UNIVERSIDADE FEDERAL DA BAHIA

FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DOS ALIMENTOS

ANA PAULA AZEVÊDO MACÊDO

EFEITO DO CONSUMO REGULAR DO CHÁ VERDE SOBRE ALTERAÇÕES METABÓLICAS INDUZIDAS POR DIETA HIPERLIPÍDICA EM CAMUNDONGOS

SALVADOR- BA

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Orientador: Prof. Dr. Ricardo David Couto

Coorientadora: Profa. Dra. Jairza Maria Barreto Medeiros

Dissertação apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Faculdade de Farmácia da Universidade Federal da Bahia, como requisito final para obtenção do título de Mestre.

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Dissertação submetida ao Programa de Pós-Graduação em Ciência de Alimentos (nível Mestrado Acadêmico) da Faculdade de Farmácia da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de mestre em Ciência de Alimentos.

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Dedico este trabalho a todos que acreditam que a educação e a ciência são capazes de mudar o mundo.

O começo de todas as ciências é o espanto de
as coisas serem o que são!

Aristóteles

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RESUMO

A obesidade é uma desordem complexa associada a consequências adversas para a saúde, incluindo dislipidemia, hipertensão arterial, diabetes mellitus tipo 2 e esteatose hepática não alcoólica. Como esta enfermidade apresenta números crescentes, tem se buscado cada vez mais tratamentos alternativos como fitoterápicos, dado ao baixo custo e fácil acesso, principalmente por consumo de chás. O objetivo desse trabalho foi avaliar o efeito do consumo regular da infusão do chá verde (*Camellia sinensis*) sobre as alterações metabólicas em camundongos submetidos ao consumo da dieta hiperlipídica. Os camundongos machos C57/BL6 (n=35) com dois meses de vida foram distribuídos em quatro grupos: dieta controle (10% de lipídios) mais água autoclavada ou chá verde e dieta hiperlipídica (58% de lipídios) mais água autoclavada ou chá verde por 16 semanas. O peso e circunferência abdominal foram aferidos quinzenalmente, a aferição da glicemia foi realizada a cada quatro semanas de experimento. Testes orais de tolerância à glicose e insulina foram realizados na última semana. Na décima sexta semana, após a eutanásia do animal, o tecido adiposo foi dissecado e realizada a pesagem do mesmo. O consumo do chá verde em camundongos alimentados com dieta hiperlipídica promoveu redução de peso na sexta e na décima sexta semana ($p<0,01$), redução significativa na circunferência abdominal na quarta semana ($p<0,05$) e continuamente a partir da oitava semana ($p<0,05$; $p<0,01$). A infusão do chá verde reduziu a deposição de tecido adiposo em todos os compartimentos analisados ($p<0,001$), inclusive do tecido adiposo marrom ($p<0,01$). A glicemia de jejum apresentou redução significativa apenas na décima sexta semana ($p<0,05$) e o pico de glicemia no tempo 15 minutos do teste de tolerância oral a glicose ($p<0,01$) apresentou redução significativa no grupo com dieta hiperlipídica que consumiu chá verde. Em conclusão, o chá verde promoveu redução de peso e circunferência da abdominal, assim como a deposição de tecido adiposo em todos os compartimentos analisados, em camundongos C57/BL6 submetidos ao consumo de dieta hiperlipídica.

Palavras-chave: *Camellia sinennis*, obesidade, tecido adiposo, glicemia e insulina.

ABSTRACT

Obesity is a complex disorder associated with adverse health consequences, including dyslipidemia, high blood pressure, type 2 diabetes, and non-alcoholic liver steatosis. As this disease is increasing, more and more alternative treatments such as herbal medicines are being sought, due to the low cost and easy access, mainly through the consumption of teas. The objective of this study was to evaluate the effect of a green tea (Camellia sinensis) infusion regular consumption on metabolic changes in mice and the use of a high-fat diet. The C57 / BL6 mice were segregated into four groups: control diet plus autoclaved water or green tea and high-fat diet plus autoclaved water or green tea for 16 weeks. Weight and waist circumference were measured every two weeks and blood glucose was measured every four weeks of experience. Oral glucose and insulin tolerance tests were performed in the last week. In the sixteenth week, after the animal was euthanized, the adipose tissue was dissected and weighed. The consumption of green tea in mice fed a high-fat diet promoted a weight reduction in the sixth and sixteenth weeks ($p < 0.01$), a significant reduction in waist circumference in the fourth week ($p < 0.05$) and continuously from the eighth week ($p < 0.05$; $p < 0.01$). The infusion of green tea reduced the deposition of adipose tissue in all analyzed compartments ($p < 0.001$), including brown adipose tissue ($p < 0.01$). Fasting blood glucose showed a significant reduction only in the sixteenth week ($p < 0.05$) and the peak of blood glucose in the 15-minute time of the oral glucose tolerance test ($p < 0.01$) showed a significant reduction for the high-fat group that consumed green tea. In conclusion, green tea promoted a reduction in weight and waist circumference, as well as the deposition of adipose tissue in all compartments analyzed in C57/BL6 mice submitted to the consumption of a high-fat diet.

Keywords: *Camellia sinennis*, obesity, adipose tissue, blood glucose and insulin.

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LISTA DE ABREVIATURAS E SIGLAS

ACC2	Acetyl-CoA Carboxylase 2
AGCL	Long-Chain Fatty Acids
BAT	Brown Adipose Tissue
BMP-7	Bone Morphogenetic Protein 7
cAMP	Cyclic Adenosine Monophosphate
COMT	Catechol-O-Methyl Transferase
CPT-1β	Carnitine Palmitoyltransferase I beta
DCNT	Doenças Crônicas Não Transmissíveis
DM	Diabetes Mellitus
ECG	Epicatechin
EGCG	Epigallocatechin 3-gallate
EGC	Epigallocatechin
EPI	Epicatechin
FASN	Fatty Acid Synthase
FGF-21	Fibroblast Growth Factor 21
GTE	Green Tea Extract
HFD	High-fat Diet
HPLC	High-Performance Liquid Chromatography
LAFTE	Laboratory of Pharmacology and Experimental Therapeutics
MUFA	Monounsaturated Fatty Acids

NAD	Nicotinamide Adenine Dinucleotide
NASH	Non-alcoholic Steatohepatitis
NRF1	Nuclear Respiratory Factor 1
PPARγ	Peroxisome Proliferator Type Gamma
PGC-1α	Peroxisome proliferator Co-activator 1-alpha
SBCAL	Brazilian Society of Science in Laboratory Animals
SIRT1	Sirtuin 1
TFAM	Mitochondrial Transcription Factor A
TLE-3	Transducin-like Enhancer Protein-3
UCP1	Uncoupling Protein-1
UCP2	Uncoupling Protein 2
WAT	White Adipose Tissue

SUMÁRIO

1. INTRODUÇÃO

A obesidade é um importante problema de saúde pública, considerada como a epidemia moderna devido aos dados epidemiológicos alarmantes e a sua associação com várias doenças crônicas não transmissíveis (DCNT) (GOOSSENS, 2017). Esta enfermidade caracteriza-se por acúmulo excessivo de tecido adiposo no corpo que apresenta impactos adversos no metabolismo e predispõe a complicações metabólicas como hiperglicemia, resistência à insulina, dislipidemia e hipertensão (XU et al., 2018).

A obesidade é reconhecida como condição multifatorial, amplamente explicada por fatores ambientais, como ingestão excessiva de energia e inatividade física apontadas como os principais responsáveis (QASIM et al., 2018). Pois desequilíbrio do balanço energético pode levar ao aumento da adiposidade e/ou obesidade (XU et al., 2018).

Atualmente, a ingestão calórica excessiva é considerada a maior causa para o surgimento desta doença (COPE, GOULD, 2017). Visto que no padrão dietético atual identifica-se maior ingestão de calorias e aumento no consumo de alimentos ricos em lipídios, principalmente em gordura saturada (NASREDDINE et al., 2018; URLACHER, KRAMER, 2018). Alimentos altamente palatáveis, como alimentos ultraprocessados, ricos em açúcares e gorduras, estão associados ao aumento da ingestão devido a suas fortes qualidades hedônicas, e podem levar a excessos e à deposição de gordura corporal (COPE, GOULD, 2017).

Dessa forma, as estratégias preventivas e terapêuticas paras essas alterações metabólicas consistem na perda de peso e normalização dos parâmetros bioquímicos e metabólicos (SAMADIAN, DALILI, JAMALIAN, 2016; TAPSELL et al., 2017). Dietas restritas em calorias juntamente com o aumento da atividade física e do apoio comportamental é a primeira linha de tratamento para moderar a obesidade e o excesso de peso, entretanto essa abordagem não tem apresentado resultados eficazes em longo prazo na população em geral (MORENO et al, 2016).

Nesse contexto, novas alternativas têm sido investigadas para o tratamento da obesidade e doenças associadas. Dentre as alternativas destaca-se o chá verde (*Camellia*

sinensis) devido às suas propriedades: antioxidante, anti-inflamatória e ação termogênica (JANSSENS, HURSEL, WESTERTERP-PLANTENGA, 2016).

O chá verde é obtido a partir da secagem de folhas de *Camellia sinensis*, e os principais itens da sua composição incluem compostos fenólicos, cafeína, carboidratos, aminoácidos e certos micronutrientes como as vitaminas E, B e C, cálcio, magnésio, zinco, potássio e ferro (CUNHA et al., 2003). Os principais flavonoides presentes no chá verde são as catequinas do chá verde, como: a catequina (C), a galocatequina (GC), a epicatequina (EC), a epigalocatequina (EGC), a epicatequinagalato (ECG) e a epigalocatequinagalato (EGCG) (ZHANG et al., 2018.). As evidências científicas mostram que as catequinas são os principais responsáveis pelos efeitos benéficos do chá verde (JURGENS et al., 2012.; TÜRKÖZÜ, TEK, 2017).

São diversos os estudos experimentais que avaliam os efeitos do chá verde sobre a obesidade e suas comorbidades associadas, entretanto a forma mais utilizada para a administração do chá verde é o extrato (CHEN et al., 2017; DEY et al., 2019). Embora o consumo do chá verde no mundo se dê principalmente sob a forma de infusão (TROISI et al., 2019) poucas pesquisas experimentais utilizam essa forma de administração do chá. Devido a isso é de suma importância avaliar os efeitos da ingestão regular da infusão do chá verde sobre as alterações metabólicas em camundongos submetidos ao consumo da dieta hiperlipídica.

