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Nutritional characterization of Cusco green tea and its synergistic effect with omega-3 on metabolic and cognitive dysfunctions in obese rats

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Abstract

BACKGROUND: Obesity and its metabolic complications, such as insulin resistance and cognitive decline, remain a global health challenge. Green tea (*Camelia sinensis*) and ω-3 polyunsaturated fatty acids (PUFAs) are recognized for their antioxidant, anti-inflammatory, and neuroprotective properties. This study aimed to qualitatively characterize green tea from Cusco, Peru, and evaluate the synergistic effects of its infusion (GTi) and ω-3 supplementation on hyperlipidemia, proinflammatory cytokines, and cognitive impairment in a high-fat diet (HFD) rat model.

RESULTS: Nutritional analysis confirmed the high quality of Cusco green tea, showing potent antioxidant activity and a unique catechin profile. In obese rats, HFD induced significant weight gain and metabolic dysfunction; supplementation with GTi, ω-3, or their combination significantly improved glucose tolerance and reduced triglyceride and very-low-density lipoprotein levels. Notably, the combined treatment (GTi + ω-3) showed the greatest reduction in plasma TNF-α. Behavioral assessments via novel object recognition tests demonstrated that supplementation alleviated obesity-related cognitive deficits, significantly enhancing both short-term and long-term memory discrimination indices.

CONCLUSION: This study highlights the potential of combining Cusco green tea and ω-3 PUFAs as a complementary nutritional intervention to mitigate hyperlipidemia and cognitive dysfunction associated with obesity. These effects are likely mediated through the modulation of proinflammatory mediators and the enhancement of antioxidant defenses, which help restore redox homeostasis and protect neuronal integrity.
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Supporting information may be found in the online version of this article.

Keywords: anti-inflammatory; antioxidant; green tea; neuroprotection; obesity; omega-3

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ABBREVIATIONS

ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical
BW	body weight
DHA	docosahexaenoic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	dry weight
EPA	eicosapentaenoic acid
GAE	gallic acid equivalent
GTi	green tea infusion
HFD	high-fat diet
IST	insulin sensitivity test
NOR	novel object recognition
OGTT	oral glucose tolerance test
STD	standard diet
TEAC	Trolox equivalent antioxidant capacity
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

INTRODUCTION

Tea infusion from the dried leaves of *Camelia sinensis* stands as one of the most widely consumed beverages globally, along with coffee and cocoa. Historical records show that tea plants have been cultivated and used in China for over 3000 years.[1] Tea has been assigned important antioxidant properties and numerous beneficial health effects such as anti-inflammatory, antimicrobial, anticancer, antihypertensive, neuroprotective, cholesterol-lowering and thermogenic effects.[2] Mainly, there are three types of tea depending on the level of fermentation: green tea, Oolong tea, and black tea.[3] Specifically, a green tea infusion is achieved by exposing the dry leaves of the plant to 70 °C for a few minutes. This process also deactivates the enzymatic activity of polyphenol oxidases, keeping the polyphenols in the infusion unaltered.[4] These polyphenolic compounds include flavonoids and phenolic acids, which give bioactive properties to green tea and other types of tea.[5] Catechins are the flavonoids with the greatest interest composing approximately 18–36% of the dry weight (DW) of the tea leaves.[6] Four catechins present in green tea have been described: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC).[1] The importance of catechins present in green tea lies in their standard reduction potential values ($E°$), which are comparable to those α-tocopherol (vitamin E) and even higher than those of ascorbate (vitamin C). Among them, EGCG has been identified as the main compound responsible for the anti-obesogenic effects of green tea, improving lipid profiles, enhancing insulin sensitivity, and promoting beta-oxidation and thermogenesis.[7]

Plentiful scientific evidence supports tea as an important source of powerful antioxidant compounds in both *in vitro* and *in vivo* studies.[8] At the cellular level, EGCG appears to be the most biologically active polyphenol in green tea.[9] There is also indication that several catechins can cross the blood–brain barrier.[10] These two aspects are related to the neuroprotective effect that polyphenols can offer, combined with their antioxidant, anti-inflammatory, and metal-chelating capacities.[11] Green tea may also contain lower levels of theaflavins. Consequently, other flavonoids with equally high antioxidant activity may be present.[12] These tea flavonoids act by regulating metabolic pathways related to angiogenesis,[5] suggesting that these tea components have further biochemical roles beyond their antioxidant properties.

Other dietary supplements widely used in the food and feed market include omega-3 (ω-3) polyunsaturated fatty acids (PUFAs), which are generally produced from fish oil. There is consistent evidence that their consumption is essential for brain development and neuronal plasticity, including the association of their deficiency with the emergence of learning problems.[13] Also, the anti-inflammatory and lipid-lowering properties of long-chain ω-3 are well known, as well as the antiobesity effects and effectiveness against metabolic syndrome.[14]

The study presented here utilized an animal model of high-calorie diet (HFD)-induced obesity in rats. In this model, diets enriched in saturated and hydrogenated fats have been shown to promote lipid dysregulation by enhancing hepatic *de novo* lipogenesis, reducing fatty acid oxidation, and increasing very-low-density lipoprotein (VLDL) secretion. These alterations frequently lead to hypertriglyceridemia and are commonly observed in HFD-induced obesity models. The aim of the study was to expand current evidence regarding the beneficial effects of green tea infusion (GTi) and ω-3 supplementation on hyperlipidemia and selected aspects of obesity-associated cognitive impairment. We propose that the antioxidant properties of green tea could help protect eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil from oxidative degradation,[15] thereby enhancing the efficacy of ω-3 when the treatment is combined. The study also included acute studies such as an oral glucose tolerance test (OGTT) and an insulin sensitivity test (IST) in healthy rats with the aim of providing an initial pharmacodynamic and proof-of-concept assessment of the immediate impact on glucose and insulin metabolism. Other objectives of the work were to qualitatively characterize specifically the green tea from Cusco, Peru.

MATERIALS AND METHODS

Obtaining green tea

Leaves of *Camellia sinensis* (L.) Kuntze were collected from plants cultivated in the Valle de la Convención (12°52′05″ S, 72°41′35″ W), Cusco, Peru. A steaming process with 100 °C water vapor was applied to freshly harvested leaves to produce green tea. This step not only inhibits oxidation but also prevents the rupture of leaf veins and the subsequent release of enzymes, thereby preserving a significant portion of the nutrients. Subsequently, the leaves were cut into rolls and dried in an oven at 25–30 °C for biochemical stabilization and preservation, protected from light, until moisture was completely removed (12–15 h). The obtained product was stored in a dry environment, protected from light, until further use.

Elemental and biochemical analysis of green tea leaves

To determine the total carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) we used 2 mg of green tea leaf sample followed by the combustion technique (600 °C), using a LECO TruSpec Micro CHNS-O elemental analyzer (St Joseph, USA). All determinations were made in triplicate ($n = 3$), and the results are expressed as percentages relative to the sample weight for each element.

The total carbohydrates were determined according to the method of Dubois *et al.*,[16] while total protein content was calculated by multiplying the total nitrogen by 6,25, according to Jones' factors.[17] Total lipids were determined by the Folch method.[18] Inorganic compounds (ash) were determined using the Ismail method by incinerating the samples in a muffle at 550 °C for 12 h to eliminate all organic matter.[19] Finally, moisture content

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was determined by placing 3 g of sample in an oven at 60 °C for 24 h and calculating the difference between the initial and final weights. All determinations were performed independently in triplicate ($n = 3$).

Fish oil fatty acid profile analysis

The fatty acid composition of fish oil from the ω-3 supplement (MaxXomega 3, Mega We Care, Lima, Peru) was determined at the Food Nutritional Evaluation Laboratory of the UNALM. Analysis was performed according to the method described in Yi *et al.*[20] Fatty acid methyl esters were separated using a gas chromatograph equipped with a Restek SP2560 capillary column (100 m × 250 μm × 0.2 μm). The oven temperature program was set as follows: initial temperature of 100 °C for 4 min, increased at 20 °C min^{-1} to 150 °C (held for 4 min), then at 20 °C min^{-1} to 200 °C (held for 4 min), at 4 °C min^{-1} to 220 °C (held for 8 min), and finally at 4 °C min^{-1} to 230 °C (held for 10 min). A split ratio of 35:1 was applied. The flame ionization detector was maintained at 200 °C. Carrier and detector gases included hydrogen (40 mL min^{-1}), air (450 mL min^{-1}), and nitrogen (30 mL min^{-1}). Fatty acids were identified by comparison with known standards and results were expressed as a percentage of total fatty acids (results are shown in Table S1).

Green tea infusion and ω-3 supplements

The GTi was prepared on the same day as administration. An amount of 4 g of green tea leaves was weighed, 20 mL of water at 70 °C was added, and kept for 3 min. The resulting infusion was filtered through a 0.45 μm filter and administered according to the DW of the green tea and the body weight (BW) of the animals. The ω-3 supplement contained 200 mg of DHA and 300 mg of EPA per gram of fish oil (3:2 EPA/DHA).

Biological properties of green tea infusion

Determination of total polyphenolic concentration

Phenolic compounds in GTi were quantified using the Folin–Ciocalteu assay and expressed as milligrams of gallic acid equivalents (GAE) per gram DW of green tea.[21] The absorbance (Abs) was measured at 760 nm and including a blank with all reagents except the extract, which was replaced with 80% MeOH. Phenolic contents were determined by constructing a standard curve using different concentrations of gallic acid (Sigma-Aldrich, St Louis, USA).

Catechin analysis

For sample preparation, 250 mg of green tea was mixed with 2 mL of 80% ethanol solution and mechanically homogenized using an Ultra-Turrax™ homogenizer. The mixture was incubated for 48 h at 4 °C in the dark and subsequently filtered through a 0.22 μm filter. The identification of catechins present in GTi was performed in triplicate using high-performance liquid chromatography coupled to high-resolution electrospray ionization mass spectrometry (HPLC–ESI-HRMS) with an Agilent-1260 HPLC system. A Hypersil GOLD™ C18 column (1,9 μm, 50 × 2.1 mm ID (Thermo Fisher Scientific, Waltham, USA, 25003-102130)) was used. The mobile phase consisted of 0.1% acetic acid in distilled water (A) and methanol (B), with initial conditions of 95% A and 5% B. The gradient program was as follows: 0–5 min, 5–15% B; 5–25 min, 15–25% B; 25–30 min, 25–50% B; 30–35 min, 50–5% B, followed by 5 min of re-equilibration at 5% B. Mass spectra were acquired in negative ionization mode over an *m/z* range of 200–600. UV–visible spectra were recorded at 280 and 210 nm. The flow rate was set at 0.3 mL min^{-1}, and the injection volume ranged from 2 to 10 μL for each analysis. Additionally, the compounds were further confirmed by comparing their mass spectra according to those available in the National Institute of Standards and Technology (NIST) Certificate of Analysis: Standard Reference Material® 3257 Catechin Calibration Solutions (Gaithersburg, MD: NIST; 2016 https://tsapps.nist.gov/srmext/certificates/archives/3257.pdf, accessed 12 February 2026).

