Differential Immunometabolic Effects of High-Fat Diets Containing Coconut, Sunflower, and Extra Virgin Olive Oils in Female Mice

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Scope: To compare the effects of three high-fat diets (HFDs) based on coconut, sunflower, or extra virgin olive oils (EVOOs) on adipose tissue, metabolism, and inflammation.
Methods and Results: Mice are fed for 16 weeks on their respective HFD. HFD based on coconut oil produces significantly lower body weight than EVOO- or sunflower oil-based HFDs. Furthermore, the coconut oil HFD leads to metabolic disturbances such as reduction of circulating leptin and adiponectin concentrations, hypertriglyceridemia, hepatomegaly, and liver triglyceride accumulation. Likewise, this diet produces an increase in serum pro-inflammatory cytokines (interleukin 6 [IL-6] and tumor necrosis factor-α [TNF-α]). In white (WAT) and brown (BAT) adipose tissue, the HFD based on coconut oil does not cause significant changes in the expression of studied proteins related to thermogenesis (uncoupling protein 1 [UCP-1]), mitochondrial biogenesis, and browning (peroxisome proliferator-activated receptor-γ coactivator 1α [PGC-1α] and nuclear factor E2-related factor 2 [Nrf2]). However, the HFD based on EVOO induces upregulation of UCP-1, PGC-1α, and Nrf2 expression in BAT, increases the expression of UCP-1 and PGC-1α in inguinal WAT, and enhances the expression of PGC-1α in epididymal WAT.
Conclusions: An HFD based on coconut oil could reduce circulating leptin and adiponectin concentrations, increase the liver fat content, raise serum triglycerides, and promote inflammation by increasing circulating pro-inflammatory cytokines, while an EVOO-based HFD could increase thermogenic activity.

1. Introduction

Nowadays, non-communicable diseases (NCDs) such as cancer, diabetes, and cardiovascular disease represent over 70% of deaths worldwide.[1] Because of its endocrine and immunomodulatory activity, adipose tissue is a central element in the development of several NCDs.[2–4] In fact, a major risk factor for NCDs is the abnormal or excessive accumulation of body fat characteristic of overweight or obesity,[5] whose prevalence has increased markedly due to the global nutritional transition.[6] This is due in part to increased availability, commercialization, low prices, and higher consumption of certain products that have contributed to the adoption of Western diets, which are characterized by high intakes of calories, fats, saturated fats, and sugars.[7,8] The transition to diets high in fats is usually marked by a rapid expansion of the consumption of edible oils.[9,10] The most widely consumed vegetable oils worldwide are olive oil in the Mediterranean region, palm and coconut oils in Asia, soybean oil in America, and sunflower oil, which is predominantly consumed in North Africa and Eastern Europe.[8,11,12]

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Historically, dietary fats and edible oils have given rise to a controversial debate about the optimal types and amounts used in the diet, their role in regulating body weight, and their importance in the etiology of NCDs. However, the source and quality of dietary fat has been recognized as a more important factor in the prevention of some NCDs than the total amount of fat.[13–15] The main difference between edible oils intake around the world is its fatty acids composition, being monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs) in the case of olive, sunflower, and coconut oils, respectively.[16,17] Currently, new nutritional trends promote coconut oil intake as the healthiest vegetable fat source. Among other properties, they assume that it has a positive impact on cardiovascular health, helps to maintain an adequate weight, or even improves the lipid profile in the blood. One of its properties that has become popular is that of its supposed thermogenic effect, which would help in weight loss.[13,18] Non-scientific articles have had a decisive influence in creating this state of opinion.

Although the impact of some oils on health has been widely analyzed, the mechanisms underlying the effects of edible vegetable oils on abnormal or excessive body fat accumulation as well as their metabolic and immunological impact remain unclear. To clarify this situation, it is important to obtain scientific evidence on the real impact of coconut oil on health. At the same time, it is important to compare these results with those obtained by other regularly consumed edible fats, such as extra virgin olive oil (EVOO) and sunflower oil.

2. Experimental Section

2.1. Materials

The following were purchased from ENVIGO (Wisconsin, WI, USA): Teklad Global 14% protein (chow diet with 13% kcal from fat, Ref. 2014S), Teklad Global 19% protein extruded rodent diet (intermediate fat diet with 22% kcal from fat, Ref. 2019S), and Teklad Custom diet (TD.170709). Ethylenediaminetetraacetic Acid (EDTA)-coated tubes and spray-coated silica tubes were obtained from BD (New Jersey, NJ, USA). Mouse Leptin enzyme-linked immunosorbent assay (ELISA) kit (Ref. RAB1111), Mouse Adiponectin ELISA kit (Ref. RAB1115), phosphate buffered saline (PBS; Ref. P4417), CellLytic MT (Ref. C3228), protease inhibitor cocktail (Ref. P8340), antifoam Y-30 (Ref. A5758), sodium dodecyl sulfate (SDS) (Ref. L3771), tetramethylethylenediamine (TEMED) (Ref. T9281), and ammonium persulfate (APS) (Ref. A3678) were from Sigma–Aldrich (Saint Louis, MO, USA). Mouse interleukin 6 (IL-6) kit (Ref. ADI-900-045), mouse tumor necrosis factor-α (TNF-α, Ref. ADI-900-047), and mouse transforming growth factor-β (TGF-β1, Ref. ADI-900-155) were obtained from ENZO Life Sciences (Barcelona, Spain). Triglyceride assay kit was purchased from Abcam (Ref. ab65336, ON, Canada). The following were purchased from Applichem Panreac (Barcelona, Spain): Bradford protein assay (Ref. A6932,0500), Tris base (Ref. A2264,1000), and NaCl (Ref. 241659.1608). M-Tubes were obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Non-fat dry milk powder was purchased from Central Lechera Asturiana (Asturias, Spain). poly(vinylidene fluoride) (PVDF) membranes (Ref. 88518) were obtained from Invitrogen (Carlsbad, CA, USA). Microcentrifuge tubes were purchased from VWR (Barcelona, Spain). Acrylamide (Ref. #1610156) was obtained from Bio-Rad (Madrid, Spain). Anti-peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α, D-5; Ref. sc-518025), anti-uncoupling protein 1 (UCP-1, 4E5; Ref. sc-293418), anti-glyceraldehyde-3-phosphate dehydrogenase (GADPH) (FL-335; Ref. sc-25778), and mouse m-IgGκ BP-horseradish peroxidase (HRP) (Ref. sc-516102) were from Santa Cruz Biotechnology (Dallas, TX, USA). The following were obtained from Merck Millipore (Burlington, MA, USA): anti-nuclear factor E2-related factor 2 (Nrf2) antibody (clone 103; Ref. MABE1799) and goat anti-Rat IgG antibody, HRP conjugate (Ref. AP136P).

2.2. Murine Experimental Model

Female CD1 mice were obtained from Charles River Laboratories (Barcelona, Spain). Four-week-old mice ($n = 44$) were housed in ventilated racks and cages (5–6 per cage) with environmental control (humidity: 55%–65%; temperature: 20 $\pm$ 2 °C; 12:12-h light–dark cycle). The trial was carried out at the Animal Production and Experimentation Centre of the University of Jaén (code ES230500000020).

Animal care and experiments were conducted following the guidelines of the Spanish Society for Laboratory Animal Science. The experimental procedures applied to these animals were approved by the Ethics Committee of the University of Jaén (Record number: CEEA-100217-1) and the Ethics Committee of Animal Experiments of the Regional Ministry of Agriculture, Fishing and Environment of the Regional Government of Andalusia, Spain (Approval number: 16/03/2017/044).