2. OBJETIVOS

2.1 GERAL

Avaliar o efeito do consumo regular da infusão do chá verde (*Camellia sinensis*) sobre as alterações metabólicas em camundongos submetidos ao consumo da dieta hiperlipídica.

2.2 ESPECÍFICOS

Avaliar os efeitos do consumo regular de chá verde (*Camellia sinensis*) em camundongos submetidos ao consumo da dieta hiperlipídica sobre:

- Evolução ponderal;
- Circunferência abdominal;
- Consumo alimentar;
- Consumo de líquidos;
- Marcadores metabólicos (glicemia, teste de tolerância oral a glicose e insulina);
- Peso dos órgãos (tecido adiposo marrom interescapsular, tecido adiposo branco visceral, epididimal e inguinal);

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3. CAPÍTULO I – REVISÃO DE LITERATURA

Este capítulo refere-se a revisão de literatura e está organizado sob formato de artigo científico que foi submetido a SN Comprehensive Clinical Medicine.

GREEN TEA INDUCES THE BROWNING OF ADIPOSE TISSUE - SYSTEMATIC REVIEW

ABSTRACT

Purpose of Review Several foods and nutrients are being studied a lot because they have a positive effect on thermogenesis and the browning of white adipose tissue. Therefore, this study aims to evaluate, through a systematic review, the effect of green tea for inducing browning of adipose tissue.

Recent Findings Ten experimental articles were included in rodents that used green tea in animals induced by obesity. Green tea reduced the weight of white and brown adipose tissue, positively regulated gene expression and microRNA that regulate the metabolism of adipose tissue and morphological changes were identified as beige tissue. According to the results found, the factors involved in this induction to browning are PPARγ, PGC-1α, UCP1, CPT, and PRDM16.

Summary Therefore, green tea promotes the browning of adipose tissue in rodents. It is important to emphasize the need for studies in obese humans to identify whether the same metabolic response occurs.

Key words: *Camellia sinensis*, brown adipose tissue, UCP1, obesity.

3.1 INTRODUTION

Adipocytes are cells specialized in the storage of lipids as triacylglycerol in their cytoplasm [1]. In mammals, there are two types of adipose tissue: white (WAT) and brown (BAT) [2]. The mature white adipocyte stores the triglycerides in a single large lipid molecule that occupies 85-90% of the cytoplasm and pushes the nucleus and a thin layer of the cytosol to the cell periphery [3]. The brown adipose tissue aims to maintain body temperature since it has a greater amount of mitochondria and the ability to metabolize large energetic substrates to produce heat [1,2].

The main difference between BAT and WAT is the presence or absence of the uncoupling protein-1 (UCP-1) activity, located in the inner mitochondrial membrane of the brown adipose tissue cells [3]. UCP1 participates in adaptive thermogenesis by decoupling the production of adenosine triphosphate from the lipids and carbohydrates catabolic pathways. The derived energy is released by the brown adipocytes as heat diffusing in the body, gratefully to the BAT rich vascularization [4].

Recently, beige adipocytes have been reported in the scientific literature. These are adipocytes located in the WAT but resemble the brown adipocyte phenotype. The appearance of beige adipose tissue is due to the "browning", a process characterized by an increase in the WAT mitochondria density and metabolic function [5].

Nutrients and foods are being studied a lot because they have a positive effect on thermogenesis and browning of WAT, such as capsaicin, vitamins A, D and E, omega 3, resveratrol, safflower and flaxseed oils [4,5,6,7]. Green tea is studied due to its different

mechanisms of anti-obesity action [8]. And this study aims to evaluate, through a systematic review, the effect of green tea to induce browning of adipose tissue.

3.2 METHODS

3.2.1 DATA SOURCES AND SEARCH STRATEGY

A search was performed in the following electronic databases: PubMed (Medline), Science Direct, Scopus, and Web of Science. The search for articles published in all years until February 2020 was delimited. The following MESH terms were used: green tea, catechin, *Camellia sinensis*, browning, brown adipose tissue, white adipose tissue. Boolean operators "AND" and "OR" were used to cross the terms as follows: (green tea OR *Camellia sinensis* OR catechin) AND (brown adipose tissue OR white adipose tissue).

3.2.2 SELECTION AND STUDY ELIGIBILITY

The evaluation of titles, abstracts, and complete papers was carried out following the steps of identification, screening, eligibility, and inclusion in February 2020. The articles selection was carried out by two researchers adopting the following inclusion criteria: experimental studies in animals (i.e. rats and mice) induced to obesity and exposed to the infusion of green tea, green tea extract or catechins; research on obese humans who consumed the infusion of green tea, green tea extract or catechins; and studies that evaluated the induction of browning of adipose tissue and published in English.

The non-inclusion criteria were: studies that did not use green tea, the association of green tea with other substances, herbal medicines or caloric restriction, review articles with humans or other animal species, experimental studies in genetically modified animals, experimental studies in which the method of obesity induction has not been the diet, animals submitted to physical exercise, even if voluntary, exposure to cold, studies that used beta-adrenergic system suppressant medication and in vitro studies.

Then, the screening was carried out, and the duplicate records were eliminated. In the next stage, the articles' eligibility was considered, bearing in mind the methodology, by eliminating those that did not correspond to experimental studies. After reading the entire consulted articles content, the records in the chosen databases were counted according to the inclusion criteria.

3.2.3 DATA EXTRACTION AND ANALYSIS

Each included article was read thoroughly, and pertinent information was extracted. Data extracted from each study were: the animal species, the green tea form of administration, the green tea dosage, extract or catechins, total catechins, duration, and the main results obtained.

The articles' quality assessment was carried out according to the ARRIVE guidelines - Animals in Research: Reporting Experiences at Vivo [9]. To assess the articles' adequacy to the ARRIVE Guidelines a scoring system (0 - no; 1 - yes) was used for the 20 listed items.

3.3 RESULTS

The systematic review flow diagram is shown in Fig. 1. The systematic search strategy retrieved 1781 records from the databases: PubMed (94), Science Direct (1423), Web of Science (250), and Scopus (14). Titles and abstracts were assessed and initially, 25 studies were considered eligible. After full-text review, 10 articles were deemed eligible is shown in Table 1. Human studies were not found.

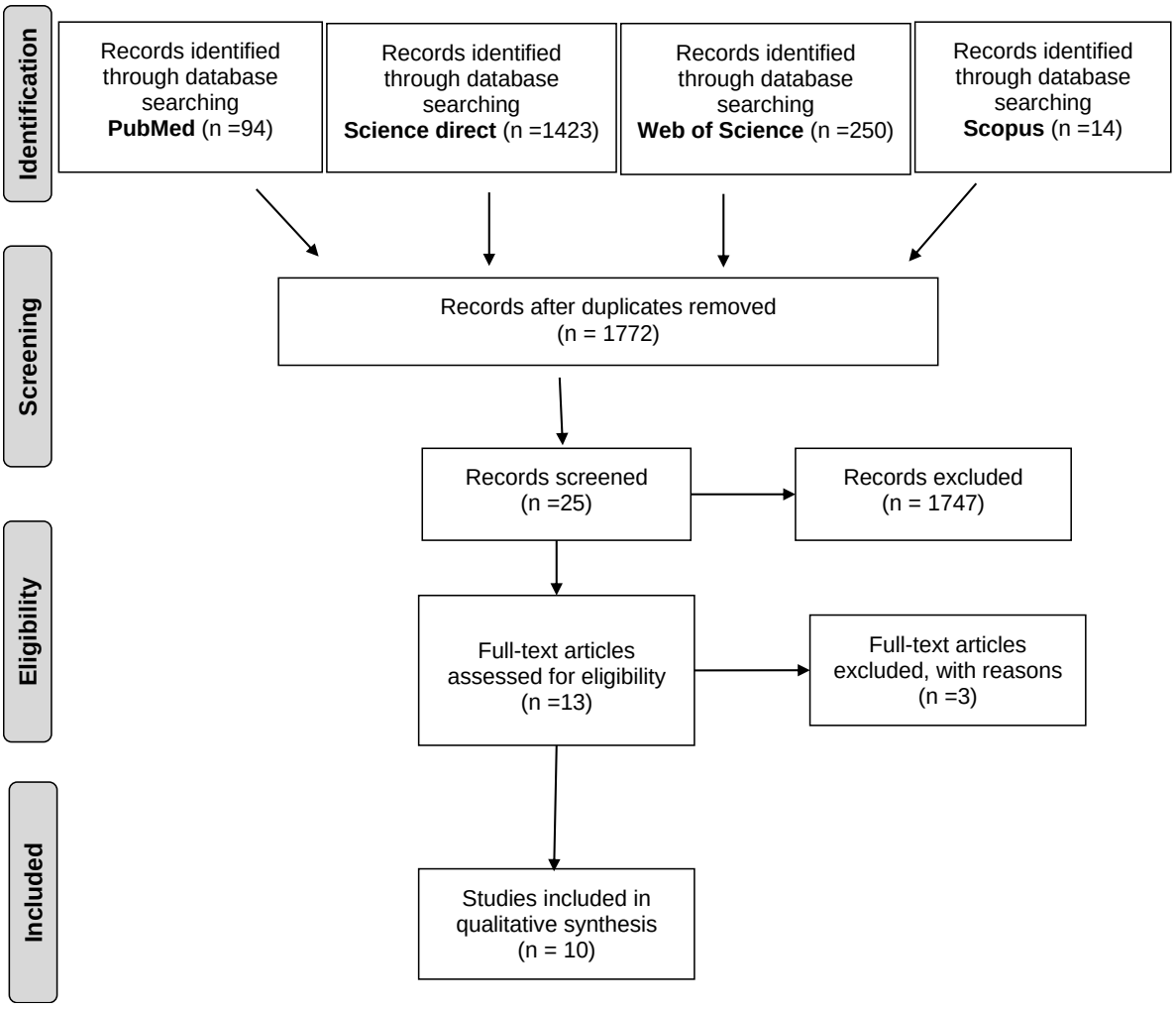

Figure 1 – Flowchart of the selection stages of articles (adapted from PRISMA).

Table 1. Experimental studies in rodents that use green tea to induce browning or adipose tissue.