DPPH radical scavenging assay

The antioxidant and radical scavenging activities of GTi were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, St Louis, USA) free radical method.[22] An amount of 0.5 mL of GTi was dissolved in 1 mL of 80% MeOH to reach dilutions so that the final concentrations in the cuvettes were from 62.5 to 2000 μg mL^{-1}. The initial Abs was measured at 517 nm, incubated for 30 min, and measured again. Ascorbic acid (125–8000 μg mL^{-1}) was used as a positive control. Each measurement was performed in triplicate ($n = 3$). The DPPH radical scavenging effect (%) was calculated as follows: [1 − (Abs1 − Abs2)/Abs0] × 100, where Abs1 is the absorbance of the GTi sample with DPPH, Abs0 is the absorbance of the control, and Abs2 is the absorbance of the sample with methanol. A calibration curve was prepared from Trolox (Sigma-Aldrich, St Louis, USA) standards and used to interpolate Abs1, determining the DPPH radical scavenging capacity expressed as μmol Trolox equivalent antioxidant capacity (TEAC) per gram DW.

Determination of antioxidant activity by ABTS assay

The ability of green tea to scavenge oxidant radicals was also evaluated using a 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) assay, as reported by Re *et al.*,[23] with a few modifications. ABTS radical cation was generated by a reaction of 7 mmol L^{-1} ABTS (Sigma-Aldrich, St Louis, USA) with 2.45 mmol L^{-1} $K_2S_2O_8$ (Sigma-Aldrich, St Louis, USA). After incubation, the mixed solution was diluted to 0.7 Abs at 734 nm with deionized water. An amount of 0.5 mL of GTi was dissolved in 1 mL of PBS to prepare dilutions, resulting in final concentrations ranging from 0.16 to 20 mg mL^{-1}. Then, 50 μL of each sample was mixed with 940 μL of PBS and 10 μL of ABTS solution in cuvettes. Then, the mixture was determined at 734 nm using a spectrophotometer, and Abs was transformed to percentage ABTS radical-scavenging capacity according to the following equation: scavenging effect (%) = [Abs(control) − Abs(sample)/Abs(control)] × 100. A calibration curve was made with different concentrations from a stock of Trolox®. The scavenging effect was reached with scavenging effect % and interpolating its value of GTi to achieve the ABTS radical scavenging capacity, expressed in μmol TEAC per gram DW. All determinations were performed in triplicate.

In vivo study

Animals and ethics statement

A total of 62 male rats were used in the study. Thirty Holtzman rats were used for chronic study being provided and maintained by the laboratory animal facility of the Department of Nutrition, Faculty of Animal Husbandry, Universidad Nacional Agraria La Molina (UNALM), Lima, Peru. Additionally, 32 Wistar rats (Charles River, Barcelona, Spain) were used for the acute studies and were maintained at the Center for Experimentation and Animal Behavior, University of Malaga, Spain. All animals, weighing approximately 250 ± 10 g at the start of the study, were housed under standard

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experimental conditions, including individual cages, ambient temperature maintained between 22 and 25 °C, 75% humidity, and a 12 h light/dark cycle with *ad libitum* water access The animals received standard rodent chow pellets (STD) (3.39 kcal g^{-1}) or a HFD (4.31 kcal g^{-1}) depending on the experimental procedures. Handling and care of animals followed the Declaration of the World Medical Association on the Use of Animals in Biomedical Research (Reaffirmed by the 203rd WMA Council Session, Buenos Aires, Argentina, April 2016) and the European Communities Council Directives 2010/63/EU (Regulation EC 86/609/ECC, 24 November 1986). Experimental animal protocols and procedures were approved by the Local Ethical Committee for Animal Research of the University of Malaga and performed in accordance with the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments).[24] The relevant authorized protocols for animal research were incorporated into the approved projects (002-2023-CEI-UNALM and 113-2023-A-CECA-UMA). The experimental design of animal study, including both acute and chronic studies, is shown schematically in Fig. 1.

Oral glucose and insulin sensitivity tests

For the OGTT, 32 Wistar rats fasted for 16 h were administered with 2 g kg^{-1} glucose by gavage in a volume of 5 mL kg^{-1}. Thirty minutes before the glucose load, animals received different acute treatments by gavage according to the following four groups ($n = 8$): GTi 900 mg kg^{-1} in a volume of 4.5 mL kg^{-1}; ω-3 fish oil 800 mg kg^{-1} in a volume of 1 mL kg^{-1} containing 160 mg of DHA and 240 mg of EPA; GTi and ω-3 combination; and control administered with water. Tail blood samples were collected at 0, 15, 30, 60, 90, and 120 min after administration of glucose overdose. Glucose levels were determined using a glucometer based on a standard glucose oxidase method (Accu-check, Roche Diagnostic, Germany).

For the IST, 32 Wistar rats fasted for 16 h were intraperitoneally (ip) administered at 0.5 IU kg^{-1} insulin in a 1 mL kg^{-1} volume. Thirty minutes before insulin administration, animals received different acute treatments via gavage following the same procedure and group settings as for the OGTT.

Obesity induction

In the first phase, 24 Holtzman rats received the HFD for 8 weeks. The obesogenic diet consisted of 83.4% standard rodent food from the Research Program and Social Projection in Food from UNALM (Alimentos Balanceados La Molina) supplemented with 16.6% of partially hydrogenated palm oil (Tropical Manoc, Industrias del Espino SA, San Martín, Peru). An additional 6 Holtzman rats were fed a STD for the same 8-week period. The nutritional compositions of the diets used are presented in Table 1. All animals were weighed weekly for 8 weeks. At the end of this period, obesity in rats was assessed using the Lee index, calculated from BW and naso-anal length.[25] A value >300 indicated obesity, whereas ≤300 indicated normal weight. The progression of BW in the rats during the obesogenic phase is shown in supporting information, Fig. S1.

Experimental groups

Once it was verified that all 24 animals fed with HFD were obese, they were randomly distributed into four groups of six animals ($n = 6$): animals exposed to HFD (Obese); animals exposed to HFD supplemented orally with GTi (Ob GTi); animals exposed

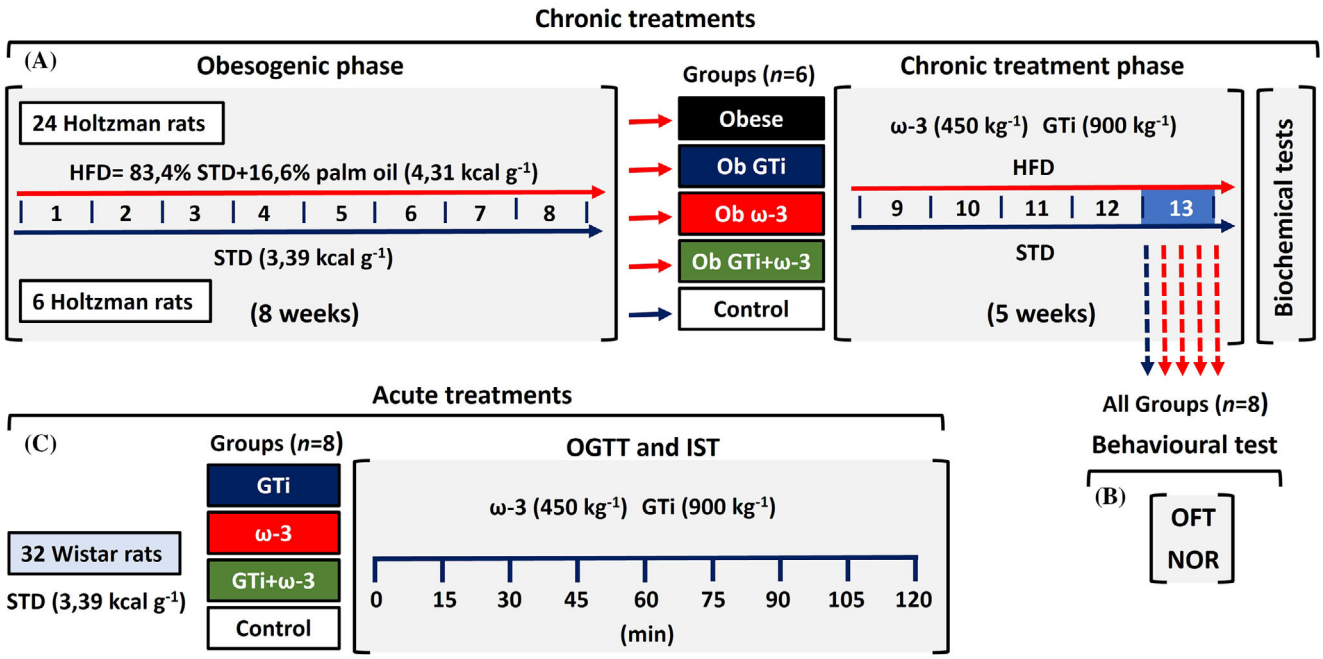

Figure 1. Schematic representation of animal experimental design. (A) For the obesity induction phase, 30 male Holtzman rats were used; 24 were fed a HFD, and 6 served as controls fed a STD. After 8 weeks, the 24 HFD-fed animals developed sufficient obesity to proceed to the chronic treatment phase. These animals were randomly divided into four groups: untreated obese (Obese), obese treated with green tea infusion (Ob GTi), obese fed a diet supplemented with omega-3-rich fish oil (Ob ω-3), and obese treated with green tea infusion combined with omega-3-rich fish oil supplementation (Ob GTi + ω-3), in addition to the 6 rats in the STD group (Control). (B) In the fifth week of treatment, OFT and NOR behavioral tests were performed. Upon completion of behavioral testing, blood samples were collected for biochemical analyses. (C) For the acute study, OGTT and IST were conducted using 32 healthy male Wistar rats randomly divided into four groups, each receiving a single dose of GTi, ω-3-rich fish oil, combined administration of GTi + ω-3, or vehicle only (Control).

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Table 1. Detail of nutritional composition for STD and HFD for rodents from the research program and social projection in food from Universidad Nacional Agraria La Molina, Lima, Peru

	STD		HFD	
Food composition	Amount (%)	Caloric contribution (%)	Amount (%)	Caloric contribution (%)
Protein	18.28	22.55	15.53	18.37
Lipids	2.89	3.56	18.53	21.92
Carbohydrates	59.90	73.89	50.47	59.71
Crude fiber	2.22	0	1.70	0
Ash	4.78	0	4.03	0
Moisture	11.93	0	9.74	0
Energy (kcal g^{-1})	3.39		4.31	

to HFD supplemented orally with fish oil (Ob ω-3); and animals exposed to HFD supplemented orally with GTi plus ω-3 (Ob GTi + ω-3). The group of six non-obese animals that were exposed to STD (Control) was also included.

