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Long-Term Palmitoleic Acid-Enriched Marine Oil Ingestion Attenuates Adiposity and Glucose Intolerance in Cafeteria Diet-Fed Mice

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Abstract

Palmitoleic acid (16:1n-7) has been associated with metabolic regulation, but the effects of long-term supplementation during consumption of a complex obesogenic diet remain poorly defined. We investigated whether palmitoleic acid-enriched marine oil modifies adiposity, glucose homeostasis, lipid metabolism, and tissue fatty-acid composition in mice exposed to a cafeteria diet. Male C57BL/6 mice were assigned in a 2 × 2 factorial design (Diet × Oil) to standard diet (SD), cafeteria diet (CAF), standard diet plus oil (SDO), or cafeteria diet plus oil (CAFO) for 16 weeks. Supplemented animals received 1 g/kg body mass/day of oil containing 558 mg/g palmitoleic acid ethyl ester. Diet × Oil interactions were observed for adiposity ($p = 0.0004$), adipocyte area ($p = 0.0062$), glucose tolerance ($p = 0.0009$), and plasma triacylglycerol ($p = 0.0070$). Compared with CAF, CAFO mice had lower adiposity, adipocyte area, glucose area under the curve, and plasma triacylglycerol, whereas hepatic lipid concentrations were not improved. Supplementation substantially increased palmitoleate in red blood cells and adipose tissue. These findings demonstrate substantial incorporation of supplemented palmitoleate and selective attenuation of metabolic alterations associated with long-term cafeteria-diet consumption.

Keywords: palmitoleic acid; omega-7 fatty acid; cafeteria diet; adiposity; glucose tolerance; adipose tissue; fatty-acid composition; marine oil

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1. Introduction

Palmitoleic acid or palmitoleate (cis-16:1n-7) is a monounsaturated omega-7 fatty acid that can be obtained from dietary sources, including some marine oils, but is also synthesized endogenously from palmitic acid through stearoyl-CoA desaturase-1 (SCD1). Seminal work by Cao et al. identified palmitoleate as a potential lipid signaling molecule,

or "lipokine," linking adipose tissue with systemic glucose and lipid metabolism [1]. Subsequent experimental studies have reported effects of palmitoleate on insulin sensitivity, glucose metabolism, adipose tissue remodeling, hepatic lipid metabolism, and inflammatory pathways [2–6]. In rodent models of obesity and diabetes, supplementation has been associated with improved glucose regulation, changes in adipocyte metabolism and morphology, and, in some studies, reduced hepatic lipid accumulation [2,3,5,6].

The metabolic significance of palmitoleate, however, remains uncertain. Circulating palmitoleate can reflect endogenous SCD1 activity and de novo lipogenesis, and higher concentrations in some lipid fractions have been associated with hypertriglyceridemia, adiposity, insulin resistance, and fatty liver [7–9]. This differs from increased palmitoleate exposure produced by dietary supplementation and complicates interpretation of the available literature. Recent human evidence has further highlighted this discrepancy [10–12]. In a randomized controlled trial, marine-derived palmitoleic acid supplementation substantially increased plasma and red blood cell palmitoleate but did not produce consistent improvements in conventional glycemic, lipid, or inflammatory outcomes [10]. A recent synthesis of the evidence therefore concluded that the metabolic effects of palmitoleate appear to depend on its source, metabolic context, background diet, and whether it reflects endogenous synthesis or exogenous intake [11].

Obesity and related metabolic disorders are strongly influenced by dietary patterns characterized by high energy density and excessive intake of fat and refined carbohydrates [13]. Chronic exposure to these diets promotes adipose tissue expansion, impaired glucose homeostasis, dyslipidemia, and ectopic lipid accumulation, contributing to the development of cardiometabolic disease [3,5,13]. The fatty-acid composition of the diet may also influence these responses, which has stimulated interest in food-derived bioactive lipids as potential nutritional strategies for modulating metabolic health [4].

Previous preclinical studies investigating the metabolic effects of palmitoleic acid have predominantly employed standardized high-fat diets or genetic models of metabolic dysfunction, frequently with relatively short supplementation periods [2,3,5,6]. Although standardized high-fat diets provide controlled experimental conditions, they do not fully reproduce the dietary heterogeneity characteristic of human obesogenic eating patterns. Cafeteria diets offer an alternative experimental model that exposes animals to a combination of energy-dense foods with different nutritional characteristics. However, this greater dietary complexity also introduces variability in food selection and nutrient exposure. The metabolic effects of long-term supplementation with palmitoleic acid-enriched marine oil under these dietary conditions remain insufficiently characterized, particularly when tissue fatty-acid incorporation is evaluated alongside adiposity, glucose homeostasis, and lipid metabolism. Therefore, the present study investigated the effects of chronic supplementation with a marine-derived palmitoleic acid-enriched oil on body composition and adipose tissue morphology, glucose homeostasis, plasma and hepatic lipid concentrations, and fatty-acid composition of red blood cells and adipose tissue in mice fed either a standard or cafeteria diet. We hypothesized that supplementation would increase palmitoleate incorporation into red blood cells and adipose tissue and attenuate selected metabolic disturbances induced by the cafeteria diet.

2. Materials and Methods

2.1. Animals and Experimental Design

Male C57BL/6 mice were obtained at 21 days of age and underwent a 9-day acclimatization period. At approximately 30 days of age, animals were allocated to experimental groups, with allocation performed to achieve similar initial total body mass among cages. Animals assigned to different experimental groups were housed in separate cages, with

a maximum of five animals per cage, under controlled environmental conditions (22 ± 2 °C; 12-h light/dark cycle) and with ad libitum access to food and water. We maintained the cages under the same environmental conditions and in close proximity. The experiment followed a 2 × 2 factorial design, with diet (standard diet vs. cafeteria diet) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as experimental factors. Animals were allocated to four groups: standard diet without oil (SD), cafeteria diet without oil (CAF), standard diet supplemented with palmitoleic acid-enriched oil (SDO), and cafeteria diet supplemented with palmitoleic acid-enriched oil (CAFO). We provided dietary treatments at the cage level, while administering oil supplementation individually to each animal. The dietary and supplementation interventions were maintained for 16 weeks (Figure 1). We conducted the experiment in two identical experimental runs to obtain the final sample sizes. The Animal Use Ethics Committee of the Federal University of Santa Catarina approved all experimental procedures (Permit No. 5855301015), and we conducted them in accordance with institutional guidelines for the care and use of laboratory animals.

