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Caffeine, but not other phytochemicals, in mate tea (*Ilex paraguariensis* St. Hilaire) attenuates high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation

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ABSTRACT

The objective was to examine the effectiveness of mate tea (MT, *Ilex paraguariensis* St. Hilaire) and caffeine from mate tea (MC) on *in vitro* lipid accumulation and *in vivo* diet-driven-obesity. MC and decaffeinated mate (DM) were obtained using supercritical CO_2 extraction and mainly composed of caffeine and caffeoylquinic acids, respectively. MC reduced lipid accumulation (41%) via downregulation of fatty acid synthase (*Fasn*) (39%) in 3T3-L1 adipocytes. Rats fed a high-fat-high-sucrose-diet and 0.1% of caffeine from MC, MT, or DM. MC attenuated weight gain (16%) and body fat accumulation (22%). MC reduced *Fasn* expression in both adipose tissue (66%) and liver (37%). MC diminished pyruvate kinase (PK, 59%) and microsomal triglyceride transfer protein (MTP, 50%) hepatic expression. *In silico*, neochlorogenic acid interacted with PK and MTP allosteric sites. FAS β-ketoacyl reductase domain showed the highest affinity to 3,5-dicaffeoylquinic acid. Caffeine suppressed lipid accumulation and body weight gain, through the modulation of lipogenic gene expression.

1. Introduction

Obesity is defined as an excess of fat accumulation that might harm health. The imbalance between energy intake and expenditure results in excessive energy storage that triggers excessive triglycerides storage in the adipose tissue (González-Muniesa et al., 2017). About 13% of the adult world population, over 640 million people, is obese (WHO, 2018). Obesity is not only a simple physical condition but also a major risk factor for chronic diseases, including type-2 diabetes (T2D), cardiometabolic disease, and fatty liver disease (Arzola-Paniagua et al., 2016). Being recognized as a chronic disease, obesity has become one of the main healthcare challenges facing us today. The loss of weight can be accomplished through different interventions (lifestyle, pharmacological, and surgical). However, sustainability in weight maintenance remains a challenge for individuals with obesity (Soleymani, Daniel, & Garvey, 2016). To combat this epidemic, safe, widely available, and affordable anti-obesity strategies are required. Numerous plants and herbs display anti-obesity and anti-diabetic effects via the modulation of appetite reduction, lipid absorption and metabolism, insulin sensitivity, thermogenesis, and gut microbiota (Martel et al., 2017).

Mate tea is an herbal infusion made from brewing the dried leaves of *Ilex paraguariensis* St. Hil. (Aquifoliaceae) consumed in most southeastern Latin American countries. People in those countries use it as a stimulant since it is a great source of caffeine among other methylxanthines (Gan, Zhang, Wang, & Corke, 2018). Several biologically active phytochemicals present in mate tea may be responsible for its health benefits such as phenolic compounds (chlorogenic acids) and methylxanthines (caffeine and theobromine), followed by flavonoids (rutin, quercetin, and kaempferol), saponins, amino acids, minerals (P, Fe, and Ca), and vitamins (C, B1, and B2) (Heck & de Mejia, 2007). Yerba mate has been described as a potential agent in the reduction of adipogenesis (Arçari, Santos, Gambero, & Ribeiro, 2013), prevention of obesity-induced inflammation and consequent insulin resistance (Arçari et al., 2011; Pimentel et al., 2013), improvement of the lipid serum profile, reduction of LDL-cholesterol (de Morais et al., 2009), reduction of LDL peroxidation (Matsumoto, Mendonça, de Oliveira, Souza, &

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Bastos, 2009), prevention of vascular endothelial dysfunction (Gao, Liu, Qu, & Zhao, 2013), and promotion of blood antioxidant defense systems (Bremer Boaventura et al., 2015).

The bioavailability of hydroxycinnamic acids and flavonols from yerba mate is low; predominantly the metabolites detected in plasma are derivatives of phase II metabolism and colonic microbiota (Gómez-Juaristi, Martínez-López, Sarria, Bravo, & Mateos, 2018). Caffeoylquinic acids have also been detected in the liver after mate tea intake (de Oliveira, Sampaio, Pinto, Catharino, & Bastos, 2017). Nonetheless, caffeine is wholly absorbed, reaching the liver at 95–99% (Gonzalez de Mejia & Ramirez-Mares, 2014) and the adipose tissue (Che, Wang, Zhang, Zhang, & Deng, 2012). The pharmacokinetics of these phytochemicals may have a crucial impact on their bioefficacy; caffeine is considered as the main active component of mate tea. A meta-analysis of randomized controlled trials proved that caffeine intake might stimulate the reduction of weight and body fat, diminishing the body mass index (Tabrizi et al., 2018). Caffeine anti-obesity effects have been mainly linked to its thermogenic effects; caffeine stimulates thermogenesis by inhibiting the phosphodiesterase-induced degradation of cAMP (Diepvens, Westerterp, & Westerterp-Plantenga, 2007) and eliciting adipose tissue browning (enhancing mitochondrial content, UPC1 expression, and cellular respiration) (Velickovic et al., 2019). Furthermore, it has been suggested that caffeine enhances lipolysis, fat oxidation, and reduces lipogenesis (Harpaz, Tamir, Weinstein, & Weinstein, 2017).

The hypothesis was that the anti-obesity effects of mate tea intake would be mostly derived from the presence of a high concentration of caffeine. The objective of this research was to determine the effect of caffeine and other components of mate tea on *in vitro* lipid accumulation, *in silico* interaction with obesity-related enzymes, and *in vivo* high-fat-high-sucrose driven weight gain, body composition, adipose tissue and liver lipid metabolism.

2. Materials and methods

2.1. Materials

Synthetic caffeine (> 99%), dexamethasone, 3-isobutyl-1-methylxanthine, insulin, fetal bovine serum, and Oil Red O were obtained from Sigma-Aldrich (St. Louis, MO, USA). Mouse 3T3-L1 cell line and Dulbecco's modified Eagle's medium (DMEM) were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Fetal bovine serum (FBS), Bovine newborn calf serum (NBS), Dulbecco's phosphate-buffered saline, 0.25% trypsin-EDTA, antibiotics (penicillin-streptomycin 100×), and TRIzol reagent were purchased from Invitrogen Co. (Carlsbad, CA, USA). Caffeine from coffee (95% caffeine) was obtained from Soaljo S.R.L. (Buenos Aires, Argentina). Male Sprague-Dawley (4 weeks old) rats were purchased from Harlan (Indianapolis, IN, USA). All diet ingredients were purchased from Dyets, Inc. (Bethlehem, PA). RNeasy Lipid Tissue Mini Kit, to extract RNA from adipose tissue was purchased from Qiagen (Valencia, CA, USA). Primers were purchased from MWG Biotech (Huntsville, AL, USA). Organic Guayaki yerba mate (*Ilex paraguariensis* St. Hil.) leaves from the Itabo rainforest preserve, in eastern Paraguay, collected in 2006, were used in this study.

2.2. Extraction of caffeine from yerba mate tea

Supercritical CO_2 extraction was done on organic yerba mate tea leaves (*Ilex paraguariensis* St. Hil.) from Paraguay to obtain caffeine from mate (MC) and decaffeinated mate. The supercritical extraction was carried out in the United States Department of Agriculture (Peoria, IL, USA). Caffeine was extracted from organic yerba mate using a water-saturated supercritical CO_2. Extraction vessel temperature was 70 °C and the pressure was 5800 psi. Receiver vessel temperature was 50 °C and the pressure was 1100 psi. The flow rate was 0.986 lb min^{-1} for a total of 510 lb of liquid CO_2. Yerba mate leaves (1.5 kg) were placed in the CO_2 supercritical extractor. The extraction lasted approximately 8 h (Assis Jacques et al., 2006). The obtained MC was stored at −20 °C and protected from light and air contact.

2.3. Preparation of mate tea and decaffeinated mate tea

Mate leaves and decaffeinated leaves were kept in sealed plastic bags and stored at 4 °C. Mate tea infusions were obtained by using the traditional American procedure of preparing the tea (Gonzalez de Mejia, Song, Ramirez-Mares, & Kobayashi, 2005). Leaves (30 g leaves L^{-1}) were boiled in water for 10 min with occasional stirring and allowed to cool to room temperature before filtration using 0.45-μm filter paper and then freeze-dried. The yield of the preparations was 28.3 g MT 100 g^{-1} leaves and 25.9 g DM 100 g^{-1} decaffeinated leaves. The freeze-dried material, mate tea (MT) and decaffeinated mate tea (DM), were stored at −20 °C and protected from light and air contact.

2.4. Quantification of caffeine

Freeze-dried powders (20 mg) were dissolved in 5 mL of deionized water at room temperature and filtered with a 13 mm filter. In a separatory funnel, the mixture was combined with chloroform in a 1:1 ratio. The mixture was gently stirred for 5 min and set for 1 min until two layers were formed. The bottom layer (chloroform with extracted caffeine) was collected into a flask and the extraction repeated two more times. Anhydrous sodium sulfate was added to the collected chloroform (15 mL) to absorb any water, and the mixture was filtered using #4 filter paper to remove it. Caffeine was quantified spectrophotometrically at an absorbance of 300 nm using a standard curve of pure caffeine (Van Atta, 1979).

2.5. HPLC-DAD-MS/MS phytochemical profile

Identification of caffeine and phenolic compounds was performed using HPLC-DAD-MS/MS, following an adaptation of a previously reported method (Chandra & Gonzalez de Mejia, 2004). The analysis was carried out using a 1050 Hewlett-Packard (Palo Alto, CA) liquid chromatograph coupled to a diode array detector (DAD). A C18 guard column and a C18 Phenomenex Prodigy ODS column (250 mm × 4.6 mm × 5 μm) were used. The temperature of the column was maintained at ambient temperature and the flow rate was 0.9 mL/min. The gradient of solvents was performed as follows: solvent A was water/methanol/formic acid (79.7/20/0.3, % v/v) and solvent B was methanol/formic acid (99.7/0.3, % v/v). Starting with 100% A, solvent B linearly increased to 52% in 50 min then to 80% B in 5 min and held at these conditions for 3 min. Finally, a linear decrease to 0% B was achieved 5 min and held at 0% B for 5 min to recover the column starting conditions. The DAD detector was set to read from 195 to 450 nm, with outputs at 260, 280, and 330 nm. After HPLC separation, mass spectra were obtained using an LCQ Deca XP mass spectrometer (Thermo Finnigan Corp., San Jose, CA) with electrospray ionization (ESI) in positive and negative modes (m/z 150–2000). Data analysis was conducted with Xcalibur software. Phytochemicals were characterized according to their UV, mass spectra and retention times and compared with standards when available.