Green tea and ω-3 administration

In the second phase of the study, beginning at week 9, chronic treatments were administered for 5 weeks to the different experimental groups. For the administration of ω-3, fish oil was added to the daily feed ration at a dose of 400 mg kg^{-1} per day providing 80 mg kg^{-1} of DHA and 120 mg kg^{-1} of EPA. The dose of fish oil used was calculated according to previous evidence, taking into account the EPA/DHA concentration,[26] and by applying the inverse allometric scaling method based on body surface area.[27] GTi was administered by gavage as an equivalent of 450 mg kg^{-1} of green tea (this dose could be considered equivalent to 400 mL day^{-1} of GTi for humans).[28] In the case of the animals included in the Obese, Ob ω-3, and Control groups, a vehicle (water) was administered, instead of GTi, via gavage. The BW and the food intake calculated as kcal g^{-1} were daily registered for 5 weeks.

Biochemical parameters

After a 5-week supplementation period, animals were fasted for 12 h, then anesthetized with an intraperitoneal injection of 75 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine. Subsequently, blood samples were collected via cardiac puncture into EDTA tubes and centrifuged (2000 × *g* for 10 min at 4 °C); the resulting plasma samples were stored at −80 °C until biochemical or pharmacokinetic analyses. Blood glucose levels were immediately measured in a blood drop using an On Call® Advanced Blood Glucose Monitoring System (ACON Laboratories Inc, USA). To determine the plasma levels of triglycerides, cholesterol, and high-density lipoprotein (HDL), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (γ-GT), and lactate dehydrogenase (LDH) were measured using a DRI-CHEM NX700 high throughput automatic clinical chemistry analyzer (Fujifilm, Tokyo, Japan). VLDL and low-density lipoprotein (LDL) were determined via modification of the Friedewald equation: VLDL = triglycerides/5; LDL = [(cholesterol/1.19) + (triglycerides/1.9) − (HDL/1.1) − 38].[29] Tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) concentrations were measured using a commercial rat ELISA kit (Rat TNF-α ELISA Kit ab236712, Abcam, UK; and Rat IL-6 ELISA Kit 17165043, Invitrogen, USA).

Behavioral tests

First, all animals were habituated in a dimly illuminated (120 lx) observation cage (65 × 45 × 45 cm^3) with a clear front panel for 5 min each day over the 5 days preceding each behavioral test. A 30% ethanol solution was used to clean the arena and eliminate odors between each animal at the end of week 5.

Open field test

The open field test (OFT) is a simple sensorimotor test used to determine general locomotor activity and exploration habits in rodents.[30] The arena was divided, by means of marks, into two zones, a central one (22.5 × 22.5) and the perimeter. Rats were transported to a noise-isolated room and habituated for at least 20 min before the behavioral assessment began. Then, each animal was placed in the OFT and monitored for 3 min. During this period, the time spent in the central and peripheral areas, the number of line crossings between areas, episodes of freezing, and the total distance traveled were recorded.

Novel object recognition paradigm

The novel object recognition (NOR) task is used to evaluate cognition, particularly recognition memory in rodents,[31] and was performed in the OFT arena. The discrimination objects were identical plastic items secured to the arena with silicone to prevent movement by the rats. Testing occurred in a sound-attenuated room under constant illumination (120 lx). Exploratory behavior was defined as the animal's nose being positioned within 2 cm of the object and/or touching it.

For short-term memory, two identical objects (O1 and O2) were placed in opposite corners of the arena. The animal was placed in the box with its back to the objects, allowing it to explore them and monitoring the time spent on each object for 3 min. At the end of the exploration time, the animal was removed and placed in its corresponding cage for 5 min. During this time, one of the objects was changed (O2 for O3), leaving one already known (O1). The box was cleaned with 70% ethanol between each trial. After a 5 min interval, the animal was placed again in the arena to explore the new object and the familiar one, monitoring the time spent in each of these. The test was concluded after 3 min of exploring.

For long-term memory, a new object was placed inside the arena while maintaining a familiar one (A1 and O4). The animal was placed in the box 48 h after the identical object recognition test, with its back to the objects, for 3 min. The time the animal spent exploring the familiar object and the novel one was monitored. Once the exploration time was over, the animal was

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removed and placed inside its corresponding cage. The box was cleaned with 70% ethanol between each trial.

To analyze the effect of supplements on short- and long-term memory, the following variables were used for data analysis:

Total exploration time (TE). Time spent with the novel object (b) and time spent with the familiar object (a'): TE = a' + b.

Absolute habituation or discrimination index (DI). This is a measure of overall exploration. It is calculated as the difference between exploration time for the new object and the exploration time for the familiar object. This is a measure of discriminatory behavior according to D1 = $b - a'$.

Relative discrimination index (RDI). Exploration time of the new object minus the time spent exploring the familiar object, divided by the total exploration time. This result can vary between +1 and −1, where a positive score indicates more time spent with the novel object, a negative one indicates more time spent with the familiar object, and a zero score indicates no preference: RDI = (DI/TE).[32]

Preference index (PI). Time spent exploring the new object divided by the total time. It is multiplied by 100 and used as a percentage value. This is a proportion of the amount of time spent exploring either of the two objects in the test phase (a' or b). Therefore, this index is interpreted as follows: above 50% indicates a preference for the new object, below 50% shows a preference for the familiar object, with 50% = no preference. PI = (b/TE) × 100.[33]

To evaluate the long-term memory, the variable b was replaced by the variable c, which represents the time the animal explores the new object, as it does with b in the short-term memory test.

Statistical analysis

Results are expressed as the mean ± SEM or SD, based on three determinations for chemical analyses (n = 3) and six determinations per *in vivo* or *ex vivo* experimental group (n = 6). Statistical data were obtained using GraphPad Prism version 9.01 (GraphPad Software Inc., San Diego, USA) and analyzed by a one-way or two-way analysis of variance (ANOVA), depending on the factors and type of analysis, followed by Bonferroni's *post hoc* test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Elemental and nutritional analysis of green tea

Elemental analysis and biochemical composition of green tea showed a high percentage of C (48.68%), followed by H (5.91%) and N (5.01%) while S content was null (Table 2). Regarding the biochemical composition of green tea, carbohydrates (36.91%) and proteins (31.28%) represented the highest contents, followed by lipids (7.98%). The remaining content consisted of ash (5.12%) and showed a low moisture content (0.29%) (Table 2).

Table 2. Elemental and nutritional analysis of green tea from Valle de la Concepción, Cusco, Peru

Element	Amount (%)
C	46.68 ± 0.11
H	5.91 ± 0.08
N	5.01 ± 0.28
S	—
Carbohydrates	36.91 ± 2.09
Proteins	31.28 ± 2.45
Lipids	7.98 ± 1.75
Ash	5.12 ± 0.03
Moisture	0.29 ± 0.02

Data are expressed as mean ± SD of three replicates per measure.

Polyphenolic content and antioxidant activity of green tea infusion

The total polyphenolic content of GTi was 55.45 mg GAE g^{-1}, which is equivalent to 5.5% of the total weight of the leaf (Table 3). The content of total phenols obtained from the GTi showed that the daily intake dose that the experimental groups received was 24.95 mg GAE kg^{-1}. The analysis of catechin proportions in GTi revealed very similar percentages of EGCG and EGC, followed by ECG and, to a lesser extent, EC. The antioxidant activity by DPPH assay yielded 3662.12 µmol TEAC g^{-1}, while the ABTS-scavenging capacity resulted in 1148.58 µmol TEAC g^{-1}. All these results are presented in Table 3.

In vivo effect of green tea infusion and ω-3 on oral glucose and insulin sensitivity tests

We analyzed the effect of acute GTi administration on both glucose tolerance after an oral glucose load and insulin sensitivity after ip insulin administration. In the OGTT, the GTi, ω-3, and GTi + ω-3 treated groups showed a significant improvement in glucose tolerance during the first 15 min compared to the Control group (Fig. 2(A)). Glycemia did not differ at subsequent time points, showing a similar trend to the Control group. The analysis of the area under the curve (AUC) showed no significant differences between treatments (Fig. 1(B)).

Regarding insulin sensitivity analysis by IST, insulin administration led to a significant increase in glycemia from 45 min onward across all treatments (Fig. 2(C)). More specifically, GTi and GTi + ω-3 showed higher blood glucose levels than the Control group while single ω-3 supplementation maintained intermediate values. The analysis of the AUC is consistent with these results (Fig. 1(D)).

Table 3. Total polyphenolic content, catechin proportions and antioxidant activity present in GTi from the Valle de la Concepción, Cusco, Peru

Polyphenolic compounds in green tea	
Polyphenolic	55.45 ± 2.61 mg GAE g^{-1}
Corresponding DW leaves	5.55 ± 0.21%
Catechin proportion	
Epigallocatechin-3-gallate (EGCG)	36.57 ± 1.53%
Epigallocatechin (EGC)	33.39 ± 2.67%
Epicatechin-3-gallate (ECG)	20.64 ± 0.78%
Epicatechin (EC)	9.40 ± 0.36%
Radical scavenging capacity	
DPPH assay	3662.12 µmol TEAC g^{-1} DW
ABTS assay	1148.58 µmol TEAC g^{-1} DW

Data for polyphenolic content are expressed as mean ± SD of three replicates per measure. Data for catechin percentage are expressed as mean ± SD of three replicates per measure.

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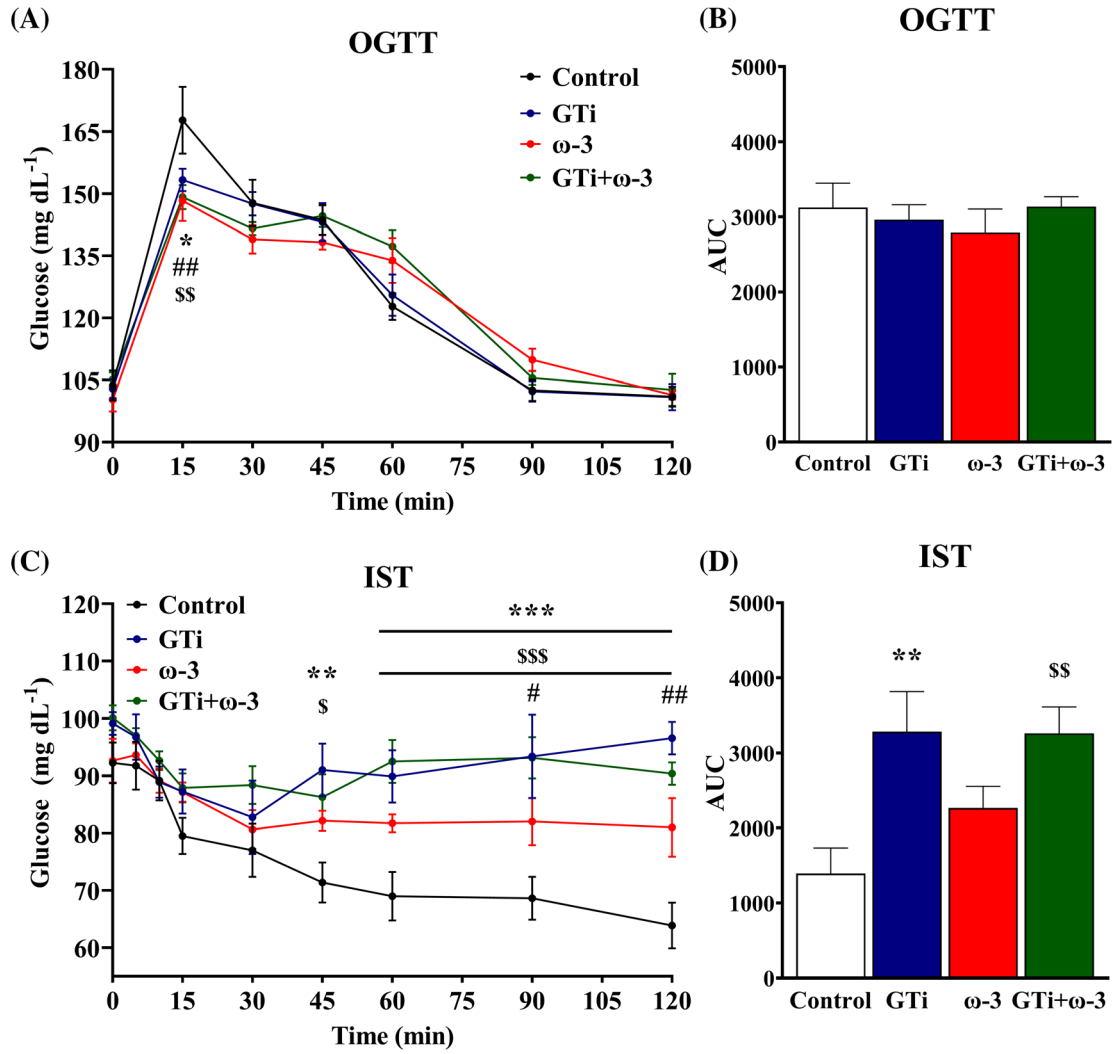