Author/year	Specie	Type of diet	Method of administration	Administered dose	Catechin content	Duration	Results
Chen et al. 2017 [15]	Rats (Sprague Dawley) induced obesity by diet	High-energy diet	Green Tea Extract	77.5mg.kg^{-1} per day of extract of green tea 155mg.kg-1 per day of extract of green tea	83,5% of catechins	8 weeks	eWAT → adipocytes were much smaller in the treated group compared to the high calorie diet; Significant increase in the expression of PPAR-γ, PRDM-16, BMP-7, FGF-21 and PGC-1α and reduction of TLE-3 in treated groups.
Klaus et al. 2005 [19]	Mice (New Zealand black - NZB) induced obesity by diet	High-fat diet (17% protein, 15% lipids and 42% carbohydrates)	EGCG	0.5% and 1% of TEAVIGO ™	94% of EGCG	4 weeks (induction obesity) + 4 weeks (treatment)	There were no changes in gene expression in BAT; WAT → SCD1 expression was reduced in groups s treated with EGCG.
Lee et al. 2017 [13]	Mice (C57BL/6J) induced obesity by diet	High-fat diet (60% lipids)	EGCG	0.2% added in the diet	-	8 weeks (induction obesity) + 8 weeks (treatment)	The weights of WAT and BAT were decreased by 45% and 34%, respectively, in the group treated with EGCG compared to the high-fat group; BAT→EGCG in the diet significantly increased the expression of UCP1, UCP2, PRDM16, PGC-1α, NRF1, TFAM and CPT-1β and decreased of ACC2, compared to the high-fat group.
Mi et al. 2017 [10]	Mice (C57BL/6J) induced obesity by diet	High-fat and high fructose diet (45% lipids and 10% fructose)	EGCG	2 g EGCG per liter of water	-	16 weeks	BAT →EGCG intake restored average cell size and distribution; increased Sirt1 and Cpt2 and reduced Fasn; WAT → EGCG prevented HFFD-induced adipocyte hypertrophy and the uneven size distribution common to iWAT and eWAT; EGCG increased Sirt, PGC-1α and Cpt2 and reduced PPARγ and Fasn.
Neyrinck et al.	Mice (C57BL/6J) induced obesity by	High-fat diet (60% lipids)	Green Tea Extract	0.5% extract of green tea added in	60% of	8 weeks	sWAT → GTE treatment reduced the weight, the size of the adipocytes, significantly increased the

2017 [11]	diet			the diet	catechins 30% of EGCG		expression of PPARγ, PGC-1α, Prdm16 and Cited1; BAT→ GTE treatment promoted normalization of weight and reduced size of lipid droplets in cells; significant reduction in the expression of C/EBPα and aP2; up-regulation of PGC-1α, Vegfa165; Beige adipocytes were defined by their multilocular lipid droplet morphology.
Nomura et al. 2008 [17]	Rats (Sprague Dawley) induced obesity by diet	High-fat diet (60% fat)	Green Tea catechin	0.5% added in the diet	81.5% of catechins (EGCG - 40.6%, ECG – 23.1%, EGC - 12.4%, EPI - 9.2%)	8 weeks	BAT - weight reduction in treated animals Control group treated with GTC showed UCP1 mRNA expression 70% higher than animals fed a control diet; High-fat group showed similar mRNA expressions from the three UCPs.
Otton et al. 2018 [12]	Mice (C57BL/6) induced to obesity by diet	High-fat diet (20% protein, 36% carbohydrates and 34% lipids)	Green Tea Extract	500 $mg.kg^{-1}$ of body weight per day	30% of catechins	4 weeks (inducation obesity) + 12 weeks (treatment)	BAT → weight and adipocyte reduction; sWAT and eWAT → weight and adipocyte reduction; eWAT → In the OB + GT group, only miR-802 was increased and 3 miRNAs were reduced (miR-335, miR-221, miR-155).
Santana et al. 2015 [18]	Swiss mice induced obesity by diet	High-fat diet	EGCG	50 $mg.kg^{-1}$ of body weight per day	-	8 weeks	It did not promote changes in the high-fat group treated with EGCG.
Yan et al. 2013 [16]	Rats (Sprague Dawley) induced obesity by diet	High-fat diet (15% saturated fat and 1% cholesterol)	Green Tea Catechin	100 $mg.kg^{-1}$ of body weight per day	50%- EGCG, 22% - ECG, 18% - EGC and 10% - EPI	6 weeks	sWAT and vWAT →GTCs increased the PPARγ and UCP-1; BAT → PPARγ level increased, significantly increased the expression of CPT1, AOX, and UCP-1.
Zhou et al.	Mice (C57BL/6)	High-fat diet	EGCG	1% EGCG of the	-	4 weeks	BAT→ increased expression of UCP1, PGC-1α and

2018 [14]	induced by diet to obesity	(60% lipids)	diet composed	PRDM16 in the HFD + EGCG group.

sWAT – subcutaneos White Adipose Tissue, eWAT- epididymal White Adipose Tissue, vWAT- visceral White Adipose Tissue BAT- Brown Adipose Tissue, EGCG – Epigallocatequinagalate, ECG - epicatechin gallate, EGC - epigallocatechin (EGC), EPI – epicatechin, GT – Green Tea, GTE- Green tea extract, HFD – High-fat diet, GTC – Green tea catechin, OB - Obese. Source: Own authorship.

Ten experimental articles were found in induced obesity rodents that used green tea. Of which, five used the C57BL/6J mouse [10,11, 12,13,14], followed by three using the Sprague Dawley rats [15,16,17], a single study using the Swiss mice [18], and another study using the New Zealand black [19].

Eight of these studies used the high-fat diet with the fat percentage ranging from 15 to 60% [11-14,16,17,19]. One of the studies did not report the percentage [18]. The other studies submitted the animals to hypercaloric [15] and high-fat diets, 45% fat combined with 10% fructose [10].

The green tea was administered in the form of an extract [11,12,15]. Only isolated epigallocatechin [10,13,14,18,19] and the combination of green tea catechins [16,17]. Green tea extract was added to the diet at a concentration of 0.5% [11]. The dose was also administered per kilo of weight, 77.5mg, and 155mg[15], and 500mg [12]. The dose of EGCG ranged from 0.2 to 1% added to the diet [13,14,19]. The dose of 50mg per kilo of the animal's weight was treated in the work of Santana et al (2015). The two-gram dose was diluted in per liter of water offered to the animal [10]. Catechins were administered at a dose of 100mg per kilo of the animal's weight [16] and five grams of catechin per kilo of feed [17]. The total concentration of catechins in the studies ranged from 30 to 100% of the extract [11, 12, 14,16,17,19].

The study duration ranged from 4 to 16 weeks. Only three studies did not give the diet concomitantly to treatment [12,13,19]. Obesity induction was made in two studies for 4 weeks. In one, the animals were subjected to treatment with green tea extract for 12 weeks, maintaining the consumption of a high-fat diet [12]. In other, they submitted the animals to

treatment for 4 weeks with EGCG but did not maintain their food intake [19]. Researchers induced obesity for 8 weeks and subjected the animals to treatment for another 4 weeks with EGCG while maintaining the high-fat diet [13].

Treatment with green tea extract (GTE) reduced the weight and the size of the adipocytes in the subcutaneous adipose tissue [11]. As well as reduced the weight and size of adipocytes of subcutaneous, epididymal, and brown tissue [12]. The weights of WAT and BAT were decreased by 45% and 34%, respectively, in the group treated with EGCG compared to those in the control group [13]. Catechins also reduced the weight of brown adipose tissue [17].

Treatment with green tea extract (GTE) significantly increased the expression of PPARγ (Peroxisome proliferator type gamma), PGC-1α (Peroxisome proliferator Co-activator 1-alpha), PRDM16 and CITED1 in subcutaneous adipose tissue [11]. In brown adipose tissue, GTE treatment promoted normalization of weight and reduced size of lipid droplets in cells; a significant reduction in the expression of C/EBPα and aP2; positive regulation of PGC-1α, and Vegfa165 [11]. The epididymal white adipose tissue treated with green tea extract showed lower adipocytes compared to a high-calorie diet. In the treated groups, was a significant expression increase in PPAR-γ, PRDM-16, BMP-7 (Bone Morphogenetic Protein 7), FGF-21 (Fibroblast Growth Factor 21) and PGC-1α, and reduced TLE-3 (transducin -like enhancer protein-3) [15].

The green tea extract after 12 weeks promoted an epididymal tissue miR-802 increase, and a 3 miRNAs reduction (miR-335, miR-221, miR-155) in the treated obese group [12].

EGCG at a concentration of 0.2% of the diet significantly increased the expression of UCP1 (Uncoupling Protein 1), UCP2 (Uncoupling Protein 2), PRDM16, PGC-1α, NRF1 (Nuclear Respiratory Factor 1), TFAM (Mitochondrial Transcription Factor A) and CPT-1β (Carnitine Palmitoyltransferase I beta) and decreased ACC2 (Acetyl-CoA carboxylase 2) compared to the high-fat group. Beige adipocytes were also identified, defined by their lipid droplets multilocular morphology after EGCG treatment [13]. EGCG added to 1% of the diet increased the expression of UCP1, PGC-1α, and PRDM16 in the high-fat group treated in brown adipose tissue [14].

While at 16 weeks, the $2g.L^{-1}$ intake of EGCG restored the average cell size and adipocyte distribution; promoted an increase in PPARγ and a decrease in Cpt2 on brown adipose tissue about the high-fat group. The EGCG also prevented adipocyte hypertrophy induced by a high-fat diet and uneven size distribution; promoted an increase in Sirt, PGC-1α, and Cpt2, also a reduction in PPARγ and FASN in white adipose tissue [10]. EGCG for eight weeks, at a dose of 50mg per kilo of the animal's weight, did not promote changes in the high-fat group, added only in the treated control group [18].

EGCG treated after obesity induction, reduced the white adipose tissue SCD1 expression. However, there were no changes in gene expression in brown adipose tissue [19].

Green tea catechins promoted a white adipose tissue increase of UCP-1 and PPAR-γ [16]. As well as the brown adipose tissue increased expression of PPAR-γ, CPT1, AOX, and UCP-1

[16]. Catechins in the treated control group showed UCP1 mRNA expression 70% higher than animals fed a control diet [17].

All selected articles are experimental studies in rodents. So, the ARRIVE guidelines were chosen to assess the studies' quality. The articles that make up this review reached 55 to 85% of the items recommended by the guidelines (Table 2).

Table 2 – Analysis of methodological adequacy according to the ARRIVE guidelines.

