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Original Communication

Potential Effects of Chia Seed (*Salvia hispanica* L.) on Adipose Tissue Gene Expression and Obesity Indicators in Cafeteria Diet-Fed Rats

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Abstract

Background: Obesity is a major global health concern, highlighting the need for effective nutritional interventions. Chia seeds (*Salvia hispanica* L.) are rich in α-linolenic acid and have recently attracted attention for potential metabolic benefits. Thus, this study aimed to investigate the effects of chia seed supplementation on obesity-related indicators in a rat model fed a cafeteria diet. **Methods**: Three-week-old male Wistar rats (n = 28) were randomly and equally divided into four groups (n = 7 each) and fed the following diets for 15 weeks: control diet (CON), CON with 20% chia seed-supplemented diet (CONC), cafeteria diet (CAF), and CAF with 20% chia seed-supplemented diet (CAFC). Food consumption and body weights were recorded daily. Tissue and plasma samples were collected at the end of the study, and body composition and gene expression levels in white adipose tissues were analyzed. **Results**: The CAF and CAFC showed significantly higher energy intake (198 $\pm$ 7.76 and 199 $\pm$ 7.76 kcal/day, respectively) and weight gain (509 $\pm$ 46.3 and 500 $\pm$ 33.8 g, respectively) compared to CON (all $p < 0.001$). However, chia seed supplementation did not significantly alter body composition ($p > 0.05$). Plasma leptin levels differed among groups ($p = 0.017$), with the CAF promoting higher levels than the CON (6.26 $\pm$ 1.78 vs. 1.20 $\pm$ 0.26 ng/mL). Sterol regulatory element binding protein-1c (*SREBP-1c*) expression was higher in the CAFC than in the CAF ($p < 0.001$). Chia seed supplementation significantly decreased uncoupling protein 2 (*UCP2*) expression in the CONC ($p < 0.05$ vs. CON), and the CAFC showed a trend toward decreased expression ($p = 0.053$ vs. CAF). Expression of the peroxisome proliferator-activated receptor α (*PPAR*α) and fatty acid desaturase 2 (*FADS2*) genes was higher in the CON than in the CAF and CAFC (both $p < 0.05$). **Conclusions**: Chia seed supplementation modified specific gene expression levels, but did not impact primary obesity indicators under obesogenic conditions.

Keywords: *Salvia hispanica*; rats; obesity; western diet; gene expression

1. Introduction

Obesity is defined as the accumulation of abnormal or excessive fat that can disrupt the maintenance of optimal health [1]. This complicated, chronic, and multifactorial disease has become a major global health concern in recent decades [2]. Sedentary lifestyles and environmental factors are considered to be primary causes of the global obesity and overweight problem [3,4]. Excessive dietary energy intake—primarily from sugars and fats (saturated and trans fatty acids)—combined with low intakes of vitamins, minerals, and dietary fiber, contributes to weight gain and increases the risk of diet-related diseases [5–8]. The urgent need for effective public health strategies to prevent obesity and overweight has emerged as a major global health priority [9].

Salvia hispanica L., commonly known as chia seed, belongs to the Lamiaceae family of plants [10]. The lipid content of chia seeds is between 25–40%, and is composed of 60% α-linolenic acid (ALA) and 20% linoleic acid (LA) [11]. This fatty acid pattern makes chia seed an important source of omega-3 (n-3) fatty acids. Besides their lipid profile, chia seeds also contain a significant amount of dietary fiber and a high protein content [12–14]. In addition, chia provides mucilage and various minerals that can influence satiety and lipid metabolism, while its soluble fiber fraction has been suggested to modulate the composition of gut microbiota [15].

Recent animal studies have shown that chia seeds positively affect lipid and glucose homeostasis, contributing to disease prevention and the management of metabolic disorders [16–18]. Chia seed supplementation of high-fat diets improved lean body mass, reduced abdominal fat, and alleviated chronic inflammation in rats [19]. In other studies, chia seeds did not prevent weight gain, but enhanced glucose and insulin tolerance and had beneficial cardiac and hepatic effects [20,21]. These findings indicate the n-3 fatty acid content of chia seeds may be an alternative dietary component against the effects of a high-fat diet [22,23].

Polyunsaturated fatty acids (PUFAs) regulate basic adipose tissue cell functions by modulating the activity of key transcription factors such as peroxisome proliferator-activated receptor (PPAR α/γ) and sterol regulatory element binding protein 1/2 (SREBP-1/2) [24]. Foods with a high ALA content have been shown to increase the accumulation of n-3 PUFA and the activity and expression of desaturases in adipose tissue [22]. However, there is still

very little known about the mechanisms underlying the beneficial effects of chia seeds on metabolic disorders caused by high-fat, high-sugar, or high-fructose diets [21,25–28].

The cafeteria diet was developed by Rothwell and Stock to improve obesity models through feeding of laboratory animals [29]. In this diet, human foods containing high energy, fat, and sugar (chocolate, biscuits, chips, etc.) are provided in addition to the normal daily feed of experimental animals. In rodent models that are resistant to the development of obesity with a normal diet, the cafeteria diet results in significantly increased food intake, body weight, and adipose tissues as the taste threshold and food variety increase [30]. The cafeteria diet has been proposed as the closest experimental model to the Western-style diet, which is considered one of the most important factors in the development of obesity in humans due to its high energy, saturated fat, salt, and sugar content, with low content of vitamins, minerals, and essential nutrients [31–33]. To our knowledge, the possible benefits of chia seed supplementation of the cafeteria diet have yet to be explored. The aim of this study was therefore to investigate the potential protective effects of chia seeds on cafeteria diet-induced obesity in rats, as well as their effect on the expression of several key genes in adipose tissue, namely *PPARα/γ*, *SREBP-1c*, uncoupling protein 2 (*UCP2*), and fatty acid desaturase 2 (*FADS2*). We hypothesized that supplementing cafeteria diet with chia seeds may exert protective effects against the development of obesity, adiposity-related markers, and gene expression levels in adipose tissue. While several studies have examined chia seed supplementation in high-fat or high-sucrose diet models, its effects in a cafeteria diet model that more closely reflects obesity-associated eating patterns in humans have yet to be explored. The present study provides new evidence on how chia seeds may influence obesity-related parameters and adipose-tissue gene expression in cafeteria diet conditions. By examining energy and nutrient intake in conjunction with anthropometric measures, biochemical markers, and gene-level changes, this study provides a better understanding of the physiological effects of chia supplementation.