Figure 2. (A) Effect of GTi (900 mg kg^{-1}), ω-3 (800 mg kg^{-1}) and their combination (GTi + ω-3) on OGTT in male Wistar rats. Glycemia were evaluated before (0 min) and after (15, 30, 45, 60, and 120 min) glucose overload (2 mg kg^{-1}). (B) AUC was also calculated for all OGTT groups. (C) Effect of GTi, ω-3 and GTi + ω-3 after ip insulin administration to analyze the IST in male Wistar rats. Glycemia was evaluated before (0 min) and after (5, 10, 15, 30, 45, 60, 90, and 120 min) of ip insulin administration (0.5 IU kg^{-1}). (D) AUC was also calculated for all IST groups. Points or bars indicate the mean ± SEM (n = 8). One-way ANOVA for OGTT and IST time course, or two-way ANOVA for OGTT and IST AUC, followed by Bonferroni *post hoc* tests, were performed: *P < 0.05, **P < 0.01 and ***P < 0.001 GTi *versus* Control. #P < 0.05 and ##P < 0.01 ω-3 *versus* Control. \$$P$ < 0.05, \$\$$P$ < 0.01 and \$\$\$$P$ < 0.001 GTi + ω-3 *versus* Control.

Kcal intake and BW gain in chronic supplementation

Cumulative kcal intake during the supplementation study was not statistically different between the GTi, ω-3, and GTi + ω-3 obese groups, although it consistently remained higher than in the Control group receiving STD (Fig. 3(A)). Regarding the comparison between the obese groups, the animals supplemented with GTi and ω-3 consumed more kcal compared to the obese group without supplementation. This difference was only observed at week 5 in the Ob GTi + ω-3 group.

Regarding the relative BW gain, all animals fed HFD (obese groups) gained more weight than those fed STD (Control) (Fig. 3 (B)). However, the GTi + ω-3 treatment showed no differences with the Control at weeks 1, 2, 3, and 5.

Biochemical parameters in plasma after supplementation

Both Obese and Ob GTi + ω-3 groups showed higher levels of glycemia after the study compared to Control group (Table 4). The Ob GTi and Ob GTi + ω-3 groups, which did not show significant differences compared with the Control, also showed lower glycemia values than the Obese group.

The analysis of lipidemia in plasma discloses, on the one hand, its increase in the obese groups and, on the other hand, that supplementation with GTi, ω-3, and GTi + ω-3 considerably reduced the levels of triglycerides and HDL in obese animals (Table 4). The administration of the supplements did not show significant changes in total cholesterol levels. Regarding the parameters used to evaluate liver damage, such as AST, ALT, γ-GT and LDH, they were not modified by obesity or supplementation (Table 4).

We also analyzed the effects of GTi, ω-3, and GTi + ω-3 on plasma TNF-α and IL-6 levels (Table 4). The Obese group exhibited significantly higher TNF-α levels compared to all other experimental groups. The groups of obese animals to which supplementation was administered showed a severe decrease in values compared to the obese group without supplementation. As expected in an animal model of obesity, IL-6 was elevated in all groups of obese animals in which neither supplementation presented an effect.

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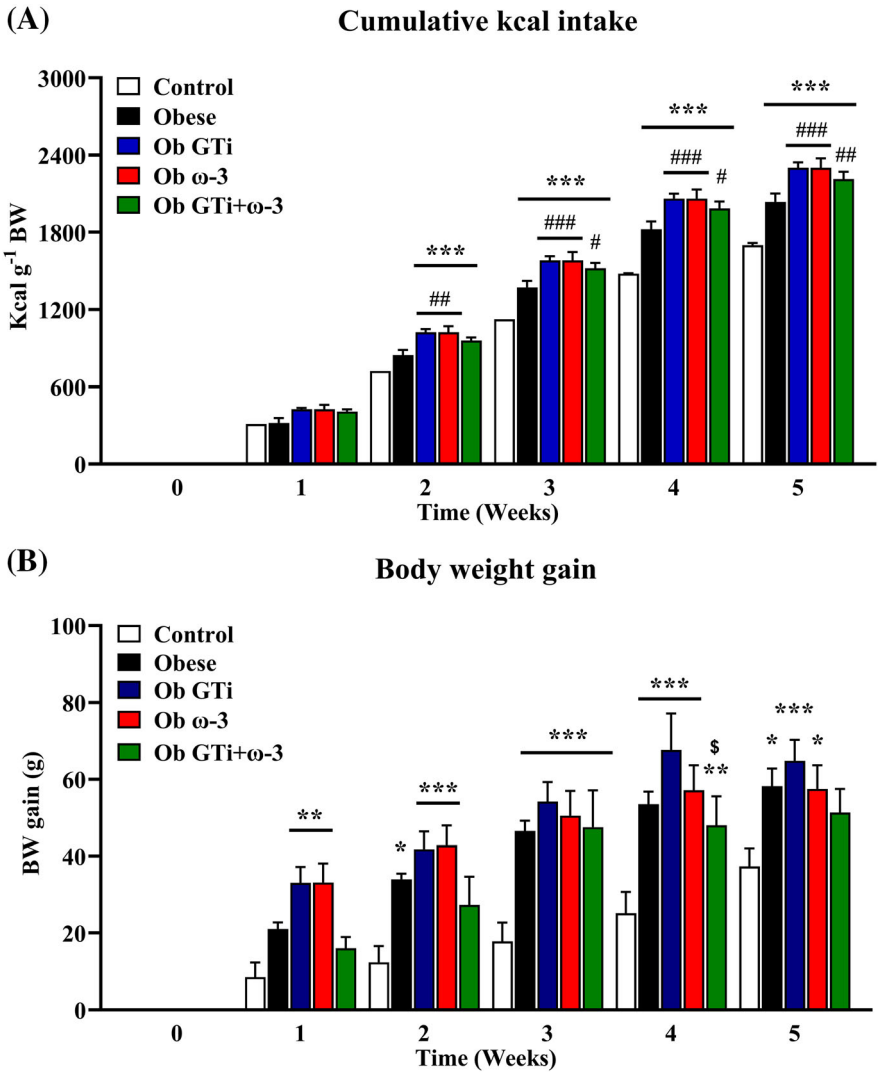

Figure 3. (A) Effects on cumulative caloric intake over 5 weeks in Holtzman male rats fed with a standard diet (Control) or a HFD: untreated (Obese), supplemented with green tea infusion (Ob GTi; equivalent to 450 mg·kg^{-1}), omega-3 fatty acids (Ob ω-3; 400 mg·kg^{-1}), or their combination (Ob GTi + ω-3). (B) Effects of GTi, ω-3, and GTi + ω-3 on BW gain. Bars indicate the mean ± SEM ($n = 6$). Two-way ANOVA, followed by Bonferroni *post hoc* tests, was performed: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ denote differences *versus* Control. #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ denote differences *versus* Obese group. \$$P < 0.05$ denotes differences *versus* Ob GTi group.

Behavioral tests

Open field test

The result of the investigation on the locomotor activity of the animals was statistically different between all the groups with the supplements, which had greater activity compared to the Control (Fig. 4(A)). Furthermore, the obese group supplemented with GTi + ω-3 presented greater locomotor activity compared to all other groups. Regarding immobilization, the times shown by the animals were statistically the same between the Control and obese supplemented groups, except for the supplementation with ω-3, which was greater (Fig. 4(B)). Finally, no differences were observed in the latency time among all the groups (Fig. 4(C)).

Novel object recognition paradigm for short-term memory

At this stage of the experiment, the effect of supplementation on cognitive performance, specifically short-term memory, of obese rats was evaluated. All obese groups exhibited longer exploration times compared to the Control group (Fig. 5(A)). Notably, the Obese group receiving GTi + ω-3 demonstrated significantly longer total exploration times than all other groups. The novel object time of exploration also showed an increase in time for all obese groups being more elevated in the groups supplemented with ω-3 and GTi + ω-3 (Fig. 5(B)). Nevertheless, the time of exploration of the known object remained unchanged (Fig. 5(C)).

The different indices used for the analysis of short-term memory, such as total and relative discrimination and preference index, were found to be always higher in the obese groups of animals supplemented with GTi and GTi + ω-3 compared with the other groups (Fig. 4(D)–(F)).

