Peer-reviewed version available at *Nutrients* **2020**, *12*, 2185; doi:10.3390/nu12082185

Article

Time-Restricted Feeding Improves Body Weight Gain, Lipid Profile, and Atherogenic Indices in Cafeteria Diet-fed Rats: Role of Browning Inguinal White Adipose Tissue

Samira Aouichat [1,2], Souhila Bouguerra-Aouichat [2] and Ahmad Agil [1,*]

[1] Department of Pharmacology, Biohealth Institute and Neuroscience Institute, School of Medicine, University of Granada, Granada, Spain

[2] Team of Cellular and Molecular Physiopathology, Faculty of Biological Sciences, Houari Boumediene University of Sciences and Technology, El Alia, Algiers, Algeria; samira_aouichat@outlook.fr (S.A.); souhila.aouichat@hotmail.fr (S.B.A.)

* Correspondence: aagil@ugr.es; Tel.: +34 958 24 35 (A.A.)

Abstract: Time-restricted feeding (TRF) showed a potent effect in preventing obesity and improving metabolic outcomes in several animal model of obesity; however, there is, as yet, scarce evidence about its effectiveness against obesogenic challenge that more accurately mimic the human Western diets, such as cafeteria diet. Moreover, the mechanism for its efficacy is poorly understood. White adipose browning has been linked to body weight loss. Herein, we tested whether TRF has the potential to induce browning of inguinal white adipose tissue (iWAT) and to attenuate obesity and associated dyslipidemia in cafeteria diet-induced obesity model. Male Wistar rats, fed normal laboratory chow (NC) or cafeteria diet (CAF) for 16 weeks, were subdivided into two groups that were subjected to either *ad libitum* (*ad lib*, A) or TRF (R) for 8 hours per day. Rats under TRF regimen had a lower body weight gain and adiposity compared with their diet-matched *ad lib* rats, despite equivalent levels of food intake and locomotor activity. In addition, TRF improved the deranged lipid profile [total cholesterol (TC); triglycerides (TG); high density lipoprotein (HDL-c); low density lipoprotein (LDL-c)] and atherogenic indices [atherogenic index of plasma (AIP); atherogenic coefficient (AC); coronary risk index (CRI)] in rats fed CAF diet. Remarkably, TRF resulted in decreased size of adipocytes and induced emergence of multilocular brown-like adipocytes in iWAT of NC- and CAF-fed rats. Protein expression of browning markers, such as uncoupling protein-1 (UCP1) and peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC1α) in iWAT were also up-regulated in time restricted NC- or CAF-fed rats. These findings suggest that TRF regimen is an effective strategy to improve obesity and associated dyslipidemia induced by CAF-diet, probably via a mechanism involving WAT browning process.

Keywords: time-restricted feeding; cafeteria diet; obesity; lipid profiles; atherogenic indices; browning adipose tissue

1. Introduction

Obesity is defined as abnormal or excessive fat accumulation that may impair health [1]. The etiology of obesity is quite complicated owing to the involvement of both genetic and environmental factors. Among the environmental factors, diet plays a significant role in the development of obesity [2]. Modern nutritional patterns termed as "Western diet", including high-fat and cholesterol, high-protein, high-sugar, and excess salt intake, as well as frequent consumption of processed and fast foods, cause a high prevalence of obesity in Western societies [3,4]. According to the data published by the World Health Organization, the worldwide prevalence of obesity increased three fold over the last 40 years, whereas is expected to reach ~30.3 billion in 2030 [1]. Obesity is often associated with a condition termed as metabolic syndrome that is characterized by insulin