2.6. In vitro cell culture study

The murine 3T3-L1 cells were cultured in DMEM containing 10% (v/v) NBS, and 1% (v/v) antibiotics (penicillin and streptomycin). The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.6.1. Adipocytes differentiation

The cells were subcultured at a density of 6×10^3 cells cm^{-2}. To

differentiate the cells into adipocytes, the previously described method was used (Zebisch, Voigt, Wabitsch, & Brandsch, 2012). Cell differentiation was induced by changing the medium to DMEM containing 10% FBS, 0.5 mmol L^{-1} IBMX, 0.25 µmol L^{-1} dexamethasone, 2 µmol L^{-1} rosiglitazone, and 1 µg mL^{-1} insulin. After 48 h, the medium was changed to DMEM containing 10% FBS and 1 µg mL^{-1} insulin. On day 7, the medium was changed to DMEM containing 10% FBS and refreshed on days 8, 10, and 12. Cells were completely differentiated at day 10–12 and then used for the experiments.

2.6.2. Experimental design and treatments

The treatments used for 3T3-L1 preadipocytes and adipocytes were synthetic caffeine (SC), caffeine from coffee (CC), caffeine from mate (MC), mate tea (MT), and decaffeinated mate tea (DM). All the treatments were dissolved in Milli-Q water and used at concentrations of 50 µmol L^{-1} caffeine. The concentration for DM was equivalent to total chlorogenic acid concentration in MT. CC and SC were used as control treatments to compare their biological activity with MC and determine the association with caffeine and not to the residual components that might be present in the extract. The doses (50–100 µmol L^{-1}) for the *in vitro* study were selected according to previous studies by authors, and the literature on adipocyte differentiation inhibition (Arçari et al., 2013; Rebollo-Hernanz, Zhang, Aguilera, Martin-Cabrejas, & Gonzalez de Mejia, 2019). The treatments were filtered, sterilized using syringe filters (0.22 µm) before being applied to cells. Fig. 1A depicts the experimental design followed.

2.6.3. Cell viability

The measurement of cell viability of 3T3-L1 adipocytes stimulated with SC, CC, MC, MT, and DM (10, 50, and 100 µM caffeine equivalents, DM dose was equivalent to MT in polyphenol content) for 24 h was carried out with the CellTiter® 96 Aqueous One Solution Proliferation assay (Promega Corporation, Madison, WI, USA) following manufacturer's instructions.

2.6.4. Determination of cellular lipid accumulation

Oil Red O lipid staining was accomplished as previously described (Rebollo-Hernanz, Zhang, Aguilera, Martin-Cabrejas, & Gonzalez de Mejia, 2019). Oil Red O is a good and cost-effective staining agent for both quantitative and qualitative measurement of lipid droplet formation (Sikkeland, Jin, & Saatcioglu, 2014). Preadipocytes were cultured in 24-well plates and induced to differentiation. SC, CC, MC, MT, and DM (50 µmol L^{-1} caffeine equivalents, DM dose was equivalent to MT in polyphenol content) were added to the culture media along the differentiation process, and lipid accumulation quantification was performed at day 10–12. Absorbance was measured at 550 nm and the values were standardized by the viability percentage. Lipid accumulation was expressed as a percentage relative to the non-treated cells value.

2.6.5. Real-time quantitative PCR (RT-qPCR) analysis

Total mRNA was extracted from matured 3T3-L1 adipocytes treated with SC, CC, and MC (100 µmol L^{-1} caffeine equivalents) using RNA easy mini-kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The three caffeine treatments (SC, CC, and MC) were selected to evaluate the gene expression of important proteins associated with lipogenesis and lipid accumulation due to their higher and significant ($p < 0.05$) influence on lipid accumulation. Preliminary results (data not shown), demonstrated no significant results for any of the treatments at 50 µmol L^{-1} caffeine equivalents; therefore, 100 µmol L^{-1} was tested in this assay. The RNA quality and concentration were determined by agarose gel electrophoresis and nanodrop spectrophotometry (ND-100 NanoDrop Tech, Wilmington, DE). RNA expression of target genes was measured using real-time quantitative PCR with SYBR Green fluorescence dye (Applied Biosystems, Foster City, CA). Briefly, 2 µg purified RNA was reverse transcribed into complementary DNA. Specific primer sequences were used for fatty acid synthase (*Fasn*) and lipoprotein lipase (*Lpl*). The 18S ribosomal RNA was used as a housekeeping control (Perez et al., 2017) (Supplementary Table 1). The plate was centrifuged with CR-422 centrifuge machine (Jouan, Inc., Frederick County, VA) and read with Taqman 7900 HT Real-time PCR System machine (Applied Biosystems, Foster City, CA). The mRNA abundance relative to 18S was determined using the comparative critical threshold method according to manufacturer's

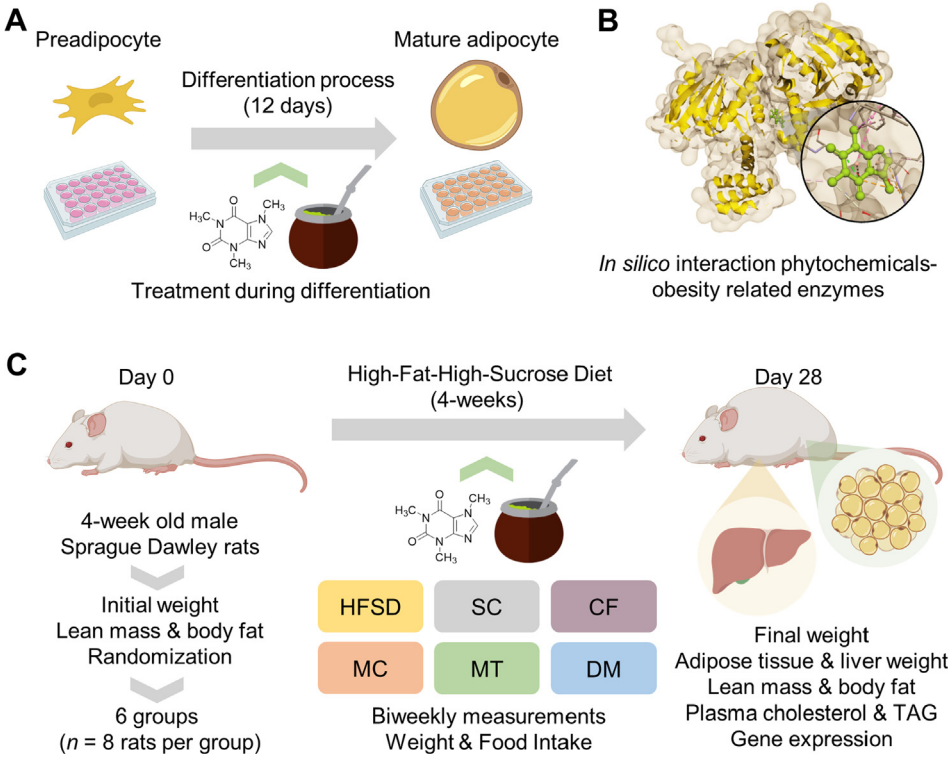


Fig. 1. Diagram of the experimental design followed in this research. The study includes an *in vitro* cell culture (3T3-L1 adipocytes) to evaluate the effects of mate tea and caffeine on adipogenesis (A), an *in silico* molecular docking to examine the potential mechanism of action of phytochemicals in mate tea (B), and an *in vivo* model of high-fat-high-sucrose-diet-driven obesity to assess the efficacy of mate tea and caffeine in fat accumulation, weight gain and the underlying mechanisms. Treatment groups included: high-fat-high-sucrose diet (HFSD), HFSD + 0.1% synthetic caffeine (SC), HFSD + 0.1% caffeine from coffee (CC), HFSD + 0.1% caffeine from mate (MC), HFSD + mate tea (0.1% caffeine) (MT), and HFSD + decaffeinated mate tea (adjusted to MT phenolic content) (DM).

instructions.

2.7. In silico molecular docking

Caffeine and phenolic compounds present in mate tea were evaluated as potential ligands for enzymes linked to lipogenesis and lipid metabolism and evaluated using molecular docking (Fig. 1B). Available protein 3D structures were acquired from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). The center of the docking area was established in the binding site of co-crystallized inhibitors or substrates (Supplementary Table 2) (Rebollo-Hernanz, Fernández-Gómez, et al., 2019). The human sequences of lipoprotein lipase (LPL) and microsomal triglyceride transfer protein (MTP) were obtained from the PubMed database (www.ncbi.nlm.nih.gov/entrez). The homology-modeling of the unavailable 3D structures was created using Swiss--Model (https://swissmodel.expasy.org/), taking the structure of the pancreatic triacylglycerol lipase (1N8S) and lipovitellin (1LSH) as templates, respectively. The binding pockets were determined using the COACH server (https://zhanglab.ccmb.med.umich.edu/COACH/). The structures of phytochemicals used as ligands were downloaded from the PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/). Verified inhibitors for each protein were also evaluated as controls (FAS: 4-methylidene-2-octyl-5-oxooxolane-3-carboxylic acid; LPL: -[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione; PK: 5-(2,5-dimethylpyrrol-1-yl)-2-hydroxybenzoic acid; MTP: 2-[[3-(4-chlorophenyl)-3-[4-(1,3-thiazole-2-carbonyl)phenoxy]propyl]-methylamino]acetic acid). Ligand gasteiger partial charges were added, and the root of each structure set rotatable bonds detected in AutoDock Tools. Docking calculations were performed using AutoDock Vina, performing 200 different runs per each ligand. The pose with the highest binding affinity (lowest binding energy) was saved and protein-ligand interactions and binding modes were visualized in the Discovery Studio 2017 R2 Client (Dassault Systemes Biovia Corp®).