Author/year	Final score (20 points)	Percentage of adequacy by ARRIVE
Chen et al. 2017	14/20	70%
Lee et al. 2017	14/20	70%
Klaus et al. 2015	11/20	55%
Mi et al. 2017	15/20	75%
Neyrinck et al. 2017	12/20	60%
Nomura et al. 2008	14/20	70%
Otton et al. 2018	17/20	85%
Santana et al. 2015	13/20	65%
Yan et al. 2013	13/20	65%
Zhou et al. 2018	15/20	75%

3.4 DISCUSSION

The results found in this systematic review suggest that green tea promotes a reduction in the weight of brown and white adipose tissue, as well as a reduction in the fat droplet of the tissues and influences the expression of genes and microRNA that regulate the metabolism of adipose tissue. In addition to promoting the browning of white adipose tissue.

It is important to note that was found only experimental works on animals, clinical research was not found on obese humans. Browning stimulation is a relatively recent therapy for the treatment of obesity. The maintenance of BAT activity and the browning of WAT has been proposed as effective strategies for the management of obesity.

Animal studies focused on the C57BL/6J mouse [10,11,12,13, 14] followed by three articles with Sprague Dawley rats [15,16, 17]. Both Sprague Dawley rats and C57BL6/J mice are animals prone to gaining weight through diet and developing obesity [20]. The Swiss mouse was used in only one study [19], but it is still a species that the high-fat diet also promotes a significant increase in weight, mainly in white adipose tissue [21].

Regarding the species, the work with New Zealand black was unexpected, as this species is used in experimental works for autoimmune models [22]. The authors' prerogative were because in his studies NZB mice was highly susceptible to the development of diet-induced obesity [19].

Most studies used the high-fat diet [11-14,16-19]. The relationship between high-fat diet and browning is already described in the literature. This diet promotes significant positive regulation of PRDM16 in rats. PRDM16 is a transcription factor that activates the differentiation of precursor cells into brown adipose cells [23].

However, little is discussed about the type and percentage of fat used in diets. This regulation is likely to differ when it comes to saturated, monounsaturated, and polyunsaturated fat. Research in this direction needs to be developed, as well as the percentage of dietary fat is another factor that can influence browning. The percentage of fat varied from 15 to 60% in the studies in this review [11-14,16,17,19]. Studies that evaluated the effect of the high-fat diet on browning used the percentage between 35.5 to 60% fat [23,24,25].

When it comes to the high-calorie, high-fructose diet, there are no reports in the scientific literature about their influence on browning and brown adipose tissue. However, they are effective diets to promote weight gain and metabolic changes [15].

Treatment with green tea extract (GTE) was able to reduce the weight, the size of the adipocytes in the subcutaneous, epididymal, and brown adipose tissue [11,12,13] and catechins reduced the weight of brown adipose tissue [17]. These findings can be attributed to the composition of green tea.

Green tea has catechins and caffeine in its composition. Both components have mechanisms to reduce body fat. Green tea catechins can stimulate thermogenesis and

oxidation of fat by inhibiting catechol-O-methyl transferase (COMT), an enzyme that degrades norepinephrine. Caffeine also inhibits the degradation of norepinephrine-induced by cyclic intracellular AMP phosphodiesterase (cAMP) [26]. Thus, green tea has anti-obesity effects.

Also, green tea increases PPARγ, PGC-1α, PRDM16, Vegfa165, BMP-7 (Morphogenetic Bone Protein 7), FGF-21 (Fibroblast Growth Factor 21) and CITED1. PPARγ activated in adipocytes ensures adequate release of adipocytokines (adiponectin and leptin), which are mediators of insulin action in peripheral tissues. As a result, the insulin sensitivity of the whole body is maintained. In addition to this adipogenic activity, PPARγ is also important in lipid metabolism and regulates genes that participate in the release, transport, and storage of fatty acids such as lipoprotein lipase (LPL) and the CD36 fatty acid transporter [27].

Green tea was able to reveal the expression of adipose tissue marker genes, FGF21, and Cited1 [11, 15]. Suggesting the induction of beige adipogenesis [28]. Fibroblast growth factor 21 (FGF21) activates PGC-1α and accelerates the function of brown adipocytes [29]. PGC-1α is highly expressed in brown adipose tissue and skeletal muscle, the two main contributing tissues in adaptive thermogenesis through the adrenergic receptor axis PGC-1α - UCP-1. It can be induced by cold or adrenergic stimuli with improved mitochondrial biogenesis. Based on the findings of this review, it can be expressed through the treatment of green tea extract in rodents [30].

Another important regulation was the increase in PRDM16. As already mentioned, it is a transcription regulator that controls the brown and beige adipocyte phenotype [23,31]. Just as

the increase in vascular endothelial growth factor (VEGFA) is an angiogenic factor in adipose tissue and is important for the development of new vessels. Overexpression of VEGFA in adipose tissue results in an increase in the number and size of blood vessels. In this way, it protects against hypoxia and obesity induced by a high-fat diet and improves insulin sensitivity and glucose tolerance [32].

It is worth mentioning the increase in BMP-7. Originally identified as a bone inducer, it is now recognized as a multifunctional cytokine and has been implicated as a potential therapeutic agent for cardiovascular, metabolic, and degenerative diseases [33]. For this bone morphogenetic protein, BMP-7, overexpressed in leaves of adipose-derived mesenchymal stem cells [34]. While BMP-2, -4, -6 and -7 are capable of inducing a massive accumulation of lipids in brown pre-adipocytes, only BMP-7 has a specific effect on inducing the brown fat specific UCP-1 protein by promoting the increased expression of PRDM16, PGC-1a, PGC-1β, and UCP-1 PPARγ, C/EBPα and aP2 [33].

Besides, green tea extract significantly reduced the expression of C/EBPα and aP2 brown adipose tissue and increased C/EBPα in subcutaneous adipose tissue [11,15]. CEBPα has an indispensable role in transcriptional activation and the increase is in line with long-term differentiation. Also, the CEBP DNA-binding protein interacts with the aP2 promoter and elevates the expression of the aP2 gene. aP2 expression is highly induced during adipocyte differentiation [35]. That is, the increase in subcutaneous adipose tissue may indicate tissue differentiation.

The transducin-3 enhancer protein (TLE-3) was reduced after treatment with the green tea extract. This protein suppresses selective genes from brown and induces selective genes from white adipose tissue. It is involved in a negative browning process regulatory pathway [15]. A summary of the possible mechanisms for the action of green tea on browning is shown in figure 2.

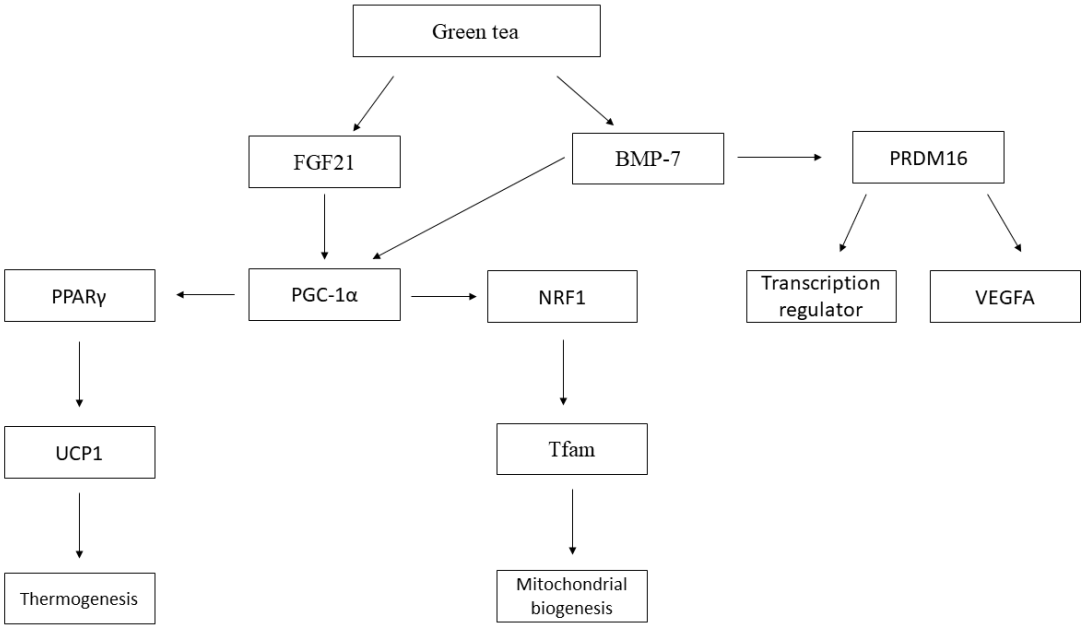

Figure 2 - Possible mechanisms by which green tea stimulates the browning of adipose tissue.

Treatment of catechins in green tea increased UCP-1, PPAR-γ in white adipose tissue and increased expression PPAR-γ, CPT1, AOX and UCP-1 in brown adipose tissue at a dose of 100mg.kg^{-1} body weight per day. The concentration of catechins used was 50% EGCG (epigallocatechin gallate), 22% ECG (epicatechin), 18% EGC (epigallocatechin), and 10% - EPI (epicatechin) for six weeks [16]. While 5g of catechins per kilo of feed in the concentration of 81.5% catechins (EGCG - 40.6%, ECG - 23.1%, EGC - 12.4%, EPI -

9.2%) promoted increased expression of UCP1 mRNA by 70% than animals fed a control diet for eight weeks, but there was no difference between the high-fat groups [17].

The found results difference can be attributed to the percentage of fat in the high-fat diet. A diet with 15% saturated fat and 1% cholesterol was used, without reporting the total percentage of fat in the diet [16]. And in the other study, they used a diet with 60% fat from coconut oil and palm oil. This reinforces the need for experimental research to standardize the type of diet use [17].

EGCG is the catechin present in greater quantity in green tea [36]. The treatment with this catechin significantly increased the expression of UCP1, UCP2, PRDM16, PGC-1α, PPARγ in brown adipose tissue [13,14]. EGCG stimulates PGC-1α and PRDM16, both regulate thermogenesis by stimulating UCP1 expression. UCPs are mitochondrial inner membrane proteins that decouple the oxidative respiratory chain, and three of these proteins have been reported to date. UCP1 is expressed mainly in adipose tissue, UCP2 is expressed ubiquitously in various tissues of the body, and UCP3 is specific for skeletal muscle and brown adipose tissue [10, 13].

PGC-1a is known as the main stimulator of mitochondrial biogenesis. It causes the activation of nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (Tfam) that increase the expression of the genes necessary for mitochondrial function [13].