resistance, dyslipidemia, hypertension, and systemic inflammation, as well as cardiovascular diseases [5]. Classical attempts to develop therapeutic strategies have mostly focused on limiting caloric intake by diet and increasing energy expenditure by physical exercise; however, these strategies is often not effective, as the results are not always satisfactory and its success is only limited to a small percentage of individuals [6]. Today, TRF, an eating pattern that involves limiting food intake to a specific time window of 12 hours or less every day without altering nutrient quality or quantity, is emerging as promising therapeutic strategy against obesity and associated metabolic disorders [7]. One outstanding study pioneered by Panda's group demonstrated that mice fed a high-fat diet (HFD) under TRF paradigm of 8 h per day during to the active phase were protected against obesity, hyperinsulinemia, hepatic steatosis, and inflammation, even though they consume equivalent calories from HFD as those with *ad lib* access [8]. Interestingly, another study by the same group showed that TRF of 8-12 h without reducing caloric intake in mice prevented and even reversed obesity and related metabolic disorders arising from a variety of obesogenic challenges, including high-fructose and high fat-high sucrose diets [9]. To date, there has been an extensive studies in both rodents and human reporting that TRF reduces body weight, improves glycemic control and insulin resistance, prevents atherogenic dyslipidemia, reduces blood pressure, prevents hepatic steatosis, and improve inflammatory markers in diet-induced obesity models [10–25]. It should be noticed that most of rodent studies have attempted to mimic the unhealthy human Western diet through numerous obesogenic diets, which generally focused on a single or a combined macronutrient(s), in particular fat and/or sugar. However, these commercial high-fat, high-sugar, or high fat-high sugar rodent diets are not analogous to the highly processed food common in Western societies and associated with increased global obesity rates. The closest comparable to the human Western foods is the cafeteria diet (CAF), as it provides animals with nutritionally varied diet of high-energy, palatable human foods that is low in fibers and contain substantial amount of fat, sugar, and salt (for example, cheese, chocolates, processed meat, chips, and cookies), thereby recapulating the key obesogenic features of the unhealthy human diet [26,27]. Furthermore, extended CAF diet in rodents produced an exaggerated phenotype of obesity with excessive body weight gain, pronounced adiposity, dyslipidemia, and liver inflammation [28–30], and has been argued to model the modern human obesity and related metabolic disorders more severely than the classical model of HFD [28]. Also, CAF diet exerted a robust impact upon appetite and energy intake due to its high amount of sugar, salt, and additives, such as appetizers and taste enhancers, in comparison to the traditional HFD [31]. To the best of our knowledge, no study has so far been conducted and published on whether TRF regimen has the potential to prevent obesity and related metabolic disarrangements in CAF diet-induced obesity models. Furthermore, the mechanism that underlies the beneficial effect of TRF paradigm on obesity is still not well-defined. Various mechanisms have been proposed to explain the beneficial effects of TRF on body weight and metabolism. For instance, TRF regimen was suggested to prevent obesity and related metabolic disorders by entraining the circadian clock to a fixed feeding time [32], altering gut microbiome [33], and increasing free fatty acids mobilization and fat oxidation [8]. However, it remains unexplored whether TRF could trigger WAT browning. Of note, WAT browning, a process of formation of brown-like adipocyte within WAT, is regarded as a potential therapeutic target for obesity due to its unique capacity to up-regulate thermogenesis and thus increasing energy expenditure through glucose and fatty acids oxidations. Brown-like adipocytes, termed as "beige cells", are characterized by multilocular lipid droplets and high amounts of UCP1-positive mitochondria, and they switch from an energy storage to an energy expenditure state by expressing thermogenic markers, including UCP1 and PGC1α, which constitute the molecular signature of brown and beige adipocytes and play an important role in metabolic thermogenesis [34]. Classically, browning of WAT can be induced in animals and humans by various physiological cues , such as exercise and pharmacological agents, such as active ingredient like melatonin, which has previously been demonstrated by our group to have an iWAT browning potential in obese diabetic animals [35]. Based on previous findings showing that TRF reduced body weight gain in diet-induced obesity models (DIO) without altering caloric intake or activity level [8,12,19], we hypothesized that TRF regimen would prevent obesity via promoting WAT browning. Therefore, the present study aims to

address whether TRF schedule of 8 h per day during the active phase can prevent excessive body weight gain in rats fed with a Western-style CAF-diet. Because there is scarce data in literature regarding the effect of TRF schedule on lipid profile and atherogenicity in DIO, it is worth investigating whether TRF regimen could prevent dyslipidemia produced by CAF-diet pattern. Additionally, the potential role of TRF paradigm as a browning inducer in the white inguinal subcutaneous adipose pads of CAF obese rats is also explored.

2. Materials and Methods

2.1. Animals

All research and animal care procedures were authorized and approved by the Institutional Animal Care Committee of the National Administration of Algerian Higher Education and Scientific Research (Ethical approval number: 981-1 law of 22 August 1998).