2.8. Animal study

2.8.1. Animals and experimental design

The experimental design followed for the *in vivo* study is shown in Fig. 1**C**. Four-week-old Sprague-Dawley male rats ($n = 48$) were purchased (Harlan Laboratories, Madison, WI, USA) and first fed a standard chow diet for a week and a half to acclimate them to their new environment. After that, they were divided randomly into six groups ($n = 8$ animals per group) each fed for four weeks a high-fat-high-sucrose diet (HFSD) consisting in 40% fat and 30% sucrose (in energy basis) and containing 0.1% caffeine from different sources (SC, CC, MC, MT, DM). DM diet was balanced to have the same total chlorogenic acid content as MT. The dose (0.1% caffeine in the diet) for the *in vivo* study was selected on the basis of a previous study using a wider caffeine range (Kobayashi-Hattori, Mogi, Matsumoto, & Takita, 2005), and considering a physiological range. Diet calories derived from 40% fat, 45% carbohydrate (30% sucrose), and 15% protein. Its composition was as follows (in g/kg): casein, 200; DL-methionine, 3; cornstarch, 150; sucrose, 350; lard, 200; cellulose, 50; mineral mix, 35; vitamin mix, 10; choline bitartrate, 2. Caffeine and yerba mate sample were included in the formulated animal diet and adjusted to 0.1% caffeine independently of the treatment.

Body weight of the rats in the six groups ranged from 150 to 180 g. The rats were checked every day and weighed twice a week. Food intake was monitored and weighed twice a week. After four weeks of feeding, rats were killed by decapitation to collect blood and tissue samples. Epididymal fat pads and liver were excised, weighed, and immediately frozen in liquid nitrogen for later analysis. The study was approved by the Institutional Animal Care and Use Committee of the University of Illinois (approval number 07126).

2.8.2. Body composition analysis

After rats were acclimated and randomized, they were sedated to analyze their body fat and lean mass using Dual-energy X-ray Absorptiometry (DXA). At least 2 scans were taken of each rat. Body fat in the six groups of rats ranged from 6.7 ± 0.1%. At the end of the study, rats were again sedated for DXA measurement. Each rat was injected subcutaneously with medetomidine (dormitor, 0.3 mg /kg^{-1} body weight) approximately 10 min before its scanning time. Immediately after scanning, each rat was injected subcutaneously with the reversal agent atipamezole (1.5 mg kg^{-1} body weight) and observed until fully awake.

2.8.3. Serum lipid concentration

Serum from blood collected at the end of the study was obtained by centrifugation at 1000*g* for 10 min and stored at −80 °C until analyzed. Cholesterol and triglyceride were quantified using commercial colorimetric kits following the manufacturer's instructions (Abcam, Boston, MA, USA; Ref. ab65336 and ab65390, respectively).

2.8.4. RT-qPCR analysis

Total mRNA was extracted from frozen adipose tissue and liver using RNA easy mini-kit and TRIzol, respectively. The procedure followed is explained in Section 2.6.5. Specific primer sequences were used for *Fasn*, adiponectin (*AdipoQ*), pyruvate kinase (*Pkm*), and microsomal triglyceride transfer protein (*Mttp*). The 18S ribosomal RNA used as a housekeeping control (Supplementary Table 1).

2.9. Statistical analysis

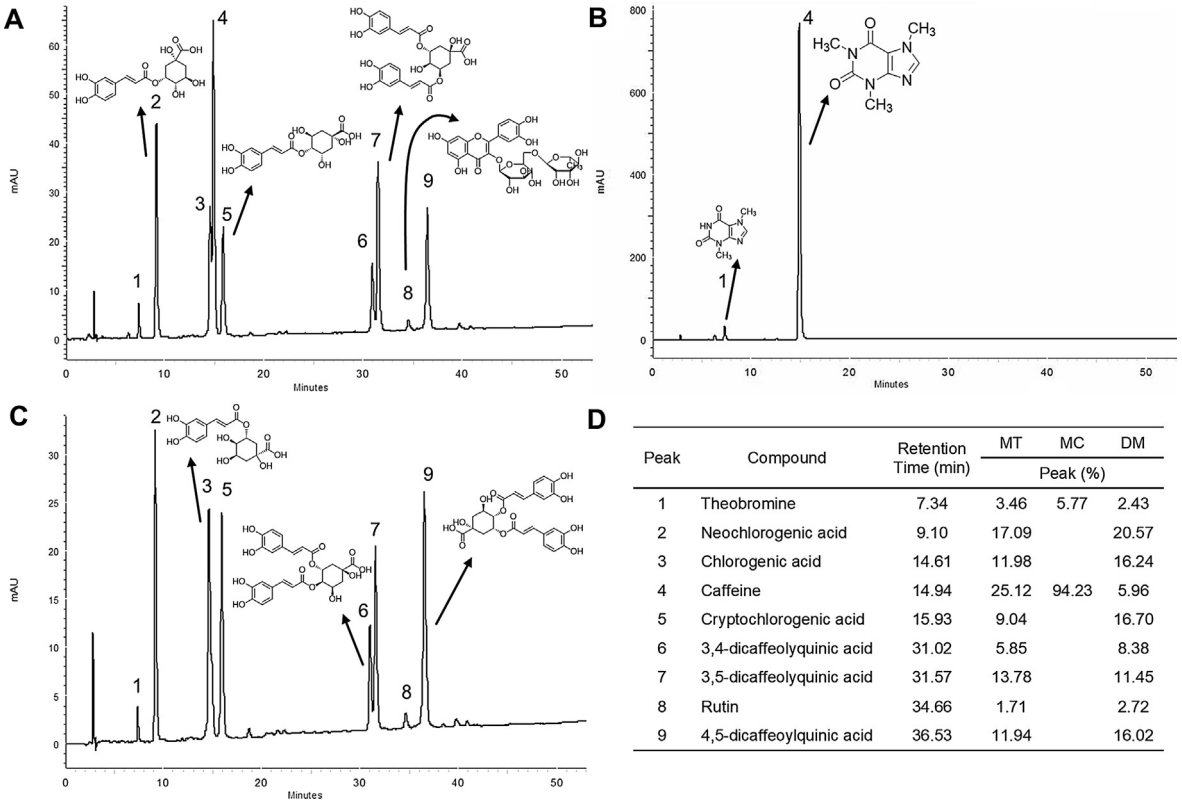
In vitro experiments were prepared and analyzed in triplicate ($n = 3$). *In vivo* samples were analyzed in triplicate; the values for each group represent the mean of eight animals ($n = 8$). Results are expressed as the mean ± standard deviation (SD) and were assessed statistically by one-way analysis of variance (ANOVA) and *post hoc* Tukey test. Differences were considered significant at $p < 0.05$. The statistical analysis was performed using SPSS 23.0. Multivariate analyses were carried out with XLSTAT 2018 for Microsoft Excel 2016.

3. Results and discussion

3.1. Mate tea was mainly composed of caffeine and caffeoylquinic acids

Mate tea has been described as a source of caffeine among other bioactive compounds (Heck & de Mejia, 2007). From mate tea (MT) leaves, a caffeine-enriched fraction (MC) was obtained and a decaffeinated mate tea (DM) was also obtained using CO_2 supercritical extraction. The concentration of caffeine in the MT and MC products was 0.22 ± 0.10, 0.91 ± 0.07 g g^{-1}, respectively. Caffeine was not detected in DM, evidencing the efficiency of the supercritical extraction method. Similar processes of extraction have been effective in the extraction of caffeine from yerba mate fruits (Fernandes et al., 2017).

To deeply investigate MT, MC, and DM phytochemical composition, the extracts were analyzed by UPLC-MS/MS (Fig. 2 and Table 1). Nine different compounds were detected. According to their retention times, maximum wavelengths, and molecular ions tentatively identified by mass spectrometry. Compound 1, eluted at 7.3 min and maximum absorbance at 275 nm, presented a pseudomolecular ion $[M-H]^+$ at *m/z* 181; then, the compounds *m/z* is 180, being identified as theobromine. Compounds 2, 3, and 5, with retention times of 9.3, 14.6, and 15.9 min, respectively, presented the same UV spectra and ion patterns in both positive ($[M-H]^+$ at *m/z* 355) and negative ($[M-H]^-$ at *m/z* 353) modes. They yielded fragment ions at *m/z* 163 ($[\text{caffeic acid}-H_2O-H]^+$), 179 (caffeic acid), 191 (quinic acid), and 372 ($[\text{caffeoylquinic acid} + NH_4]^+$), and 707 ($[2M-H]^-$). These results evidenced the presence of three chlorogenic acid isomers. According to literature (Bravo, Goya, & Lecumberri, 2007), they were identified as 5-