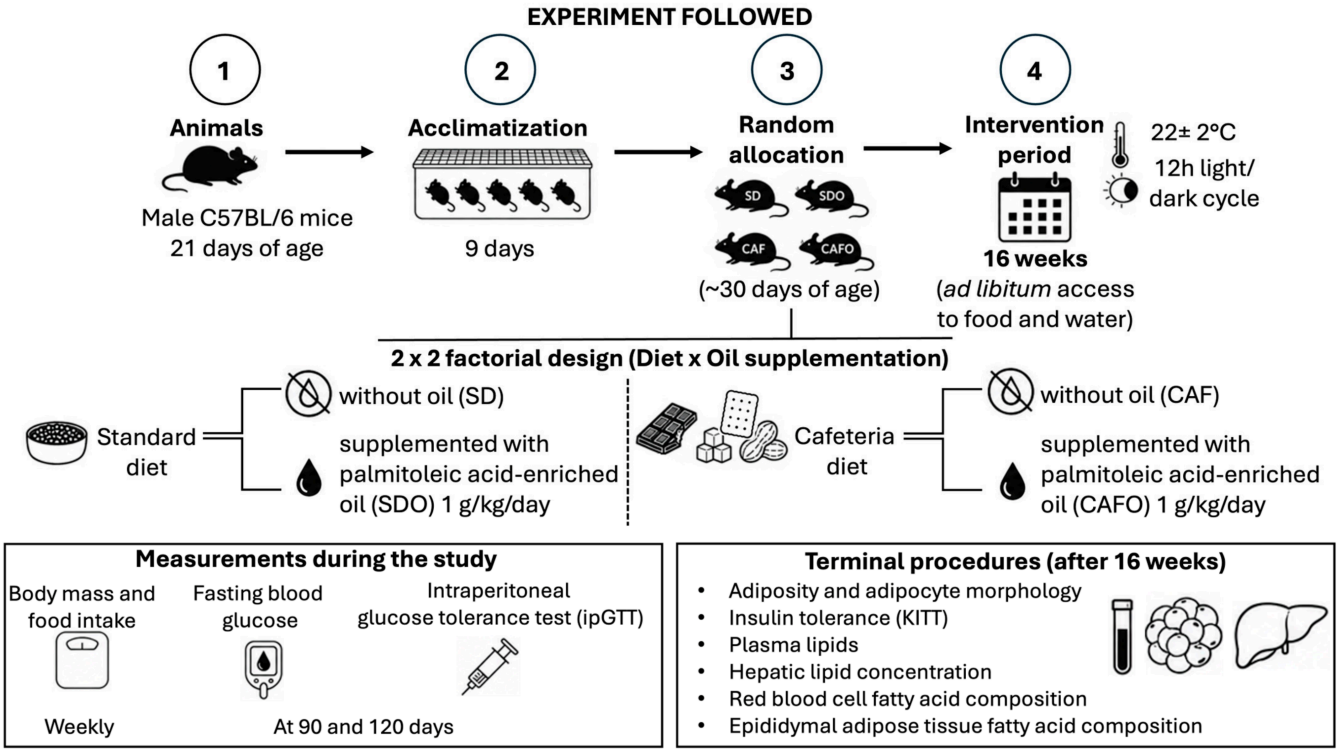

Figure 1. Summary of the experimental design, group allocation, interventions and assessed outcomes.

2.2. Experimental Diets

The standard chow diet provided approximately 29% of energy from protein, 59% from carbohydrate, and 12% from fat, with an energy density of approximately 3 kcal/g. The cafeteria diet consisted of standard chow diet (15 g), milk chocolate (10 g), peanuts (10 g), cornstarch crackers (5 g), and sugar (5 g), corresponding to a 3:2:2:1:1 ratio [12]. Ingredients were combined and formed into pellets of approximately 10 g. The cafeteria diet provided approximately 14% of energy from protein, 47% from carbohydrate, and 39% from fat, with an energy density of approximately 4.41 kcal/g. Analysis of the fatty-acid composition of the diets showed marked differences between the standard and cafeteria diets, particularly for palmitic, oleic, linoleic, α-linolenic, arachidonic, EPA, and DHA (Supplementary Table S1). Food intake was measured weekly at the cage level from the difference between the amount of food provided and the amount remaining. Cage-level food consumption was divided

by the number of animals housed in each cage and expressed as g/mouse/week. Food-derived energy intake was calculated from food consumption and the energy density of the respective diets.

2.3. Palmitoleic Acid-Enriched Oil Supplementation

The palmitoleic acid-enriched oil (AlaskOmega Palmitoleic 500; lot 115116, Coshocton, OH, USA) was derived from Alaska walleye pollock (*Theragra chalcogramma*). According to the manufacturer's certificate of analysis, the product contained 558 mg/g palmitoleic acid ethyl ester and 257 mg/g palmitic acid ethyl ester, with 1 mg/g oleic acid ethyl ester, and contained 0.25% natural mixed tocopherols. Gas chromatographic analysis confirmed palmitoleic acid (16:1) as the predominant fatty acid, accounting for approximately 55.75% of the analyzed fatty-acid composition. Animals in the SDO and CAFO groups received the palmitoleic acid-enriched oil orally at 1 g/kg body mass/day throughout the 16-week intervention. The oil was administered directly into the mouth using a pipette, and the administered amount was adjusted according to body mass. Animals in the SD and CAF groups received water by pipette using the same route of administration.

2.4. Body Mass, Tissue Collection, and Adiposity

Body mass was measured weekly throughout the 16-week experimental period. At the end of the intervention, mice were fasted for 6 h and anesthetized with inhaled isoflurane (1–2%). Blood was collected by cardiac puncture, after which tissues were rapidly excised and weighed. Samples intended for biochemical analyses were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Epididymal, retroperitoneal, and inguinal white adipose tissue depots were collected and weighed. Total white adipose tissue mass was calculated as the sum of the collected white adipose tissue depots. Adiposity index was calculated as the combined mass of the epididymal, retroperitoneal, and inguinal white adipose tissue depots relative to final body mass and expressed as a percentage. Individual tissue masses were expressed relative to final body mass as mg/g body mass. Interscapular adipose tissue, liver, soleus, gastrocnemius, and visceral tissue masses were also determined.

2.5. Adipocyte Morphometry

Immediately after euthanasia, samples from the right epididymal adipose tissue depot were transferred to histological cassettes and fixed in 4% paraformaldehyde for 48 h at 8 °C. Samples were subsequently dehydrated in ethanol and embedded in paraffin. Tissue sections were mounted on glass slides, deparaffinized in xylene, rehydrated, stained with hematoxylin and eosin, and cover-slipped with Canada balsam. Slides were digitized using an automated ZEISS slide scanner (Oberkochen, Germany), and adipocyte morphometry was performed using ZEN Lite software (Blue Edition; ZEISS). Adipocytes with intact plasma membranes were manually outlined to determine cell area. Ten to fifteen randomly selected fields, corresponding to approximately 150 adipocytes per tissue section, were analyzed in a blinded manner to obtain the mean adipocyte area for each animal.

2.6. Plasma and Hepatic Lipid Analyses

Blood obtained by cardiac puncture was centrifuged at 400× *g* for 10 min at 4 °C. Plasma triacylglycerol and total cholesterol concentrations were measured using commercial enzymatic colorimetric assays (Biotécnica, Varginha, MG, Brazil) according to the manufacturer's instructions. For hepatic lipid analysis, approximately 100 mg of liver tissue obtained from the same anatomical region in all animals was transferred to tubes containing 1 mol/L NaCl and homogenized using an Ultra-Turrax homogenizer (IKA, Staufen,

Germany). Chloroform: methanol (2:1, *v*/*v*) was added, and samples were centrifuged at 5000× *g* for 5 min. Following phase separation, the lower phase was collected and dried in a boiling water bath. The dried extract was resuspended in Triton X-100: methanol (2:1, *v*/*v*), vortexed, and triacylglycerol and total cholesterol were quantified using enzymatic colorimetric assays (Biotécnica), according to a procedure adapted from Trinh et al. and Barbosa et al. [13,14]. Hepatic triacylglycerol and total cholesterol concentrations were expressed as mg/g of liver tissue.