2. Materials and Methods

2.1 Animals, Diets and Interventions

The experimental group was comprised of 28 male *Wistar albino* rats (3-weeks old) provided by Kobay Experimental Animals Laboratory Inc. All animals were individually housed on shavings in plastic cages at the Laboratory Animals Research and Application Centre, Hacettepe University, under controlled conditions (22 ± 1 °C temperature, 45% humidity, 12 h light-dark cycle). Rats were given *ad libitum* access to water and chow diet for one week to achieve habituation. Subsequently, the rats (average body weight 105 ± 2.61 g) were randomly assigned to four groups (7 in each group) based on body weight using the online software program Research Randomizer (https://www.randomizer.org/) [34]. The four experimental groups were: control diet (CON), control diet supplemented with chia seed (CONC), cafeteria diet (CAF) and cafeteria diet with chia seed (CAFC). Rats were fed these diets for 15 weeks, with the researchers not blinded to the treatment groups. CON and CONC diets were based on the AIN-93G diet [35], and their nutrient composition (shown in Table 1) was calculated and reported by the manufacturer (ARDEN Research & Experiment, Ankara, Turkiye). The CONC diet was made isocaloric with the CON diet by lowering the amounts of corn starch, soybean oil, casein and replacing them with 200 g chia seed/kg diet (Table 1), as previously described [36]. The percentage of chia inclusion (20%, w/w) was therefore based on an earlier experimental study demonstrating that this level is well tolerated by rodents and sufficient to elicit measurable physiological effects [36], with additional support from studies using higher dietary proportions of chia seed in rat models [16–18]. Although a range of chia seed inclusion levels has been used in rodent studies, the selected proportion represents a moderate and biologically relevant level. Chia seed was obtained from Yayla Agro Food Industry (Ankara, Turkiye) and Transportation Inc.(Ankara, Turkiye), and its energy and macronutrient composition was provided by the supplier. Fatty acid and mineral compositions were determined by an external laboratory (Nanolab Food Control Laboratory, Istanbul, Turkiye) using gas chromatography with a flame ionization detector (GC-FID) (Trace 1310 GC, Thermo Fisher Scientific, Milan, Italy) and inductively coupled plasma-mass spectrometry (ICP-MS) (7850 ICP-MS, Agilent Technologies, Santa Clara, CA, USA), respectively [36,37]. The nutritional composition of chia seeds is presented in Table 2 (Ref. [38]); selected values were derived from previous reports [38]. CAF diet included both a control diet and an arbitrary mix of high-energy, palatable foods. Information on cafeteria diet was obtained from previous reports [39,40]. In brief, the high-energy, palatable foods consist of peanuts, potato and corn crisps, milk chocolate, cheese stick crackers, chocolate bars, kashar cheese, and biscuits filled with chocolate cream, milk-chocolate, and orange jelly. Five of these foods were offered in excess every day in a cup on the cage floor. To ensure variation, three of these foods were substituted with new ones every day, meaning the rats were not provided with identical foods for more than two consecutive days. CAFC diet consisted of CAF supplemented with chia in the same way as the CONC diet. Food and water consumption were recorded daily for each rat between 13:00 and 15:00 throughout the 15-week experimental period. Calculations for daily energy, macronutrient, and micronutrient intake were based on supplier data for the cafeteria diet (**Supplementary Table 1**). The cafeteria diet provided an average energy distribution of 40.3% from carbohydrates, 8.1% from protein, and 50.8% from fat.

Table 1. Composition of the CON and CONC diets.

Energy and nutrients	CON diet		CONC diet	
	g (%)	kcal (%)	g (%)	kcal (%)
Macronutrients				
Carbohydrate	63.9	63.9	63.9	63.9
Corn starch	39.7	39.7	32.5	32.5
Dextrinized corn starch	13.2	13.2	13.2	13.2
Sucrose	10.0	10.0	10.0	10.0
Chia seed[1]	-	-	7.2	7.2
Fat[2]	7.0	15.8	7.0	15.8
Soybean oil	7.0	15.8	1.1	2.5
Chia seed	-	-	5.9	13.3
Protein	20.3	20.3	20.3	20.3
Casein	20.0	20.0	15.4	15.4
L-cysteine	0.3	0.3	0.3	0.3
Chia seed	-	-	4.6	4.6
Energy (kcal/g of diet)	4		4	
Sugar (g/g diet)	0.11		0.11	
Dietary fiber (g/g diet)	0.05		0.09	
Saturated fat (g/g diet)	0.01		0.01	
Sodium (mg/g diet)	1.02		1.02	

CON diet, control diet; CONC diet, control+chia diet. Both diets also contained vitamin mix (V10001) 1%, mineral mix (S10026) 1%, choline bitartrate 0.25%, methyl paraben 0.0014%, dicalcium phosphate 1.3%, calcium carbonate 0.55%, and potassium citrate 1.7%. [1]Chia seed: 200 g/kg diet. [2]Saturated fat content (g/100 g diet) of the CON and CONC diets was 1.12 and 0.7, respectively as stated by the manufacturer.

2.2 Blood and Tissue Collection

After an overnight fast, rats were terminally anesthetized with isoflurane at the end of the intervention period. Blood samples were obtained using cardiac puncture and collected into heparinized capillary tubes. Samples were then transferred to Eppendorf Tubes for centrifugation at 3000 ×g for 15 min. Plasma was isolated and stored in polypropylene microtubes at –80 °C prior to testing.

Body cavities of animals were opened and major organs were removed and weighed, including the liver, brain, kidneys, gonadal (GWAT) and peri-renal white adipose tissues (PWAT), and interscapular brown adipose tissue (IBAT). The organs were placed in punctured sterile bags and immersed in liquid nitrogen, followed by storage at –80 °C for subsequent assays. The weight of adipose tissue and organs are presented as a percentage of the animal's total weight (relative).

2.3 Anthropometric Measurements and Carcass Composition

The body length (naso-anal distance) of anaesthetized rats was recorded before euthanasia. Body weight and length were used to determine the Lee obesity index, calculated as $\sqrt[3]{\text{body weight } (g)}$/length (cm) [41].