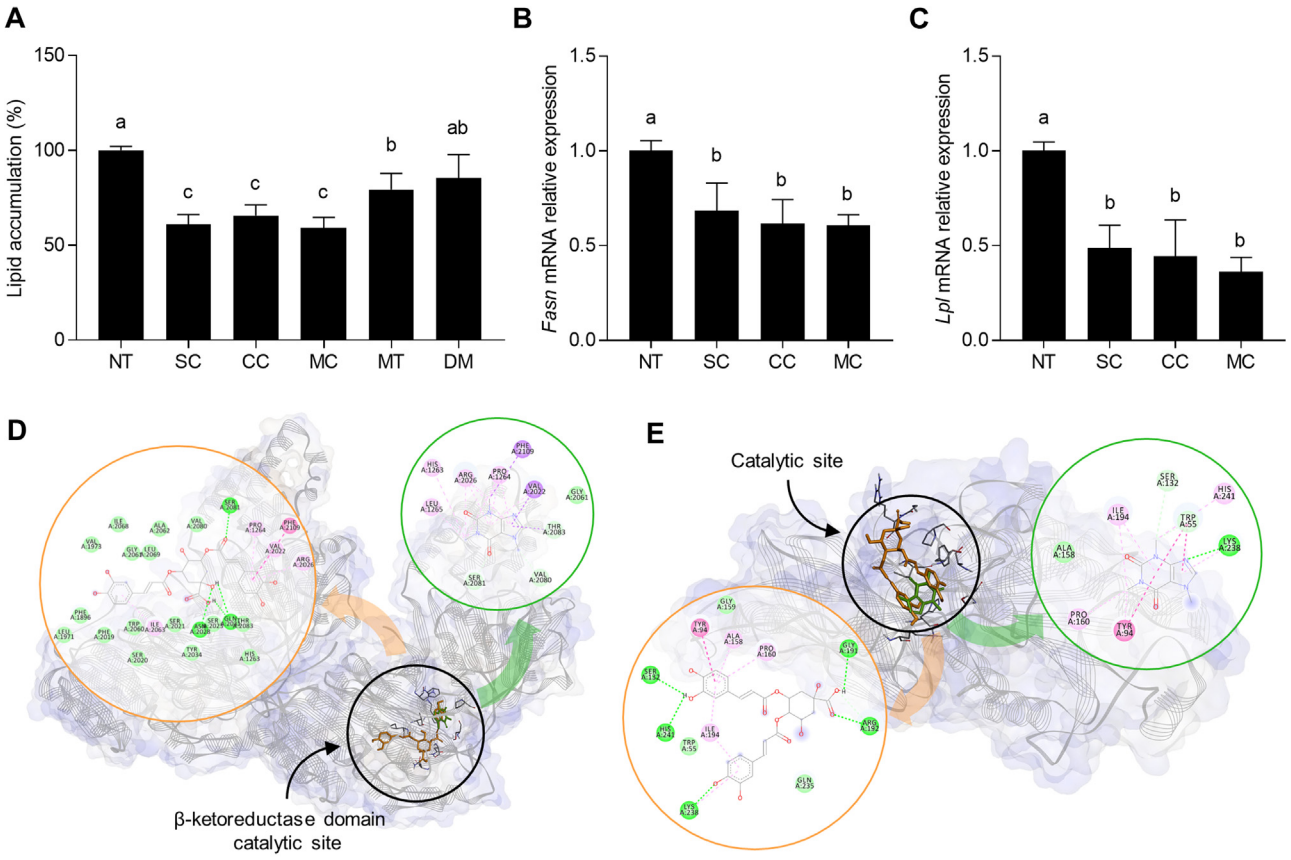
Peak	Compound	Retention Time (min)	MT	MC	DM
			Peak (%)		
1	Theobromine	7.34	3.46	5.77	2.43
2	Neochlorogenic acid	9.10	17.09		20.57
3	Chlorogenic acid	14.61	11.98		16.24
4	Caffeine	14.94	25.12	94.23	5.96
5	Cryptochlorogenic acid	15.93	9.04		16.70
6	3,4-dicaffeolyquinic acid	31.02	5.85		8.38
7	3,5-dicaffeolyquinic acid	31.57	13.78		11.45
8	Rutin	34.66	1.71		2.72
9	4,5-dicaffeoylquinic acid	36.53	11.94		16.02

Fig. 2. Representative chromatograms of the phytochemical HPLD-DAD-MS/MS analysis recorded at 280 nm of mate tea (MT) (A), caffeine from mate (MC) (B), and decaffeinated mate (DM) (C) extracts, and the corresponding compound identified and percentage of area for each peak (D).

caffeoylquinic acid (neochlorogenic acid), 3-caffeoylquinic acid (chlorogenic acid), and 4-caffeoylquinic acid (cryptochlorogenic acid), respectively. The compound eluting at 14.9 min (peak 4) showed maximum absorbance at 275 nm, the characteristic maximum of methylxanthines (Belay, Ture, Redi, & Asfaw, 2008). Generating a pseudomolecular ion at a $[M-H]^+$ at *m/z* 195 it was identified as caffeine. Compounds 6, 7, and 9 retention times were 31.0, 31.6, and 34.7 min, respectively, and presented maximum absorption at 325 nm, and yielded ions $[M-H]+$ at *m/z* 517 and $[M-H]^-$ at *m/z* 515, suggesting the compounds molecular weight was 516 g/mol. Fragments ions for these peaks were similar to those found in peaks 2, 3, and 5; these compounds produced fragment ions at *m/z* 163 ($[M-\text{quinic acid}-H_2O-H]^+$), 179 (caffeic acid), 191 (quinic acid), 353 ($[M-\text{caffeic acid}-H_2O]$, [chlorogenic acid $-H]^-$), 354 ($[M-\text{caffeic acid}-H_2O]^+$, chlorogenic acid), 1031 (di-caffeoylquinic acid dimer), and 1050 ($2M+H_2O$). Hence, these compounds were identified as di-caffeoylquinic acid, namely, 3,4-dicaffeoylquinic acid, 3,5-di-caffeoylquinic acid, and 4,5-dicaffeoylquinic acid (Bravo et al., 2007; Schutz, Schutz, Kammerer, Carle, & Schieber, 2004). Finally, compound 8 eluted at 34.7 min and showed a UV spectra characteristic of flavonols with maximum absorption at 355 nm. Fragment ions were observed in the positive ($[M-H]+$ at *m/z* 611) and negative ($[M-H]^-$ at *m/z* 610) modes. The molecular weight of the compounds was estimated as 610.5 g/mol. Moreover, the compound displayed one more fragment in the negative mode at *m/z* 301 ($[M-H-\text{rutinose}]^-$, [quercetin $-H]^-$). This compound was identified as quercetin-3-*O*-rutinoside or rutin. A similar profile of phytochemicals was found independently of the growing and drying conditions of yerba mate leaves (Heck, Schmalko, & Gonzalez de Mejia, 2008). Comparing chromatograms in Fig. 2A–C, it was observed that the higher proportion of caffeine in MT against the other phytochemicals (25.1%) (Fig. 2D). In MC, 94.2% was caffeine, whereas only 6.0% was found in DM. Previously, yerba mate decaffeination generated products with similar composition: significantly reduced concentration of caffeine in the leaves while preserved concentration of caffeoylquinic acids in the decaffeinated product and a supercritical extract mainly composed by caffeine with a low proportion of theobromine (Cassel et al., 2010). Consequently, it was concluded that the supercritical CO_2 extraction process efficiently produced two fractions from mate tea, caffeine from mate (MC) and decaffeinated mate (DM), completely different between them, and

Table 1
Retention time (R_t), wavelength of maximum UV–VIS absorption, and molecular ions of the identified phytochemicals in the HPLC-DAD-MS/MS analysis.

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^+$ (m/z)	MS^2 (m/z)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification
1	7.3	275	181	–	–	–	3,7-dimethylxanthine (theobromine)
2	9.1	325	355	163, 372	353	179, 191, 707	5-caffeoylquinic acid (neochlorogenic acid)
3	14.6	325	355	163, 372	353	179, 191, 707	3-caffeoylquinic acid (chlorogenic acid)
4	14.9	275	195	–	–	–	1,3,7-trimethylxanthine (caffeine)
5	15.9	325	355	163, 372	353	179, 191, 707	4-caffeoylquinic acid (cryptochlorogenic acid)
6	31.0	325	517	163, 354, 1050	515	179, 191, 353, 1031	3,4-dicaffeoylquinic acid
7	31.6	325	517	163, 354, 1050	515	179, 191, 353, 1031	3,5-dicaffeoylquinic acid
8	34.7	355	611	–	610	301	Quercetin-3-*O*-rutinoside (rutin)
9	36.5	325	517	163, 354, 1050	515	179, 191, 353, 1031	4,5-dicaffeoylquinic acid



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Binding energy (kcal/mol)	THEO	NEO	CGA	CAF	CRYPTO	3,4diCGA	3,5diCGA	RUT	4,5diCGA
FAS, TE domain	–6.3	–7.5	–7.4	–5.5	–7.4	**–9.2**	–8.5	–6.7	–9.0
FAS, ENR domain	–5.5	–7.2	–7.1	–5.7	–7.1	–6.9	–6.9	**–7.3**	–6.9
FAS, βKR domain	**–6.4**	**–8.5**	**–8.7**	**–6.4**	**–8.8**	**–8.8**	**–10.2**	**–9.5**	**–9.5**
LPL	–6.0	–7.4	–7.3	–5.5	–7.6	–8.2	–7.9	–7.9	**–8.4**

Fig. 3. Anti-adipogenic effects of synthetic caffeine (SC), caffeine from coffee (CC), caffeine from mate (MC), mate tea (MT), and decaffeinated mate (DM). 50 or 100 μmol L^{-1} caffeine equivalents, DM was dosed to be equivalent to MT in polyphenol content. Suppressive effects on lipid accumulation in 3T3-L1 adipocytes (50 μmol L^{-1} caffeine equivalents) (A), regulation of the mRNA expression of fatty acid synthase (*Fasn*) (100 μmol L^{-1} caffeine equivalents) (B) and lipoprotein lipase (*Lpl*) (100 μmol L^{-1} caffeine equivalents) (C), potential interaction of mate tea phytochemicals (caffeine in green, the phytochemical with higher affinity in orange) with fatty acid synthase (FAS) (D) and lipoprotein lipase (LPL) (E) expressed as binding energies (F). The phytochemical with the highest affinity to each protein is bolded. Inhibitors exhibited the following binding energies: FAS, TE domain: –6.8 kcal mol^{-1}; ENR domain: –6.6 kcal mol^{-1}; βKR domain: –7.0 kcal mol^{-1}; LPL: –7.0 kcal mol^{-1}. TE: thioesterase; ENR: enoyl-acyl carrier-protein reductase; βKR: β-ketoacyl reductase. THEO: theobromine; NEO: neochlorogenic acid; CGA: chlorogenic acid; CAF: caffeine; CRYPTO: cryptochlorogenic acid; 3,4diCGA: 3,4-dicaffeoylquinic acid; 3,5diCGA: 3,5-dicaffeoylquinic acid; RUT: rutin; 4,5diCGA: 4,5-dicaffeoylquinic acid. The results were expressed as mean ± SD ($n = 3$). Different letters among columns denote significant ($p < 0.05$) differences according to ANOVA and Tukey's multiple range test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

composed of mainly caffeine and caffeoylquinic acids, respectively.