2.3. Diets

Mice were fed with a maintenance chow diet (defined as chow diet), with 13% kcal from fat, an intermediate fat diet with 22% kcal from fat, and a custom ready-to-use (fat-free) base for high-fat diets (HFDs) with 60% kcal from fat. The custom fat-free base was created exclusively by ENVIGO for this study (TD.170709). To prepare each of the HFDs, one of the following edible oils were added to the custom base: sunflower oil, coconut oil, or EVOO from the picual olive variety. The HFDs were made (as pellets) and administered daily under sterile conditions. The composition of the HFDs is provided in **Tables 1** and **2**.

2.4. Experimental Design

Upon arrival, the mice were divided randomly into four groups ($n = 11$) and assigned to the different diets:

- Group 1: Chow diet (chow)
- Group 2: HFD with coconut oil (coconut-HFD)
- Group 3: HFD with sunflower oil (sunflower-HFD)
- Group 4: HFD with EVOO (EVOO-HFD)

Before the start of the dietary intervention, mice were maintained in an acclimation phase for 3 weeks. In the first week, all mice were fed with a chow diet, followed by 2 weeks with an

Table 1. Composition of experimental high fat diets.

	Coconut	Sunflower	EVOO
Diet component	g kg^{-1}		
Custom diet mix[a]	666.6	666.6	666.6
Coconut oil	333.4	–	–
Sunflower oil	–	333.4	–
Extra virgin olive oil	–	–	333.4
% kcal			
Protein	18	18	18
Carbohydrate	22	22	22
Fat	60	60	60
Fatty acid composition	g per 100 g total fatty acids		
Σ SFA[b]	90.4	7.7	16.04
Σ MUFA	7.2	39.7	78.54
Σ PUFA	1.9	52.7	4.46
Fatty acids			
Caprylic acid (C8:0)	5.8	–	–
Capric acid (C10:0)	6.2	–	–
Lauric acid (C12:0)	46.2	–	–
Myristic acid (C14:0)	19.3	–	0.01
Palmitic acid (C16:0)	10	5.1	13.61
Palmitoleic acid (C16:1)	–	0.1	1.21
Stearic acid (C18:0)	2.9	2.6	2.42
Oleic acid (C18:1)	7.2	39.6	77.33
Linoleic acid (C18:2ω6)	1.9	52.6	3.64
α-linolenic acid (C18:3ω3)	–	0.1	0.82

EVOO, extra virgin olive oil; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. [a] Custom diet mix supplied and formulated by ENVIGO. [b] SFAs.

Table 2. Minority compounds found in extra virgin olive oil (EVOO).

Minority compounds	
	mg kg^{-1}
Squalene	9701
Total tocopherols	378.2
α-Tocopherol	369.1
β-Tocopherol	3.6
γ-Tocopherol	5.6
δ-Tocopherol	<1.0
Tyrosol	2.5
	mg kg^{-1} tyrosol
Total biophenols	265.1
Hydroxytyrosol	0.6
Oleocanthal	34
	mg caffeic acid per kg
Total polyphenols	372

intermediate diet for acclimation to HFD transition, except for the chow group, which continued with the chow diet throughout all the assays/interventions. Once the intervention phase started, mice were fed either a chow diet, or one of the EVOO-, coconut-, or sunflower-HFDs with ad libitum access to water and food for 16 weeks (**Figure 1**). Food was monitored indirectly through daily monitoring of intake.

2.5. Measurement of Body Weight

Mice were weighed individually at the start of the intervention phase and weekly until the end of the study, using an analytical electronic balance with 0.01 g precision.

2.6. Sample Collection

For plasma determination, a pool of blood from the submandibular veins of all mice in the same group was collected monthly in EDTA-coated tubes. Blood was quickly centrifuged at 1600g at 4 °C for 15 min and the supernatants stored at −80 °C until further analysis.

For serum determination, blood was collected in spray-coated silica tubes as a pool of all mice in the same group by puncture of

the aorta in the thoracic cavity at the moment of sacrifice. Blood was quickly centrifuged at 1600g at 4°C for 15 min and supernatants stored at −80 °C until further analysis.

At 16 weeks of intervention, mice were sacrificed using a euthanasia mixture of ketamine (160 mg kg^{-1}) and xylazine (10 mg kg^{-1}). Liver, epididymal WAT (gonadal region; eWAT), inguinal WAT (subcutaneous region; iWAT), and interscapular BAT were surgically removed individually from each mouse. All samples were weighed on an analytical electronic balance with 0.001 g precision, individually dissected, immediately frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.7. Metabolic and Inflammatory Markers

2.7.1. Plasma

Leptin and adiponectin were determined in each intervention group using a mouse leptin ELISA kit and mouse adiponectin ELISA kit, respectively. Data were measured at 450 nm with a Tecan GENios Plus microplate reader (Tecan Group Ltd, Zürich, Switzerland). All assays were performed according to the manufacturers' protocols, and each sample was assayed in triplicate.

2.7.2. Serum

IL-6, TNF-α, and TGF-β were determined in the serum of each intervention group. IL-6 levels were estimated using the mouse IL-6 ELISA kit, TNF-α levels by the mouse TNF-α ELISA kit, and TGF-β levels by using the mouse TGF-β ELISA kit. Triglyceride levels were quantified using the triglyceride assay kit. Data were obtained by absorbance measurement at 450 nm with a microplate reader (Tecan GENios Plus) except in the triglyceride assay, where fluorescence was measured at $\lambda Ex/Em = 535/587$ nm. Each sample was assayed in quadruplicate.
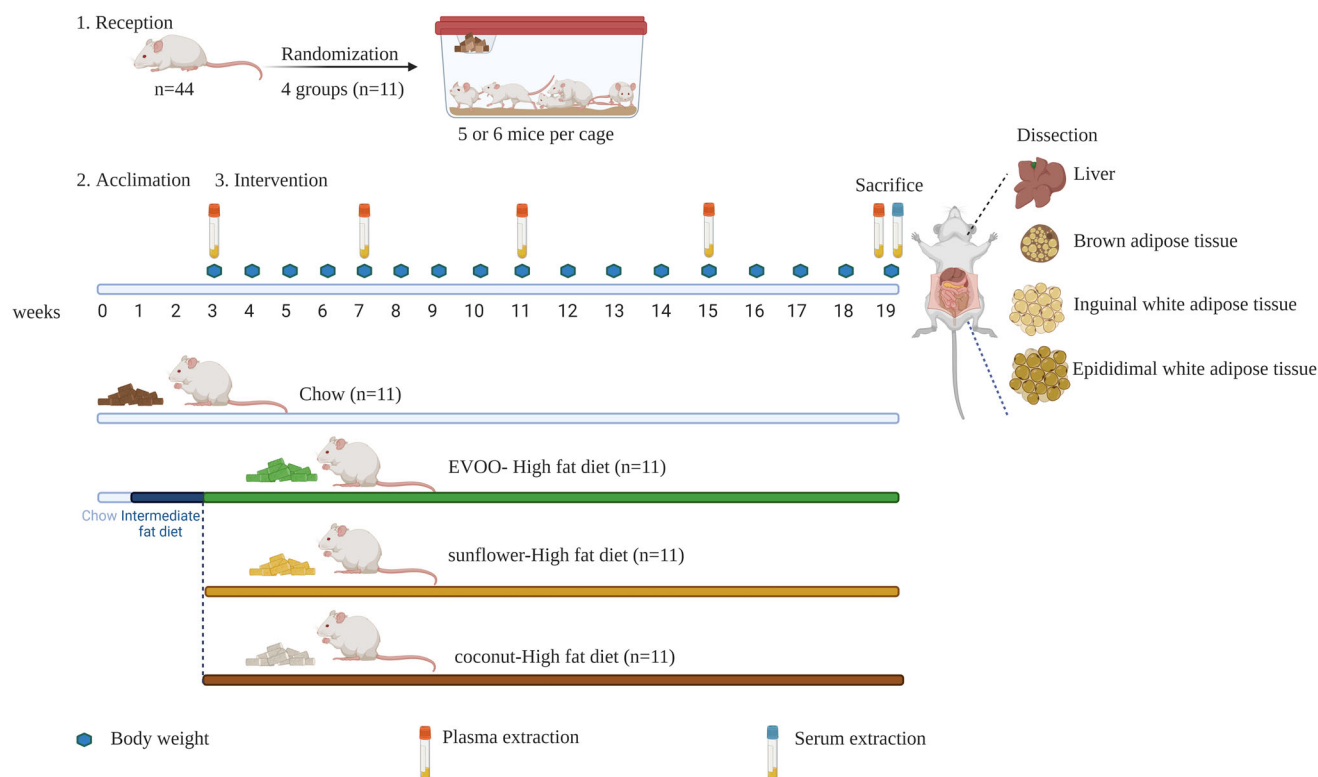
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Figure 1. Experimental design.