The body composition was assessed through chemical analysis of the carcass, which is the reference method for determining rat body composition [42]. Whole carcasses were oven-dried to assess body water levels. The dried carcasses were then homogenized and sampled to estimate nitrogen content using the Kjeldahl method, and fat content using Soxhlet extraction.

2.4 Plasma Biochemical Indicators

Commercially available ELISA kits were used to assess plasma total cholesterol (Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China; Cat. No. 201-11-0785), triglycerides (Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China; Cat. No. 201-11-0250), insulin (Crystal Chem Inc., Elk Grove Village, IL, USA; Cat. No. 90060), leptin (Crystal Chem Inc., Elk Grove Village, IL, USA; Cat. No. 90040) and C-peptide (Crystal Chem Inc., Elk Grove Village, IL, USA; Cat. No: 90055), as per the manufacturers' instructions [39]. Plasma glucose concentration was measured with the glucose oxidase method and a fully automated biochemical analyzer (BS-300, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) [43].

2.5 Gene Expression Assays

Gene expression levels of *SREBP-1c*, *UCP2*, *PPARα*, *PPARγ* and *FADS2* in GWAT were evaluated by real-time polymerase chain reaction (RT-PCR) with an Applied

Table 2. Nutritional composition of chia seed.

Energy and nutrients	Chia seed
Energy (kcal/100 g)	434
Carbohydrate (g/100 g)	35.8
Sugar (g/100 g)	0
Fiber (g/100 g)	31.9
Protein (g/100 g)	22.9
Fat (g/100 g)	29.3
Fatty acid composition (% of total lipid)[1]	
Palmitic acid (C16:0)	8.13
Stearic acid (C18:0)	4.32
Oleic acid (C18:1)	7.05
Linoleic acid (C18:2)	17.0
Linolenic acid (C18:3)	58.3
Total saturated fatty acids	5.79
Total monounsaturated fatty acids	2.38
Total polyunsaturated fatty acids	24.9
Omega-3	19.2
Omega-6	5.61
Omega-3/Omega-6 ratio	3.43
Mineral composition (mg/100 g)	
Sodium (mg)	0
Potassium (mg)	641
Calcium (mg)	538
Iron (mg)	9.54
Magnesium (mg)	277
Phosphorus (mg)	594
Zinc (mg)	4.76
Copper (mg)	1.64
Manganese (mg)	12.4
Total phenolic content (mg GAE/g)	3.42[2]

GAE, Gallic acid equivalent. Energy and nutrient values were provided by the supplier; fatty acid and mineral compositions were analyzed by an external laboratory.

[1]Fatty acid composition was analyzed as fatty acid methyl esters derived from lipids extracted from chia seeds.

[2]Total phenolic content was obtained from the literature [38].

Biosystems (ABI) 7500 Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). RNA was extracted using a total RNA isolation kit (Vivantis Total RNA Extraction Kit, Vivantis Technologies Sdn. Bhd., Selangor, Malaysia; Cat. No. GF-TR-100), as per the manufacturer's protocol. OneScript Plus Reverse Transcriptase (Applied Biological Materials Inc. Richmond, BC, Canada; Cat. No. G237) was used to synthesize cDNA from the extracted mRNA, which was subsequently amplified with the BrightGreen qPCR MasterMix (Applied Biological Materials Inc. Richmond, BC, Canada). Primers for the *SREBP-1c*, *UCP2*, *PPARα*, *PPARγ* and *FADS2* genes, and for the housekeeping gene glyceraldehyde 3- phosphate dehydrogenase (*GAPDH*), were supplied by Biomers.net Gmbh (Ulm, Germany). The primer sequences (forward and reverse) were as follows: *SREBP-1c* (5′-CAACAACCAAGACAGTGA-3′) and (5′-AGAGAAGCAGGAGAAGAG-3′); for *UCP2* (5′-CACTTCACTTCTGCCTTC-3′) and (5′-TATCTCGTCTTGACCACAT-3′); for *PPARα* (5′-TCTGAATGAGCACTTCTAAG-3′) and (5′-CTGTAATTGTCTGAATCCTACTA-3′); for *PPARγ* (5′-CACCAACTTCGGAATCAG-3′) and (5′-TCATAGTGTGGAGCAGAA-3′); for *FADS2* (5′-CCTTCTTCAATGACTGGTT-3′) and (5′-CGGCTTCTCTTGGTATTC-3′); and for *GAPDH* (5′-ATGACAATGAATATGGCTACA-3′) and (5′-TCTTGCTCTCAGTATCCTT-3′). A total of 10 µL of mixture containing 5 µL MasterMix, 0.5 µL primer, and 4.5 µL RNase-free water was added to each well, followed by 20 ng of cDNA. Cycle conditions were 95 °C for 15 min, 40 cycles of 95 °C for 15 min, annealing at 57 °C for 60 s, and finally 40 °C for 30 s. All target gene expression levels were normalized using *GAPDH* as an internal control. Fold changes between groups were calculated using the $2^{-\Delta\Delta Ct}$ equation.

2.6 Statistical Analysis

The data were analyzed with SPSS version 23.0 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). Results are expressed as the mean ± standard error. Pooled standard errors were used for variables analyzed with repeated measures analysis of variance (ANOVA). A p-value < 0.05 was considered statistically significant. The water and diet consumption of animals was recorded daily throughout the 15-week experimental period. Energy and nutrient intakes were calculated from these records and analyzed using repeated measures ANOVA. Anthropometric measurements, body composition, organ and adipose tissue weights, and plasma analyses were evaluated using one-way ANOVA. In repeated measurements, differences between groups were revealed by examining dietary intervention, treatment time, and the interaction of diet and treatment time. For pairwise comparisons in one-way ANOVA, Bonferroni or Tamhane *post hoc* tests were performed according to the homogeneous distribution of variances. The Shapiro-Wilk test revealed a normal distribution of gene expression levels in all groups. The independent samples t-test was used for pairwise comparison of groups. Data from all animals was included in the analyses. Power analysis indicated that at least 6 animals per group was sufficient to detect a minimum 12% difference in energy intake with a power of 80% and alpha of 0.05 [40].