Novel object recognition paradigm for long-term memory

We analyzed the effect of supplementation in the obese groups on cognitive performance, specifically long-term memory. The total exploration time was statistically longer in the Obese, Obese ω-3 and Obese GTi + ω-3 groups, compared to the Control and Obese GTi groups (Fig. 6(A)). Furthermore, comparing the obese

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Table 4. Plasma biochemical parameters in plasma of Holtzman male rats

Plasma biochemical parameters (mg dL^{-1})	STD	HFD			
	Control	Obese	Ob GTi	Ob ω-3	Ob GTi + ω-3
Glucose	79.80 ± 2.20	91.20 ± 1.39**	83.20 ± 2.15$^{\#}$	84.00 ± 1.52$^{\#}$	89.40 ± 1.69**
Triglycerides	61.20 ± 4.55	100.20 ± 2.77***	84.60 ± 5.64***$^{\#\#\#}$	65.08 ± 4.08$^{\#\#\#\$\$\$}$	60.20 ± 4.02$^{\#\#\#\$\$\$}$
Cholesterol	56.67 ± 2.87	67.83 ± 3.07	55.40 ± 3.84	54.40 ± 4.53$^{\#}$	64.2 ± 2.87
HDL	37.80 ± 0.58	18.20 ± 1.27***	18.80 ± 1.96***	19.40 ± 0.37***	16.20 ± 0.08***
LDL	47.07 ± 3.65	82.23 ± 1.12***	73.47 ± 2.99***	63.03 ± 2.46***$^{\#\#\#}$	62.99 ± 1.05***$^{\#\#\#\$}$
VLDL	12.24 ± 0.41	20.04 ± 0.23***	16.92 ± 0.46***$^{\#\#\#}$	13.16 ± 0.33$^{\#\#\#\$\$\$}$	12.04 ± 0.33$^{\#\#\#\$\$\$}$
Transaminase (IU L^{-1})					
AST	34.33 ± 1,91	33.50 ± 1.57	27.60 ± 2.46	26.8 ± 2.97	± 29.40 ± 1.40
ALT	23.67 ± 1.49	25.83 ± 1.59	23.40 ± 2.11	25.00 ± 1.92	25.25 ± 2.29
γ-GT	2.33 ± 0.36	2.68 ± 0.52	3.00 ± 0.32	2.00 ± 0.45	2.25 ± 0.43
LDH	157.68 ± 30.68	129.83 ± 44.19	96.67 ± 11.45	110.60 ± 16.29	97.00 ± 11.51
Cytokines (pg mL^{-1})					
TNF-α	36.38 ± 1.07	338.38 ± 14.53***	155.13 ± 9.57***$^{\#\#\#}$	182.63 ± 7.20***$^{\#\#\#}$	147.13 ± 5.15***$^{\#\#\#\&}$
IL-6	280.87 ± 25.37	382.87 ± 23.50*	393.53 ± 123.81*	408.87 ± 47.53*	402.20 ± 39.27*

Effects of GTi supplementation (equivalent to 450 mg kg^{-1}), ω-3 (400 mg kg^{-1}), and their combination (GTi + ω-3) on metabolic biochemical parameters in plasma after 5 weeks in Holtzman male rats with a STD as Control, or HFD: all Obese animals (Ob). Values are expressed as the mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* tests for multiple comparisons: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ denote differences with Control. #$P < 0.05$ and ###$P < 0.001$ denote differences with Obese. $$P < 0.05$ and $$$$P < 0.001$ denote differences with Obese GTi. &$P < 0.05$ denotes differences with Obese ω-3.

groups, the supplements with GTi and GTi + ω-3 presented longer exploration times of the novel object (Fig. 6(B)). In turn, the exploration of the known object also presented longer exploration times by the groups of obese animals compared with the Control group (Fig. 6(C)).

In the case of the absolute and relative discrimination indices, the results showed similar values for the supplemented obese groups and the Control group and were higher than the obese group (Fig. 6(D),(E)). The preference index also showed no differences between the supplemented obese groups and the Control (Fig. 6(F)).

DISCUSSION

The chemical and nutritional composition of tea depends on factors such as geography, climate, season, cultivation, processing, and leaf age. Green tea's minimal processing effectively halts enzymatic polyphenol degradation, preserving its bioactivity.[34] However, few studies have examined the elemental and nutritional composition of freshly processed leaves used for green tea production. This study is the first to analyze the elemental and nutritional profile of green tea from Valle de la Concepción, Cusco, Peru. The dry leaves show carbohydrate, protein, lipid, ash, and moisture contents similar to those of tea from other regions,[35] and both dry leaves and infusions display bioactive compounds consistent with established quality indicators.[36]

Green tea's strong antioxidant properties mainly derive from its high polyphenol content, which serves as primary antioxidants and free radical scavengers.[37] Our analysis confirmed a high total polyphenol concentration in the GTi, consistent with previous studies,[38] and indirect assays showed that both phenolic content and antioxidant capacity exceeded average values reported elsewhere.[39] Regarding the analysis of catechins present in the GTi, the main difference compared to the proportions generally reported in previous chemical analyses is that the green tea from Cusco shows a lower proportion of EGCG and a relatively higher proportion of EGC. The percentages of ECG and EC are very similar to and consistent with previous evidence.[40]

Our main objective was to investigate whether the combined effect of Cusco green tea and ω-3 PUFAs from fish oil on hyperlipidemia and selected aspects of obesity-associated cognitive impairment in an HFD-induced obesity animal model is greater than the effects of these compounds when administered separately. ω-3 PUFAs, particularly EPA and DHA, are vital dietary components that maintain physiological homeostasis and mental health by enhancing membrane fluidity, regulating inflammation, and modulating signaling and gene expression. They support cardiovascular, immune, metabolic, and brain functions, promoting optimal neurodevelopment, neurotransmission, and neuroplasticity. Adequate intake is linked to improved cognition and reduced risk or severity of depression and anxiety via anti-inflammatory and neuroprotective effects.[41] Additionally, our own analysis of a commercial ω-3-rich fish oil product did not yield data indicating the presence of other fatty acids in sufficient quantities to exert a significant biological effect or be relevant to our study.

Two different rat strains were used to align the biological model with the specific aims of each experimental phase and to reduce strain-specific bias. Holtzman rats were selected for the chronic study because they provide a robust model for long-term follow-up of diet-induced obesity and associated metabolic alterations. In contrast, Wistar rats were chosen for the acute tests (OGTT and IST) as they are a well-established reference strain in metabolic research, with extensively characterized glycemic and insulin responses. Using both strains strengthens the external validity of the findings by minimizing the possibility that the observed anti-obesogenic effects are limited to the genetic background of a single rat line.

The acute study using either single GTi, single ω-3, or the combined GTi + ω-3 treatment in healthy Wistar rats provides pharmacodynamic proof-of-concept for immediate effects on

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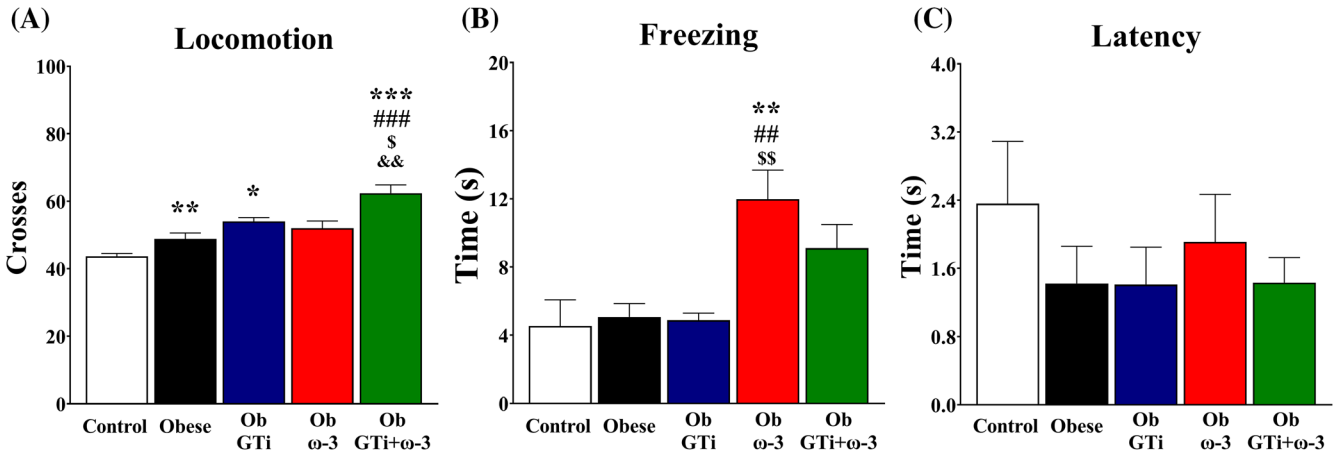

Figure 4. Effects GTi supplementation (equivalent to 450 mg kg^{-1}), ω-3 (400 mg kg^{-1}), and their combination (GTi + ω-3) on metabolic biochemical parameters in plasma after 5 weeks in Holtzman male rats with a STD as Control, or HFD: all Obese animals (Ob), on the OFT parameters. (A) Total number of crossings between the central area and the external area of the arena, (B) duration of freezing state and (C) duration of time spent in the central area of the arena. Bars indicate the mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* tests: *P < 0.05, **P < 0.01, and ***P < 0.001 denote significant differences compared with the Control. ##P < 0.01 and ###P < 0.001 denote significant differences compared with Obese. \$$P$ < 0.05 and \$\$$P$ < 0.01 denote significant differences compared with Ob GTi. &&P < 0.01 denotes significant differences compared with Ob ω-3.