2.8. Hepatic Triglycerides

A hepatic pool was made from each group after defrosting tissues (100 mg of each mouse liver). Livers were washed with cold PBS, re-suspended in 1% Tween 20, and dissociated in a gentleMACS dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany). Samples were immersed in a water bath at 80–100 °C for 5 min and the previous steps repeated until all tissues were homogenized. Samples were collected and centrifuged for 2 min at 13 200g using a microcentrifuge (Eppendorf, Hamburg, Germany). Supernatants were quantified using a triglyceride assay kit. Fluorescence was measured at $\lambda Ex/Em = 535/587$ nm with Tecan GENios Plus. All samples were assayed in quadruplicate.

2.9. Western Blotting

Prior to sample processing, the eWAT, iWAT, and BAT of each mouse were removed from storage at −80 °C and kept immediately on ice. A lysis buffer (CellLytic MT, protease inhibitor cocktail and antifoam Y-30) was used with all adipose tissues for homogenization in a gentleMACS dissociator. Then, all tissues were centrifuged at 13 200g for 10 min at 4 °C. Finally, supernatants were collected and the proteins stored at −80 °C until further analysis.

Protein (25 ng) was separated by electrophoresis through 12% acrylamide gels. Proteins were transferred to PVDF membranes and incubated for 1 h with blocking buffer (1× Tris-buffered saline with 0.1% Tween (TBS-T) with 5% non-fat dry milk powder w/v). After blocking, membranes were washed three times with TBS-T and incubated with one of the following primary antibodies O/N at 4 °C: anti-PGC-1α (D-5; Ref. sc-518025), anti-UCP-1 (4E5; Ref. sc-293418), anti-GADPH (FL-335; Ref. sc-25778), or anti-Nrf2 (clone 103; Ref. MABE1799), diluted in 1× TBS-T containing 1% non-fat dry milk powder w/v. Membranes were washed three times with TBS-T and incubated with the appropriate HRP-conjugated secondary antibodies against mouse (m-IgGκ BP-HRP, sc-516102) or rabbit (Gt X Rat IgG HRP, AP136P) at room temperature for 1 h. Finally, membranes were washed in 1× TBS-T three times for 5 min. Immunoblots were analyzed with ChemiDoc XRS+ Imaging System (Bio-Rad, Madrid, Spain), and protein levels were quantified using Image Lab Software (Bio-Rad Imaging Systems, Madrid, Spain).

2.10. Statistical Analysis

All results, unless otherwise specified, were reported as means of at least three independent experiments (±SEM). Some results were expressed as a percentage relative to the chow group, which was set as 100%. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's LSD test. Differences between groups were considered significant at p-values less than or equal to 0.05, 0.01, or 0.001. Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). ANOVA of
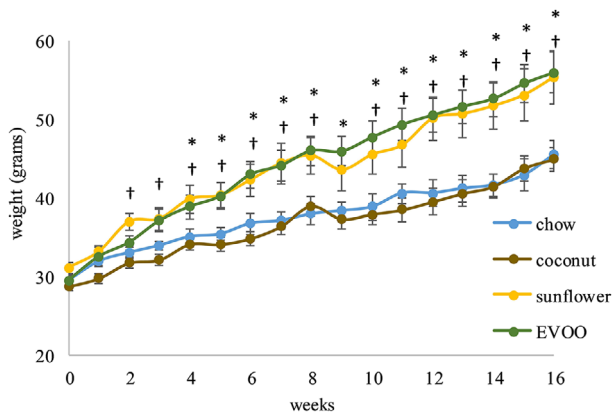
Figure 2. Bodyweights of chow- and HFD-fed groups ($n = 44$). Data are represented as mean $\pm$ SEM of the weekly weights of mice ($n = 11$). For statistical analysis, two-way ANOVA, followed by Fisher's LSD test were performed; $*p < 0.05$ for EVOO-HFD, and $\dagger p < 0.05$ for sunflower-HFD versus chow. ANOVA, analysis of variance; EVOO, extra virgin olive oil; HFD, high-fat diet; LSD, least significant difference; SEM, standard error of the mean.

eWAT, iWAT, or BAT weights versus bodyweight was carried out with Stata version 12.0 (Stata Corporation, College Station, TX, USA).

3. Results

3.1. Coconut-HFD Did Not Promote Weight Gain

Mice fed sunflower-HFD or EVOO-HFD showed significant weight gain compared to those fed chow diet (**Figure 2**). In the case of mice fed sunflower-HFD, the differences became significant from the second week of the nutritional intervention and were maintained until the end of the experiment (**Table 3**). In mice fed EVOO-HFD, the differences were significant from the fourth week onwards and remained so until the end of the trial. However, mice fed coconut-HFD maintained an even lower weight than those fed chow diet, although there were no statistically significant differences.

3.2. Coconut-HFD Reduced Leptin and Adiponectin Plasma Levels

In chow-fed mice, there was an increase in leptin and adiponectin levels as body weight increased (**Figure 3**a). However, at the end of the intervention, mice fed sunflower-HFD or EVOO-HFD showed a reduction in circulating adiponectin concentrations and an increase in leptin concentrations (Figure 3c, d) while mice fed coconut-HFD developed a reduction in both circulating leptin and adiponectin concentrations (Figure 3b, **Table 4**).

3.3. Consumption of EVOO-, Sunflower-, and Coconut-HFD Led to Different Alterations in Adiposity

To study the effect of each of the diets administered on adipose tissue, three different types of indices were calculated as follows: (fat weight/body weight) $\times$ 100, where "fat" was replaced

Table 3. Body weight (g).