2.7. Fasting Blood Glucose and Intraperitoneal Glucose Tolerance Test

Fasting blood glucose was assessed after 90 and 120 days of intervention following a 6-h fast. At 120 days, glucose tolerance was assessed by an intraperitoneal glucose tolerance test (ipGTT), also following a 6-h fast. In conscious mice, approximately 0.5 mm of the tail tip was removed for blood sampling. The first drop of blood was discarded, and the subsequent sample was used to determine baseline blood glucose using an Accu-Chek Performa glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Immediately after baseline measurement, a 50% glucose solution was administered intraperitoneally at 2 g/kg body mass. Blood glucose was subsequently measured at 30, 60, 90, and 120 min after glucose administration. The area under the glucose concentration-time curve (AUC) was calculated for each animal [15].

2.8. Insulin Tolerance Test

The insulin tolerance test was performed in fed animals. After measurement of baseline blood glucose, insulin was administered intraperitoneally at 0.75 U/kg body mass. Blood glucose was measured from the tail tip at 10, 15, 20, and 60 min after insulin administration. The glucose disappearance rate (KITT) was calculated as −100 times the slope of the linear regression of the natural logarithm of blood glucose concentrations from 0 to 20 min after insulin administration and expressed as %/min [15].

2.9. Fatty-Acid Composition of Red Blood Cells and Epididymal Adipose Tissue

Fatty-acid composition was determined in epididymal adipose tissue and red blood cells. Approximately 50 mg of epididymal adipose tissue and 200 µL of red blood cells were used for analysis. Total lipids were extracted according to the Folch method [16], and fatty acids were subsequently analyzed by high-performance liquid chromatography following the method of Nishiyama-Naruke et al. [17]. Extracted lipids were incubated with 1 mol/L NaOH in 90% methanol at 37 °C for 2 h with agitation. Samples were then acidified to approximately pH 3 with 1 mol/L HCl. Fatty acids were extracted three times with 2 mL hexane, and the organic solvent was evaporated under nitrogen. Free fatty acids were derivatized with bromomethyl-methoxycoumarin in acetonitrile, with 18-crown-6 used as catalyst, at 60 °C for 15 min. [18]. Derivatized fatty acids were analyzed using a Varian ProStar HPLC system equipped with a ProStar 363 fluorescence detector and a Phenomenex C8 column (5-µm particle size, 100-Å pore size). Acetonitrile and water were used as the mobile phase at a flow rate of 0.7 mL/min. Fluorescence was monitored at an excitation wavelength of 325 nm and an emission wavelength of 395 nm, and chromatographic data were acquired using ProStar LC Workstation version 6.0. The following fatty acids were quantified: myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1n-7), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), α-linolenic acid (18:3n-3), arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). Fatty-acid composition was expressed as a percentage of total measured fatty acids.

2.10. Statistical Analysis

Data are presented as mean ± SD unless otherwise indicated. Data distributions were assessed using the Shapiro–Wilk test. End-point outcomes were analyzed using two-way analysis of variance (ANOVA), with diet (standard vs. cafeteria diet) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as between-subject factors. Models included the main effects of Diet and Oil and the Diet × Oil interaction. When a significant Diet × Oil interaction was detected, pairwise comparisons among the four experimental groups were performed using the Holm–Šídák multiple-comparisons procedure. Statistical significance was accepted at $p < 0.05$. The individual animal was considered the experimental unit for the outcomes included in the analyses. Sample sizes varied among outcomes because of sample availability, assay-specific data availability, and exclusions, and the number of animals included in each analysis is reported in the corresponding table. Statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Body Mass, Food Intake, Adiposity, and Tissue Mass

Diet did not significantly affect final body mass, and no Diet × Oil interaction was detected, although oil had a main effect ($p = 0.0097$; Table 1). Diet significantly affected food intake ($p < 0.001$), with lower intake in cafeteria diet-fed mice, whereas oil supplementation had no significant effect ($p = 0.889$) and the Diet × Oil interaction was not significant ($p = 0.572$; Table 1). Despite the lower food intake, calculated energy intake was higher in cafeteria diet-fed mice because of the greater energy density of the cafeteria diet (Diet, $p < 0.001$). There was no significant effect of oil supplementation on energy intake ($p = 0.974$) and no Diet × Oil interaction ($p = 0.563$; Table 1). Cafeteria-diet feeding markedly increased adiposity, and palmitoleic acid-enriched oil supplementation modified this response. Adiposity index increased from 1.60 ± 0.43% in SD to 3.84 ± 0.98% in CAF mice, whereas CAFO mice had an adiposity index of 2.77 ± 0.81%. There was a significant effect of diet ($p < 0.0001$) and a Diet × Oil interaction ($p = 0.0004$). Post hoc analysis showed greater adiposity in CAF than SD and CAFO mice, although adiposity remained greater in CAFO than SD mice (Table 1). Similar Diet × Oil interactions were observed for epididymal ($p = 0.0011$), retroperitoneal ($p = 0.0001$), inguinal ($p = 0.0277$), total white ($p = 0.0004$), and interscapular ($p < 0.0001$) adipose tissue mass. Total white adipose tissue mass was 15.95 ± 4.28 mg/g in SD, 38.39 ± 9.75 mg/g in CAF, 22.03 ± 5.69 mg/g in SDO, and 27.67 ± 8.12 mg/g in CAFO mice. CAF mice had greater total white adipose tissue mass than SD, SDO, and CAFO mice (Table 1). Cafeteria-diet feeding was also associated with lower relative liver mass ($p < 0.0001$), without significant Diet × Oil interactions. Relative soleus and gastrocnemius masses were not significantly affected by diet, oil supplementation, or their interaction (Table 1).

Table 1. Body mass, adiposity, adipocyte area, and selected tissue masses.

