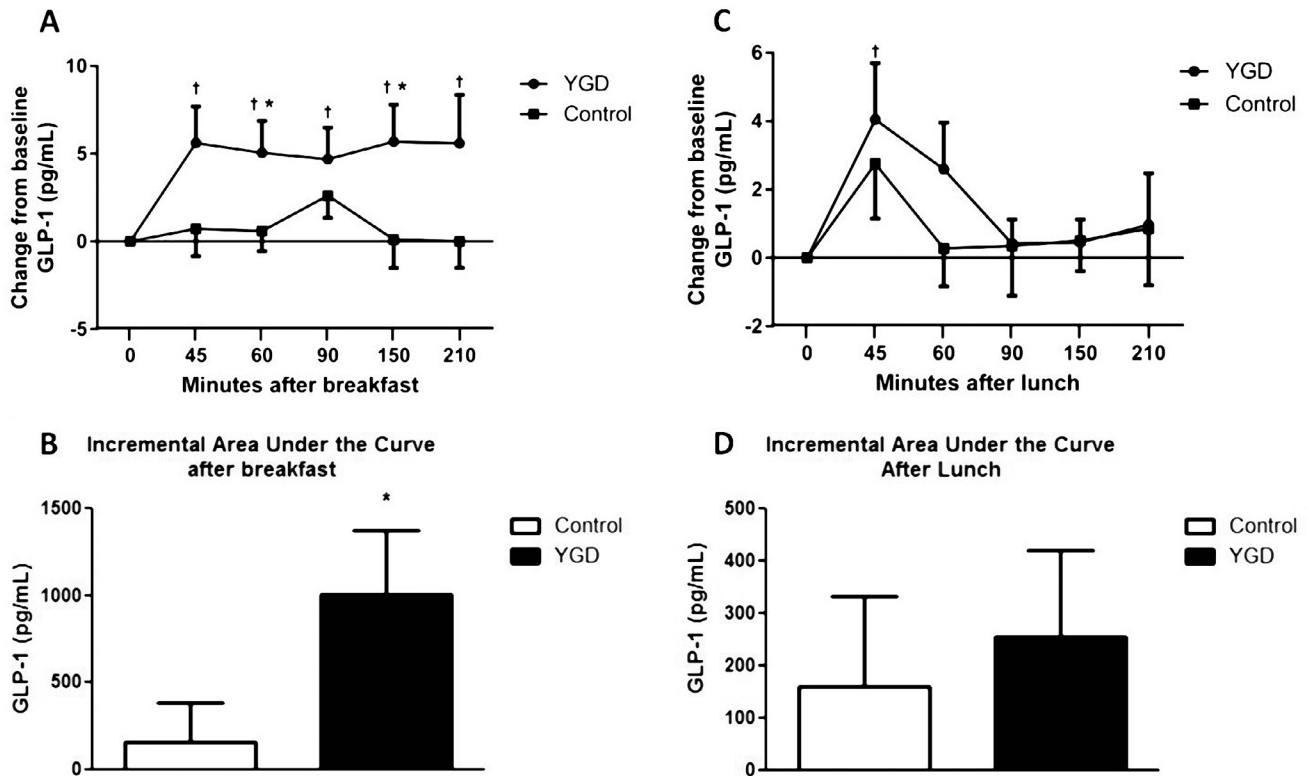


Fig. 4. Postprandial profiles of GLP-1 after breakfast (A) and after lunch (C). Incremental area under the curve for GLP-1 after breakfast (B) and after lunch (D). * $P < 0.05$ vs. control group; † $P < 0.05$ vs. baseline.

example, may be able to reduce palatable food ingestion in rats (Pettenuzzo et al., 2008) and type 2 diabetes mellitus risk in humans (Jessen, Bumann, Toubro, Skovgaard, & Astrup, 2005) and increase weight loss through thermogenesis and fat oxidation (Kim et al., 2015; Pimentel, Zemdegs, Theodoro, & Mota, 2009). Caffeine acts by inhibiting phosphodiesterase, an enzyme that degrades intracellular cyclic AMP, and by antagonizing the negative modulatory effect of adenosine on increased noradrenaline, dopamine and serotonin concentrations (Hursel & Westerterp-Plantenga, 2013; Nehlig, Daval, & Debry, 1992).

Dietary supplementation with phenolic compounds was associated with reduced food intake in obese and/or metabolic syndrome subjects (Panickar, 2013). Törrönen et al. (2012) found that the consumption of berries, rich in phenolic acids and flavonoids, had a beneficial impact on glucose, insulin and GLP-1 responses. Furthermore, polyphenols have the ability to affect the neuro regulatory factors, neural signaling pathways and peripheral mechanisms that modulate food intake (Gómez-Pinilla, 2008). In the present study, YGD negatively modulated acylated ghrelin and positively modulated GLP-1; however, we also observed that there were differences in the hormone response profiles for breakfast and lunch. The lack of significance in the AUC for acylated ghrelin after breakfast may be associated with a higher amount of carbohydrate at lunch compared with breakfast. It is known that carbohydrates have greater ability to suppress ghrelin compared with lipids and proteins (Koliaki, Kokkinos, Tentolouris, & Katsilambros, 2010). However, the absence of changes in GLP-1 AUC after lunch may be due to its high concentration before the meal, which remained high until lunch. Subjects that consumed more fat exhibited higher concentrations of GLP-1 (Di Francesco et al., 2010), corroborating the results of this study, because the amount of fat in breakfast was higher than the amount of fat in lunch. As previously mentioned, breakfasts and lunches were based on the eating habits

of the participants so as not to affect food consumption. The lack of a resting metabolic rate measurement is a limitation of this study.

The results of this study are restricted to the meals offered, and cannot be extrapolated to situations where these meals are not controlled. Therefore, we concluded that the synergism between the South American herbal extracts that containing Yerba Mate (*Ilex paraguariensis*), Guarana (*Paullinia cupana*) and Damiana (*Turnera diffusa*) may be responsible to reduce energy and macronutrient intake due to lower release acylated ghrelin and enhancing GLP-1 concentrations in overweight and obese women.

5. Conflicts of interest

The authors declare no conflicts of interest.

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