Weeks	Chow	Coconut-HFD	Sunflower-HFD	EVOO-HFD
0	29.49 $\pm$ 0.37	28.65 $\pm$ 0.48	31.05 $\pm$ 0.77	29.43 $\pm$ 0.76
1	31.9 $\pm$ 0.49	29.71 $\pm$ 0.59	33.09 $\pm$ 0.81	32.45 $\pm$ 1.19
2	33.01 $\pm$ 0.76	31.67 $\pm$ 0.69	36.9 $\pm$ 1.05**	34.26 $\pm$ 0.87
3	33.92 $\pm$ 0.53	32.07 $\pm$ 0.65	37.26 $\pm$ 1.48*	37.03 $\pm$ 1.41
4	35.06 $\pm$ 1.00	34.05 $\pm$ 0.77	39.77 $\pm$ 1.76*	38.81 $\pm$ 1.48*
5	35.36 $\pm$ 0.83	34.04 $\pm$ 0.83	40.24 $\pm$ 1.61*	40.14 $\pm$ 1.69*
6	36.71 $\pm$ 1.33	34.78 $\pm$ 0.94	42.21 $\pm$ 2.01*	42.89 $\pm$ 1.70**
7	37.06 $\pm$ 1.08	36.32 $\pm$ 0.98	44.31 $\pm$ 2.52**	44.05 $\pm$ 2.02**
8	37.91 $\pm$ 1.40	38.9 $\pm$ 1.20	45.3 $\pm$ 2.32**	45.93 $\pm$ 1.90**
9	38.32 $\pm$ 1.08	37.27 $\pm$ 1.31	43.51 $\pm$ 2.62	45.85 $\pm$ 1.86**
10	38.8 $\pm$ 1.68	37.87 $\pm$ 1.28	45.42 $\pm$ 2.44*	47.65 $\pm$ 2.16**
11	40.55 $\pm$ 1.25	38.43 $\pm$ 1.58	46.65 $\pm$ 2.78*	49.28 $\pm$ 2.07**
12	40.56 $\pm$ 1.72	39.45 $\pm$ 1.72	50.05 $\pm$ 2.76**	50.47 $\pm$ 2.13**
13	41.14 $\pm$ 1.73	40.52 $\pm$ 1.69	50.63 $\pm$ 3.05**	51.59 $\pm$ 2.17**
14	41.57 $\pm$ 1.45	41.41 $\pm$ 1.53	51.64 $\pm$ 2.88**	52.57 $\pm$ 2.23**
15	42.83 $\pm$ 2.06	43.7 $\pm$ 1.55	53.02 $\pm$ 3.26**	54.49 $\pm$ 2.48**
16	45.48 $\pm$ 1.85	44.88 $\pm$ 1.48	53.31 $\pm$ 3.34**	55.9 $\pm$ 2.65**

Data are represented as mean $\pm$ SEM of $n = 11$ mice for each group. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed; $*p < 0.05$ and $**p < 0.01$ for groups versus chow. ANOVA, analysis of variance; EVOO, extra virgin olive oil; HFD, high-fat diet; LSD, least significant difference; SEM, standard error of the mean.

by iWAT, eWAT, or BAT (**Figure 4**). Mice fed sunflower oil-HFD showed a significant increase in the relative weight of iWAT ($p = 0.018$). Furthermore, sunflower-HFD and EVOO-HFD produced a relative increase in eWAT ($p < 0.01$). None of the diets administered altered BAT weight. On the other hand, coconut-HFD had no impact on any of the adipose tissues studied.

3.4. Coconut-HFD Appeared to Promote Low-Grade Systemic Inflammation, While Sunflower-HFD and EVOO-HFD May Modulate the Inflammatory Process

To assess inflammatory status, serum levels of two pro-inflammatory cytokines (IL-6 and TNF-α) and one anti-inflammatory cytokine (TGF-β) were determined at the end of the dietary intervention (**Figure 5**). Coconut-HFD produced a statistically significant increase in serum levels of IL-6 and TNF-α and a minor increase in TGF-β. EVOO-HFD produced a moderate increase in IL-6, no change in serum TNF-α levels, and a very significant increase in TGF-β levels. Sunflower-HFD induced a moderate decrease in IL-6 levels, no change in plasma TNF-α levels, and a moderate increase in TGF-β.

3.5. Coconut-HFD Promoted Hypertriglyceridemia

Determination of serum triglyceride levels showed that coconut-HFD significantly increased triglyceride values ($p < 0.001$). On the other hand, EVOO-HFD increased triglycerides very slightly, while sunflower-HFD had no effect (**Figure 6**).
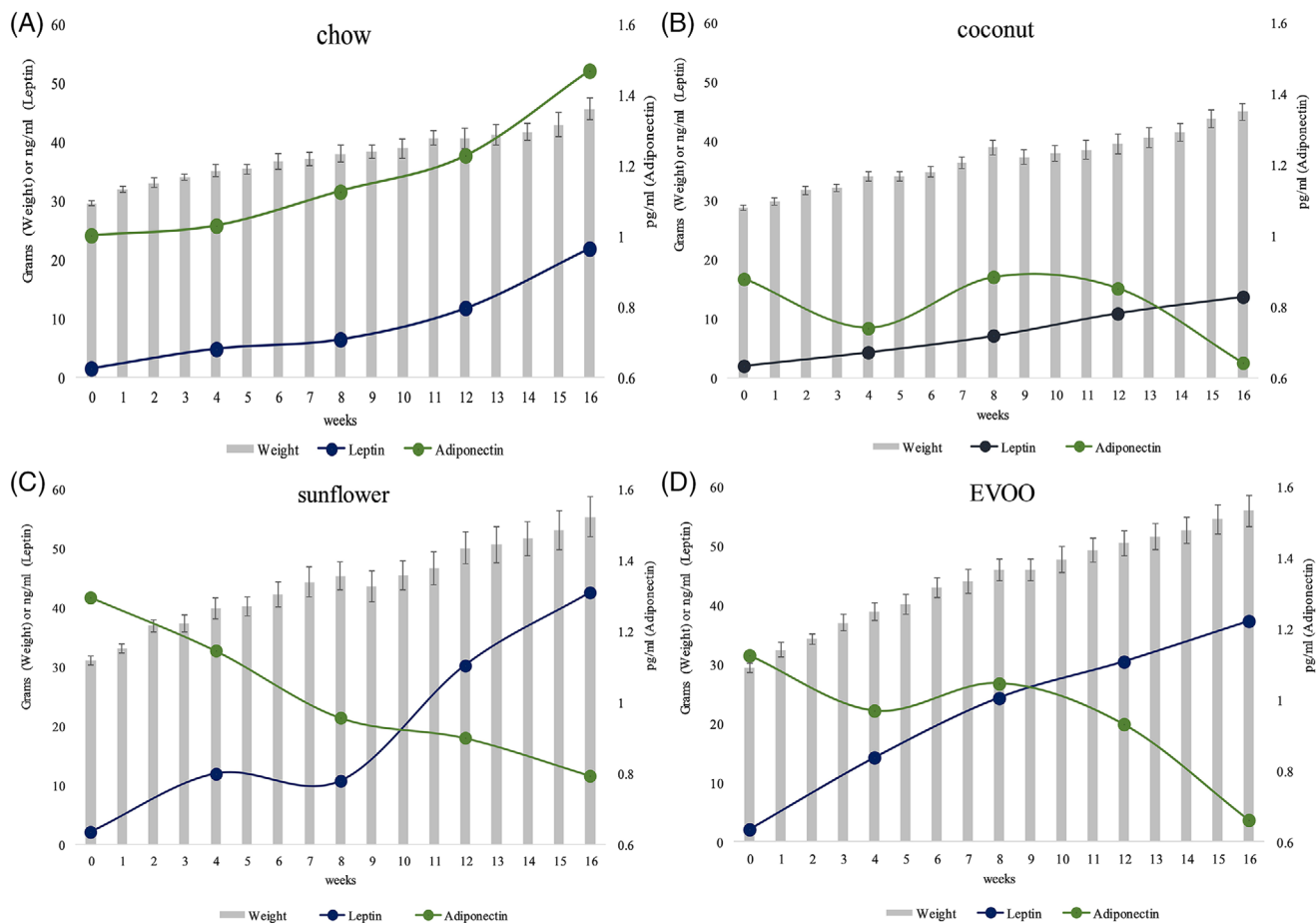
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Figure 3. Evolution of weight, leptin, and adiponectin. Data are represented as mean ± SEM of body weight (g) and triplicate replies of pooled serum ($n = 11$) for each group obtained in ELISA. Leptin and adiponectin data of HFD groups are expressed relative to that of the chow-fed group, which was established as 1. a) Chow, b) coconut-HFD, c) sunflower-HFD, and d) EVOO-HFD. ELISA, enzyme-linked immunosorbent assay; EVOO, extra virgin olive oil; HFD, high-fat diet; SEM, standard error of the mean.