3. Results

3.1 Food and Water Consumption, Energy and Nutrient Intake

Food consumption and nutrient intake were the primary outcomes of this study (Table 3). Diet significantly influenced food consumption ($p < 0.001$). The level of food consumption was significantly different between groups

Table 3. Average food and water consumption, and energy and nutrient intake by the four groups.

Parameter	Group				SE	*p*
	CON n = 7	CONC n = 7	CAF n = 7	CAFC n = 7		
Food consumption (g/day)	25.4^{a}	32.3^{b}	41.8^{c}	42.2^{c}	1.64	<0.001
Water consumption (g/day)	21.2^{a}	26.3^{ab}	31.9^{b}	28.7^{ab}	1.89	0.004
Energy (kcal/day)	102^{a}	129^{a}	198^{b}	199^{b}	7.76	<0.001
Carbohydrate (g/day)	16.2^{a}	20.6^{b}	20.5^{b}	20.4^{b}	0.86	0.003
Fiber (g/day)	1.27^{a}	1.61^{b}	1.78^{b}	1.84^{b}	0.07	<0.001
Sugar (g/day)	2.56^{a}	3.26^{a}	6.30^{b}	5.82^{b}	0.35	<0.001
Protein (g/day)	5.15^{a}	6.55^{b}	5.64^{ab}	5.93^{ab}	0.24	0.003
Fat (g/day)	1.78^{a}	2.26^{b}	10.0^{c}	10.1^{c}	0.49	<0.001
Saturated fat (g/day)	0.28^{a}	0.23^{b}	4.09^{c}	3.93^{c}	0.21	<0.001
Sodium (mg/day)	25.9^{a}	32.8^{b}	231^{c}	247^{c}	14.6	<0.001

CON, control diet; CONC, control+chia diet; CAF, cafeteria diet; CAFC, cafeteria+chia diet; SE, pooled standard error. Values shown are the mean with pooled standard error. Repeated measures analysis of variance (ANOVA) was used for the analysis, and Bonferroni or Tamhane *post hoc* tests were used for pairwise comparisons according to the homogeneity of variances. Mean values with non-identical superscript letters were significantly different ($p < 0.05$).

over the study period (diet and time interaction, $p = 0.09$). *Post hoc* analysis showed that CAF and CAFC had higher total food consumption during treatment than CON and CONC. Water consumption was also significantly influenced by diet ($p = 0.004$), with the CAF group consuming more than the CON group.

The type of diet affected energy intake, with the CAF and CAFC groups having higher intakes than CON and CONC groups ($p < 0.001$). The energy intake of the different groups over time is shown in **Supplementary Fig. 1A**. CON had the lowest carbohydrate and fiber intakes ($p = 0.003$, and $p < 0.001$, respectively). Diet also influenced sugar intake ($p < 0.001$), with the CAF and CAFC groups having a higher intake than CON and CONC groups. Similarly, protein intake was influenced by diet ($p = 0.003$), with CON having a lower intake than CONC. Diet also influenced the intake of fat and saturated fat (both $p < 0.001$). CON had the lowest fat intake, whereas saturated fat intake was lowest in CONC. The carbohydrate, protein, and fat intakes of the groups over time are presented in **Supplementary Fig. 1B–D**. Sodium intake was also significantly influenced by diet ($p < 0.001$), and varied across groups over time (diet and time interaction, $p = 0.01$). Sodium intake was higher in the CAF and CAFC groups than in the CON and CONC groups.

3.2 Anthropometric Measurements and Body Composition

Changes in rat body weight over time are shown in Fig. 1A. Diet significantly influenced the body weight of animals ($p < 0.001$). The average body weight during treatment was significantly higher in CAF and CAFC compared to CON, and lower in CONC compared to CAFC. The highest and lowest weight gains were observed in CAF and CON, respectively (Fig. 1B). Total weight gain at the end of treatment was significantly lower in CON than in CAF and CAFC ($p < 0.001$). At the end of the study, the Lee index was significantly higher in CONC, CAF and CAFC compared to CON ($p < 0.001$; Fig. 1C).

Fig. 1D shows the body composition distribution of the four groups. Diet significantly influenced body water and body fat (both $p = 0.001$). CON had higher body water than CAF and CAFC, whereas it was lower in CAFC than CONC. CAF and CAFC both had higher body fat than CON.

3.3 Adipose Tissue and Organ Weights

Table 4 shows the adipose tissue and major organ weights of animals relative to their body weight. Diet had a significant influence on white adipose tissue (WAT) (all $p < 0.001$). Nevertheless, the IBAT and other major organs were similar among the different groups ($p > 0.05$). CON had lower PWAT, GWAT and total WAT compared to the CAF and CAFC groups. In addition, CAFC had higher PWAT, GWAT and total WAT compared to CONC.

3.4 Plasma Biochemical Indicators

The plasma parameters measured at the end of the study are shown in Table 5. Dietary treatment had no impact on the levels of plasma cholesterol, triglycerides, glucose, insulin or C-peptide. Only plasma leptin levels showed a significant difference between groups ($p = 0.017$), with CAF having significantly higher levels compared to CON.

3.5 Gene Expression Assays

The relative gene expression levels of *SREBP-1c*, *UCP2*, *PPARα*, *PPARγ*, and *FADS2* in GWAT are shown in Fig. 2. These genes were selected because they play key roles in lipid metabolism and adipose tissue function,