Outcome	SD	CAF	SDO	CAFO	Diet *p*	Oil *p*	Diet × Oil *p*
Final body mass (g)	28.20 ± 2.44	29.00 ± 3.30	27.08 ± 2.50	26.00 ± 1.65	0.765	0.0097	0.221
Food intake (g food/mouse/week)	27.98 ± 1.82	22.59 ± 2.09	28.63 ± 3.27	22.15 ± 1.70	<0.001	0.889	0.572
Food-derived energy intake (kcal/mouse/week)	83.95 ± 5.47	99.62 ± 9.23	85.90 ± 9.82	97.69 ± 7.49	<0.001	0.974	0.563
Adiposity index (%)	1.60 ± 0.43 ^{a}	3.84 ± 0.98 ^{b}	2.20 ± 0.57 ^{ac}	2.77 ± 0.81 ^{c}	<0.0001	0.295	0.0004
Epididymal fat (mg/g)	11.78 ± 2.92 ^{a}	26.87 ± 7.07 ^{b}	15.80 ± 3.69 ^{ac}	20.09 ± 5.62 ^{c}	<0.0001	0.374	0.0011
Retroperitoneal fat (mg/g)	4.18 ± 1.44 ^{a}	11.53 ± 2.87 ^{b}	6.23 ± 2.03 ^{ac}	7.58 ± 2.66 ^{c}	<0.0001	0.185	0.0001
Inguinal fat (mg/g)	9.33 ± 3.74 ^{a}	21.86 ± 7.67 ^{b}	12.51 ± 4.42 ^{a}	16.33 ± 8.05 ^{ab}	0.0002	0.542	0.0277
Total white adipose tissue (mg/g)	15.95 ± 4.28 ^{a}	38.39 ± 9.75 ^{b}	22.03 ± 5.69 ^{ac}	27.67 ± 8.12 ^{c}	<0.0001	0.297	0.0004
Interscapular fat (mg/g)	3.60 ± 0.75 ^{a}	7.04 ± 1.09 ^{b}	4.71 ± 1.11 ^{c}	4.81 ± 1.01 ^{c}	<0.0001	0.074	<0.0001
Epididymal adipocyte area (μm^2)	2227 ± 433 ^{a}	4298 ± 657 ^{b}	2717 ± 492 ^{a}	3530 ± 958 ^{c}	<0.0001	0.864	0.0062
Liver (mg/g)	50.87 ± 3.51	37.12 ± 5.25	47.29 ± 3.70	37.79 ± 4.86	<0.0001	0.280	0.118
Soleus (mg/g)	0.94 ± 0.29	0.94 ± 0.25	1.03 ± 0.20	0.94 ± 0.24	0.500	0.539	0.530
Gastrocnemius (mg/g)	11.53 ± 0.60	11.71 ± 1.24	11.62 ± 0.55	12.05 ± 0.87	0.230	0.403	0.641

Values are presented as mean ± SD. Data were analyzed by two-way ANOVA with diet (standard diet vs. cafeteria diet) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as factors. When a significant diet × oil interaction was detected, pairwise comparisons among experimental groups were performed using the Holm–Šídák multiple-comparisons procedure. Within each row, means without a common superscript letter differ significantly ($p < 0.05$). SD, standard diet; CAF, cafeteria diet; SDO, standard diet plus palmitoleic acid-enriched oil; CAFO, cafeteria diet plus palmitoleic acid-enriched oil. Sample sizes: SD n = 10, CAF n = 10, SDO n = 12, CAFO n = 12 for tissue outcomes. For adipocyte area: SD n = 10, CAF n = 8, SDO n = 12, CAFO n = 11.

3.2. Epididymal Adipocyte Morphology

Epididymal adipocyte area increased from 2227 ± 433 μm^2 in SD to 4298 ± 657 μm^2 in CAF mice, whereas adipocyte area was 2717 ± 492 μm^2 in SDO and 3530 ± 958 μm^2 in CAFO mice. There was a significant effect of diet ($p < 0.0001$) and a Diet × Oil interaction ($p = 0.0062$), with no main effect of oil. Adipocyte area was greater in CAF than SD, SDO, and CAFO mice, while CAFO remained greater than SD and SDO mice (Table 1). Thus, palmitoleic acid-enriched oil supplementation attenuated, but did not completely prevent, cafeteria-diet-induced adipocyte hypertrophy. Supplementary Figure S1 presents representative hematoxylin and eosin-stained sections of epididymal adipose tissue from the four experimental groups.

3.3. Plasma and Hepatic Lipids

Plasma triacylglycerol concentrations showed a significant Diet × Oil interaction ($p = 0.0070$). Concentrations were 57.99 ± 15.17 in CAF compared with 41.80 ± 7.82 in CAFO mice ($p = 0.0105$), whereas no other pairwise comparison was significant (Table 2). Plasma total cholesterol was significantly affected by diet ($p = 0.0002$), without significant effects of oil or a Diet × Oil interaction. Similarly, hepatic triacylglycerol and total cholesterol showed significant effects of diet ($p = 0.0004$ and $p < 0.0001$, respectively), but no significant Diet × Oil interactions. The main effect of oil on hepatic triacylglycerols did not reach statistical significance ($p = 0.055$; Table 2).

3.4. Fasting Glucose and Glucose Tolerance

Diet significantly affected fasting glucose at both 90 and 120 days ($p = 0.0005$ and $p < 0.0001$, respectively), with no significant Diet × Oil interactions. At 120 days, fasting glucose was 140.0 ± 16.9 mg/dL in SD, 170.9 ± 15.9 mg/dL in CAF, 139.6 ± 13.4 mg/dL in SDO, and 154.8 ± 16.4 mg/dL in CAFO mice (Table 2). Thus, oil supplementation did not significantly affect fasting glucose concentrations. In contrast, glucose tolerance showed a significant Diet × Oil interaction ($p = 0.0009$). GTT AUC was 10,013 ± 2129 in SD, 14,861 ± 2927 in CAF, 12,686 ± 3295 in SDO, and 11,130 ± 2841 in CAFO mice. GTT AUC was greater in CAF than in SD mice ($p = 0.0040$) and lower in CAFO than in CAF mice ($p = 0.0291$), with no other significant pairwise differences (Table 2).

3.5. Response to Insulin

KITT was significantly affected by diet ($p < 0.0001$), but there was no significant effect of oil or Diet × Oil interaction (Table 2). Thus, cafeteria-diet feeding altered the glycemic response to insulin, without evidence that palmitoleic acid-enriched oil modified this response.

The main outcomes showing significant Diet × Oil interactions, adiposity index, epididymal adipocyte area, glucose tolerance AUC, and plasma triacylglycerol concentrations, are summarized in Figure 2.

3.6. Red Blood Cell Fatty-Acid Composition

Red blood cell fatty-acid composition was markedly influenced by diet and oil supplementation (Table 3). Most notably, palmitoleic acid (16:1n-7) increased from 0.578 ± 0.075% and 0.488 ± 0.015% in SD and CAF mice to 4.452 ± 0.367% and 4.030 ± 0.138% in SDO and CAFO mice, respectively. Oil had a pronounced main effect ($p < 0.0001$), and diet also had a main effect ($p = 0.0046$), but the Diet × Oil interaction was not significant ($p = 0.072$). Significant Diet × Oil interactions were also detected for palmitic, stearic, oleic, linoleic, α-linolenic, arachidonic, EPA, and DHA ($p \leq 0.0446$; Table 3). The complete fatty-acid profile and post hoc comparisons are presented in Table 3.