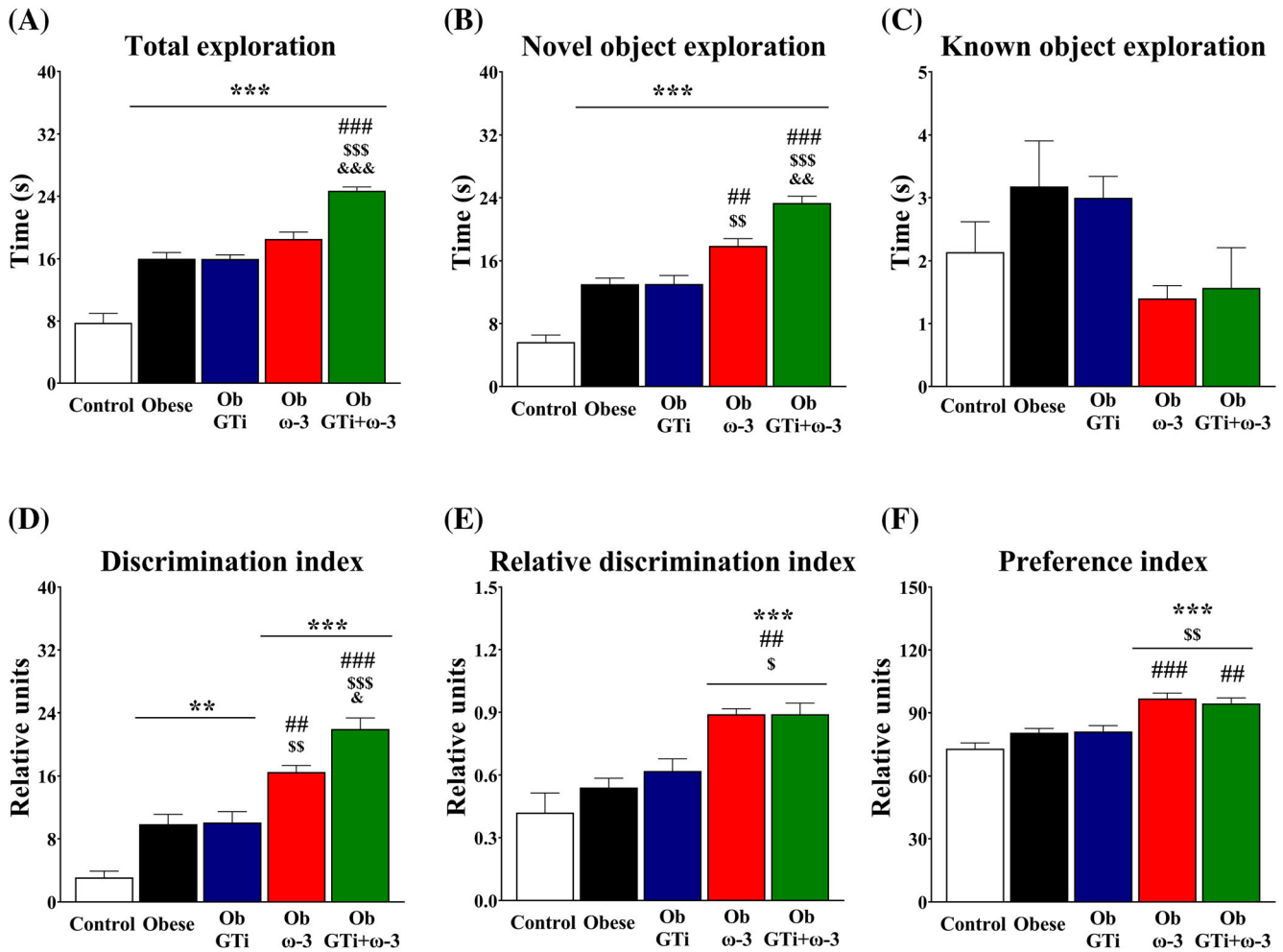

Figure 5. Effects of GTi supplementation (equivalent to 450 mg kg^{-1}), ω-3 (400 mg kg^{-1}) and their combination (GTi + ω-3) on metabolic biochemical parameters in plasma after 5 weeks in Holtzman male rats with a STD as Control, or HFD: all Obese animals (Ob), on the short-term memory by NOR tests. (A) Total time of exploration, (B) time of novel object exploration, (C) time of known object exploration, (D) discrimination index, (E) relative discrimination index and (F) preference index. Bars indicate the mean ± SEM (n = 6). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* tests: **P < 0.01 and ***P < 0.001 denote significant differences compared with the Control. ##P < 0.01 and ###P < 0.001 denote significant differences compared with Obese. \$$P$ < 0.05, \$\$$P$ < 0.01 and \$ \$\$$P$ < 0.001 denote significant differences compared with Ob GTi. &P < 0.05 and &&P < 0.01 denote significant differences compared with Ob ω-3.

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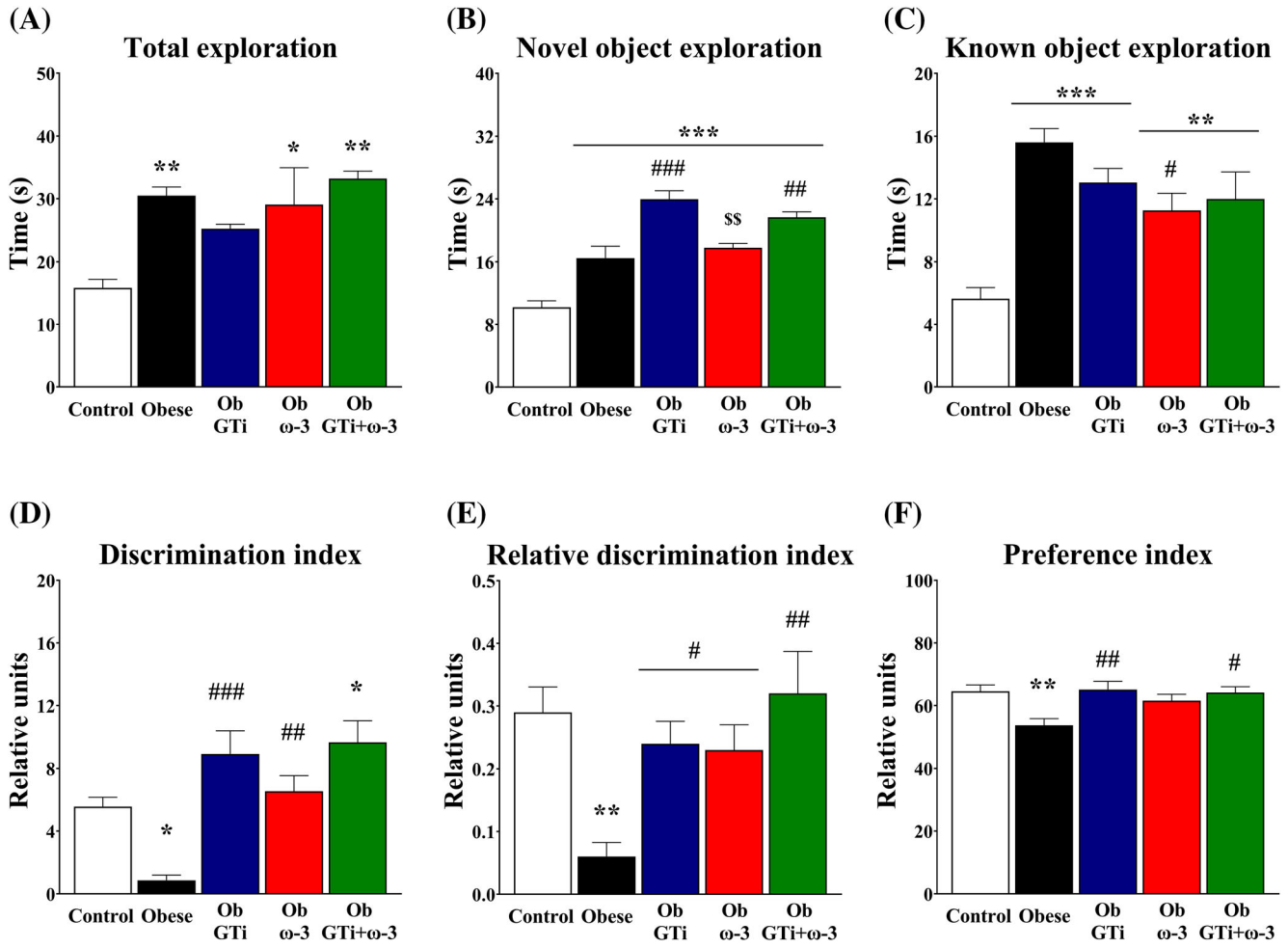

Figure 6. Effects of GTi supplementation (equivalent to 450 mg kg^{-1}), ω-3 (400 mg kg^{-1}) and their combination (GTi + ω-3) on metabolic biochemical parameters in plasma after 5 weeks in Holtzman male rats with a STD as Control, or HFD: all Obese animals (Ob), on the long-term memory by novel object recognition tests. (A) Total time of exploration, (B) time of novel object exploration, (C) time of known object exploration, (D) discrimination index, (E) relative discrimination index, and (F) preference index. Bars indicate the mean ± SEM ($n = 6$). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* tests: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ denote significant differences compared with the Control. #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ denote significant differences compared with Obese. $$$P < 0.01$ denotes significant differences compared with the Ob GTi.

glucose and insulin metabolism, assessed by OGTT (glycemia curve: AUC, peak, and baseline return) and IST (hypoglycemic response slope), revealing direct actions on insulin secretion, glucose uptake, and hepatic homeostasis. This mechanistic screening validates the combination before chronic obesity testing, distinguishing acute responses from long-term adaptations. It supports the chronic hypothesis: short-term modulation of normal dynamics may correct obesogenic dysfunctions. Although previous studies have linked tea consumption to improved glycemic control in healthy individuals,[42] the underlying mechanisms remain to be fully elucidated. Our OGTT results in healthy rats show that GTi administered either singly or in combination with ω-3 significantly attenuated the initial plasma glucose peak observed within 15 min post-glucose administration. Since the plasma glucose level at 30 min post-load in the OGTT is predictive of type 2 diabetes risk in humans,[43] our findings are consistent with previous animal studies reporting that green tea extract enhances glucose tolerance potentially by restoring the activity of key glucose-metabolizing enzymes and stimulating insulin synthesis in the liver and pancreas.[44] Such effects are likely mediated by GTi catechins through the upregulation of glucose transporter type 4 (GLUT4)-mediated glucose uptake in skeletal muscle tissue.[45] Both the administration of GTi and ω-3, separately and in combination, improved the results of OGTT by highly probable improved insulin in response to peak of hyperglycemia. Enhanced OGTT outcomes indicate that dietary incorporation of PUFAs may be effective in combating HFD-induced insulin resistance. In the healthy rat model, the IST revealed maintained normal blood glucose levels following exogenous insulin administration across all supplemented groups during 120 min of tracking. This improvement in insulin sensitivity, without causing hypoglycemia, may derive from coordinated actions of GTi catechins[46] and EPA/DHA on GLUT4 transporter expression and translocation,[47] facilitating a more regulated intracellular glucose uptake and preventing excessive insulin-induced glucose decreases. Evidence from human studies also indicates that green tea extracts and ω-3 supplementation improve glycemic parameters, consistent with our findings in the animal model and suggesting that the incorporation of PUFAs and/or GTi may contribute to improved insulin sensitivity.[48,49]

During the five weeks of chronic supplementation, as expected, the Control group consumed fewer calories than all obese groups.

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All supplemented obese groups, in turn. consumed more calories than the obese Control group; however, this increase was modest in the GTi + ω-3 combination group. These findings are partly consistent with previous studies in animal models of obesity showing no significant differences in food intake with green tea supplementation,[44,50] including a rodent study by Shirai and Suzuki that combined green tea with ω-3.[51] However, some studies using ω-3-enriched fish oil have reported reduced intake in diet-induced obese rats.[52]

The relative BW gain during supplementation was partially consistent with the caloric intake data. On average, all obese groups exhibited increased BW compared to the Control. Interestingly, the combined GTi + ω-3 treatment showed no significant differences in BW gain compared with either the Control or Obese groups during the first 2 weeks, and even by week 5. Similar findings were reported by Chen *et al.*[44] and Shen *et al.*,[53] who observed that green tea supplementation alone did not reduce BW, whereas Bajerska *et al.*[50] reported reductions in visceral fat with ω-3 supplementation. Thus, we can assume that the combination of GTi and ω-3 maintained BW despite an HFD for at least 2 weeks. Notably, few studies have investigated the effect of green tea combined with other natural compounds. Kwon *et al.*[54] demonstrated antiobesity effects of green tea paired with black rice bran in preclinical models. Although, our study did not show a significant reduction in caloric intake or BW it appears to be one of the first to provide preliminary evidence on the combination of green tea supplementation with PUFAs. EGCG seems to be the catechin most strongly associated with the antiobesity effects of green tea,[7] and according to our analysis, its proportion is lower in green tea from Cusco. This may partly explain the limited manifestation of the extract's anti-obesogenic potential. Nonetheless, our findings highlight the need for further research to determine the optimal doses and treatment durations to achieve significant antiobesity effects.

Regarding lipid metabolism, the influence of green tea and ω-3 remains unclear in human trials due to variable factors such as differences in administration timeframes and individual heterogeneity.[14,55] In our controlled animal study, supplementation with GTi and ω-3, either individually or in combination, improved blood lipid profiles. GTi supplementation primarily reduced triglyceride and VLDL levels, whereas ω-3 supplementation also decreased total cholesterol and LDL levels. The hypotriglyceridemic effects appear to be mediated by the polyphenolic and polysaccharide fractions in GTi and by the PUFAs in ω-3. These findings are consistent with previous research showing that such supplementation reduces hypertriglyceridemia in obese animals, partly by lowering hepatic diacylglycerol levels, which contribute to triglyceride synthesis.[56,57] However, the combined treatment did not produce greater effects than ω-3 supplementation alone. Regarding the analysis of different cholesterol types, the combination of GTi and ω-3 showed a reduction only in VLDL compared with ω-3 alone.