3.2. Caffeine, independently of its origin, abrogated lipid accumulation via regulation of lipogenic genes in vitro

Mate tea and fractionated caffeine and decaffeinated samples were assayed for their anti-adipogenic potential in 3T3-L1 adipocytes (Fig. 3). Synthetic caffeine (SC) and caffeine extracted (CO_2 supercritical extraction) from coffee (CC) were also evaluated. All samples but DM significantly ($p < 0.05$) reduced lipid accumulation (from 20.6 to 40.7%) (Fig. 3A). Caffeine (50 μM), synthetic, from coffee or mate, exhibited the highest potential to reduce adipogenesis. Thus, these treatments were selected to evaluate the *Fasn* and *Lpl* gene expression (Fig. 3B, C), enzymes associated with the synthesis of fatty acids from glucose and the hydrolysis of the triglycerides from circulating chylomicrons and very-low-density lipoproteins (VLDL), thereby providing free fatty acids transport to the cell (Berndt et al., 2007; Zechner et al., 2000). *Fasn* expression was reduced by 31.5–39.3% while *Lpl* expression was diminished by 51.1–63.8%. All the caffeine treatments, independently of their origin, significantly downregulated the gene expression of those two lipogenic proteins. Nonetheless, MT treatments, with the same caffeine concentration, prompted less sharped effects. The modulation of *Fasn* expression could be facilitated by a hormone-mediated modulation in the expression of SREBP-1c via PI3K/AKT and MAPK transduction cascades (Menendez, Vazquez-Martin, Ortega, & Fernandez-Real, 2009). Likewise, *Lpl* expression has been shown to be regulated by PI3K/AKT and AMPK activation (Kersten, 2014). Our research group, previously evaluated the effects of both 3-caffeoylquinic acid (chlorogenic acid) and caffeic acid in the accumulation of fat in 3T3-L1 adipocytes (Rebollo-Hernanz, Zhang, Aguilera, Martin-Cabrejas, & Gonzalez de Mejia, 2019a,b). Results demonstrated the lower effects of these phenolic compounds in comparison with caffeine.

It was also revealed the effects of caffeine on lipid accumulation, presenting outstanding contribution in complex mixtures of phytochemicals (Rebollo-Hernanz, Zhang, Aguilera, Martin-Cabrejas, & Gonzalez de Mejia, 2019b). A comparison of mate tea, chlorogenic acid, rutin, and quercetin also confirmed that the inhibitory effects of mate on lipid accumulation were higher than those of the phenolic compounds (Arçari et al., 2013). Therefore, the *in vitro* results suggested that the effect of MT were mainly due to the presence of caffeine rather than phenolic compounds. Previous studies demonstrated the effects of caffeine on 3T3-L1 adipogenesis; caffeine was able to diminish differentiation via a decrease in the protein expression of CCAAT/enhancer-binding protein (C/EBP) β, C/EBPα, peroxisome proliferator-activated receptor (PPAR) γ, and fatty acid synthase (FAS) (Kim et al., 2016).

To gain insights on the mechanism of action of caffeine and the phenolics from mate in the adipogenic process, the potential interaction of these compounds with the enzymes associated, FAS and lipoprotein lipase (LPL) were assessed. Using *in silico* molecular docking, the different protein-phytochemicals interactions and their binding energies (Fig. 3D-F) were observed. As can be appreciated, caffeine binding with FAS was weaker than the binding of theobromine and the different phenolic compounds. The binding interaction for the FAS thioesterase domain ranged from –5.5 to –9.2 kcal mol^{-1}, being 3,4-dicaffeoylquinic acid the compound with the highest affinity. In the enoyl-acyl carrier-protein reductase domain, the affinity varied from –5.5 to –7.3 kcal mol^{-1}, being, rutin the phytochemical with the strongest potential interaction. Lastly, the β-ketoacyl reductase domain showed the highest affinity to 3,5-dicaffeoylquinic acid (–10.2 kcal mol^{-1}), being theobromine and caffeine the compounds with the weakest potential interactions (–6.4 kcal mol^{-1}, both). Thus, the β-ketoacyl reductase domain would be the most likely to be regulated by the interaction of phytochemicals due to its higher interaction with all the studied compounds. Besides, the phytochemicals interacted with LPL (from –5.5 to –8.4 kcal mol^{-1}) being 4,5-dicaffeoylquinic acid the compound with a strongest potential interaction. The results demonstrated that caffeine, being a smaller and more polar compound, presented weaker interaction with both FAS and LPL (independently of the domain). Phenolic compounds presented a higher number of strong interactions (hydrogen bonds) and multiple van der Waals, mainly due to the multiple hydroxyl groups in their structures. However, caffeine showed predominantly hydrophobic (alkyl) and carbon-hydrogen bonds. Multiple natural and synthetic compounds have been investigated as FAS inhibitors in its three domains (Viegas, Neves, Ramos, & Fernandes, 2018). Conversely, there are no reports on the interaction of LPL with caffeoylquinic acids. However, it has been demonstrated their inhibitory activity on pancreatic lipase and LPL homologous protein through the interaction in the catalytic site which demonstrates a correlation between the binding affinity and the *in vitro* inhibitory capacity (Hu et al., 2015). Consequently, these results suggest that the mechanism of action of caffeine is associated to the modulation of gene expression rather than to its interaction with the adipogenesis-related proteins.

3.3. Caffeine reduced body weight gain and fat accumulation in vivo

The *in vitro* results pointed out the beneficial effects of mate and caffeine on adipogenesis. Consequently, the impact of mate and caffeine intake on the attenuation of HFSD-driven lipogenesis and body fat accumulation in rats was evaluated. The intake of 0.1% caffeine in the diet produced significant ($p < 0.05$) outcomes in terms of body weight gain and percentage of body fat (Table 2). Even if the lean mass at the end of the 4-week treatments was not significantly different among groups ($p > 0.05$), the change in the percentage of lean body mass (Fig. 4A) significantly ($p < 0.05$) differ in SC, CC, and MC groups in contrast to the HFSD group. These treatments promoted lean mass in 3.0, 2.2, and 2.7%, respectively. Conversely, the change in body fat (Fig. 4B) was less pronounced in these treatments. SC, CC, and MC exhibited 2.9, 2.3, and 2.7% less increase in body fat. Therefore, the final percentage of body fat (Table 2) was significantly ($p < 0.05$) lower in SC, CC, and MC groups. Likewise, the epididymal fat pad weight seemed to be lower in 0.1% caffeine-fed rats. When the percentage of body fat was measured by a more precise technique than organ weighing, in this case DXA, no differences ($p > 0.05$) were observed in the total body fat accumulation in the DM group. Thus, authors presented both values but mainly referred to body fat accumulation as the gold marker of fat accumulation. Yerba mate previously proved its effects on attenuating weight gain and epididymal fat accumulation in a high-fat-diet obesity model. This anti-adipogenic potential was associated with decreased gene expression of cAMP-responsive element-binding protein (*Creb*) 1, *C/EBPα,* and *Pparγ* (Arçari et al., 2013). Moreover, caffeine has also evidenced its potential in the prevention of weight and fat gain (Kobayashi-Hattori et al., 2005). Nevertheless, the weight of the liver did not suffer any change among treatments; only DM seemed to trigger an increase in the liver. The concentration of serum lipids (Supplementary Fig. 2) did not suffer significant ($p > 0.05$) changes. Both total cholesterol and total triglycerides were similar among groups. Zheng, Qiu, Zhang, and Li (2014) demonstrated the modulation of lipogenic gene expression in the liver and adipose tissue without significant changes in the lipid serum profile following chlorogenic acid and caffeine treatments. These results agree with the observations of this research. The intake of mate tea for 4 weeks along with a high-fat-high-sucrose diet resulted in diminished total triglycerides and total cholesterol without affecting the levels of HDL cholesterol (Gao, Long, et al., 2013). Also, consuming 0.1% caffeine for 8 weeks reduced the serum concentration of triglycerides and cholesterol (Liu & Sayama, 2018). However, another study showed no efficacy in reducing serum lipids with the consumption of mate tea, polyphenols, or saponins from mate (de Resende et al., 2015). Hence, the difference in preparation, dose or time could be responsible for the diverse observed effects.

3.4. Caffeine reduced fatty acid synthase expression in the adipose tissue

Fasn and *AdipoQ* gene expression was measured in the adipose tissue of the rats to understand the molecular mechanisms underlying the reduction in fat accumulation (Fig. 4C, D). *Fasn* gene overexpression has been linked to increasing visceral fat accumulation and insulin resistance (Angeles & Hudkins, 2016). Inhibiting FAS activity has been shown to block adipocyte differentiation and reduce adipocyte number while eliciting thermogenesis and diminishing activation of PPARγ, which resulted in increased energy expenditure (Lodhi et al., 2012). As observed *in vitro*, the mRNA expression of *Fasn* in the adipose tissue was significantly ($p < 0.05$) lower in the group fed SC (57.4%), CC (56.5%), or MC (65.5%) in comparison to the control (Fig. 4C). There were no significant ($p > 0.05$) beneficial effects in *Fasn* mRNA expression in MT and DM treatments. Recently, it was demonstrated that supplementing high-fat diets with 0.6% yerba mate, a higher dose in comparison with this study, was able to reduce *Fasn* expression (Choi, Park, Kim, Kim, & Jung, 2017). To the best of our knowledge, this is the first report on the association of caffeine intake with the reduction of *Fasn* expression in the adipose tissue.