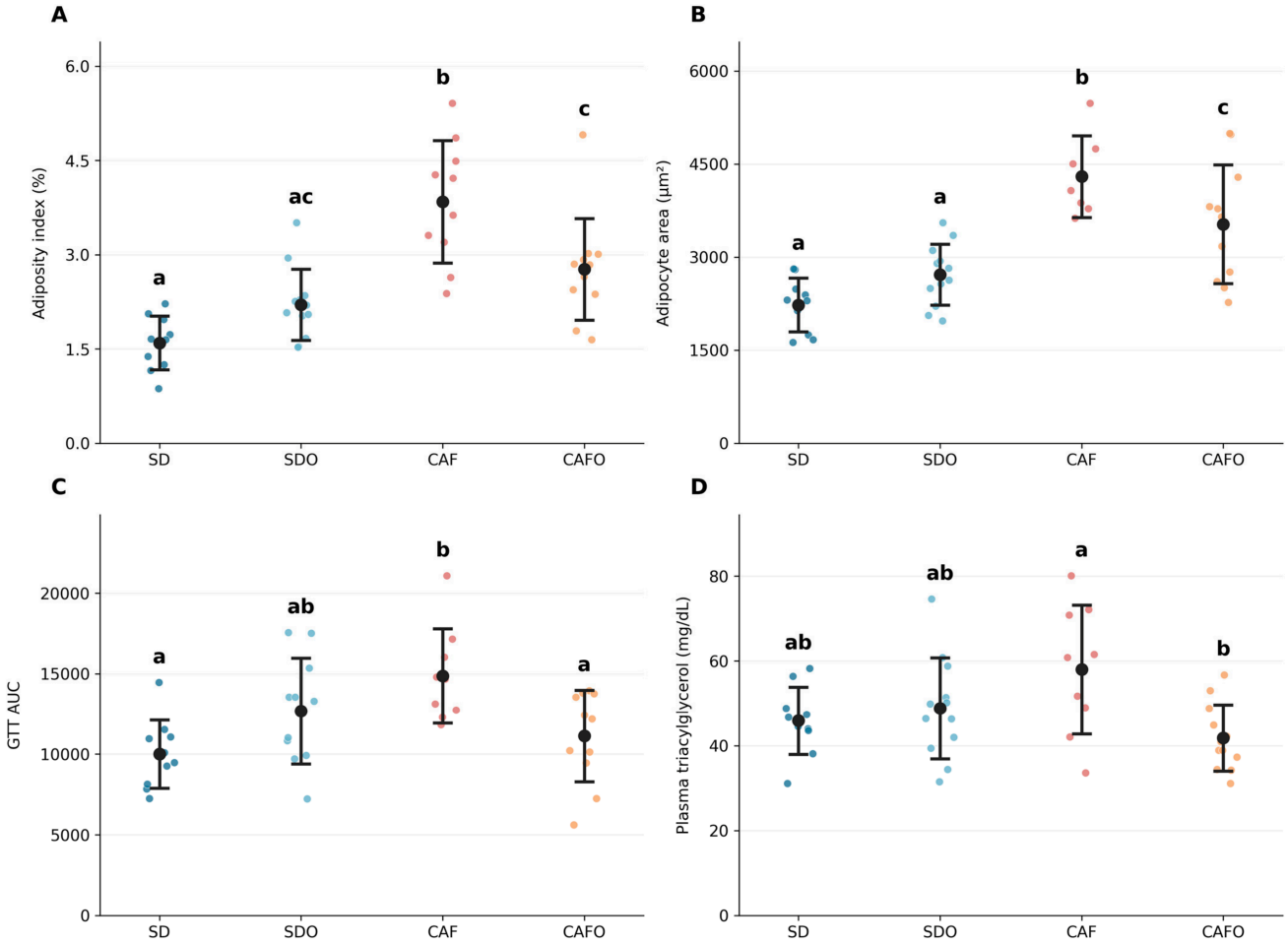

Figure 2. Effects of long-term palmitoleic acid-enriched marine oil supplementation on selected metabolic outcomes in mice fed standard or cafeteria diets. (**A**) Adiposity index, (**B**) epididymal adipocyte area, (**C**) glucose tolerance test area under the curve (AUC), and (**D**) plasma triacylglycerol concentrations. Individual observations are shown, with black symbols and error bars representing mean ± standard deviation. Groups that do not share a letter differ significantly according to the Holm–Šídák multiple-comparisons procedure ($p < 0.05$). SD, standard diet; SDO, standard diet supplemented with palmitoleic acid-enriched marine oil; CAF, cafeteria diet; CAFO, cafeteria diet supplemented with palmitoleic acid-enriched marine oil.

3.7. Epididymal Adipose Tissue Fatty-Acid Composition

Palmitoleic acid incorporation was particularly pronounced in epididymal adipose tissue, increasing from 0.545 ± 0.019% in SD and 1.457 ± 0.034% in CAF mice to 7.085 ± 0.221% in SDO and 5.764 ± 0.152% in CAFO mice. Diet ($p = 0.0054$), oil ($p < 0.0001$), and the Diet × Oil interaction ($p < 0.0001$) had significant effects, and all four groups differed significantly from one another (Table 4). The adipose tissue fatty-acid profile was more broadly remodeled, with significant Diet × Oil interactions also observed for myristic, palmitic, oleic, linoleic, and DHA ($p \leq 0.0146$). Stearic, α-linolenic, arachidonic, and EPA showed significant main effects of diet and/or oil without significant interactions (Table 4).

Table 2. Effects of palmitoleic acid-enriched oil supplementation on glucose homeostasis and lipid metabolism in mice fed standard or cafeteria diets.

Outcome	SD	CAF	SDO	CAFO	Diet *p*	Oil *p*	Diet × Oil *p*
Fasting glucose, 90 d (mg/dL)	139.7 ± 8.4 (10)	174.4 ± 28.8 (10)	156.1 ± 16.7 (12)	171.3 ± 24.1 (12)	0.0005	0.303	0.131
Fasting glucose, 120 d (mg/dL)	140.0 ± 16.9 (10)	170.9 ± 15.9 (10)	139.6 ± 13.4 (12)	154.8 ± 16.4 (12)	<0.0001	0.0897	0.107
GTT AUC	10,012.5 ± 2128.5 ^{a} (10)	14,860.8 ± 2927.2 ^{b} (9)	12,685.9 ± 3294.6 ^{ab} (11)	11,130.0 ± 2841.1 ^{a} (11)	0.121	0.625	0.0009
KITT (%/min)	1.72 ± 0.57 (10)	3.90 ± 1.02 (10)	1.80 ± 0.56 (12)	3.88 ± 1.16 (11)	<0.0001	0.892	0.858
Plasma triacylglycerol (mg/dL)	45.91 ± 7.93 ^{ab} (10)	57.99 ± 15.17 ^{a} (9)	48.83 ± 11.96 ^{ab} (12)	41.80 ± 7.82 ^{b} (12)	0.676	0.0657	0.0070
Plasma cholesterol (mg/dL)	97.72 ± 13.74 (10)	114.31 ± 14.98 (8)	97.06 ± 13.71 (12)	125.25 ± 22.95 (8)	0.0002	0.428	0.287
Hepatic triacylglycerol (mg/g tissue)	7.32 ± 4.35 (10)	11.09 ± 4.11 (10)	4.26 ± 1.70 (12)	9.45 ± 4.88 (12)	0.0004	0.0553	0.551
Hepatic cholesterol (mg/g tissue)	1.36 ± 0.51 (9)	2.66 ± 0.65 (10)	1.50 ± 0.53 (12)	2.83 ± 0.81 (12)	<0.0001	0.452	0.944

Values are presented as mean ± SD, with sample size (n) shown in parentheses. Data were analyzed by two-way ANOVA with diet (standard vs. cafeteria) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as factors. When a significant Diet × Oil interaction was detected, pairwise comparisons among experimental groups were performed using the Holm–Šídák multiple-comparisons procedure. Within each row, means without a common superscript letter differ significantly ($p < 0.05$). SD, standard diet; CAF, cafeteria diet; SDO, standard diet plus palmitoleic acid-enriched oil; CAFO, cafeteria diet plus palmitoleic acid-enriched oil; GTT, glucose tolerance test; AUC, area under the curve; KITT, glucose disappearance rate during the insulin tolerance test.