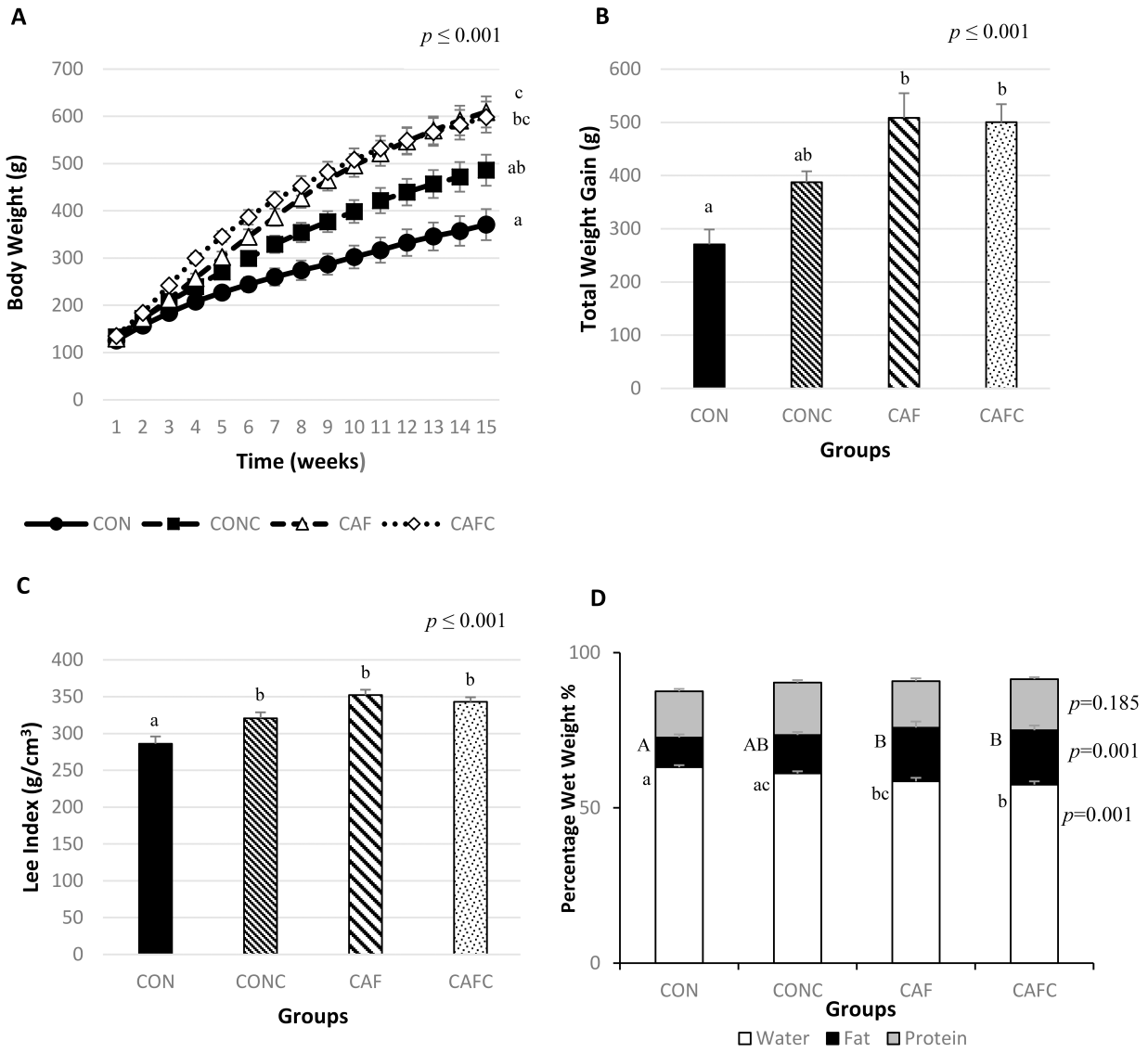

Fig. 1. Body weight changes, total weight gain, Lee index, and body composition in rats. CON, control diet; CONC, control+chia diet; CAF, cafeteria diet; CAFC, cafeteria+chia diet; n = 7 per group. Data are presented as the mean ± SEM. Significant differences between groups are indicated by different letters. (A) Body weight changes over the study period. Repeated measures analysis of variance (ANOVA) was performed. (B) Total weight gain at the end of the study. One-way ANOVA was performed. (C) Lee index at the end of the study. One-way ANOVA was performed. (D) Body composition (percentage wet weight of body water, fat, and protein) at the end of the study. One-way ANOVA was performed. Uppercase letters indicate group differences for fat, and lowercase letters indicate group differences for water. Mean values with non-identical superscript letters were significantly different.

which are central mechanisms in the development of obesity. *SREBP-1c* regulates lipogenic gene expression involved in fatty acid and triglyceride synthesis; alterations in its activity have been associated with adipose tissue expansion and obesity-related metabolic changes [44]. *UCP2* is involved in the regulation of mitochondrial energy efficiency and plays an important role in oxidative metabolism and reactive oxygen species regulation [45]. *PPARγ* enhances insulin sensitivity, lipogenesis, and adipocyte function and is essential for normal adipose tissue development, whereas *PPARα* promotes fatty acid oxidation, adipocyte differentiation, and anti-inflammatory pathways, thereby contributing to reduced adipocyte hypertrophy, improved insulin sensitivity, and protection against obesity-related metabolic dysfunction, particularly in rodent models [46]. *FADS2* is a key enzyme in PUFA metabolism [47]. Alterations in the expression of these genes have been widely associated with obesity and diet-induced metabolic dysfunction. The expression level of *SREBP-1c* was significantly higher in CAFC compared to CON and CAF, and lower in CAF compared to CONC (Fig. 2A). *UCP2* expression was significantly higher in CON compared to CONC

Table 4. Adipose tissue and organ weights relative to body weight.

Adipose tissue and organ weight (% BW)	Group				*p*
	CON n = 7	CONC n = 7	CAF n = 7	CAFC n = 7	
IBAT	0.12 ± 0.03	0.08 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.301
PWAT	1.19 ± 0.24^{a}	2.05 ± 0.12^{ab}	4.25 ± 0.79^{bc}	3.71 ± 0.34^{c}	<0.001
GWAT	0.69 ± 0.14^{a}	1.07 ± 0.11^{ab}	2.37 ± 0.41^{bc}	2.05 ± 0.14^{c}	<0.001
Total WAT (PWAT + GWAT)	1.88 ± 0.37^{a}	3.12 ± 0.20^{ab}	6.62 ± 1.19^{bc}	5.76 ± 0.47^{c}	<0.001
Liver	2.53 ± 0.12	2.36 ± 0.03	2.42 ± 0.09	2.54 ± 0.06	0.368
Brain	0.54 ± 0.03	0.40 ± 0.02	0.44 ± 0.12	0.35 ± 0.02	0.211
Right kidney	0.33 ± 0.02	0.30 ± 0.01	0.30 ± 0.02	0.30 ± 0.01	0.275
Left kidney	0.32 ± 0.01	0.30 ± 0.01	0.30 ± 0.02	0.29 ± 0.01	0.210

CON, control diet; CONC, control+chia diet; CAF, cafeteria diet; CAFC, cafeteria+chia diet; BW, body weight; IBAT, interscapular subcutaneous brown adipose tissue; PWAT, peri-renal white adipose tissue; GWAT, gonadal white adipose tissue; WAT, white adipose tissue. Values shown are the mean ± SEM. One-way analysis of variance (ANOVA) was performed. Tamhane *post hoc* test was used for pairwise comparisons. Mean values with non-identical superscript letters were significantly different ($p < 0.05$).