Fig. 4D shows the mRNA expression of *AdipoQ* in the adipose tissue after the 4-week HFSD and 0.1% caffeine supplementation. There were no significant ($p > 0.05$) differences in *AdipoQ* mRNA expression in comparison to the control. *AdipoQ* expression seemed to be higher under MC treatment in comparison to CC and MT treatments. Adiponectin expression in the adipose tissue has been associated with a lower weight, higher insulin sensitivity, and lower TNF-α expression (Kern, Di Gregorio, Lu, Rassouli, & Ranganathan, 2003). Moreover, adiponectin diminished lipogenesis and increased β-oxidation in the liver through the activation of AMP protein kinase (AMPK) and PPARα (Stern, Rutkowski, & Scherer, 2016). A mate extract previously augmented the levels of *AdipoQ* in mice adipose tissue (Arçari et al., 2009). Caffeine demonstrated to maintain adiponectin levels in mice until the second

Table 2
Food intake, body weight, food efficiency, body fat, and epididymal fat pad of rats fed a high-fat high-sucrose-diet containing 0.1% synthetic caffeine (SC), caffeine from coffee (CC), caffeine from mate (MC), mate tea (MT), and decaffeinated mate (DM).

Treatment	Daily food intake (g)	Bodyweight gain (g)	FER*	Lean mass (%)	Body fat (%)	Epididymal fat pad (g)	Liver (g)
HFSD	15.9 ± 0.7^{a}	161.9 ± 13.7^{a}	0.36 ± 0.02^{a}	87.7 ± 3.2^{a}	12.3 ± 1.0^{a}	4.1 ± 0.4^{b}	10.9 ± 0.9^{ab}
SC	14.1 ± 0.8^{b}	136.1 ± 16.4^{b}	0.34 ± 0.02^{ab}	90.7 ± 2.5^{a}	9.3 ± 0.8^{b}	3.1 ± 0.5^{c}	9.9 ± 1.4^{b}
CC	15.7 ± 0.9^{a}	145.4 ± 12.1^{ab}	0.33 ± 0.02^{b}	89.9 ± 2.7^{a}	10.1 ± 1.1^{b}	3.7 ± 0.5^{bc}	11.6 ± 1.0^{ab}
MC	14.5 ± 0.8^{b}	135.2 ± 8.5^{b}	0.33 ± 0.01^{b}	90.4 ± 2.6^{a}	9.6 ± 0.9^{b}	3.5 ± 0.6^{bc}	11.5 ± 2.1^{ab}
MT	16.0 ± 0.7^{a}	161.2 ± 10.0^{a}	0.36 ± 0.01^{a}	88.7 ± 3.0^{a}	11.3 ± 1.2^{a}	4.1 ± 0.4^{b}	11.5 ± 0.7^{ab}
DM	15.8 ± 1.0^{a}	163.9 ± 16.1^{a}	0.37 ± 0.02^{a}	86.9 ± 3.4^{a}	13.1 ± 1.3^{a}	5.1 ± 0.5^{a}	12.1 ± 0.9^{a}

* FER (Food Efficiency Ratio) = (Total weight gain/total food intake). The results are expressed as mean ± SD (n = 8). Different letters among rows denote significant ($p < 0.05$) differences according to ANOVA and Tukey's multiple range test.

week of a high-fat diet, but the effects were not significant from this week to the eighth week (Yun et al., 2008). Thus, the effects of both caffeine and mate on adiponectin expression are still not well understood.

3.5. Caffeine reduced de novo fatty synthesis and lipid transport in the liver

Fig. 5A shows *Pkm* mRNA expression in the liver, a gene from the glycolysis pathway. Pyruvate kinase catalyzes the last but rate-limiting step of glycolysis, producing pyruvate from phosphoenolpyruvate (Gupta & Bamezai, 2010). Pyruvate synthesized in the glycolysis is an intermediary in the conversion of carbohydrates into fatty acids and cholesterol (McCommis & Finck, 2015). *Pkm* expression was significantly ($p < 0.05$) reduced in SC, CC, and MC groups in contrast to the HFSD group. There was no significant difference in PK expression between purified caffeine treatments (SC, CC, and MC reduced the expression in 55.4, 36.4, and 58.5%, respectively). Besides, *Fasn* mRNA expression in the liver was significantly ($p < 0.05$) lowered with purified caffeine treatments (26.5–35.4%) (Fig. 5B). There was no significant ($p > 0.05$) difference in *Fasn* mRNA expression for MT and DM, from the control. The effects of long-term yerba mate intake (1–4% of the diet) on *Fasn* hepatic expression showed decreases in comparison with control high-fat-diets (Choi et al., 2017; Gao, Liu, Wan, Qu, & Chen, 2013). Even if in the present study, MT did not exert a significant effect on *Fasn* expression in the liver, the effect of caffeine (0.1% for 4 weeks) on hepatic lipid synthesis was demonstrated. Similarly, other authors evidenced the impact of caffeine on the decrease of *Fasn* expression in liver (Yamauchi et al., 2010). Carbohydrate response element-binding protein (ChREBP) up-regulates glycolytic genes (*Pkm*) and lipogenic genes (*Fasn*) in response to a high-carbohydrate diet (Iizuka, 2017). Similarly, sterol regulatory element-binding protein (SREBP)-1 can induce *Fasn* expression (Kim et al., 2010). SREBP-1 is down-regulated in the presence of caffeine (Quan, Kim, & Chung, 2013). Thus, ChREBP could also be a potential mediator of the caffeine effect on the expression of *Pkm* and *Fasn* in the liver. Fig. 5C shows *Mttp* mRNA expression in the liver. Microsomal triglyceride transfer protein

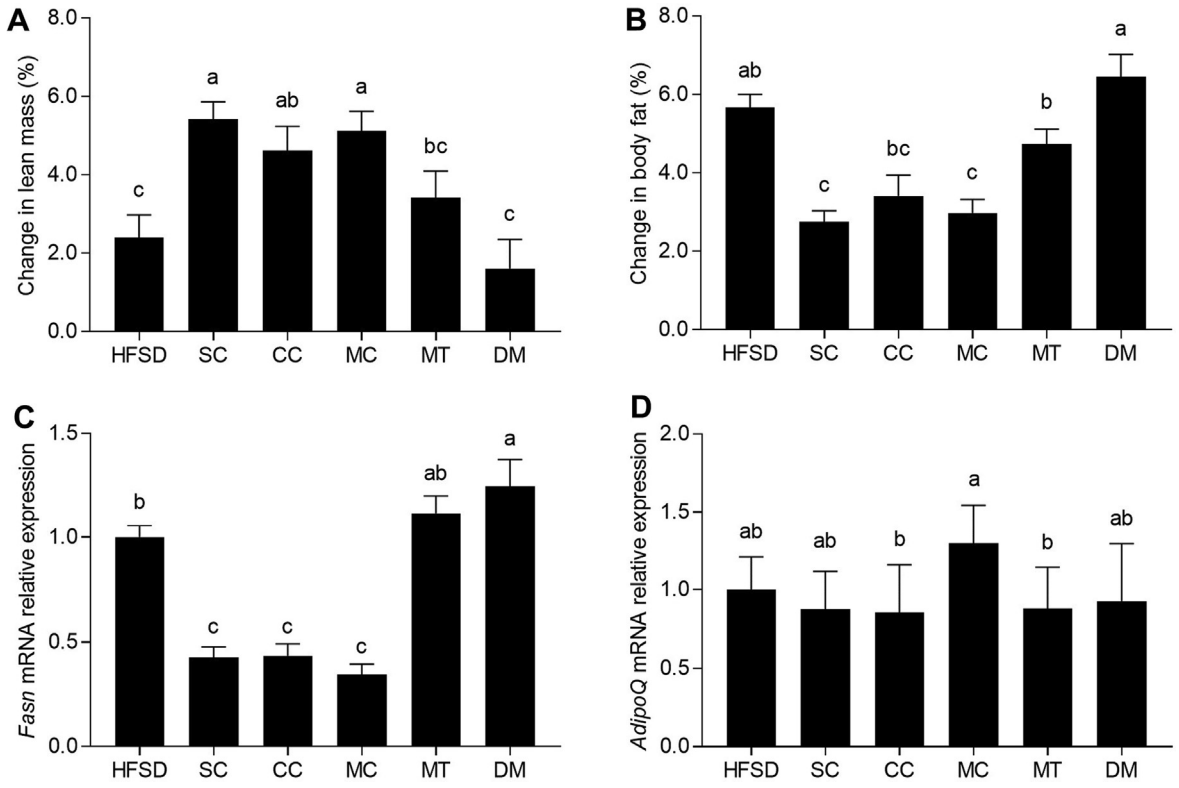
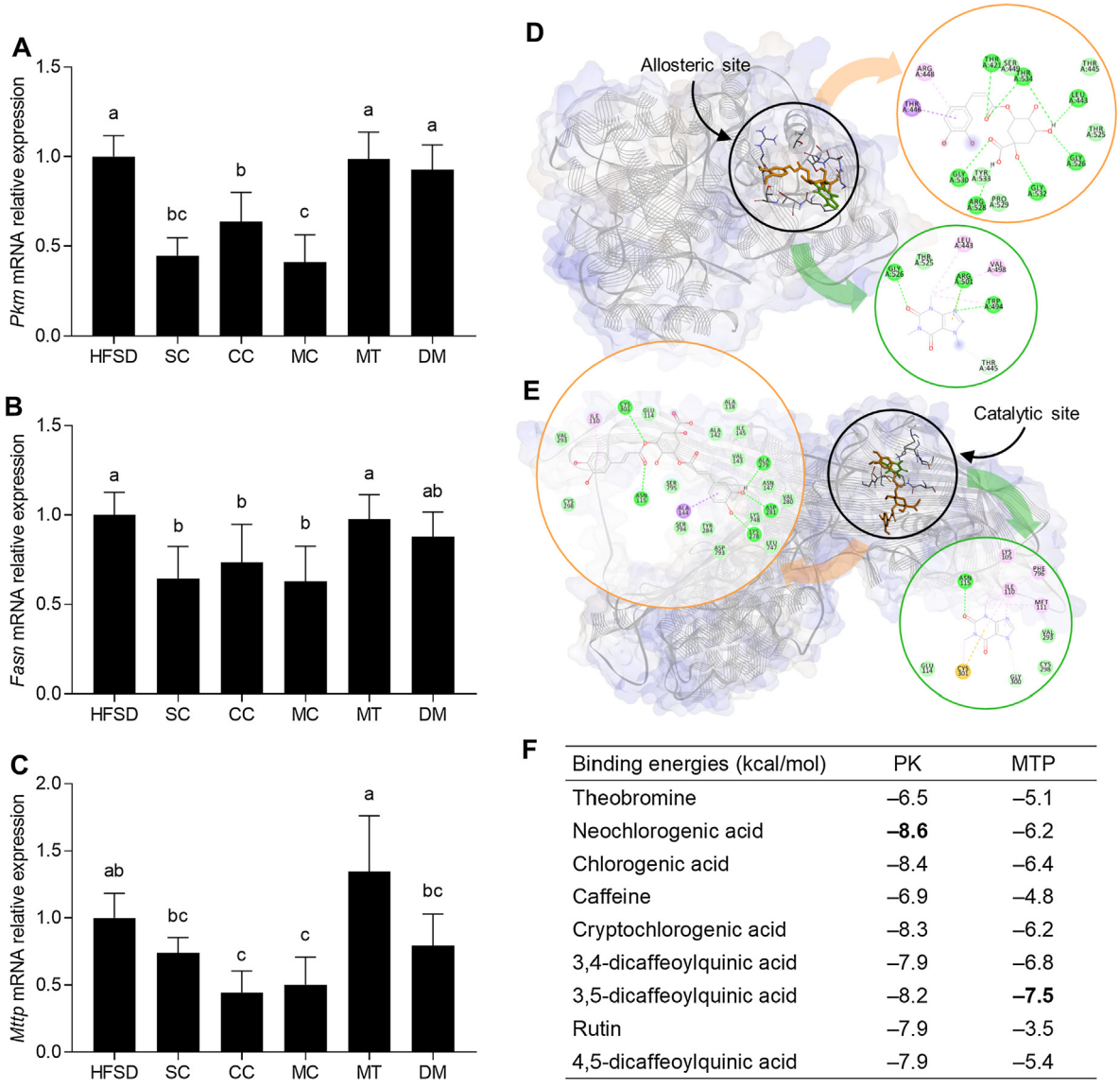