3.6. Coconut-HFD Promoted Hepatomegaly and Liver Triglyceride Accumulation

As **Figures 7** and **8** show, coconut-HFD produced a very significant increase in liver weight relative to body weight ($p < 0.001$) as well as an increase in the quantity of triglycerides stored in the liver ($p < 0.05$). Therefore, this diet could lead to hepatomegaly and hepatic steatosis. Sunflower-HFD did not change the relative liver weight compared to the chow diet but did induce an increase in liver triglycerides ($p < 0.05$). Finally, EVOO-HFD produced a moderate increase in relative liver weight ($p < 0.05$), although there was no increase in hepatic triglycerides.

3.7. EVOO-HFD Increased UCP-1 Expression in BAT and iWAT

UCP-1 is the protein responsible for thermogenesis in adipocytes. Determination of UCP-1 expression by western blotting showed that EVOO-HFD produced a significant increase in its expression in both iWAT and BAT but not in eWAT (**Figure 9**.1a, 2a, 3a). Sunflower-HFD produced an increase in UCP-1 expression only in BAT, although to a lesser extent than

EVOO-HFD. In contrast, coconut-HFD did not modify UCP-1 expression in any of the adipose tissues studied.

3.8. EVOO-HFD Appeared to Promote Mitochondrial Biogenesis in WAT and BAT

PGC-1α is a protein involved in mitochondrial biogenesis and thermogenesis in WAT and BAT. As shown in Figure 9.1b, 2b, and 3b EVOO-HFD was the only diet administered that significantly increased PGC-1α expression in all adipose tissues. Neither coconut-HFD nor sunflower-HFD modified PGC-1α expression in any of the adipose tissues studied.

3.9. Sunflower-HFD and EVOO-HFDs Enhanced Nrf2 Expression in Adipose Tissue

Nrf2 is involved indirectly in adipocyte differentiation and has antioxidant activity in response to oxidative stress. As shown in Figure 9.1c, 2c and 3c, none of the diets administered modified Nrf2 expression in iWAT. However, sunflower-HFD was the only diet

Table 4. Weight (g), leptin (ng mL^{-1}), and adiponectin (pg mL^{-1}) plasma levels throughout the intervention.

Weeks		Chow	Coconut-HFD	Sunflower-HFD	EVOO-HFD
0	Weight	29.49 ± 0.37	28.65 ± 0.48	31.05 ± 0.77	29.43 ± 0.76
	Leptin	1.43 ± 0.02	2.07 ± 0.07***	2.09 ± 0.05***	2.09 ± 0.08***
	Adiponectin	1 ± 0.06	0.88 ± 0.05*	1.29 ± 0.04***	1.12 ± 0.02*
4	Weight	35.06 ± 1.00	34.05 ± 0.77	39.77 ± 1.76*	38.81 ± 1.48*
	Leptin	4.79 ± 0.13	4.33 ± 0.03***	11.95 ± 0.72***	14.19 ± 0.46***
	Adiponectin	1.03 ± 0.03	0.74 ± 0.02***	1.14 ± 0.02*	0.97 ± 0.03
8	Weight	37.91 ± 1.40	38.9 ± 1.20	45.3 ± 2.32**	45.93 ± 1.90**
	Leptin	6.39 ± 0.4	7.04 ± 0.09	10.71 ± 0.78***	24.32 ± 0.58***
	Adiponectin	1.13 ± 0.06	0.88 ± 0.03	0.96 ± 0.004	1.04 ± 0.007
12	Weight	40.56 ± 1.72	39.45 ± 1.72	50.05 ± 2.76**	50.47 ± 2.13**
	Leptin	11.68 ± 0.06	10.84 ± 0.16	30.24 ± 2.48***	30.44 ± 1.51***
	Adiponectin	1.27 ± 0.04	0.85 ± 0.01***	0.9 ± 0.02***	0.92 ± 0.004***
16	Weight	45.48 ± 1.85	44.88 ± 1.48	53.31 ± 3.34**	55.9 ± 2.65**
	Leptin	21.92 ± 1.12	13.58 ± 0.64***	42.52 ± 1.07***	37.25 ± 1.44***
	Adiponectin	1.46 ± 0.11	0.64 ± 0.02***	0.79 ± 0.008***	0.66 ± 0.005***

Data are represented as mean ± SEM of $n = 11$ mice for each group. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for groups versus chow. ANOVA, analysis of variance; EVOO, extra virgin olive oil; HFD, high-fat diet; LSD, least significant difference; SEM, standard error of the mean.

that increased Nrf2 expression in eWAT. On the other hand, both EVOO-HFD and sunflower-HFD significantly increased Nrf2 expression in BAT ($p < 0.01$ and $p < 0.05$, respectively).

4. Discussion

Given the controversy in the literature regarding the impact of vegetable oils on health and the growing epidemic among the population caused by excess and abnormal accumulation of body fat related to a high fat intake the aim of this study was to analyze the effect of different HFDs based on coconut oil, EVOO, or sunflower oil on adipose tissue, alterations in metabolism, and modulation of inflammation in mice.

Adipose tissue is responsible for energy storage but also plays an important role as an endocrine organ in the regulation of biological processes such as immunity and metabolic homeostasis.[19] It secretes adipokines that have a significant role in the pathogenesis of fat accumulation disturbance and its metabolic complications.[20,21]

Regarding fat accumulation and metabolism, our results showed that mice fed coconut-HFD had a significantly lower mean body weight than those fed EVOO- or sunflower-HFDs. Coconut oil has been recommended for its weight reducing properties although these effects remain controversial.[18,22] It has been suggested that this property of coconut oil could be related to its constituent medium-chain fatty acids (MCFAs), which are quickly used as an energy substrate and are less susceptible to accumulation in adipose tissues.[23,24] Traditionally, the main fatty acid in coconut oil, lauric acid, has been considered as an MCFA. However, recent evidence has shown that it should be classified as a long-chain fatty acid due to its metabolic destination following gut absorption.[25,26] In this sense, the effects of coconut-HFD on body weight regulation may be due to its satiating activity through leptin, which is directly related to appetite regulation because of its action on hypothalamic centers.[27] However, our results showed that coconut-HFD was the only diet that produced low circulating leptin levels.

In relation to adiponectin, whose plasma levels are inversely correlated with fat mass,[28] all mice fed HFDs showed low circulating adiponectin concentrations. This disturbance is related to insulin resistance and metabolic complications.[29] Regardless of their effects on body weight, all HFDs resulted in altered adipokine levels.