Table 5. Comparison of fasting plasma biochemical parameters.

Biochemical parameter	Group				*p*
	CON n = 7	CONC n = 7	CAF n = 7	CAFC n = 7	
Cholesterol (mg/dL)	87.9 ± 5.37	86.5 ± 8.42	91.2 ± 5.54	86.7 ± 5.28	0.947
Triglycerides (mg/dL)	104 ± 5.86	114 ± 1.68	103 ± 5.64	104 ± 3.45	0.322
Glucose (mg/dL)	198 ± 13.2	177 ± 23.4	198 ± 20.0	226 ± 18.8	0.377
Insulin (ng/mL)	8.04 ± 0.23	8.18 ± 0.35	8.34 ± 0.36	8.07 ± 0.31	0.910
Leptin (ng/mL)	1.20 ± 0.26^{a}	2.26 ± 0.48^{ab}	6.26 ± 1.78^{b}	5.67 ± 1.60^{ab}	0.017
C-peptide (ng/mL)	1.15 ± 0.42	1.68 ± 0.27	2.77 ± 0.97	2.85 ± 0.85	0.248

CON, control diet; CONC, control+chia diet; CAF, cafeteria diet; CAFC, cafeteria+chia diet. Values shown are the mean ± SEM. One-way analysis of variance (ANOVA) was performed. The Bonferroni *post hoc* test was used for pairwise comparisons. Mean values with non-identical superscript letters were significantly different ($p < 0.05$).

and CAFC (Fig. 2B). Furthermore, CAFC showed a trend for lower *UCP2* gene expression than CAF ($p = 0.053$). The expression levels of *PPARα* and *FADS2* were significantly higher in CON compared to CAF and CAFC (Fig. 2C,E). *PPARγ* expression did not differ significantly between groups (Fig. 2D). Additionally, CONC showed a trend for lower *PPARα* gene expression than CON ($p = 0.051$).

4. Discussion

The aim of this study was to investigate whether chia seed has protective effects against the progression of diet-induced obesity in a rat model. Several studies have reported beneficial effects from supplementing various obesogenic diets with chia seeds, chia oil, or chia flour [20,21,28,48–51]. However, the influence of chia seeds on the cafeteria diet, which has greater similarity with a multifactorial human diet, has so far not been investigated in studies of obesity development.

Chia seed supplementation had no effect on daily food or water consumption by the CAF group, as previously reported [21]. The CAF and CAFC groups were found to consume a significantly greater quantity of food than the CON and CONC groups. This result is similar to previous studies in which obesity was induced by cafeteria diet in rats [33,39,52]. Consumption of highly palatable cafeteria foods increased the fat mass and resulted in hyperphagia and rapid weight gain in male *Wistar* rats [33]. Cafeteria diet groups showed higher daily energy intake compared to CON and CONC groups. Studies on chia seed supplementation in obesogenic diets for rodents have yielded conflicting results, with some finding increased energy intake with chia seed supplementation of high-fat diets [21], while others found no short- or long-term differences [28,53]. In the present study, chia showed only a minimal contribution (5.3%, data not shown) to total energy intake in the CAFC group. Moreover, the CAFC and CAF groups derived almost the same proportion of energy from cafeteria diet (75.7% and 75.8%, respectively, data not shown), indi-