Despite previous reports suggesting that fish oil increases HDL,[58] our study found no variation beyond a consequent decrease in the obesity group. Established obesity, together with continued HFD feeding, may cause severe dysregulation in lipid metabolism, impeding reversal by supplementation, which started after the obesity onset. We can also highlight the improvement in blood glucose level observed with both green tea from Cusco and fish oil administration, as well as the maintenance of transaminase levels after repeated supplementation with these agents suggesting that they are safe for liver function.

Inflammatory markers, specifically plasma TNF-α and IL-6 concentrations, were evaluated due to their known involvement in obesity-induced inflammation.[59] As expected, their levels were elevated in obese rats, and only TNF-α was reduced by supplementation. This finding reflects IL-6's complex role: adipocyte-derived IL-6 recruits macrophages to adipose tissue, helping to maintain homeostasis and limit excessive adipocyte hypertrophy.[60] Elevated local TNF-α can disrupt insulin signaling in adipose tissue through insulin receptor substrate-1.[61] In this study, HFD-induced inflammation was evidenced by increased plasma TNF-α across all obese rat groups. Although levels did not fully normalize compared with Control, supplementation lowered TNF-α, with the GTi + ω-3 combination showing greatest effect. Consistently, green tea supplementation has been reported to decrease plasma TNF-α, likely through the downregulation of Toll-like receptor 4 and subsequent inhibition of proinflammatory cytokine expression.[62] Similarly, ω-3 reduced TNF-α in diet-induced obese rats by suppressing kappa-light-chain-enhancer of activated B cells pathway activity.[52] These results align with the well-documented anti-inflammatory effects of green tea and ω-3, supporting their potential benefits in obesity.[63]

Animal models provide valuable insight into cognitive and emotional processes relevant to humans, although with limitations.[64] This study investigated the effects of GTi and ω-3 supplementation on locomotion, short-term memory, and long-term memory in diet-induced obese rats using standardized tests such as OFT and NOR. OFT results showed increased locomotor activity in all obese groups, with the GTi + ω-3 group exhibiting the highest activity. These findings align with previous studies reporting enhanced locomotor activity in ω-3-supplemented rats[65] and in rats treated with catechin-rich extracts like *Gnidia glauca*.[66] Freezing and latency times were similar across groups, suggesting no differences in stress or anxiety. While locomotor activity may be expected to decrease due to obesity-related physical limitations, obesity can alter neuronal circuits and neurochemical signaling, affecting spontaneous behavior and activity.[67,68]

Obesity is also associated with cognitive deficits in humans, including impaired learning, memory, brain atrophy, and increased risk of dementia, such as Alzheimer's disease.[69] Animal models show similar impairments under HFDs.[70,71] Evidence for the protective effects of green tea or ω-3 against obesity-related cognitive decline is limited.[72] Using the NOR test to assess memory, we observed that obese rats supplemented with GTi + ω-3 displayed enhanced exploratory behavior, object discrimination, and preference for novel objects, indicative of improved short-term memory as shown in previous similar studies.[73]

Evaluation of long-term memory revealed that supplemented rats showed increased exploratory tendencies and greater interest in familiar objects. Discrimination indices indicated improved long-term memory in groups receiving GTi and GTi + ω-3, partially agreeing with studies where ω-3 did not enhance long-term memory but supported object recognition.[74] These effects may relate to enhanced hippocampal neurogenesis and progenitor cell proliferation, as seen with theanine supplementation in tea.[75] The integrity of the hippocampus and perirhinal cortex is critical for long-term object recognition, with the perirhinal cortex also supporting perceptual processing and short-term recognition memory.[33,76] EGCG from green tea reduces oxidative stress and hepatic fat accumulation, though bioavailability and age, dose, and treatment duration may limit cognitive benefits.[77-79]

TNF-α, a central proinflammatory cytokine, mediates brain inflammation, and its elevated levels in obesity may serve as a

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marker of neuroinflammation.[80] EPA and DHA reduce proinflammatory cytokines in humans,[81] and in the central nervous system, they limit microglial activation, mitigating neuroinflammation.[65,75,82,83] In our study, plasma TNF-α levels decreased with supplementation, suggesting that GTi and ω-3 attenuate obesity-induced neuroinflammation and oxidative stress, supporting cognitive function. Collectively, these findings indicate that dietary supplementation with green tea and ω-3 can improve locomotor activity, enhance short- and long-term memory, and reduce inflammation in obese rats, highlighting their potential neuroprotective effects.

This study provides an overview of the effects of green tea and/or ω-3 supplementation on food intake, BW, glycemia, lipidemia, anti-inflammatory cytokines, and cognitive impairments in an animal model of obesity. Although reductions in food intake and BW were observed only with the combined green tea and ω-3 supplementation, all supplementation strategies improved insulin sensitivity and obesity-related memory deficits, supporting their potential as complementary nutritional interventions for obesity and associated conditions. It is plausible that the antioxidant properties of green tea polyphenols synergistically protect EPA and DHA in fish oil against oxidative degradation, enhancing efficacy of the combined treatment.

Additionally, green tea from Valle de la Concepción, Cusco, demonstrated notable nutritional quality, with balanced levels of carbohydrates, proteins, lipids, ash, and moisture comparable to high-quality green teas elsewhere. Both the dry leaves and their infusion, particularly when combined with ω-3, meet established quality indicators, highlighting the product's nutritional and health potential. Future studies should explore longer supplementation periods to better assess the production of biochemical compounds involved in homeostasis, energy regulation, and behavior. They should also include female animals to determine whether sex-related differences exist and to increase the translational value of this type of research.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

MRP, PFC, RAD, NBO, and JD: Investigation, Conceptualization, Data curation. PFC, RAD, JD, and NBO: Methodology. PFC, MRP, AM, RAD, NBO, and JD: Formal analysis, Visualization. RAD, NBO, and JD: Resources, Funding acquisition. RAD, NBO, and JD: Project administration. RAD, NBO, and JD: Validation. RAD, NBO, and JD: Supervision. PFC, NBO, and JD: Writing – original draft. PFC, RAD, NBO, and JD: Writing – review and editing.

CONFLICT OF 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.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Hinojosa-Nogueira D, Perez-Burillo S, Pastoriza de la Cueva S and Rufian-Henares JA, Green and white teas as health-promoting foods. *Food Funct* **12**:3799–3819 (2021).
2 Hayat K, Iqbal H, Malik U, Bilal U and Mushtaq S, Tea and its consumption: benefits and risks. *Crit Rev Food Sci Nutr* **55**:939–954 (2015).
3 Chan EW, Soh EY, Tie PP and Law YP, Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. *Pharm Res* **3**:266–272 (2011).
4 Babu PV and Liu D, Green tea catechins and cardiovascular health: an update. *Curr Med Chem* **15**:1840–1850 (2008).
5 Wiseman S, Mulder T and Rietveld A, Tea flavonoids: bioavailability in vivo and effects on cell signaling pathways in vitro. *Antioxid Redox Signal* **3**:1009–1021 (2001).
6 Khan N and Mukhtar H, Tea polyphenols in promotion of human health. *Nutrients* **11**:39 (2018).
7 Wolfram S, Wang Y and Thielecke F, Anti-obesity effects of green tea: from bedside to bench. *Mol Nutr Food Res* **50**:176–187 (2006).
8 Prasanth MI, Sivamaruthi BS, Chaiyasut C and Tencomnao T, A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients* **11**:474 (2019).
9 Lorenz M, Cellular targets for the beneficial actions of tea polyphenols. *Am J Clin Nutr* **98**:1642S–1650S (2013).
10 Halliwell B, Oxidative stress and neurodegeneration: where are we now? *J Neurochem* **97**:1634–1658 (2006).
11 Sun S, Liu Z, Lin M, Gao N and Wang X, Polyphenols in health and food processing: antibacterial, anti-inflammatory, and antioxidant insights. *Front Nutr* **11**:1456730 (2024).
12 Leung LK, Su Y, Chen R, Zhang Z, Huang Y and Chen ZY, Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J Nutr* **131**:2248–2251 (2001).
13 Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ *et al*., Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev* **11**:CD003177 (2018).
14 Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-McComb J *et al*., Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *J Nutr Biochem* **58**:1–16 (2018).
15 Nain CW, Berdal G, Thao PTP, Mignolet E, Buchet M, Page M *et al*., Green tea extract enhances the oxidative stability of DHA-rich oil. *Antioxidants (Basel)* **10**:982 (2021).
16 DuBois M, Gilles KA, Hamilton JK, Rebers PA and Smith F, Colorimetric method for determination of sugars and related substances. *Anal Chem* **28**:350–356 (1956).
17 Mariotti F, Tome D and Mirand PP, Converting nitrogen into protein – beyond 6.25 and Jones' factors. *Crit Rev Food Sci Nutr* **48**:177–184 (2008).
18 Folch J, Lees M and Sloane Stanley GH, A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**:497–509 (1957).
19 Ismail BP, Ash content determination, in *Food Analysis Laboratory Manual*. Springer, Cham, pp. 117–119 (2017).
20 Yi T, Li SM, Fan JY, Fan LL, Zhang ZF, Luo P *et al*., Comparative analysis of EPA and DHA in fish oil nutritional capsules by GC-MS. *Lipids Health Dis* **13**:190 (2014).
21 Folin O and Ciocalteau V, Tyrosine and tryptophane in proteins. *J Biol Chem* **73**:627–648 (1927).
22 Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* **28**:25–30 (1995).
23 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231–1237 (1999).
24 Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M *et al*., The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *Br J Pharmacol* **177**:3617–3624 (2020).