Fig. 4. Effects of 0.1% synthetic caffeine (SC), caffeine from coffee (CC), caffeine from mate (MC), mate tea (MT), and decaffeinated mate (DM) on a high-fat-high-sucrose-diet driven obesity model. Treatments modulated the change in lean mass (A) and body fat (B) percentages from the beginning to the end of the intervention and regulated the mRNA expression of fatty acid synthase (*Fasn*) (C) and adiponectin (*AdipoQ*) (D) in the adipose tissue. HFSD: indicated non-supplemented control diet. The results are expressed as mean ± SD (n = 8). Different letters among columns denote significant ($p < 0.05$) differences according to ANOVA and Tukey's multiple range test.



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Binding energies (kcal/mol)	PK	MTP
Theobromine	–6.5	–5.1
Neochlorogenic acid	**–8.6**	–6.2
Chlorogenic acid	–8.4	–6.4
Caffeine	–6.9	–4.8
Cryptochlorogenic acid	–8.3	–6.2
3,4-dicaffeoylquinic acid	–7.9	–6.8
3,5-dicaffeoylquinic acid	–8.2	**–7.5**
Rutin	–7.9	–3.5
4,5-dicaffeoylquinic acid	–7.9	–5.4

Fig. 5. Impact of 0.1% synthetic caffeine (SC), caffeine from coffee (CC), caffeine from mate (MC), mate tea (MT), and decaffeinated mate (DM) on a high-fat-high-sucrose-diet driven obesity model. Treatments modulated the mRNA expression of pyruvate kinase (*Pkm*) (A), fatty acid synthase (*Fasn*) (B), and microsomal triglyceride transfer protein (*Mttp*) (C) in the liver. HFSD: indicated non-supplemented control diet. The results are expressed as mean ± SD ($n = 8$). Different letters among columns denote significant ($p < 0.05$) differences according to ANOVA and Tukey's multiple range test. Phytochemicals from mate tea exhibited a potential interaction with the lipid metabolism-related enzymes pyruvate kinase (PK) (D) and microsomal triglyceride transfer protein (MTP) (E) with different binding energies (F). Caffeine is depicted in green and the phytochemical with higher affinity in orange and bolded in the table. Inhibitors exhibited the following binding energies: PK: –6.5 kcal mol^{-1}; MTP: –5.7 kcal mol^{-1}. The results were expressed as mean ± SD ($n = 8$). Different letters among columns denote significant ($p < 0.05$) differences according to ANOVA and Tukey's multiple range test.

(MTP) is a transporter protein that has a regulatory role in VLDL synthesis. MTP transfers lipids to apolipoprotein B to form lipoproteins (Walsh & Hussain, 2017). Only CC and MC groups exhibited significantly ($p < 0.05$) reduced *Mttp* expression (55.4 and 49.6%, respectively). There were no significant ($p > 0.05$) lowering effects in SC, MT, and DM groups concerning the control. This is the first report of caffeine and yerba mate intake on *Mttp* hepatic expression to date. The inhibition of MTP results in markedly reduced plasma triglyceride and cholesterol contents, which is proposed as beneficial for the treatment of atherosclerosis (Walsh & Hussain, 2017).

The potential mechanisms of caffeine and the phenolic compounds from mate in the lipogenic process in the liver were evaluated by their potential interaction with pyruvate kinase (PK) and microsomal triglyceride transfer protein (MTP) using *in silico* molecular docking (Fig. 5D–F). Phytochemicals interacted in the allosteric site of PK with binding energies from –6.9 to –8.6 kcal mol^{-1}, being neochlorogenic acid the compound showing the strongest potential interaction. The blockade of the allosteric activation site of PK, by phenolic compounds, could inhibit PK interaction with fructose-1,6-bisphosphate, therefore reducing PK activity (Jurica et al., 1998). The interaction of phytochemicals with MTP was weaker (from –3.5 to –7.5 kcal mol^{-1}). The highest potential was observed for 3,5-dicaffeoylquinic acid. The lower number of hydrogen bonds resulted in weaker binding energies in MTP. Results suggested that phenolic compounds were better potential inhibitors of both PK and MTP. Phytochemicals from traditional Chinese herbs were previously assessed as potential natural MTP inhibitors *in silico*, exhibiting a potential role in MTP regulation (Jiang et al., 2016). The interaction of phenolic compounds in the lipid binding domain of MTP could avoid the transfer of lipids to apolipoprotein B and then hinder lipoprotein assembly (Bradbury et al., 1999). Hence, results

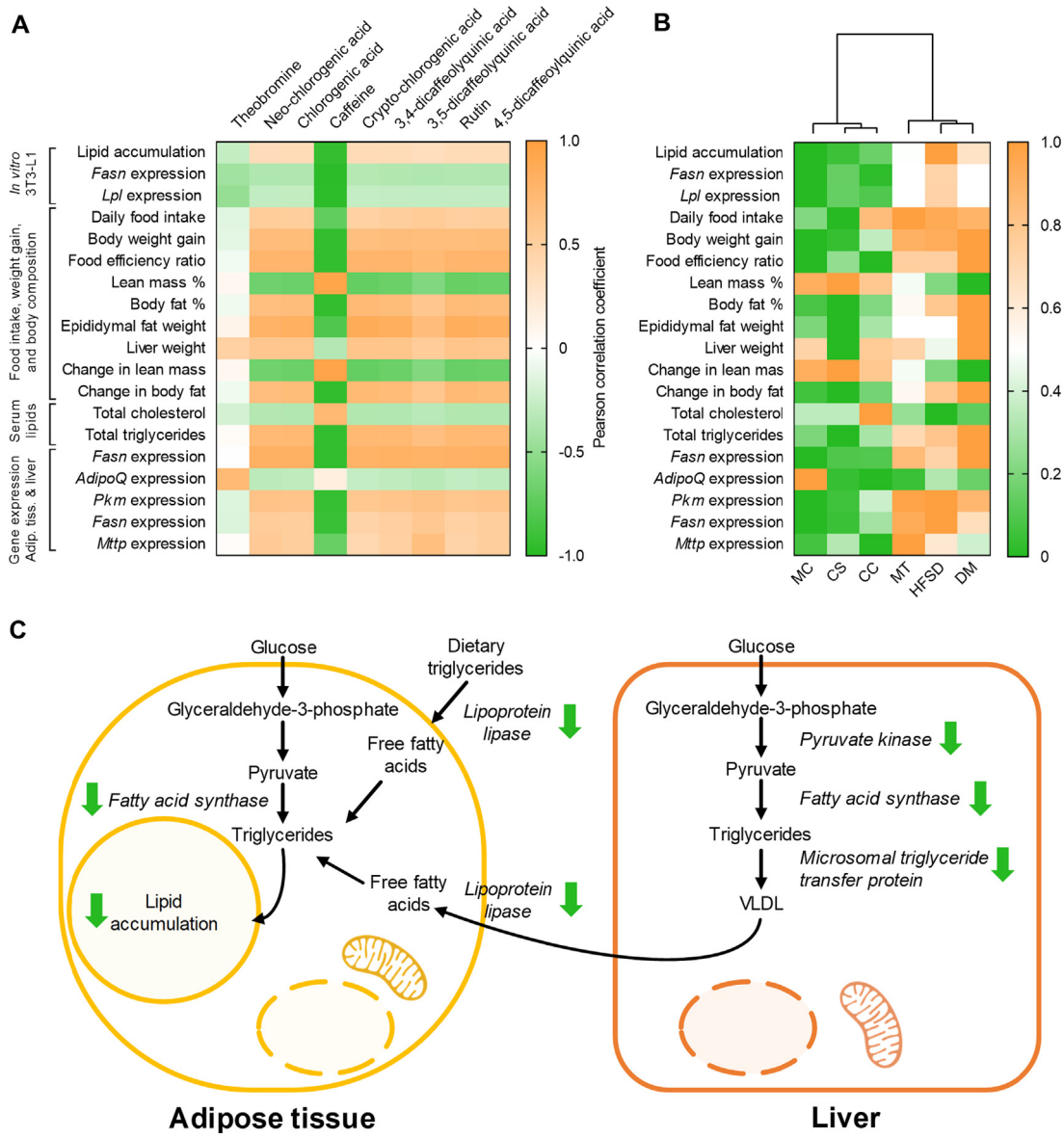

Fig. 6. Heat maps of the Pearson correlation coefficients among the biomarkers measured both *in vitro* and *in vivo* and the area of the compounds in chromatograms (A), hierarchical cluster analysis and heat map of the statistical aggrupation of the different treatments according to their relative biomarkers levels (B), and descriptive diagram of the effects of caffeine from mate tea in the adipose tissue and the liver under high-fat-high-sucrose diet conditions. SC: synthetic caffeine, CC: caffeine from coffee, MC: caffeine from mate, MT: mate tea, DM: decaffeinated mate.

suggest that caffeine mechanisms are not related to its binding capacity to the protein; its possible regulation and its effects in the liver might be due to the regulation of gene expression.