The intake of a high-calorie diet should promote excessive fat accumulation. However, depending on the source of fat, the HFDs used in this study resulted in different types of fat accumulation. Mice fed the coconut-HFD showed no differences in fat accumulation compared to mice fed a chow diet. The sunflower-HFD increased subcutaneous and visceral fat, while the EVOO-HFD only increased subcutaneous fat significantly.

With respect to immune modulation, the serum levels of various pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory (TGF-β) cytokines were analyzed. Our data showed that mice fed the coconut-HFD had high levels of pro-inflammatory cytokines, while serum TGF-β levels were highest in mice fed the EVOO-HFD, followed by the sunflower-HFD and coconut-HFD. This cytokine has an essential role in the suppression of inflammation.[30] These results are in concordance with previous data published by our group, where gut microbiota analysis showed that sunflower-HFD and coconut-HFD promoted a pro-inflammatory intestinal microenvironment in these mice while EVOO-HFD produced an anti-inflammatory microenvironment.[31] Collectively, these data suggest that coconut-HFD could induce a low-grade systemic inflammation. These alterations may be associated with the previous observation that TLR4 modification induced by a diet supplemented
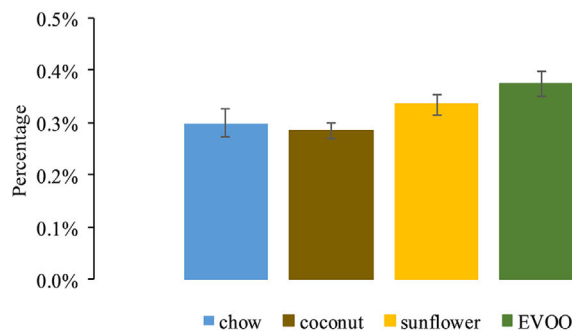
(A) iWAT weight/ body weight index (%)
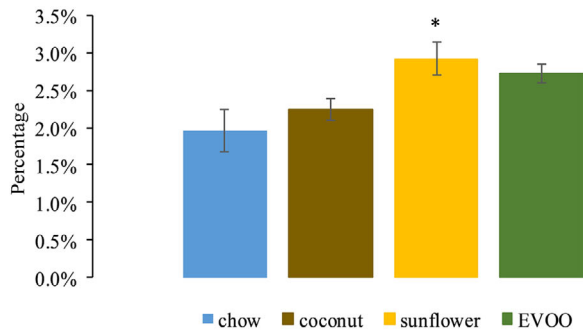
iWAT weight /body weight index (%)			
Group	Mean	SD	P value
Chow	1.98	0.94	
Coconut	2.24	0.45	1.000
Sunflower	2.92	0.75	*0.018*
EVOO	2.73	0.40	0.093

(B) eWAT weight/ body weight index (%)
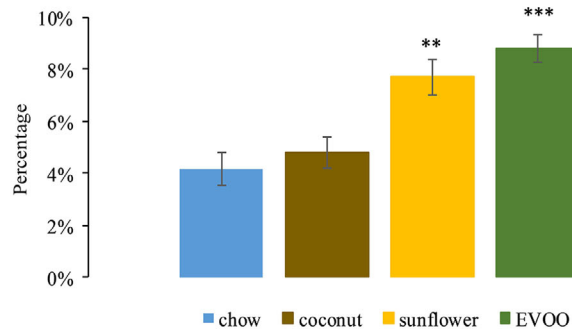
eWAT weight/body weight index (%)			
Group	Mean	SD	P value
Chow	4.17	2.14	
Coconut	4.79	1.99	1.000
Sunflower	7.69	2.28	*0.002*
EVOO	8.79	1.83	*0.000*

(C) BAT weight/ body weight index (%)

BAT weight/ body weight index (%)			
Group	Mean	SD	P value
Chow	0.30	0.08	
Coconut	0.28	0.05	1.000
Sunflower	0.33	0.06	1.000
EVOO	0.37	0.08	0.107

Figure 4. Ratio of adipose tissue/body weight. a) iWAT, b) eWAT, and c) BAT. Data are represented as mean ± SD of $n = 11$ mice for each group and are expressed in percentages. ANOVA followed by Fisher's LSD test comparing all groups versus chow. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. ANOVA, analysis of variance; BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue; LSD, least significant difference; SD, standard deviation.

with coconut oil in healthy mice.[24] TLR4 plays a key role in the activation of inflammatory pathways.[24]

Furthermore, concerning the effects on lipid metabolism, mice fed coconut-HFD developed serum hypertriglyceridemia. Likewise, liver triglycerides were significantly elevated in coconut-HFD and sunflower-HFD-fed mice. Moreover, the mice with the highest ratio of liver weight to body weight were those fed coconut-HFD, suggesting that this diet, despite not increasing body weight, could produce hepatomegaly and metabolic alterations.

Adipocytes can be classified as white, brown, and beige. White adipocytes are distributed in subcutaneous and visceral adipose tissue, and their function is to store excess energy in the form of triglycerides. Brown adipocytes are located in limited areas of the body, and their primary physiological function is energy dissipation. Finally, beige adipocytes are inducible brown-like thermogenic adipocytes found sporadically in WAT deposits.[32,33] Brown and beige adipocytes trigger non-shivering thermogenesis in response to cold through increased activity of UCP-1.[34]
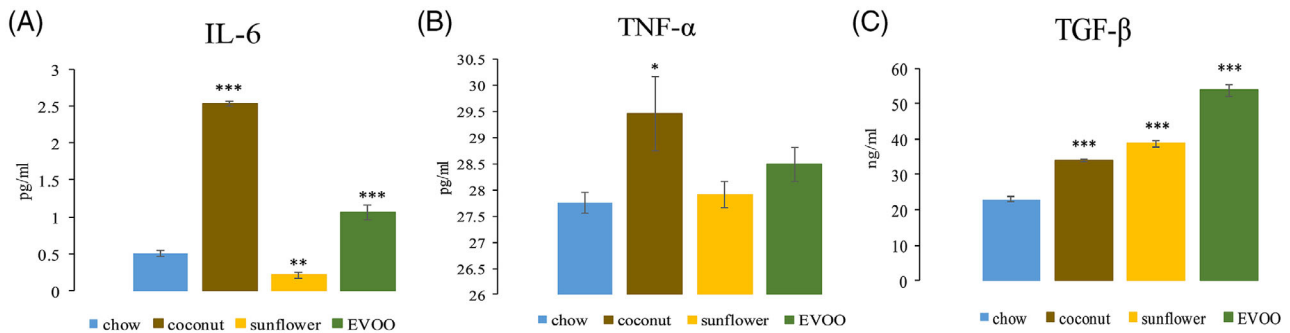
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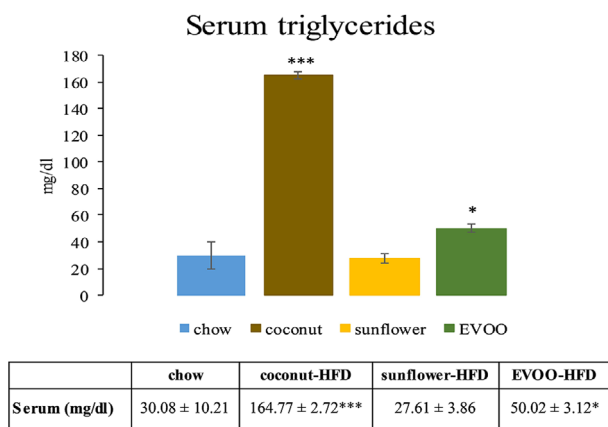Food Research