EGCG at a diet concentration of 0.2% significantly increased the expression of CPT-1β (carnitine palmitoyltransferase I β) and decreased of ACC2 in brown adipose tissue. CPT-

1β is a rate-limiting enzyme in the regulation of fatty acids uptake and oxidation by the mitochondria in BAT, playing an important role in stimulating BAT thermogenesis. On the other hand, ACC2 inhibits CPT-1β activity by catalyzing the formation of malonyl-coenzyme (CoA) from acetyl-CoA [13]. Thus, green tea regulates thermogenesis by yet another metabolic pathway, CPT-1β.

In white adipose tissue, EGCG promoted increased expression of PGC-1α, CPT2, PPARγ, SIRT1 (Sirtuin 1) [10]. Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD) dependent deacetylase protein, acting as a regulator of fatty acid oxidation and mitochondrial biogenesis by deacetylating the activated receptors gamma coactivator by peroxisome 1a proliferation (PGC1α). Activation of SIRT1 has been proposed as a key regulator to prevent obesity and obesity-related metabolic dysfunction [37]. Also CPT2 codes for the enzyme carnitine palmitoyltransferase 2. This enzyme is involved in the transfer of long-chain fatty acids (AGCL) from the cytosol to the mitochondria, allowing greater oxidation and energy generation [38].

EGCG also promoted the reduction of FASN in white adipose tissue [10,18]. FASN encodes the enzyme fatty acid synthase (FASN) responsible for the synthesis of fatty acids. The reduction of FASN in white adipocytes from mature mice increases the sympathetic activity and induces the browning of white adipose tissue and improves glucose homeostasis in obese mice [39].

Treated EGCG after obesity induction reduced SCD1 expression in white adipose tissue [19]. SDC1 is the gene that encodes stearoyl-CoA desaturase, an enzyme responsible for the

synthesis of monounsaturated fatty acids (MUFA) [40]. There are reports in the literature that overexpression of SDC1 increases leptin levels [41]. Corroborating the findings, leptin expression in WAT also decreased due to EGCG treatment. As the expression of leptin in white fat is highly correlated with fat mass, this decrease may be a consequence of the reduction in the amount of fat, as it may be related to the lower expression of SDC1[19].

Besides, EGCG promoted morphological changes in adipose tissue as it prevented adipocyte hypertrophy induced by a high-fat diet and uneven size distribution [10]. And browning was confirmed, beige adipocytes were identified, defined by their multilocular morphology of lipid droplets after EGCG treatment [13].

The duration of the studies ranged from 4 to 16 weeks. This wide variation in the study period has a direct impact on the results found. Three-week studies showed an increase in adiposity with the high-fat diet, the percentage of fat accumulated in the liver, and an increase in triglycerides. Only after eight weeks were identified changes in weight and biochemical parameters [42].

As for the induction method, most studies have induced obesity concomitantly with treatment. Only three studies did not submit the diet to treatment together. Both studies showed positive results for the regulation of brown adipose tissue and the reduction of white adipose tissue. Green tea extract and EGCG were shown to be efficient in the expression of markers involved in browning even with concomitant use or after the induction of obesity [12,13,19].

The main study's innovation was the microRNAs analysis, which has been a trend in current science. Treatment of green tea extract at a dose of 500mg.kg^{-1}, with 30% catechins for 12 weeks, promoted a reduction of 3 miRNAs (miR-335, miR-221, miR-155) in the treated obese group [12]. MiR-335 is positively regulated in adipocytes by pro-inflammatory cytokines, and this is prevented by catechins in green tea. MiR-155 is significantly increased in atherosclerosis and decreased in NAFLD, type 2 diabetes/insulin resistance (T2DM / IR), and obesity [43]. MiR-221 adipose is over-regulated in obesity [44]. Also, the tea extract increased the expression of miR-802 [12]. The expression of miR-802 in obese mice contributed to the induction of obesity and impaired metabolism, while inhibition of miR-802 in obese mice improved glucose tolerance [45]. The green tea was not able to inhibit an increase in miR-802.

The limitation of this review comprises the evident methodological differences observed in the research carried out, such as the great variation in the percentage of fat in the high-fat diet, the green tea form of preparation and dosage, and the study time. It is important to point out that no studies were found with the infusion of green tea and it is known that this corresponds to the greatest form of use by humans.

3.5 CONCLUSION

The effect of green tea in obesity is proposed by different mechanisms. There are mechanisms elucidated by the reduction of body fat via COMT. The induction of browning was identified by the morphological changes in the white adipose tissue. However, there is no defined mechanism, but it seems to involve different genes and microRNA expression.

According to the results found, the factors involved in this induction to Browning are PPARγ, PGC-1α, UCP1, CPT, and PRDM16. Because, the studies do not follow a methodological pattern to evaluate the green tea utilization, including the duration, type of diet, dose administration, way of use, and the genes to be analyzed, this makes it difficult to outline the mechanism of action, as there are several possibilities. However, the most likely mechanism is the PGC-1α - UCP1 axis. It is important to emphasize the need for studies in obese humans to identify whether the same metabolic response occurs.

DECLARATIONS

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Conflicts of interest/Competing interests

All authors declare that they have no conflicts of interest.

Ethics approval

This study was exempted from approval by the Ethics Committee because there was no direct involvement of animals or humans.

Consent to participate

Not applicable.

Consent for publication

All authors have approved the version of the manuscript submitted and agreed to publish this in Current Obesity Reports. All authors also declare that this study is not published or submitted elsewhere for peer review.

Availability of data and material (data transparency)

The study data and materials of this study are available upon request.

Code availability

Not applicable.

Authors' contributions

Ana Paula Azevedo Macêdo - Drafting the article and substantial contributions to conception and design, data acquisition, analysis and interpretation.

Mariane Gonçalves dos Santos - Substantial contributions to conception and design, data acquisition, analysis and interpretation.

Jairza Maria Barreto Medeiros - Revising it critically for important intellectual content.

Jorge Mauricio David - Revising it critically for important intellectual content.

Cristiane Flora Villarreal - Revising it critically for important intellectual content.

Simone Garcia Macambira - Revising it critically for important intellectual content.

Milena Botelho Pereira Soares - Revising it critically for important intellectual content.

Ricardo David Couto - Revising it critically for important intellectual content and final approval of the version to be published.

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4. CAPÍTULO II – PESQUISA EXPERIMENTAL

Este artigo refere-se a pesquisa experimental desenvolvida no mestrado e está sob formato de artigo que foi submetido ao International Journal of Food Sciences and Nutrition.

Green tea infusion promotes body fat reduction in mice fed a high-fat diet

This study aims to evaluate the effects of green tea (*Camellia sinensis*) infusion on mice and its action on glycemic parameters and adipose tissue in an induced high-fat diet obesity model. C57/BL6 mice male (n = 35) with two months of life were distributed into four groups: control diet plus autoclaved water or green tea, and high-fat diet plus autoclaved water or green tea for 16 weeks. Murinometric and glucose levels measurements were done during the experiments. Oral glucose and insulin tolerance tests were performed in the last week. Afterward, adipose tissue was dissected. The green tea promotes weight and waist circumference reductions, by reducing the adipose tissue deposition in all analyzed compartments. The fasting blood glucose showed a significant reduction only in the sixteenth week. In conclusion, green tea reduced weight, abdominal circumference, and the accumulation of adipose tissue in C57/BL6 mice exposed to a high-fat diet.

Keywords: *Cammelia sinnensis*, obesity, adipose tissue, glucose, insulin.

Subject classification codes: Ethics Committee of the School of Veterinary Medicine – CEUA under protocol 03/2019.

4.1 INTRODUCTION

Obesity is a public health problem that has reached epidemic levels, constitutes the main global burden of diseases and chronic complications, including type 2 diabetes mellitus (DM), cardiovascular diseases, risk of non-alcoholic steatohepatitis (NASH) and even certain types of cancer (MĂRGINEAN et al. 2020).

Obesity treatment includes dietary control, exercise, lifestyle changes, medications, and weight loss surgery. However, many clinical and epidemic studies have shown that maintaining a long-term lifestyle change is a major challenge. Therefore, strategies to assist weight loss can inspire the obese patient's confidence to achieve their goals and give them more motivation to change their lifestyle behavior (SUN et al. 2016).

In this sense, green tea consumption has been studied because it is associated with weight loss and modulation of fat metabolism and energy expenditure (OKLA 2017). Different mechanisms have been proposed for anti-obesity action promoted by green tea, such as appetite suppression, inhibition of pancreatic lipase activity, suppressing adipogenesis and lipid synthesis, and increasing energy expenditure via thermogenesis (GAMBOA-GÓMEZ et al. 2015).

Thus, Neyrinck et al. (2017) evaluated that the anti-obesity effect of green tea leaf extract was associated with the activation of the browning of white adipose tissue (WAT) or the inhibition of whitening in brown adipose tissue (BAT) in obese mice with a high-fat diet (HFD). Green tea extract significantly reduced diet-induced adiposity in both tissues and reduced inflammation in WAT, as well as reduced the size of adipocytes in BAT and the

size of lipid droplets in WAT. Chen et al. 2011 developed a study with a similar methodological design in rats and obtained the same result.

Several in vitro or in vivo studies show that green tea inhibits fat accumulation in adipose tissue, in these studies performed by Lin et al. (2005); Chen et al. (2011) and Neyrinck et al. (2017) the green tea was used as a polyphenols extract and not as an infusion. However, green tea is generally consumed as an infusion (LEE et al. 2015).

To date, no study has been identified to assess the effect of green tea infusion on the adipose tissue, including brown adipose tissue, in rodents induced by obesity by a high-fat diet. Therefore, this study aimed to evaluate the effects of the green tea (*Camellia sinensis*) infusion on an obesity-induction model in mice by the high-fat diet (HFD) on glycemic levels and adipose tissue.

4.2 MATERIAL AND METHODS

4.2.1 STUDY DESIGN AND SAMPLE SIZE CALCULATION

Was realized an interventional animal model study, and the sample size was calculated by using the Winpepi program (version 1.69) to estimate the sample necessary to find 40 cases since the literature suggests 8 to 10 animals per group (UEDA et al., 2012; Li et al. 2017). The sample size was calculated to assume the null hypothesis test with a minimal power of 0.99, considering a 15% common animal loss rate. The initial calculation showed 48 animals, so each group should be composed of at least 12 animals. However, as a pilot study was carried out initially (i.e. to adjust the model conditions, to verify whether or not the intended changes were observed) because the possibility of experiments' repetition, the first estimated animals' number double plus 20%, to be exact, our final number shows the

need of 116 animals. The number was inflated due to the expectation of animal loss, and the need for experiments repetition. If we had an animal loss, this could impact the study significantly, by reducing the number of predicted animals per group.