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25 Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F *et al.*, Anthropometrical parameters and markers of obesity in rats. *Lab Anim* **41**:111–119 (2007).
26 Pirillo A and Catapano AL, Omega-3 polyunsaturated fatty acids in the treatment of hypertriglyceridaemia. *Int J Cardiol* **170**:S16–S20 (2013).
27 Reagan-Shaw S, Nihal M and Ahmad N, Dose translation from animal to human studies revisited. *FASEB J* **22**:659–661 (2008).
28 Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR and West SG, Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. *Am J Clin Nutr* **93**:243–252 (2011).
29 Warnick GR, Knopp RH, Fitzpatrick V and Branson L, Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* **36**:15–19 (1990).
30 Stanford SC, The open field test: reinventing the wheel. *J Psychopharmacol* **21**:134–135 (2007).
31 Grayson B, Leger M, Piercy C, Adamson L, Harte M and Neill JC, Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. *Behav Brain Res* **285**:176–193 (2015).
32 Aggleton JP, Albasser MM, Aggleton DJ, Poirier GL and Pearce JM, Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behav Neurosci* **124**:55–68 (2010).
33 Hammond RS, Tull LE and Stackman RW, On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* **82**:26–34 (2004).
34 Xu N and Chen Z, Green tea, black tea and semi-fermented tea, in *Tea: Bioactivity and Therapeutic Potential*, ed. by Zhen Y. Taylor & Francis, New York, pp. 35–36 (2002).
35 Czernicka M, Zagula G, Bajcar M, Saletnik B and Puchalski C, Study of nutritional value of dried tea leaves and infusions of black, green and white teas from Chinese plantations. *Rocz Panstw Zakl Hig* **68**: 237–245 (2017).
36 Megias-Perez R, Shevchuk A, Zemedie Y and Kuhnert N, Characterization of commercial green tea leaves by the analysis of low molecular weight carbohydrates and other quality indicators. *Food Chem* **290**: 159–167 (2019).
37 Gramza A, Khokhar S, Yoko S, Gliszczynska-Swiglo A, Hes M and Korczak J, Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur J Lipid Sci Technol* **108**: 351–362 (2006).
38 Shannon E, Jaiswal AK and Abu-Ghannam N, Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: a kinetic study. *Food Res* **1**:1–11 (2017).
39 Armoskaite V, Ramanauskiene K, Maruska A, Razukas A, Dagilyte A, Baranauskas A *et al.*, The analysis of quality and antioxidant activity of green tea extracts. *J Med Plants Res* **5**:811–816 (2011).
40 Radeva-Ilieva M, Stoeva S, Hvarchanova N and Georgiev KD, Green tea: current knowledge and issues. *Foods* **14**:745 (2025).
41 Lakshimi VI and Kavitha M, New insights into prospective health potential of ω-3 PUFAs. *Curr Nutr Rep* **12**:813–829 (2023).
42 Xu R, Bai Y, Yang K and Chen G, Effects of green tea consumption on glycemic control: a systematic review and meta-analysis of randomized controlled trials. *Nutr Metab (Lond)* **17**:56 (2020).
43 Hirakawa Y, Hata J, Yoshinari M, Higashioka M, Yoshida D, Shibata M *et al.*, 30-minute postload plasma glucose levels during an oral glucose tolerance test predict the risk of future type 2 diabetes: the Hisayama study. *BMJ Open Diabetes Res Care* **8**:8 (2020).
44 Chen N, Bezzina R, Hinch E, Lewandowski PA, Cameron-Smith D, Mathai ML *et al.*, Green tea, black tea, and epigallocatechin modify body composition, improve glucose tolerance, and differentially alter metabolic gene expression in rats fed a high-fat diet. *Nutr Res* **29**:784–793 (2009).
45 Ueda-Wakagi M, Nagayasu H, Yamashita Y and Ashida AH, Green tea ameliorates hyperglycemia by promoting the translocation of glucose transporter 4 in the skeletal muscle of diabetic rodents. *Int J Mol Sci* **20**:2436 (2019).
46 Yan J, Zhao Y, Suo S, Liu Y and Zhao B, Green tea catechins ameliorate adipose insulin resistance by improving oxidative stress. *Free Radic Biol Med* **52**:1648–1657 (2012).
47 Zhang Y, He L, Chen X, Shentu P, Xu Y and Jiao J, Omega-3 polyunsaturated fatty acids promote SNAREs mediated GLUT4 vesicle docking and fusion. *J Nutr Biochem* **101**:108912 (2022).
48 El-Fayoumi SH, Mahmoud AAA, Fahmy A and Ibrahim I, Effect of omega-3 fatty acids on glucose homeostasis: role of free fatty acid receptor 1. *Naunyn Schmiedebergs Arch Pharmacol* **393**:1797–1808 (2020).
49 Venables MC, Hulston CJ, Cox HR and Jeukendrup AE, Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. *Am J Clin Nutr* **87**:778–784 (2008).
50 Bajerska J, Wozniewicz M, Jeszka J, Drzymala-Czyz S and Walkowiak J, Green tea aqueous extract reduces visceral fat and decreases protein availability in rats fed with a high-fat diet. *Nutr Res* **31**:157–164 (2011).
51 Shirai N and Suzuki H, Effects of simultaneous intakes of fish oil and green tea extracts on plasma, glucose, insulin, C-peptide, and adiponectin and on liver lipid concentrations in mice fed low- and high-fat diets. *Ann Nutr Metab* **52**:241–249 (2008).
52 Liu S-H, Chen Y-C, Tzeng H-P and Chiang M-T, Fish oil enriched ω-3 fatty acids ameliorates protein synthesis/degradation imbalance, inflammation, and wasting in muscles of diet-induced obese rats. *J Funct Foods* **87**:104755 (2021).
53 Shen CL, Han J, Wang S, Chung E, Chyu MC and Cao JJ, Green tea supplementation benefits body composition and improves bone properties in obese female rats fed with high-fat diet and caloric restricted diet. *Nutr Res* **35**:1095–1105 (2015).
54 Kwon KS, Lee ES, Lee KH, Hwang WS, Lee WY, Kim JJ *et al.*, Anti-obesity and other health benefits of bioprocessed black rice bran in combination with green tea extract in 3T3-L1 preadipocyte cells and in mice on a high-fat diet. *Food Funct* **15**:12083–12100 (2024).
55 Xu R, Yang K, Li S, Dai M and Chen G, Effect of green tea consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials. *Nutr J* **19**:48 (2020).
56 Xu Y, Zhang M, Wu T, Dai S, Xu J and Zhou Z, The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. *Food Funct* **6**:297–304 (2015).
57 Bays HE, Tighe AP, Sadovsky R and Davidson MH, Prescription omega-3 fatty acids and their lipid effects: physiologic mechanisms of action and clinical implications. *Expert Rev Cardiovasc Ther* **6**:391–409 (2008).
58 Chiu CY, Wang LP, Liu SH and Chiang MT, Fish oil supplementation alleviates the altered lipid homeostasis in blood, liver, and adipose tissues in high-fat diet-fed rats. *J Agric Food Chem* **66**:4118–4128 (2018).
59 Tzanavari T, Giannogonas P and Karalis KP, TNF-alpha and obesity. *Curr Dir Autoimmun* **11**:145–156 (2010).
60 Han MS, White A, Perry RJ, Camporez JP, Hidalgo J, Shulman GI *et al.*, Regulation of adipose tissue inflammation by interleukin 6. *Proc Natl Acad Sci USA* **117**:2751–2760 (2020).
61 Hotamisligil GS, Budavari A, Murray D and Spiegelman BM, Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest* **94**:1543–1549 (1994).
62 Molina N, Bolin AP and Otton R, Green tea polyphenols change the profile of inflammatory cytokine release from lymphocytes of obese and lean rats and protect against oxidative damage. *Int Immunopharmacol* **28**:985–996 (2015).
63 Bogdanski P, Suliburska J, Szulinska M, Stepien M, Pupek-Musialik D and Jablecka A, Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res* **32**:421–427 (2012).
64 Bovenkerk B and Kaldewaij F, The use of animal models in behavioural neuroscience research. *Curr Top Behav Neurosci* **19**:17–46 (2015).
65 Tyrtyshnaia AA and Manzhulo IV, The effect of omega-3 polyunsaturated fatty acids on neuroinflammation in the hippocampus. *Neurochem J* **12**:168–179 (2018).
66 Arika WM, Kibiti CM, Njagi JM and Ngugi MP, Effects of DCM leaf extract of *Gnidia glauca* (Fresen) on locomotor activity, anxiety, and exploration-like behaviors in high-fat diet-induced obese rats. *Behav Neurol* **2019**:7359235 (2019).
67 Appleton KM, Voyias PD, Sallis HM, Dawson S, Ness AR, Churchill R *et al.*, Omega-3 fatty acids for depression in adults. *Cochrane Database Syst Rev* **11**:CD004692 (2021).

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68 Vignes M, Maurice T, Lante F, Nedjar M, Thethi K, Guiramand J *et al.*, Anxiolytic properties of green tea polyphenol (−)-epigallocatechin gallate (EGCG). *Brain Res* **1110**:102–115 (2006).

69 Miller AA and Spencer SJ, Obesity and neuroinflammation: a pathway to cognitive impairment. *Brain Behav Immun* **42**:10–21 (2014).

70 Gergerlioglu HS, Oz M, Demir EA, Nurullahoglu-Atalik KE and Yerlikaya FH, Environmental enrichment reverses cognitive impairments provoked by Western diet in rats: role of corticosteroid receptors. *Life Sci* **148**:279–285 (2016).

71 Winocur G, Greenwood CE, Piroli GG, Grillo CA, Reznikov LR, Reagan LP *et al.*, Memory impairment in obese Zucker rats: an investigation of cognitive function in an animal model of insulin resistance and obesity. *Behav Neurosci* **119**:1389–1395 (2005).

72 Onishi S, Meguro S, Pervin M, Kitazawa H, Yoto A, Ishino M *et al.*, Green tea extracts attenuate brain dysfunction in high-fat-diet-fed SAMP8 mice. *Nutrients* **11**:821 (2019).

73 Faccinetto-Beltran P, Aguirre-Lopez LO, Banuelos-Pineda J, Reza-Zaldivar EE, Santacruz A, Hernandez-Brenes C *et al.*, Fish oil and probiotics supplementation through milk chocolate improves spatial learning and memory in male Wistar rats. *Front Nutr* **9**:1023653 (2022).

74 de Andrade AM, Fernandes MDC, de Fraga LS, Porawski M, Giovenardi M and Guedes RP, Omega-3 fatty acids revert high-fat diet-induced neuroinflammation but not recognition memory impairment in rats. *Metab Brain Dis* **32**:1871–1881 (2017).

75 Takeda A, Sakamoto K, Tamano H, Fukura K, Inui N, Suh SW *et al.*, Facilitated neurogenesis in the developing hippocampus after intake of theanine, an amino acid in tea leaves, and object recognition memory. *Cell Mol Neurobiol* **31**:1079–1088 (2011).

76 Reger ML, Hovda DA and Giza CC, Ontogeny of rat recognition memory measured by the novel object recognition task. *Dev Psychobiol* **51**:672–678 (2009).

77 Huang J, Li W, Liao W, Hao Q, Tang D, Wang D *et al.*, Green tea polyphenol epigallocatechin-3-gallate alleviates nonalcoholic fatty liver disease and ameliorates intestinal immunity in mice fed a high-fat diet. *Food Funct* **11**:9924–9935 (2020).

78 Nakagawa K and Miyazawa T, Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. *J Nutr Sci Vitaminol (Tokyo)* **43**:679–684 (1997).

79 Flores MF, Martins A, Schimidt HL, Santos FW, Izquierdo I, Mello-Carpes PB *et al.*, Effects of green tea and physical exercise on memory impairments associated with aging. *Neurochem Int* **78**:53–60 (2014).

80 Olmos G and Llado J, Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. *Mediators Inflamm* **2014**:861231 (2014).

81 Tan A, Sullenbarger B, Prakash R and McDaniel JC, Supplementation with eicosapentaenoic acid and docosahexaenoic acid reduces high levels of circulating proinflammatory cytokines in aging adults: a randomized, controlled study. *Prostaglandins Leukot Essent Fatty Acids* **132**:23–29 (2018).

82 Chung IY and Benveniste EN, Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. *J Immunol* **144**:2999–3007 (1990).

83 Smith JA, Das A, Ray SK and Banik NL, Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res Bull* **87**:10–20 (2012).

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