3.6. Caffeine, but not other phytochemicals, in mate tea, attenuated high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation

In this study, the anti-obesity effects of mate tea were mainly due to its concentration of caffeine. Besides observing the effects of the three mate fractions (MT, MC, and DM), Pearson correlations were obtained to observe the relationship of the concentration of each compound on each of the measured biomarkers (Fig. 6A). The concentration of caffeine showed significant negative correlations ($r \leq -0.815$, $p < 0.05$) with all parameters of the *in vitro* study and most of the *in vivo*, excluding food intake, liver weight, total serum cholesterol, and *AdipoQ* and *Mttp* expression. A significant positive correlation was observed with the percentage of lean mass and the change on lean mass during the study ($r = 0.947$ and $r = 0.948$, $p < 0.05$, respectively). No significant correlations were observed with the other phytochemicals found in mate. Hence, results suggest that the effects of yerba mate on lipogenesis and fat accumulation would mainly be associated with the content of caffeine in samples. Furthermore, the effects of caffeine were similar independently of its source (Fig. 6B). MT and DM intake were not efficacious in the prevention of the changes triggered by the high-fat-high-sucrose diet. The loss of function of the decaffeinated mate might be associated with the elimination of caffeine from this treatment. Nonetheless, decaffeinated mate may still have different biological activities not associated with its caffeine content. In previous research, there was an effect of polyphenols from yerba mate extract on markers of insulin resistance and inflammation in mice with high fat diet-induced obesity (Arçari et al., 2011; Riachi & De Maria, 2017).

There were no reports on the use of decaffeinated yerba mate on the prevention of obesity and related diseases. Caffeine action is mediated through the activation of A_2 adenosine receptors and the inhibition of phosphodiesterase. These processes trigger an increase in cAMP content and PKA activity, which evokes an increase in lipolysis in the adipose

tissue (da Silva et al., 2017). Hence, caffeine could not only be inhibiting the synthesis of lipids in adipocytes but also prompting a mild lipolysis. The combined results would be a lower lipid accumulation in the adipose tissue (Carrageta et al., 2018). Moreover, it has been proposed that some bioactive compound could antagonize the effects of caffeine. Several reports indicated that at equal caffeine intake, pure caffeine has exerted more potent metabolic effects than coffee (Graham, Hibbert, & Sathasivam, 1998; Hodgson, Randell, & Jeukendrup, 2013). These results were attributed to the antagonistic effect of chlorogenic acid on the adenosine receptor (de Paulis et al., 2002). Additionally, the lower effects of MT in comparison with MC could be explained by the different metabolism of caffeine in the presence of phenolic compounds. Caffeine is metabolized in the liver by CYP1A2, an isoenzyme of cytochrome P450. CYP1A2 can be modulated by the intake of different foods and phytochemicals (Hodges & Minich, 2015). According to previous studies, chlorogenic acids present in yerba mate could be abating caffeine effects by eliciting its metabolism and degradation into other molecules via the modulation of CYP1A2 activity (Carrillo & Benitez, 2000; Nehlig, 2018; Pang, Sheng, Jiang, Wei, & Ji, 2015).

Moreover, the correlation between biomarkers from the *in vitro* and *in vivo* studies (Supplementary Fig. 3) highlighted the positive relationship among *in vitro* and *in vivo* measurements. Lipid accumulation in 3T3-L1 adipocytes was significantly associated with body weight gain, and the percentage of body fat ($r \geq 0.893$, $p < 0.05$). Therefore, the results support the use of *in vitro* models for the evaluation of natural products with potential against adipogenesis. Likewise, the body weight gain and the percentage of body fat were associated with *Fasn* expression in the adipose tissue ($r \geq 0.930$, $p < 0.05$) and the liver ($r \geq 0.831$, $p < 0.05$). Increased *Fasn* gene expression in adipose tissue has been linked to visceral fat accumulation (Berndt et al., 2007). However, the contribution of the adipose tissue to whole-body lipogenesis is considered to be lower than that of the liver (Menendez et al., 2009).

Fig. 6C shows the mechanism of action of caffeine in lipid metabolism of male Sprague-Dawley rats fed with a high-fat-high-sucrose diet. The consumption of mate, caffeine from mate, or caffeine from other sources alleviated the negative impact of a high-fat-high-sucrose diet on body composition due to the modulation of certain lipogenic enzymes in both adipose tissue and liver. The decreased of *Fasn* and *Lpl* expression conduced to a lower synthesis and accumulation of triglycerides in the adipose tissue. Concomitantly, the diminished expression of *Pkm*, *Fasn*, and *Mttp* in liver evoked a lower synthesis of triglycerides and its posterior secretion as VLDL. Although *in silico* results could not explain why caffeine had stronger effects on lipogenesis, these experiments suggested that the effects might not be associated with the inhibition of enzymes activity, since caffeine presented potential low binding energies, in comparison with the other phytochemicals.

The intake of 0.1% caffeine in the different treatments is equivalent to drinking 4 cups of coffee per day in humans. The amount of caffeine per serving in mate tea is 65–130 mg. A typical cup of brewed coffee contains approximately 30–300 mg of caffeine, and a cup of tea contains 15–60 mg of caffeine (Gonzalez de Mejia & Ramirez-Mares, 2014). Caffeine consumption from different sources reaches 210–238 mg/person per day in the USA (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014). When consumed in amounts below 400 mg a day, caffeine exhibits no adverse effects on human health (Heckman, Weil, & de Mejia, 2010). Thus, the results of this research could be scaled to humans supporting the intake of mate and caffeine as dietary strategies in the prevention of overweight and obesity, as well as the subsequent metabolic disorders associated. Although some studies have been performed, there are limited controlled clinical trials on the effects of yerba mate on human health. Phytochemicals from yerba mate should also be assessed to comprehend their contribution to physiological effects (Riachi & De Maria, 2017).

4. Conclusions

In summary, caffeine from natural (mate and coffee) and synthetic sources promoted reduction of body fat accumulation in animals fed with a high-fat-high-sucrose diet. This study presents the composition of three mate products (mate tea, caffeine from mate, and decaffeinated mate) and their effects *in vitro*, *in silico*, and *in vivo*. Considering the results, mate tea and caffeine can be considered as anti-obesity agents, being caffeine the most active compound in mate tea attenuating high-fat-high-sucrose-diet-driven lipogenesis and body fat accumulation *in vitro* and *in vivo*. In conclusion, caffeine is defined as responsible for the effects of yerba mate tea on adipogenesis, lipogenesis, and body fat accumulation based on *in vitro*, *in silico* and *in vivo* results. The limitations of this research were the lack of hepatic triglyceride levels and the effects on all genes regulating lipid metabolism; these will be the objective of future communications. Future studies will unravel the synergisms among caffeine and the other phytochemicals found in mate tea.

Abbreviations

3,4diCGA	3,4-dicaffeoylquinic acid
3,5diCGA	3,5-dicaffeoylquinic acid
4,5diCGA	4,5-dicaffeoylquinic acid
AdipoQ	adiponectin
AMPK	AMP protein kinase
βKR	β-ketoacyl reductase
C/EBP	CCAAT/enhancer-binding protein
CC	caffeine from coffee
CAF	caffeine
CGA	chlorogenic acid
ChREBP	carbohydrate response element-binding protein
CREB	cAMP-responsive element-binding protein
CRYPTO	cryptochlorogenic acid
CYP1A2	cytochrome P450 family 1 subfamily A member 2
DM	decaffeinated mate tea
DMEM	Dulbecco's modified Eagle's medium
ENR	enoyl-acyl carrier-protein reductase
FAS	fatty acid synthase (protein)
Fasn	fatty acid synthase (gene)
FBS	fetal bovine serum
FER	food efficiency ratio
HDL	high-density lipoprotein
HFSD	high-fat-high-sucrose diet
LDL	low density lipoprotein
Lpl	lipoprotein lipase (gene)
LPL	lipoprotein lipase (protein)
MC	caffeine from mate
MT	mate tea
MTP	microsomal triglyceride transfer protein (protein)
Mttp	microsomal triglyceride transfer protein (gene)
NBS	newborn bovine calf serum
NEO	neochlorogenic acid
PK	pyruvate kinase (protein)
Pkm	pyruvate kinase (gene)
PPAR	peroxisome proliferator-activated receptor
RUT	rutin
SC	synthetic caffeine
SREBP	sterol regulatory element-binding protein
T2D	type 2 diabetes
TE	thioesterase
THEO	theobromine
TNF-α	tumor necrosis factor α
VLDL	very low-density lipoprotein

Ethics Statements File

Authors followed the guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2019.103646.

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