Table 3. Red blood cell fatty acid composition.

Fatty Acid (% Total FA)	SD	CAF	SDO	CAFO	Diet *p*	Oil *p*	Diet × Oil *p*
Myristic acid (14:0)	9.172 ± 0.890	5.952 ± 0.277	8.715 ± 0.369	5.170 ± 0.411	<0.0001	0.0103	0.469
Palmitic acid (16:0)	29.024 ± 0.990 ^{a}	26.676 ± 1.279 ^{a}	23.273 ± 2.377 ^{b}	26.937 ± 0.894 ^{a}	0.116	0.0005	0.0002
Palmitoleic acid (16:1n-7)	0.578 ± 0.075	0.488 ± 0.015	4.452 ± 0.367	4.030 ± 0.138	0.0046	<0.0001	0.072
Stearic acid (18:0)	8.292 ± 0.562 ^{a}	9.736 ± 0.215 ^{b}	9.010 ± 0.291 ^{ab}	8.861 ± 0.800 ^{ab}	0.0276	0.654	0.0027
Oleic acid (18:1n-9)	13.016 ± 0.336 ^{a}	21.672 ± 0.756 ^{b}	13.760 ± 1.019 ^{a}	20.887 ± 0.708 ^{b}	<0.0001	0.887	0.0272
Linoleic acid (18:2n-6)	16.188 ± 0.827 ^{a}	10.368 ± 0.419 ^{b}	15.482 ± 0.465 ^{a}	11.646 ± 0.602 ^{c}	<0.0001	0.217	0.0008
α-Linolenic acid (18:3n-3)	0.208 ± 0.031 ^{a}	0.038 ± 0.004 ^{b}	0.185 ± 0.052 ^{a}	0.070 ± 0.006 ^{b}	<0.0001	0.676	0.0446
Arachidonic acid (20:4n-6)	23.074 ± 0.361 ^{ab}	24.788 ± 0.478 ^{a}	24.585 ± 1.776 ^{a}	22.171 ± 0.979 ^{b}	0.198	0.195	0.0003
Eicosapentaenoic acid (EPA; 20:5n-3)	0.298 ± 0.052 ^{a}	0.164 ± 0.065 ^{b}	0.465 ± 0.086 ^{c}	0.101 ± 0.053 ^{b}	<0.0001	0.096	0.0005
Docosahexaenoic acid (DHA; 22:6n-3)	0.144 ± 0.017 ^{a}	0.118 ± 0.004 ^{a}	0.070 ± 0.006 ^{b}	0.123 ± 0.024 ^{a}	0.0115	<0.0001	<0.0001

Values are presented as mean ± SD. Data were analyzed by two-way ANOVA with diet (standard diet vs. cafeteria diet) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as factors. When a significant diet × oil interaction was detected, pairwise comparisons among experimental groups were performed using the Holm–Šídák multiple-comparisons procedure. Within a row, means without a common superscript letter differ significantly ($p < 0.05$). SD, standard diet; CAF, cafeteria diet; SDO, standard diet plus palmitoleic acid-enriched oil; CAFO, cafeteria diet plus palmitoleic acid-enriched oil. Sample sizes were SD, n = 5; CAF, n = 5; SDO, n = 6; and CAFO, n = 7.

Table 4. Epididymal adipose tissue fatty acid composition.

Fatty Acid (% Total FA)	SD	CAF	SDO	CAFO	Diet *p*	Oil *p*	Diet × Oil *p*
Myristic acid (14:0)	1.217 ± 0.173 [a]	0.877 ± 0.039 [b]	1.233 ± 0.047 [a]	1.108 ± 0.106 [a]	<0.0001	0.0078	0.0146
Palmitic acid (16:0)	14.770 ± 0.626 [a]	9.897 ± 0.549 [b]	14.981 ± 0.659 [a]	12.661 ± 0.361 [c]	<0.0001	<0.0001	<0.0001
Palmitoleic acid (16:1n-7)	0.545 ± 0.019 [a]	1.457 ± 0.034 [b]	7.085 ± 0.221 [c]	5.764 ± 0.152 [d]	0.0054	<0.0001	<0.0001
Stearic acid (18:0)	1.370 ± 0.168	1.126 ± 0.106	1.587 ± 0.086	1.456 ± 0.093	0.0004	<0.0001	0.227
Oleic acid (18:1n-9)	32.690 ± 1.199 [a]	70.780 ± 1.034 [b]	30.994 ± 1.111 [c]	61.554 ± 0.944 [d]	<0.0001	<0.0001	<0.0001
Linoleic acid (18:2n-6)	38.579 ± 1.461 [a]	9.274 ± 0.605 [b]	35.001 ± 1.296 [c]	11.328 ± 1.214 [d]	<0.0001	0.0687	<0.0001
α-Linolenic acid (18:3n-3)	1.339 ± 0.170	0.224 ± 0.009	1.316 ± 0.145	0.108 ± 0.010	<0.0001	0.138	0.302
Arachidonic acid (20:4n-6)	8.723 ± 0.281	5.877 ± 0.140	7.568 ± 0.208	5.322 ± 0.877	<0.0001	<0.0001	0.099
Eicosapentaenoic acid (EPA; 20:5n-3)	0.573 ± 0.103	0.208 ± 0.015	0.502 ± 0.020	0.100 ± 0.004	<0.0001	0.0003	0.387
Docosahexaenoic acid (DHA; 22:6n-3)	0.193 ± 0.016 [a]	0.083 ± 0.005 [b]	0.356 ± 0.010 [c]	0.194 ± 0.012 [a]	<0.0001	<0.0001	<0.0001

Values are presented as mean ± SD. Data were analyzed by two-way ANOVA with diet (standard diet vs. cafeteria diet) and palmitoleic acid-enriched oil supplementation (without vs. with oil) as factors. When a significant diet × oil interaction was detected, pairwise comparisons among experimental groups were performed using the Holm–Šídák multiple-comparisons procedure. Within a row, means without a common superscript letter differ significantly ($p < 0.05$). SD, standard diet; CAF, cafeteria diet; SDO, standard diet plus palmitoleic acid-enriched oil; CAFO, cafeteria diet plus palmitoleic acid-enriched oil. Sample sizes were SD, n = 7; CAF, n = 7; SDO, n = 7; and CAFO, n = 6.