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Figure 5. Serum inflammatory cytokine levels. Data are represented as mean ± SEM of quadruplicate replicates of pooled serum ($n = 11$) for each group obtained in ELISA for a) IL-6, b) TNF-α, and c) TGF-β. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for groups versus chow. ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin 6; LSD, least significant difference; SEM, standard error of the mean; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

	chow	coconut-HFD	sunflower-HFD	EVOO-HFD
Serum (mg/dl)	30.08 ± 10.21	164.77 ± 2.72***	27.61 ± 3.86	50.02 ± 3.12*

Figure 6. Serum triglycerides. Data are represented as mean ± SD of quadruplicate replies of pool serum ($n = 11$) for each group obtained in ELISA. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for groups versus chow. ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; LSD, least significant difference; SD, standard deviation.
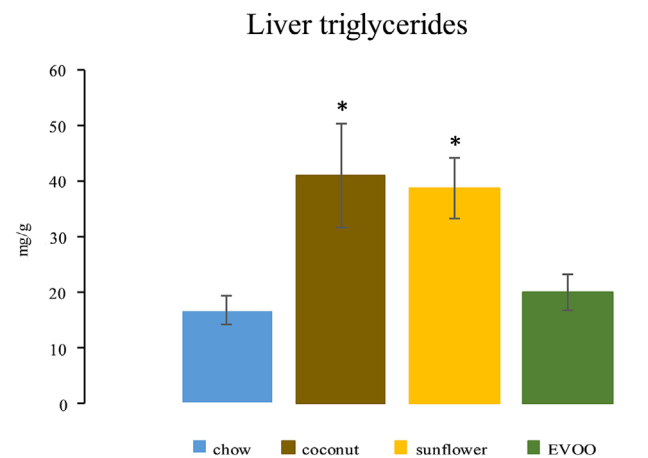

Figure 8. Liver triglycerides. Data are represented as mean ± SEM of quadruplicate replies of pool livers ($n = 11$) for each group obtained in ELISA. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed. *$p < 0.05$ for groups versus chow. ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; LSD, least significant difference; SEM, standard error of the mean.
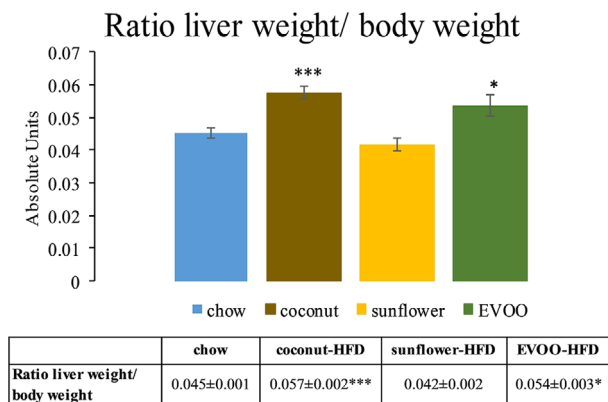

	chow	coconut-HFD	sunflower-HFD	EVOO-HFD
Ratio liver weight/ body weight	0.045±0.001	0.057±0.002***	0.042±0.002	0.054±0.003*

Figure 7. Ratio liver weight/body weight. Data are represented as mean ± SEM of $n = 11$ mice for each group. For statistical analysis, one-way ANOVA followed by Fisher's LSD test were performed; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for groups versus chow. ANOVA, analysis of variance; LSD, least significant difference; SEM, standard error of the mean.Fi

We observed that the EVOO-HFD significantly increased UCP-1 expression in iWAT and BAT, in agreement with the results obtained by Oi-Kano et al.[35] Furthermore, they found that EVOO increased triglyceride catabolism and thermogenesis in BAT by increasing the UCP-1 level, suggesting that oleuropein (a minor compound in EVOO) was responsible for this activity. Similarly, Rodríguez et al.[36] found that olive oil induces an increase in UCP-1 mRNA expression in the BAT of rats. Reinforcing our results, a recently published clinical trial observed that a dietary intervention with EVOO was able to increase the thermogenic activity of BAT and increase leptin levels.[37]

On the other hand, the results obtained in the present study showed that a coconut-HFD did not produce any significant change in UCP-1 expression. Therefore, the mechanism that ameliorates weight gain after a coconut-HFD is not associated with an increase in thermogenic activity.

PGC-1α is a cold-inducible transcription coactivator of adaptive thermogenesis in adipose tissue. Furthermore, its expression is necessary to promote differentiation to the brown-adipocyte
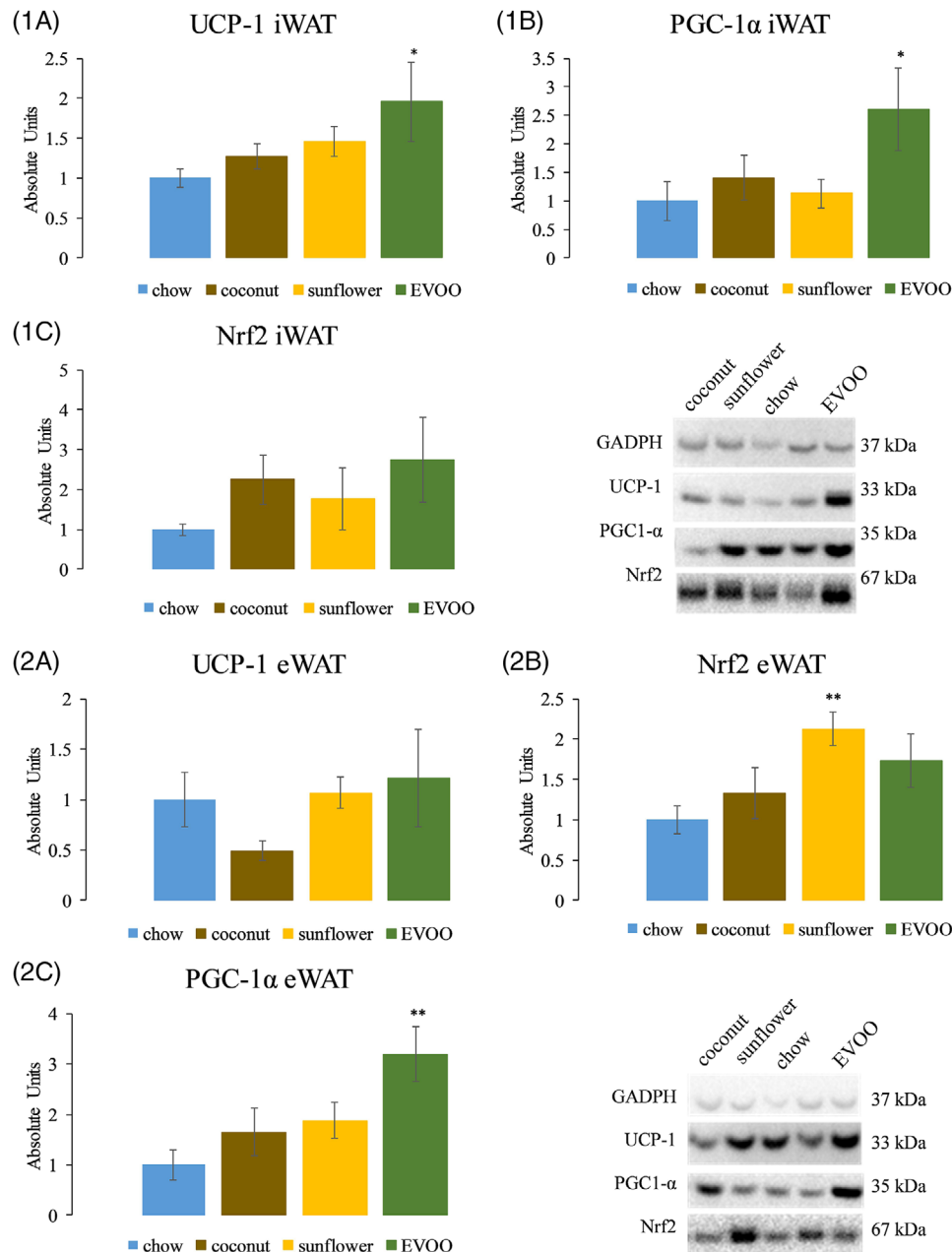
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Figure 9. Quantitative analysis (left) and representative western blot (right) of a) UCP-1, b) PGC-1α, and c) Nrf2 protein expression in 1) iWAT, 2) eWAT, and 3) BAT. GADPH, loading control. Data are represented as the mean of $n = 11$ per group with triplicate independent experiments. For statistical analysis, one-way ANOVA followed by Fisher's LSD test was performed. *$p < 0.05$ and **$p < 0.01$ for groups versus chow. ANOVA, analysis of variance; BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue; GADPH, glyceraldehyde-3-phosphate dehydrogenase; LSD, least significant difference; Nrf2, nuclear factor E2-related factor 2; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; UCP-1, uncoupling protein 1.