Most likely fewer animals than was estimated here was used, so, the study was carried out with 9 animals per group, except for the control diet + water group, as described here: control diet + water, 8 animals; control diet + green tea, 9 animals; HFD + water, 9 animals; HFD + green tea, 9 animals. This study was approved by the Ethics Committee of the School of Veterinary Medicine under the protocol number 03/2019. All activities were carried out by the standards guideline suggested by the SBCAL - Brazilian Society of Science in Laboratory Animals and with the international standards established by the National Institute of Health Guide for Care Use of Laboratory Animals.

4.2.2 ANIMALS AND DIET

C57/BL6 mice fed a standard diet up to two months of age were used. The animals were kept in the Laboratory of Pharmacology and Experimental Therapeutics (LAFTE) of the Faculty of Pharmacy of the Federal University of Bahia (Salvador, Brazil), under the following conditions: temperature of 22 ± 2 º C, under controlled humidity (50 %), with unrestricted access to food and exposed a constant light–dark cycle of 12 h and 12 h.

After 2 months of age, the mice were kept in polypropylene boxes randomly (3-5 animals per box) divided into four groups: fed with a control diet plus autoclaved water or green tea and fed a high-fat diet plus autoclaved water or green tea (Figure 1).

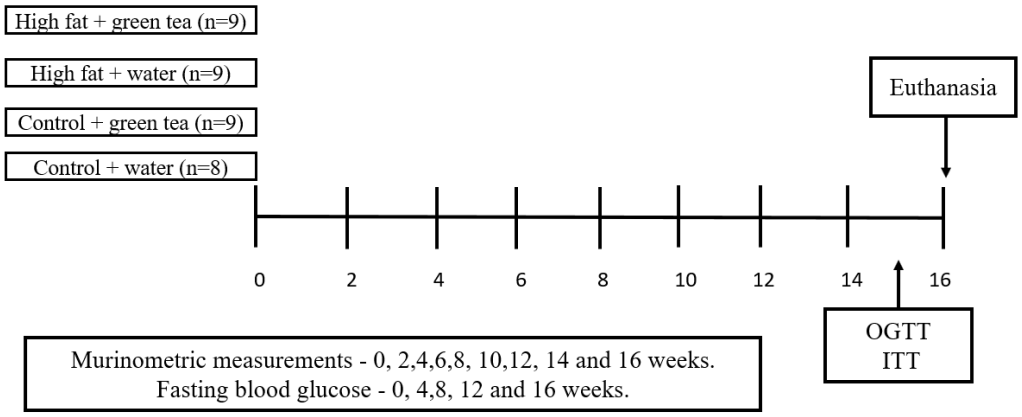

Figure 1 - Experimental design. OGTT – Oral glucose tolerance test. ITT – Insuline Tolerance Test.

The diet was offered to the mice for 16 weeks to induce metabolic changes. This induction was an adaptation from the method carried out by Jang et al. (2018). The high-fat diet offered had a lipid content of 58%, as shown in Table 1. The control group fed the AIN-93M diet containing 4.07 kcal/g ready diet, and the group high-fat (HFD) fed a modified AIN-93 diet with an increased 33.35% hydrogenated fat, containing 5.56 kcal/ g. The composition of the control and HF diets is shown in Table 1.

Table 1- Nutritional composition of the high-fat diet (HFD) and control diet.

	HFD – High-fat Diet		Control diet	
	G	Kcal %	G	Kcal%
Protein	23.0	16.4	16.8	16.4
Carbohydrate	35.5	25.5	74.3	73.1
Lipid	35.8	58.0	4.8	10.5
$kcal.g^{-1}$	5.56		4.07	

Source: Adapted from DALTRO and collaborators (2015)

The green tea was daily available to mice through drinking fountains as the only hydration

source, at a concentration of 2%, during 16 weeks (WANG et al. 2017).

4.2.3 GREEN TEA

The green tea (*Camellia sinensis*) was acquired from the Viva Natureza® brand, and before starting the experiments, the phytochemical analysis was performed. The presence of alkaloids, glycosides, saponins, tannins, and terpenoids was identified by standard methods (Brito et al., 2008). In the green tea extract, the presence of caffeine, epicatechin gallate, and epigallocatechin was identified through High-Performance Liquid Chromatography (HPLC). The infusion was prepared by immersing 2 g of dried tea leaves into 100 mL of hot water (100 ° C) for 5 minutes. The infusion was prepared daily (WANG et al. 2017).

4.2.4 CONSUMPTION OF LIQUIDS

A daily 200 mL of water or green tea per box was offered. The consumption of water and green tea was checked daily using a millimeter measuring cylinder. The difference between the initially placed solution and the volume leftovers divided by the number of animals per box were admitted as the amount consumed. The measurements of liquid consumption have always been made at the same time.

4.2.5 FOOD CONSUMPTION

The food consumption of the experiment was measured from the first to the last day as follows: every two days, 100 g of the control diet or high-fat diet were placed in each polypropylene box and the individual daily consumption of the animals was obtained by the average consumption (discounting the remaining ration) for two days, divided by the number of animals per box (PEREIRA et al. 2018).

4.2.6 MURINOMETRIC MEASURES

The murinometric measurements were taken every two weeks. The animals body mass was recorded with the aid of an electronic scale (Toledo® - Model 9094C/4). The abdominal circumference was determined by the circumference of the midpoint between the anterior and posterior legs (PINI et al. 2016).

4.2.7 GLYCEMIC CONTROL

Fasting glycemia was performed monthly from the first to the last week of experimentation, following the protocol by MONACO et al. (2017). The On-Call Plus II® glucometer was used, the blood glucose measurements were obtained in milligrams per deciliter.

The oral glucose tolerance test (OGTT) was performed with the animals in the last week of the experiment. After a six-hour fast, the first blood collection was made (time 0), by cutting the end of the animals' tail to remove a 10 µL blood aliquot. Then, a 20% glucose solution was administered by gavage at a dose of 1mg/kg of the animals' weight. Blood collection was held in times 30, 60, 90, and 120 minutes. Blood glucose concentrations were determined using the On-Call Plus II® glucometer.

The insulin tolerance test was performed 48 hours after the glucose tolerance test, and animals have fasted for 6 hours. The first blood collection (time 0) was done by cutting the end of the animals' tail, to remove a 10 µL blood aliquot. Then, an insulin solution (0.75 U insulin of the animals' weight) was administered intraperitoneally. Blood collection was performed at times 15, 30, 45, and 60 minutes. Blood glucose concentrations were determined as above described.

4.2.8 ADIPOSE TISSUE

The animals were euthanized by decaptation. After euthanizing the animals, the epididymal, inguinal, visceral, and interscapular adipose tissues were dissected, and later the weighing was performed. The weight was measured with an analytical balance.

4.2.9 STATISTICAL ANALYSIS

The data were expressed as mean ± standard error of the mean (SEM) for the number of animals in each group. For tests of extreme values (the presence of an outlier), the Grubb test was applied. Two-way ANOVA analysis of variance with the Bonferroni post-test (data with two independent variables) and one-way ANOVA with the Tukey post-test (for only one independent variable) was used for the parametric data distribution. In all cases, to consider statistical significance, a critical level of 5% was assumed within a 95% confidence interval (C.I.). All statistical tests were applied with a minimum discriminatory power of 80%. Statistical analysis was performed using the Graph Pad Prism 5.0 software (San Diego, CA, USA).

4.3 RESULTS

Food consumption by animals was significantly reduced in the HFD + water group compared to the other groups. The HFD + green tea group showed a significant increase in food intake compared all group. The HFD + green tea group had significantly higher tea consumption compared to Control + green tea. As for water consumption, the HFD + water group had significantly lower water intake than Control + water as shown in Table 2.

Table 2. Consumption of diet, water and green tea by C57Bl / 6 mice (Mean ± SD).

	Control + Water	Control + Green Tea	HFD + Water	HFD + Green Tea
Food intake, g/day	2.90 ± 0.28**	3.03 ± 0.26*	2.33 ± 0.21	2.51 ± 0.45***
Tea consumption, mL/day	-	5.69 ± 0.82	-	10.47 ± 3.7†
Water consumption, mL/day	5.67 ±1.07	-	4,98 ± 0.91#	-

*Control + Green Tea vs HFD + Water, $p<0.05$, ** Control + Water vs HFD + Water, $p<0.01$; ***HFD + Water vs HFD + Green Tea, $p<0.001$; † Control + Green tea vs HFD + Green Tea, $p<0.001$; #Control + Water vs HFD + Water, $p<0.001$. HFD – High Fat Diet.

The animals body weight showed a significant increase from the fourth week when compared between the control + water and the HFD + water groups. In the sixth and sixteenth weeks, the weight of the HFD + green tea group, showed a significant reduction when compared to the HFD + water group. The Control + green tea group showed weight reduction in the second week and from the sixth week until the end of the experiment compared with the HFD + water group (Figure 2).

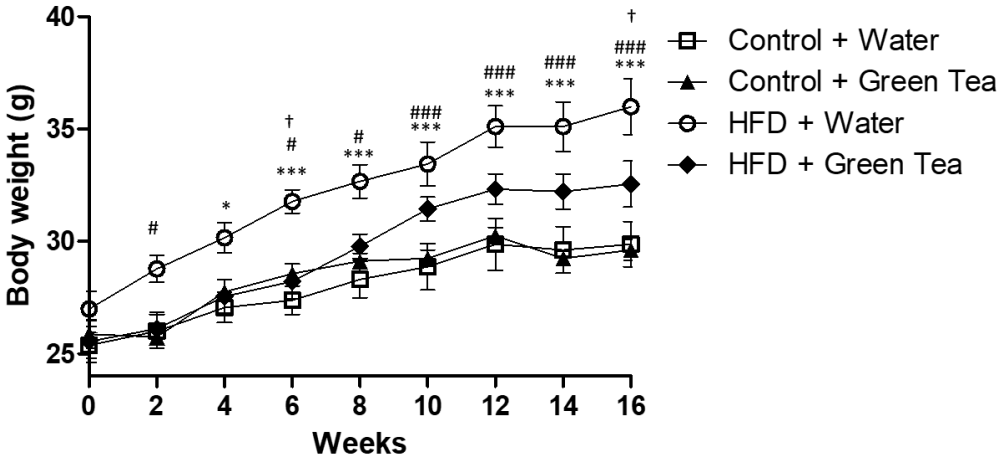

Figure 2 - Body weight of C57Bl / 6 mice. * Control + Water vs HFD + Water, p <0.05; *** Control + Water vs HFD + Water, p <0.01; # Control + Green tea vs HFD + Water, p <0.05 ## Control + Green tea vs HFD + Water, p <0.001; † HFD + Water vs HFD + Green Tea, p <0.01. HFD – High Fat Diet.