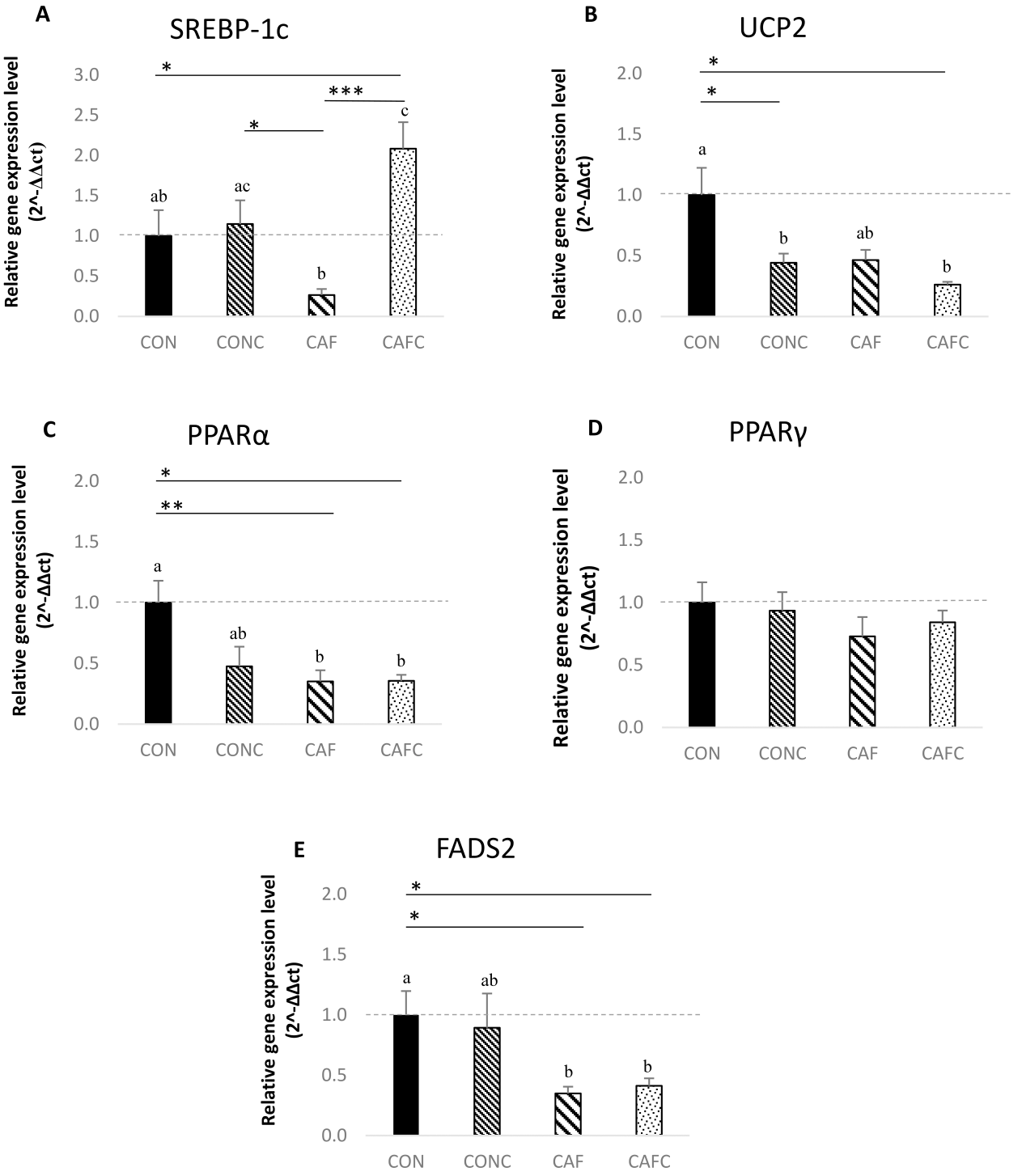

Fig. 2. Relative gene expression levels in WAT at the end of the study. CON, control diet; CONC, control+chia diet; CAF, cafeteria diet; CAFC, cafeteria+chia diet. n = 7 in each group. Data are presented as the mean ± SEM and normalized to the CON group. Asterisks indicate statistically significant differences as determined by *t*-tests (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Gene expression levels for (A) *SREBP-1c*, (B) *UCP2*, (C) *PPARα*, (D) *PPARγ*, and (E) *FADS2*.

cating that both groups consumed similar amounts of cafeteria diet foods and that chia seed supplementation had no effect on energy intake [54].

The CAFC group had the highest fiber intake in this study, likely due to its high food consumption and the 32.5% fiber content of chia seeds (Table 3). Taken together, our findings suggest that the potential satiating or hypocaloric effects of fiber may be overridden [55]. However, this effect was not observed in our study, likely because of the higher energy, fat, carbohydrate, and salt con-

tent of the cafeteria diet. Despite the low fiber content of cafeteria foods, the CAF group had the second-highest fiber intake due to increased food consumption. In addition to fiber, chia also contains mucilage and minerals that may contribute to satiety [15]. However, such effects were not evident under the obesogenic conditions of the cafeteria diet. These results suggest that the potential satiating or hypocaloric effects of fiber may be overridden in the presence of an extremely energy-dense and palatable dietary background [55,56]. Therefore, the high fiber content of chia alone may be insufficient to counteract the hyperphagic drive induced by the cafeteria diet.

Chia seed supplementation did not significantly alter saturated fat intake in rats fed the cafeteria diet, whereas it substantially reduced saturated fat intake in the control group. High consumption of saturated fats is known to increase oxidative stress and the production of free radicals [57], which contribute to obesity by promoting preadipocyte proliferation and increasing the size of differentiated adipocytes [58]. Excessive intake of saturated fat is therefore a key risk factor for the development of obesity. A study on three-week-old male *Wistar* rats demonstrated that adding 41.7% chia seeds to a high-fat diet significantly reduced saturated fat intake [59]. In contrast, our study suggests that supplementation of the cafeteria diet with a lower dose (20%) of chia seeds may not result in the same reduction in saturated fat. This finding indicates the lipid-modulating effects of chia may depend on both its proportion in the diet and the severity of the obesogenic background. The high fat and energy content of the cafeteria diet is likely to mask potential benefits from chia that have been observed in simpler, high-fat models [56,59].

Chia seed supplementation of the cafeteria diet did not exert a significant effect on body weight. However, the final body weight and overall weight gain in the CAFC group were marginally lower compared to the CAF group, although this difference did not reach statistical significance. There are contradictory findings in the literature regarding the effect of chia seeds on weight gain. Several studies found no difference in body weight or weight gain when different forms of chia (seed, flour, or oil) were added to high-fat or high-sucrose diets at different doses (3–36.2%) and for different times in rodents and rabbits [26,28,53,60,61]. However, other studies reported that supplementation of a high-fat or high-fructose diet with 1.5–4% chia oil reduced weight gain [61,62]. This discordance in research results highlights the complexities of the effects of chia seed supplementation on weight management, and suggests that dosage, duration, and dietary context can affect its physiological impact. The absence of a measurable effect of chia seeds on body weight in our study could reflect the relatively short consumption period and moderate dose, which may not have been sufficient to counteract the strong obesogenic drive of the cafeteria diet.

Analysis of the carcass at the end of this study revealed similar body fat ratios in both the CAF and CAFC groups. There are conflicting findings in the literature regarding the effect of chia seeds on body fat in obese rodents. Oliva *et al*. [37] and Fonte-Faria *et al*. [49] found that chia seed and oil treatments led to a reduction in the percentage of body fat. In contrast, de Miranda *et al*. [26] and Poudyal *et al*. [21] reported no significant changes in total body fat with 3% chia flour and 5% chia seed supplementation, respectively. These studies utilized different experimental models, with various forms of chia given in varying amounts and for different times. In our model, supplementation with chia seed showed no effect on body fat, suggesting that its impact on adiposity may depend on the dietary context and could be more apparent under less extreme nutritional conditions. The complex and variable composition and high nutrient density of the cafeteria diet might attenuate the capacity for chia to modulate fat accumulation [56].

PWAT, GWAT, and total WAT in the CAF and CAFC groups were 2–3-fold higher than in the CON group, consistent with other reports in the literature [33,39,63–66]. Chia seed supplementation of the cafeteria diet did not affect the weight of adipose tissues in our study, although some studies found that addition of chia seed to a sucrose-rich diet reduced visceral adipose tissue [37,67]. The cafeteria diet is considered to be more obesogenic than diets in which energy is increased by the addition of a single nutrient, possibly explaining why no effect on adipose tissue weight was observed [56].