4. Discussion

This study investigated whether 16 weeks of supplementation with a marine-derived palmitoleic acid-enriched oil could modify the metabolic effects of a cafeteria diet in mice. Supplementation attenuated cafeteria diet-induced adiposity and adipocyte hypertrophy, improved glucose tolerance, and reduced plasma triacylglycerol concentrations. These effects coincided with substantial enrichment of palmitoleic acid in red blood cells and epididymal adipose tissue. However, supplementation did not improve all metabolic outcomes, particularly hepatic lipid concentrations or insulin-mediated glucose disappearance assessed by KITT. Overall, the metabolic response to the palmitoleic acid-enriched oil was selective and depended on both the dietary background and the outcome examined.

Effects on adipose tissue were among the most consistent findings. As expected, cafeteria diet feeding markedly increased the adiposity index, individual white adipose tissue depots, and adipocyte area. Significant Diet × Oil interactions for several of these outcomes indicated that the response to supplementation depended on dietary background. In cafeteria diet-fed mice, the adiposity index was lower in CAFO than in CAF animals, and supplementation also attenuated the increase in adipocyte area. These differences occurred without a significant effect of oil supplementation on food or calculated food-energy intake, suggesting that reduced energy consumption was unlikely to explain the lower adiposity in CAFO mice. However, neither adiposity nor adipocyte area was fully restored to values observed in SD mice. The present data therefore support attenuation, rather than prevention, of cafeteria diet-induced adipose tissue expansion.

These findings are consistent with experimental evidence that palmitoleate can influence adipose tissue metabolism and remodeling. In high-fat diet-fed mice, Cruz et al. [5] reported that palmitoleic acid supplementation altered both lipogenesis and fatty acid oxidation in adipose tissue, while Simão et al. [6] found that palmitoleate attenuated body mass gain and prevented epididymal adipocyte hypertrophy, although it did not prevent the increase in epididymal fat mass. The latter finding is particularly relevant to the present study, as it suggests that changes in adipocyte morphology may occur without proportional changes in total adipose tissue mass. Our observation of lower adiposity and attenuated adipocyte hypertrophy in CAFO mice is therefore consistent with palmitoleate-enriched oil affecting adipose tissue expansion and remodeling.

The relationship between palmitoleate and adiposity is complex. Palmitoleate can be derived from dietary sources or synthesized endogenously from palmitate via stearoyl-CoA desaturase-1. Circulating palmitoleate can therefore reflect endogenous fatty-acid synthesis and de novo lipogenesis rather than dietary intake. Indeed, observational studies have associated circulating palmitoleate with triglyceridemia, abdominal adiposity, and other metabolic abnormalities [8,9]. These associations may appear inconsistent with experimental studies reporting favorable effects of palmitoleate supplementation. However, endogenous palmitoleate, as a marker of metabolic flux, and palmitoleate provided through dietary supplementation represent distinct biological exposures [4,11]. This distinction is particularly relevant here, as supplementation produced substantial increases in palmitoleate in both red blood cells and adipose tissue.

The glucose findings provide further evidence that supplementation modified the metabolic response to the cafeteria diet. Cafeteria diet feeding increased fasting glucose, and GTT AUC was greater in CAF than in SD mice. However, GTT AUC was lower in CAFO than in CAF mice, indicating that supplementation attenuated the impairment in glucose tolerance associated with cafeteria diet feeding. Similar effects have been reported in other experimental models. Yang et al. [2] found that oral palmitoleic acid administration attenuated hyperglycemia in KK-Ay mice, while other studies have reported favorable effects of palmitoleate on glucose homeostasis in models of diet-induced metabolic dysfunction [3].

However, the lower GTT AUC in CAFO mice should not be interpreted as evidence of improved insulin sensitivity, as oil supplementation did not significantly affect KITT. KITT was affected by diet, but neither the Oil main effect nor the Diet × Oil interaction was significant. GTT and KITT assess different aspects of glucose regulation: the GTT reflects the integrated contributions of insulin secretion, hepatic glucose production, peripheral glucose uptake, and insulin-independent glucose disposal, whereas KITT reflects the rate of glucose disappearance following exogenous insulin administration. Thus, the lower GTT AUC observed in CAFO mice may reflect changes in several components of glucose homeostasis that KITT did not capture. The present study was not designed to identify the mechanisms responsible for this response.

The effects on circulating lipids also differed by outcome. Plasma triacylglycerol concentrations showed a significant Diet × Oil interaction and were lower in CAFO than in CAF mice. This finding is consistent with the reduction in hypertriglyceridemia reported by Yang et al. [2] following chronic palmitoleic acid administration in diabetic mice. In contrast, plasma total cholesterol was affected by diet but was not significantly modified by oil supplementation. Thus, supplementation affected circulating triacylglycerol but not total cholesterol.

Cafeteria diet feeding increased hepatic triacylglycerol and cholesterol concentrations, neither of which oil supplementation significantly reduced. This differs from studies reporting reduced hepatic lipid accumulation or steatosis following palmitoleic acid supplementation in diabetic or high-fat diet-fed mice [2,5]. However, hepatic responses to palmitoleate have not been consistent across experimental models. Guo et al. [7], for example, reported improved insulin sensitivity despite increased hepatic steatosis in high-fat diet-fed mice, accompanied by increased hepatic lipogenic signaling and reduced inflammatory responses. These findings illustrate that hepatic lipid accumulation and systemic glucose regulation can respond differently to palmitoleate exposure. Accordingly, the lower GTT AUC observed in CAFO mice without a corresponding reduction in hepatic lipid concentrations is not necessarily contradictory and is consistent with the heterogeneous responses reported across experimental models.

Differences among studies may be partly explained by the experimental model, dose, formulation, and exposure duration. Many previous rodent studies used purified palmitoleic acid over relatively short supplementation periods. Yang et al. [2], for example, administered 300 mg/kg/day for four weeks, while similar doses have been used in other high-fat diet models [3,5,6]. In the present study, animals received 1 g/kg/day of a marine oil containing approximately 558 mg/g of palmitoleic acid ethyl ester, corresponding to approximately 558 mg/kg/day of the target fatty acid ester, throughout the 16-week dietary intervention. The cafeteria diet also represents a different nutritional challenge from the purified high-fat diets commonly used in previous studies, with differences in macronutrient composition, fatty-acid profile, and energy density. These differences in intervention and dietary context may contribute to the heterogeneous metabolic responses reported across studies. Another distinction is that supplementation began concurrently with the cafeteria diet and continued throughout the 16-week intervention. This design allowed evaluation of whether supplementation modified the development of diet-associated metabolic alterations during prolonged dietary exposure, rather than whether it reversed an established metabolic phenotype. The duration of the intervention is therefore relevant to interpreting the observed differences in adiposity, glucose tolerance, and circulating triacylglycerol concentrations.

Fatty-acid profiling provided direct evidence of substantial palmitoleate incorporation following supplementation. In red blood cells, palmitoleate increased from less than 1% of measured fatty acids in unsupplemented mice to approximately 4% in supplemented ani-

mals. In epididymal adipose tissue, the increase was even greater, reaching approximately 6–7% in supplemented groups. These findings demonstrate that chronic administration of the palmitoleic acid-enriched oil substantially increased palmitoleate content in both red blood cells and adipose tissue.