The abdominal circumference showed a significative difference between the HFD + water and the control + water group, after the second week of diet consumption, and between the HFD + water and HFD + green tea group, in the fourth week, and continuously from the eighth week, suggesting an effective action of the green tea infusion on the reduction of the abdominal circumference (Figure 3).

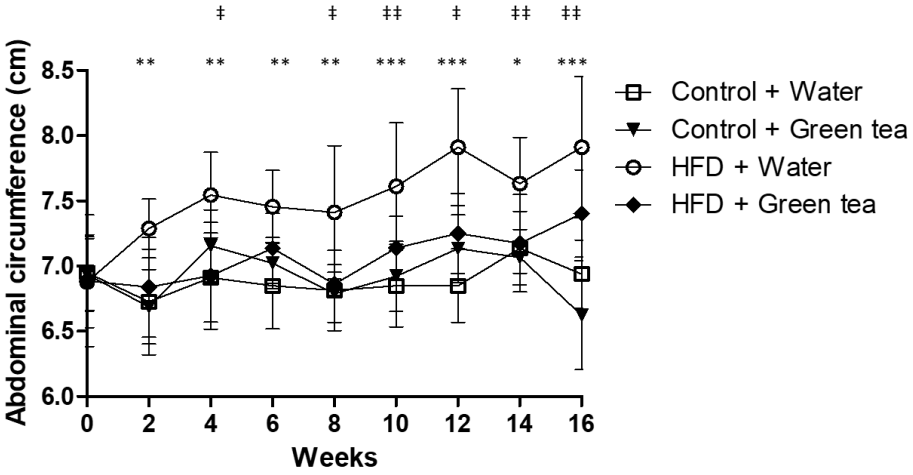

Figure 3 - Abdominal circumference of C57Bl / 6 mice. * Control + Water vs HFD + Water, p <0.05; ** Control + Water vs HFD + Water, p <0.01; *** Control + Water vs HFD + Water, p <0.001. ‡ HFD + Water vs HFD + Green Tea, p <0.05; ‡‡ HFD + Water vs HFD + Green Tea p <0.01. HFD – High Fat Diet.

The blood glucose determined monthly showed a significant difference only in the sixteenth week. There was a significant increase in the HFD + water group compared to the control + water group. Also was observed significant blood glucose decreased in the HFD + green tea group when compared to the HFD + water group (Figure 4).

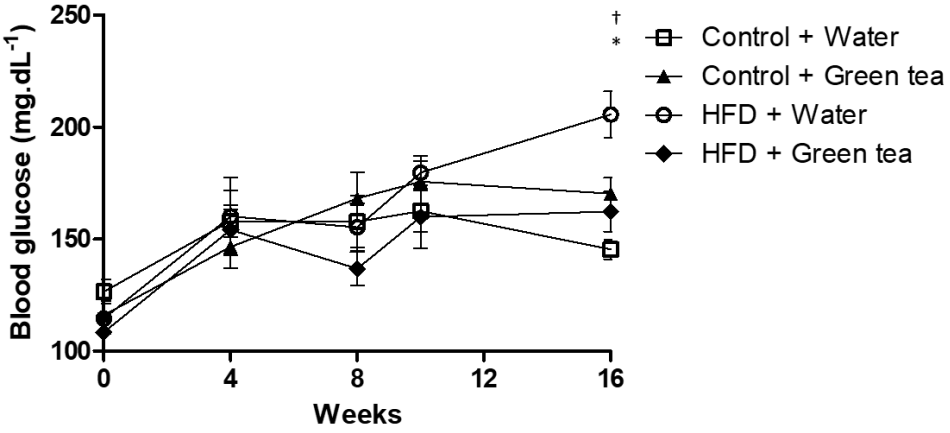

Figure 4 - Monthly glycemia of C57BL/6 mice. * Control + Water vs HFD + Water, p <0.01. † HFD + Green tea vs HFD + Water, p <0.05. HFD – High Fat Diet.

By evaluating the oral glucose tolerance test, at a time of 15 minutes, the HFD + green tea group showed a significant reduction when compared to the control + water group. At the same time, the HFD + green tea shows a glucose significant reduction when compared to the HFD + water group. At a time of 60 minutes, the HFD + water group shows a significant increase when compared to the control + water group (Figure 5).

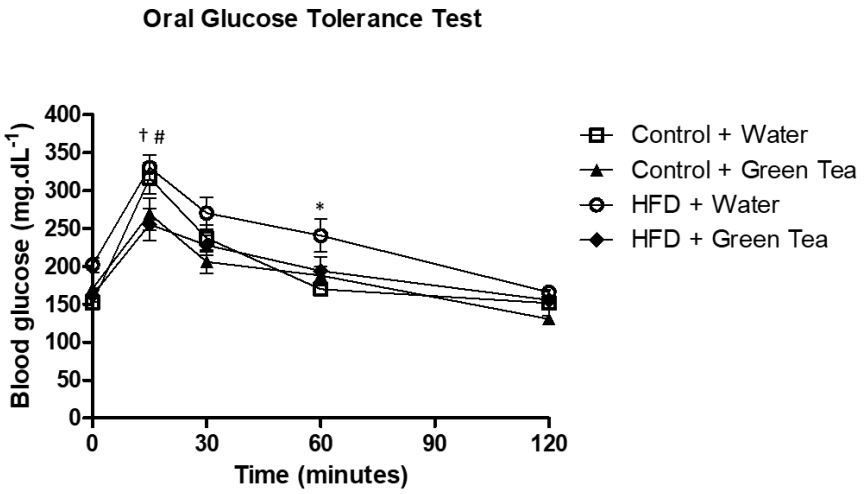

Figure 5 - Oral glucose tolerance test of C57BL/6 mice. * Control + Water vs HFD + Water, p <0.01; † Control + Water vs HFD + Green Tea, p <0.05; #HFD + Water vs HFD + Green Tea, p <0.01.

The insulin tolerance test comparison between the control + water and the HFD + water group showed a significative blood glucose reduction only after 15 minutes. On the other hand, the control + green tea showed a significant reduction when compared to the HFD + green tea group at all insulin tolerance test times (Figure 6).

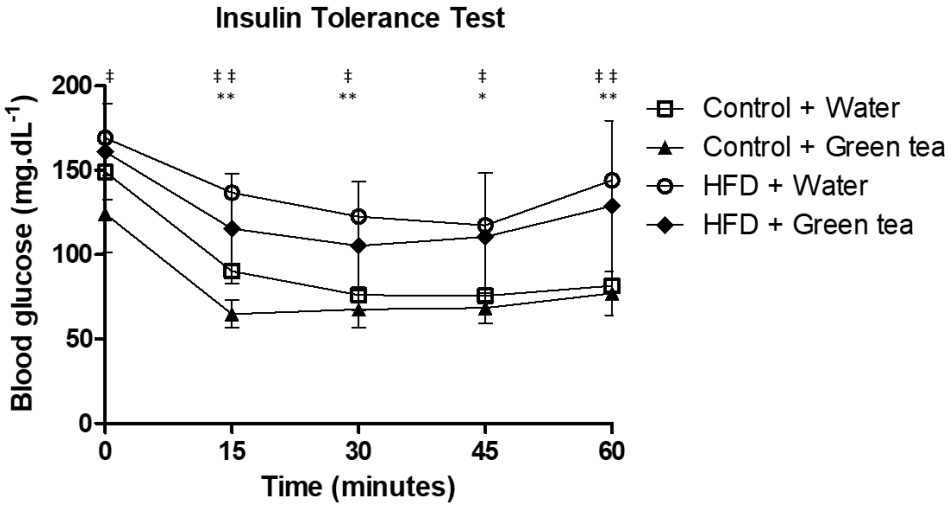

Figure 6 - Insulin tolerance test of C57BL/6 mice. * Control + Water vs HFD + Water, p <0.01; ** Control + Water vs HFD + Water, p <0.001; ‡ Control + Green tea vs HFD + Green tea, p <0.01; ‡‡ Control + Green tea vs HFD + Green tea, p <0.001.

The interscapular (brown) adipose tissue had a significant increase in the HFD + water group when compared to the other groups as shown in Figure 7. As with brown adipose tissue, in white adipose tissue in the HFD + water group increased significantly compared to other groups (Figure 7). Similarly, significant increases were showed in inguinal and epididymal adipose tissue in the HFD + water group when compared to the other groups.