lineage, and it is the major regulator of mitochondrial biogenesis and oxidative metabolism in BAT.[38,39]

Our results showed that only the EVOO-HFD was able to significantly increase PGC-1α expression in all adipose tissues studied. Interestingly, Zhang et al.[40] reported that berberine, a plant-derived compound, induced development of murine beige adipocytes in iWAT through PGC-1α signaling. Similarly, there have been described other dietary factors that promote

the development of brown and beige adipocytes and induce thermogenesis.[41] In line with our results, some authors have suggested that phenolic compounds, which are present in high amounts in EVOO, may be responsible for this browning effect via PGC-1α activation.[42,43]

Nrf2 is a transcription factor that plays a critical role in mitochondrial biogenesis and controls the capacity for adipose tissue expansion.[44] Nrf2 also protects cells against oxidative stress and
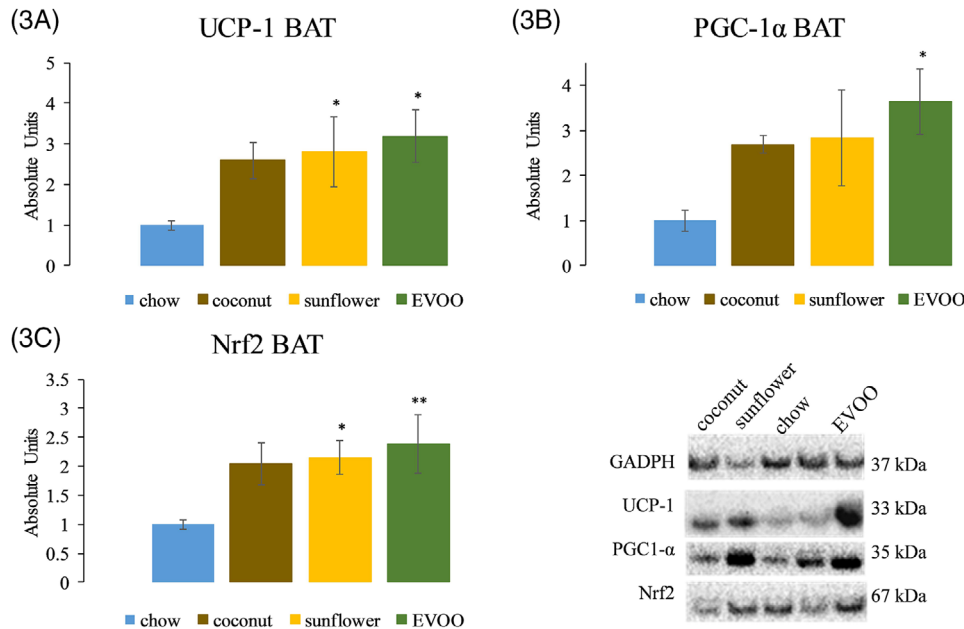
Figure 9. *Continued*

Table 5. Summary of the effects of an HFD.

Parameters		Groups		
		Coconut-HFD	EVOO-HFD	Sunflower-HFD
Body weight		–	↑	↑
Leptin		↓	↑	↑
Adiponectin		↓	↓	↓
IL-6		↑↑↑	↑↑	↓↓
TNF-α		↑	–	–
TFG-β		↑	↑↑↑	↑↑
Triglyceridemia		↑↑↑	↑	–
Liver weight		↑↑↑	↑	–
Liver triglycerides		↑	–	↑
iWAT	Weight	–	–	↑
	UCP-1	–	↑	–
	PGC-1α	–	↑	–
	Nrf2	–	–	–
eWAT	Weight	–	↑↑↑	↑↑
	UCP-1	–	–	–
	PGC-1α	–	↑↑	–
	Nrf2	–	↑↑	↑↑
BAT	Weight	–	–	–
	UCP-1	–	↑	↑
	PGC-1α	–	↑	–
	Nrf2	–	↑↑	↑

↑ or ↓ indicate difference compared to control. –, no significant changes. BAT, brown adipose tissue; EVOO, extra virgin olive oil; eWAT, epididymal white adipose tissue; HFD, high-fat diet; IL-6, interleukin 6; iWAT, inguinal white adipose tissue; Nrf2, nuclear factor E2-related factor 2; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator 1α; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; UCP-1, uncoupling protein 1.

has a potent anti-inflammatory effect.[45] Expression of Nrf2 protein in BAT was upregulated in EVOO-HFD mice. Taking into account UCP-1 and PGC-1α data, EVOO-HFD could have a positive function in the promotion of thermogenesis by increasing mitochondrial synthesis in adipocytes.

Existing literature shows that an HFD leads to metabolic and inflammatory disorders as well as alterations in adipose tissue function that could be mediated by TLR4 modulation.[24] On the other hand, coconut oil is rich in myristic and palmitic acids that could induce lipoapoptosis,[46] which could explain the results obtained with coconut-HFD. However, more studies are needed to understand the mechanisms of action of different edible vegetable oils.

In summary, as shown in **Table 5** coconut-HFD caused numerous metabolic and inflammatory disorders, such as reduced circulating leptin and adiponectin concentrations, an increased in hepatic lipid content, elevated serum triglycerides, and increased circulating pro-inflammatory cytokines. In addition, an HFD based on coconut oil ameliorated body weight gain relative to diets that included EVOO or sunflower oil. Our results exclude the possibility that this effect was due to the thermogenic effect of coconut oil. On the other hand, results showed that EVOO-HFD increases thermogenic activity and could promote browning of WAT.

Acknowledgements

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

J.J.G. and C.S.-Q. conceived and designed the research; C.R.-G. performed the experiments and analyzed the data; J.J.G. and C.R.-G. drafted the manuscript; J.J.G., I.A., and C.S.-Q. edited and revised the manuscript; and all authors read and approved the final version of the manuscript.

Data Availability Statement

All data are available in the manuscript or upon request to the authors.

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