Adipose tissue enrichment is particularly relevant because this tissue is both a site of endogenous palmitoleate production and a potential target of its biological actions [1,4]. The concurrent increase in adipose tissue palmitoleate and attenuation of adipocyte hypertrophy in cafeteria diet-fed mice is consistent with a relationship between palmitoleate exposure and adipose tissue remodeling. However, these findings do not establish that increased tissue palmitoleate directly caused reduced adipocyte size. The relationship should therefore be considered associative, as the molecular pathways linking fatty-acid incorporation to adipocyte morphology were not examined.

The effects of supplementation on tissue fatty-acid composition were not limited to palmitoleate. Multiple fatty acids in red blood cells and adipose tissue were affected by diet, oil supplementation, or their interaction. Given the substantial differences in fatty-acid composition between the standard and cafeteria diets, the resulting tissue profiles likely reflect the combined influence of background diet, the supplemented oil, and endogenous fatty-acid metabolism.

The intervention composition is also important when interpreting these findings. The marine-derived product contained approximately 558 mg/g of palmitoleic acid ethyl ester and 257 mg/g of palmitic acid ethyl ester. The intervention should therefore be considered a palmitoleic acid-enriched oil rather than purified palmitoleic acid, and contributions from other oil components to the observed metabolic effects cannot be excluded. This distinction is particularly important when comparing the present findings with studies using purified palmitoleate.

Human intervention studies provide important context for interpreting the metabolic effects observed in the present experiment. In a 12-week randomized, double-blind, placebo-controlled trial involving 123 adults, Bridges et al. [10] investigated supplementation with 500 or 1000 mg/day of marine-derived palmitoleic acid. Although supplementation increased circulating and red blood cell palmitoleate concentrations, it did not significantly improve fasting glucose, insulin, HbA1c, or plasma lipid concentrations, including triacylglycerols. Similarly, Huang et al. [12] investigated sea buckthorn oils containing cis- and trans-palmitoleate in a randomized crossover dose-escalation study involving 13 adults. Supplementation increased the concentrations of the corresponding palmitoleate isomers in circulating phospholipids but did not significantly affect fasting glucose, insulin, or plasma lipid concentrations. However, this study primarily assessed fatty-acid incorporation and was not powered to detect changes in metabolic outcomes.

These findings contrast with the reductions in adiposity and plasma triacylglycerol concentrations and the improvement in glucose tolerance observed in the present study. Nevertheless, several differences limit direct comparisons between experimental and human findings. The present experiment involved mice exposed to a cafeteria diet, with supplementation initiated concurrently with dietary exposure, whereas the human trials investigated adults without experimentally induced metabolic dysfunction. Furthermore, the dose administered to mice was substantially higher relative to body mass, and differences in oil composition, baseline metabolic status, and intervention duration may influence the observed responses. Importantly, the improvement in glucose tolerance in our experiment was not accompanied by improved insulin-mediated glucose disappearance assessed by KITT, indicating selective metabolic effects. Collectively, the available human intervention evidence does not establish consistent improvements in glucose regulation or lipid

metabolism following palmitoleate supplementation, and further clinical studies are needed to determine whether the effects observed in experimental models translate to humans.

Most previous mouse studies have evaluated purified palmitoleate in high-fat diets or genetic models of obesity and diabetes [2,3,5–7]. In contrast, the present study administered a palmitoleic acid-enriched marine oil throughout a 16-week cafeteria diet intervention. The 2 × 2 factorial design allowed the effects of supplementation to be evaluated under both standard and obesogenic dietary conditions. This distinction was important because several key outcomes, including adiposity, adipocyte area, glucose tolerance, and plasma triacylglycerol, showed Diet × Oil interactions rather than uniform responses to supplementation.

Several limitations should be considered. Only male mice were studied, limiting extrapolation across sex. The intervention used a palmitoleic acid-enriched oil rather than purified palmitoleic acid, and the unsupplemented groups received water rather than a matched control oil; therefore, the observed effects cannot be attributed specifically to palmitoleate, and contributions from other oil components cannot be excluded. The dose was also relatively high compared with exposures achievable through usual human diets or supplementation, limiting direct translation of the findings. Animals were group-housed, with diet allocation and food intake assessed at the cage level, whereas the oil was administered individually. The experiment was conducted in two experimental runs to achieve the final sample size. However, detailed information linking individual animals to cages within each run was not available for the present analysis, precluding statistical adjustment for potential cage or run effects. The study was designed to characterize phenotypic and metabolic responses rather than identify the molecular mechanisms underlying the observed effects. We did not perform gene-expression analyses or measure proteins or enzymatic activities involved in adipose tissue metabolism and glucose regulation. Consequently, the present findings cannot establish which molecular pathways contributed to the attenuation of adiposity, adipocyte hypertrophy, or glucose intolerance. Although the substantial incorporation of palmitoleate into red blood cells and adipose tissue confirms changes in tissue fatty-acid composition following supplementation, it does not establish a causal relationship between fatty-acid incorporation and the observed metabolic outcomes. Future studies incorporating molecular and functional measurements are needed to investigate these mechanisms. Finally, sample sizes differed among some outcomes because of sample availability and assay-specific exclusions, particularly for fatty-acid analyses. These limitations should be considered when interpreting the magnitude and generalizability of the observed effects.

5. Conclusions

In conclusion, chronic supplementation with a marine-derived palmitoleic acid-enriched oil substantially altered red blood cell and adipose tissue fatty-acid composition and selectively attenuated several metabolic effects of cafeteria diet feeding in mice. Supplementation was associated with lower adiposity and adipocyte hypertrophy, improved glucose tolerance, and lower plasma triacylglycerol concentrations in cafeteria diet-fed animals, whereas hepatic lipid concentrations and KITT were not improved. These findings extend previous work with purified palmitoleate and high-fat diet models by showing that long-term supplementation with a palmitoleic acid-enriched marine oil can modify selected metabolic responses during cafeteria diet feeding while substantially incorporating palmitoleate into circulating cells and adipose tissue. Together with emerging human evidence, the findings suggest that tissue incorporation of dietary palmitoleate does not necessarily translate into uniform metabolic effects, which appear to depend on dietary context, formulation, dose, and metabolic state.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app162110598/s1, Figure S1: Representative hematoxylin and eosin (H&E)-stained histological images of epididymal adipose tissue (EAT) from mice assigned to the four experimental groups: standard diet (SD), cafeteria diet (CAF), standard diet supplemented with palmitoleic acid-enriched marine oil (SDO), and cafeteria diet supplemented with palmitoleic acid-enriched marine oil (CAFO). Scale bar = 50 μm. Table S1: Fatty-acid composition of the standard and cafeteria diets.

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Abbreviations

The following abbreviations are used in this manuscript:

AUC	Area under the curve
CAF	Cafeteria diet
CAFO	Cafeteria diet plus palmitoleic acid-enriched oil
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GTT	Glucose tolerance test
ipGTT	Intraperitoneal glucose tolerance test
KITT	Glucose disappearance rate during the insulin tolerance test
SCD1	Stearoyl-CoA desaturase-1
SD	Standard diet
SDO	Standard diet plus palmitoleic acid-enriched oil

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