Among the plasma parameters, only the leptin level was significantly different between groups. Chia seed supplementation of the cafeteria diet had no significant impact on the leptin level compared to the CAF diet alone; however, the leptin level in CAFC group was also similar to those in the CON and CONC groups, suggesting a trend toward control values. Although some studies have reported that chia oil reduced leptin levels in mice [49,62], the present study found that chia seeds did not alter leptin levels in *Wistar* rats. This partial normalization of leptin levels suggests a modest improvement in leptin sensitivity or regulation, even if not statistically significant.

Supplementation with chia seeds was associated with significant changes in the expression of some genes in WAT. Several studies have reported that ALA in chia seeds can either increase or decrease the expression of *SREBP-1c* [18,22,37,68–70]. The expression level increased when adipocyte cells were treated with chia seeds [68], and when rats were fed with chia oil [69], consistent with our findings. These effects are mainly attributed to the high ALA content of chia, which is the principal bioactive component that influences lipid metabolism [71]. The higher *SREBP-1c* expression level observed in the CAFC and CONC groups compared to the CAF group may be related to the high ALA content of chia seeds. However, chia seed reduced the expression of *UCP2* in our study. Creus *et al*. [72] reported

that *UCP2* expression in the heart muscle of obese rats was not affected by chia seed supplementation, and this gene has not been investigated in other studies of chia seeds. Fish oil supplementation of a high-fat diet was shown to reduce *UCP2* gene expression in adipose tissue [73,74]. Chia seeds are rich in plant-derived n-3 ALA, but deficient in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [23]. Since ALA serves as a precursor to both EPA and DHA [75], this could potentially explain the reduced *UCP2* expression observed in our study. The levels of *PPAR*α and *FADS2* gene expression were lower in the CAF and CAFC groups compared to the controls. However, chia seeds given with an obesogenic diet did not affect the expression level of these genes in WAT. Collectively, these molecular findings imply that chia seed supplementation under obesogenic conditions may not be sufficient to counteract the lipogenic gene activation induced by the cafeteria diet. However, the observed alterations in *SREBP-1c* and *UCP2* expression may point to early changes in lipid metabolism that have yet to result in measurable physiological effects.

To our knowledge, this is the first study to examine the effects of chia seed supplementation in a cafeteria diet-induced obesity model, which is a good model for Western-type eating patterns. We performed physiological, biochemical, and molecular assessments to obtain a broad view of the effect of chia seeds on obesity-related outcomes. The cafeteria diet is not a standardized approach and there are challenges associated with its implementation and accurate energy and nutrient estimation. Nevertheless, it is a well-established method for increasing adiposity parameters [39] and may better resemble Western diets compared to conventional high-fat models [56]. These aspects highlight the translational relevance of this work, as it bridges experimental findings with complex human dietary behaviors. Our results highlight the importance of evaluating nutritional interventions within realistic dietary contexts to better understand their physiological potential.

A key strength of this work is the integrated evaluation of dietary intake, body composition, plasma biomarkers, and gene expression in adipose tissue, thus providing a multidimensional understanding of chia seed supplementation. However, several limitations should be acknowledged: the cafeteria diet model is inherently variable, only one form and dose of chia seed was tested, and the relatively short duration may have limited the ability to detect subtle metabolic changes.

Unexpectedly, chia seed supplementation did not significantly alter energy and nutrient intake, body composition, or plasma parameters under obesogenic conditions. The most plausible explanation is that the dosage used was insufficient to counteract the strong obesogenic effects of the cafeteria diet. Future studies using different chia seed doses, longer intervention periods, and combined metabolic and gut microbiota assessments should help to clarify whether the observed changes in gene expression translate into meaningful metabolic improvements. Since the soluble fiber and mucilage fractions in chia may influence gut microbial composition, the integration of microbiota analysis would provide a more complete understanding of its physiological actions.

5. Conclusions

We hypothesized that supplementing a cafeteria diet with chia seed (20% w/w) may exert protective effects against the development of obesity, adiposity-related markers, and changes in gene expression levels in adipose tissue. This study found that chia seed supplementation in rats fed an obesogenic diet increased *SREBP-1c* expression and showed a trend toward decreased *UCP2* expression in WAT, possibly mediated by the high ALA content of chia. In summary, this is the first study to explore the effects of chia seed supplementation in a cafeteria diet-induced obesity model, providing new insights into how plant-based n-3 sources can modulate adipose tissue gene expression under complex dietary conditions. The findings of this study should inform future research into the effects of chia seeds on obesity and lipid metabolism, focusing on both short- and long-term supplementation with varying doses. Future studies should incorporate dietary models that are more reflective of human nutrition, such as the cafeteria diet, to provide deeper insights into the potential benefits of chia seeds.

Abbreviations

ALA, Alpha-linolenic acid; CAF, Cafeteria diet; CAFC, Cafeteria diet with chia seed; CON, Control diet; CONC, Control diet supplemented with chia seed; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; *FADS2*, Fatty acid desaturase 2; *GAPDH*, Glyceraldehyde 3- phosphate dehydrogenase; GWAT, Gonadal white adipose tissue; IBAT, Interscapular brown adipose tissue; LA, Linoleic acid; n-3, Omega-3; *PPAR* α/γ, Peroxisome proliferator activity receptors α/γ; PUFAs, Polyunsaturated fatty acids; PWAT, Peri-renal white adipose tissues; RT-PCR, Real-time polymerase chain reaction; *SREBP-1/2*, Sterol regulatory element binding protein 1/2; *UCP2*, Uncoupling protein 2; WAT, White adipose tissue.

Availability of Data and Materials

All data supporting the findings of this study are available upon reasonable request from the corresponding author.

Author Contributions

CC, AAkyol, and AAyaz contributed to conceptualization, investigation, formal analysis, and data curation. AAkyol and AAyaz contributed to methodology. CC contributed to software and writing—original draft preparation.

AAkyol and AAyaz contributed to review, editing, and supervision. AAyaz contributed to funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All experimentation was conducted in accordance with Directive 2010/63/EU and ARRIVE guidelines, with a license from the Ethics Board on Animal Experimentations of Hacettepe University, Ankara, Turkiye (protocol number: 2016/33-04).

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGpt-5 to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR46718.

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