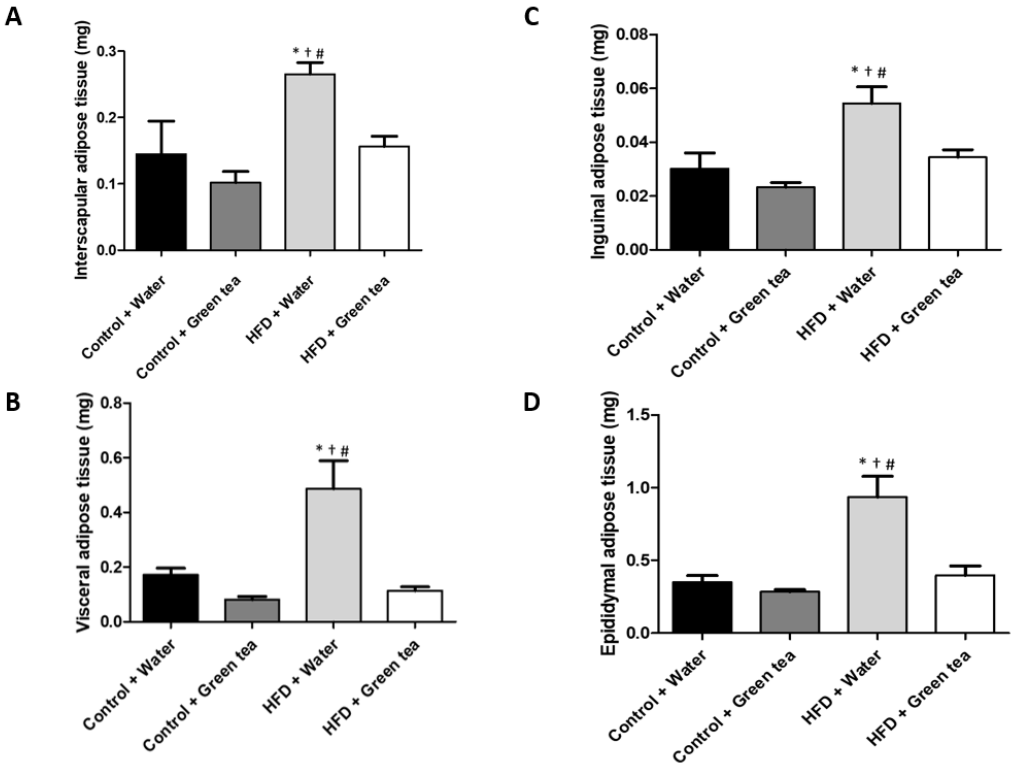

Figure 7 - Weight of adipose tissue in C57BL/6 mice. (A) Interscapular - brown adipose tissue * Control + Water vs HFD + Water, p <0.05; #Control + Green tea vs HFD + Water, p <0.001; † HFD + Green tea vs HFD + Water, p <0.01. (B) Visceral - white adipose tissue weight in C57BL6 mice. * Control + Water vs HFD + Water, p <0.001; # Control + Green tea vs HFD + Water, p <0.001; † HFD + Green tea vs HFD + Water, p <0.001. (C) Inguinal adipose tissue * Control + Water vs HFD + Water, p <0.01; # Control + Green tea vs HFD + Water, p <0.001; † HFD + Green tea vs HFD + Water, p <0.001. (D) Epididymal adipose tissue * Control + Water vs HFD + Water, p <0.001; # Control + Green tea vs HFD + Water, p <0.001; † HFD + Green tea vs HFD + Water, p <0.001.

4.4 DISCUSSION

In the present study, the mice consumption of the high-fat diet during 16 weeks induced weight gain, accumulation of adipose tissue, and changes in glucose metabolism. The infusion of green tea was able to reverse the changes caused by the consumption of the high-fat diet, among them: weight reduction and abdominal circumference, adipose tissue weight normalization, the blood glucose peaks reduction after the oral glucose tolerance test. The high-fat diet as evidenced in the literature is capable of inducing obesity metabolic changes in the animals (MATSUZAWA-NAGATA et al. 2008). Just as green tea has been studied for its benefits to human health, its main use purpose is the prevention of obesity (LIU et al. 2016).

Diets high-fat for prolonged periods can promote the basal increase of glucagon-like peptide-1 (GLP-1). GLP-1 acts with a signal of satiety and increased insulin release (RICHARDS et al. 2016). Therefore, the consumption of a high-fat diet was significantly reduced in the HFD + water group compared to the other groups, because the high-fat diet promoted satiety. This finding corroborates the data on food consumption in the literature (LEE et al. 2015). Probably, due to the satiety promoted by the high-fat diet, the HFD + water group ingested less water when compared to the control.

However, green tea showed an increase in food intake compared to HFD + water. In the literature, the effect of green tea on satiety and appetite suppression is still controversial. Green tea intake was higher in the HFD + green tea group compared to Control + green tea. To date, no data has been found to compare the consumption of infusion by mice. In a study with rats submitted to the consumption of decoction, there is a description of the amount consumed (SNOUSSI et al. 2014). However, the authors did not perform inferential

statistics.The work found that analyzed metabolic parameters and tea infusion were performed in a 13-week study in C57/BL6J mice in which the animals were exposed to a high-fat diet, and treatment with three infusions, including green tea. The animals presented weight reduction, retroperitoneal, and epididymal adipose tissue, as well as plasma glucose (LIU et al. 2016). Corroborating with our findings, however, the brown adipose tissue and the insulin tolerance test were not analyzed.

The infusion of green tea was able to promote a reduction in the body weight of animals exposed to the high-fat diet in the sixth and sixteenth weeks compared to the water high-fat group. The animals' weight reduction by green tea treatment is well established in the literature. C57BL/6J mice showing a reduced body weight and the amount of total adipose tissue after a 28 weeks consumption of a high-fat diet with an aqueous tea extract supplementation (LIU et al. 2019). Even after the induction of obesity with the high-fat diet, supplementation with green tea extract significantly attenuated body weight gain (ZHU et al. 2020).

Green tea is effective in reducing abdominal circumference. Abdominal circumference is the main murinometric indicator of fat accumulation in rodents (NOVELLI et al. 2007; AVTANSKI et al. 2019). Corroborating this, when the adipose tissue was evaluated, the infusion of green tea had reduced the fat deposits in all analyzed compartments.

The white adipose tissue weight reduction is well regarded in the scientific literature. There is a green tea already know established mechanisms of fat reducing deposition, such as decreasing the intestinal absorption of lipids. The epigallocatequinagallate (EGCG) present in green tea decreases the levels of intestinal bile acids and, consequently, decreases the

absorption of lipids (HUANG et al. 2018). Although the EGCG not identified in the green tea of this study.

As well as, the regulation of the sympathetic nervous system involved in lipolysis, which plays an important role in the total body fat regulation. The green tea catechins and caffeine can inhibit catechol-O-methyl transferase (COMT), an enzyme that breaks down norepinephrine in the synaptic cleft. By prolonging the action of norepinephrine, it amplifies its action on target tissues. This leads to the activation of cyclic adenosine monophosphate (cAMP), which promotes increased lipolysis and heat production in skeletal muscle (GAHREMAN et al. 2016).

Besides, new mechanisms have been elucidated for the activation of AMPK (i.e. protein kinase activated by 5′-AMP), such as the increase in adiponectin mRNA expression in white adipose tissue. Adiponectin promotes the oxidation of lipids and decreases muscle accumulation of lipids, reduces the plasma levels of free fatty acids, and increases the sensitivity to insulin (ROCHA et al. 2016).

The reduction of brown adipose tissue was already expected according to the mechanisms described above. Thus, green tea reduced total body fat (Figure 7, 8, 9 and 10). Thus, Neyrinck et al. (2017) observed, after a green tea extract supplementation, the normalization of an obese mice adipose tissue. While Lee et al. (2017) and Chen et al. (2011) observed a reduction in brown adipose tissue in the same species under similar conditions. It is important to note that these tissues can present morphological differences such as in the size of fat droplets and tissue color.

The infusion of green tea acted by regulating blood glucose peaks in the oral glucose

tolerance test and fasting glucose after sixteen weeks of tea consumption (Figure 4 and 5). In agreement with our findings, a study with rats fed a high-fat diet and subjected to the ingestion of green tea decoction for six weeks, consumption of green tea reduced body weight, and improved glucose tolerance. The improvement in glucose tolerance can be attributed to the display of a significant decrease in the sodium-glucose transport protein mRNA (SGLT-1) and increased levels of the mRNA glucose transporter 2 (GLUT2) (fasting), as well as increased expression of the mRNA 4 glucose transporter (GLUT4) in adipose tissue, green tea has been shown to reduce the intestinal proportion of SGLT-1 / GLUT2, a characteristic of regulating glucose absorption in the enterocyte and improving adipose GLUT4 (SNOUSSI et al. 2014).

4.5 CONCLUSION

The daily consumption of green tea infusion had beneficial effects in obese mice. The consumption of green tea in mice fed a high-fat diet promoted weight reduction in the sixth and sixteenth weeks, a significant reduction in abdominal circumference by the fourth week and continuously from the eighth week. The green tea infusion reduced the adipose tissue deposition in all analyzed compartments, including brown adipose tissue. Fasting glycemia showed a significant reduction only in the sixteenth week, and the oral glucose tolerance test 15 minutes glycemia peak was lower for the high-fat group consuming the tea. Therefore, this study brings enough pieces of evidence of green tea infusion attenuating the metabolic changes caused by the high-fat diet and obesity on C57BL/6J mice.

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DISCLOSURE STATEMENT

There are no conflicts of interest.

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5. CONCLUSÕES E PERSPECTIVAS FUTURAS

O chá verde é estudado principalmente devido ao seu efeito antiobesidade. Entretanto, nas pesquisas experimentais, a principal forma de administração é o extrato, conforme resultado da revisão sistemática. Contrapõe-se a forma mais consumida pela população é a infusão. Dessa forma, a análise do efeito da infusão no estudo experimental foi necessária.

Ambas formulações apresentam resultados positivos evidenciados por meio da revisão sistemática e da pesquisa experimental. O extrato promoveu a indução de escurecimento no tecido adiposo branco, identificado por alterações histológicas. E modulou os genes envolvidos nessa indução como PPARγ, PGC-1α, UCP1, CPT e PRDM16. E o consumo diário de infusão de chá verde atenuou as alterações metabólicas causadas pela dieta hiperlipídica C57BL/6J.

Com base no potencial terapêutico do chá verde na obesidade, as perspectivas futuras para a pesquisa experimental desenvolvida consistem em análise histológica do tecido adiposo dos animais submetidos ao consumo da infusão, bem como a análise da expressão de genes específicos neste tecido. Além disso, há a necessidade do desenvolvimento de estudos em seres humanos obesos para identificar se há a mesma resposta metabólica.

ANEXO

ANEXO A – CERTIFICADO DE APROVAÇÃO DO COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE LABORATÓRIO - CEUA

Universidade Federal da Bahia
Escola de Medicina Veterinária e Zootecnia
Comitê de Ética no Uso de Animais
Av. Ademar de Barros, 500 – Ondina-40170-110 Salvador-BA
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CERTIFICADO

Certificamos que a proposta intitulada **"Efeito do consumo regular do chá verde sobre alterações metabólicas, inflamatórias e hepáticas induzidas por dieta hiperlipídica em camundongos"**, registrada com o nº **03/2019**, sob a responsabilidade do(a) **Prof. (a) Ricardo David Couto**, e que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Escola de Medicina Veterinária da Universidade Federal da Bahia, **em reunião de 24.04.2019.**

Finalidade	( )Ensino (X) Pesquisa Científica
Vigência da autorização	24/04/2019 à 18/11/2019
Espécie/linhagem/raça	*Camundongo C57BL/6*
Nº de aninais	116
Peso/Idade	25-35g / 5 meses
Sexo	Macho
Origem	Biotério

Salvador, 25/04/2019.

Claudio de Oliveira Romão
Prof. Claudio de Oliveira Romão
Coordenador CEUA/EMEVZ-UFBA