A total of 24 male albino Wistar rats (*Rattus norvegicus*) weighting 120 - 130g were obtained from the Pasteur Institute of Algiers, Algeria. Rats were single-housed in polypropylene cages with a stainless steel lid under thermoneutrality condition (28 - 30 °C) and 12 h light: 12 h dark schedule, with lights on at 08:00 a.m (Zeitgeber time 0, ZT0). Normal rat chow pellets (supplied by ONAB, Algiers) and water were provided *ad lib*. All animals were acclimatized to their new environment for 15 days before starting the experiment.

2.2. Experimental Feeding Schedule and Diets

Upon initiation of the experimental, rats (initial body weight 150 ± 1.5 g) were randomized by weight into four groups (n = 6 per group) to ensure equivalent starting body weight. They were maintained on the normal chow diet (NC) or switched to a pre-selection of highly palatable, energy-dense human foods consisting of cookies, cereals, chocolate, crackers, cheeps, cheese, processed meat, etc. (cafeteria diet; CAF) either with *ad lib* (A) or temporally restricted access to food (R), for 16 wks. Rats under TRF regimen were allowed to access the diet for 8 h per day during the active phase (dark period) from ZT 13 (1 hours after lights off) to ZT 21 (3 hours before lights on). Food access was monitored by manually switching the rats between cages with food and water and clean cages for fasting period with water only, so as to avoid foraging and coprophagia. The CAF-diet protocol used in this study was adapted from previous study [28] and consisted of 19 different highly palatable and energy-rich unhealthy human snack foods purchased from supermarket of Algeria, accompanied by normal rat chow pallets. The list of cafeteria diet food items with nutrient compositions is listed in Supplementary Table S1. Three different items plus normal rat chow pallets were provided daily in excess quantities to each animal. To maintain variety and thus induce sustained hyperphagia, the food items provided were altered daily by replacing the three food items with new fresh ones. The menu was varied daily in a manner that ensures relatively similar proportion of fat, protein, sugar and carbohydrate in each daily set of CAF foods. Normal chow-diet and CAF-diet food items were pre-weighted individually and presented to the rats at ZT 13, and the left over was weighted after 24 and 8 h in *ad lib* and time-restricted fed rats, respectively. To carefully monitor the CAF food consumption, food spillage were meticulously collected from the cage floor, and the difference between presented and recovered food items was corrected for drying, as previously described By Shafat et al. [31]. Energy intake was calculated as weekly intake, based on nutritional information provided by the manufacturer (Supplementary Table S1). The body weight was recorded weekly over 16 wks between 09:00 and 10:00 a.m.

2.3. Locomotor Activity Assessment

At week fifteen, the locomotor activity was evaluated in the open field by counting the number of squares crossing and occurrences of rearing behavior. The test was performed in a silent room at night, 2 h after light off, in a four-sided 90 × 90 × 45 cm varnished wood box, and the floor of the open field was divided into 25 equal-size squares (18 cm). Each rat were placed singly in the center of the open field and was given 5 min to explore. A rat was recorded as crossing a square when

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 June 2020 doi:10.20944/preprints202006.0134.v1

Peer-reviewed version available at Nutrients 2020, 12, 2185; doi:10.3390/nu12082185

more than three paws or half of the body crossed the boundary into the nearby squares. Rearing was defined from the moment when both forelimbs raised at least 1 cm above the floor. At the end of each trial, the box was cleaned with water and wiped dry before introducing the next animal [36].

2.4. Blood and Tissue Collection

At the end of the experimental period, rats were fasted for ~16 hours starting from ZT 21, anesthetized by urethane (1 g/kg b.w, i.p.), and killed. Blood were collected via heart puncture and serum was obtained by centrifugation at 3000 r.p.m for 15 minutes at 4°C. The recovered serum was frozen at –20°C until analysis. White adipose tissue depots from mesenteric (mWAT), epididymal (eWAT), retroperitoneal (rWAT), and inguinal subcutaneous (iWAT) sites were rapidly removed, rinsed with phosphate buffer (PBS (phosphate - buffered saline), and then weighed, followed by immediate freezing in liquid nitrogen. Serum and iWAT specimens were shipped on dry ice to the laboratory of Prof. Ahmad Agil (UGR, Spain), and then they were stored at –80°C for lipid profiles and Western blot analysis. The experimental protocol is described in Figure 1.

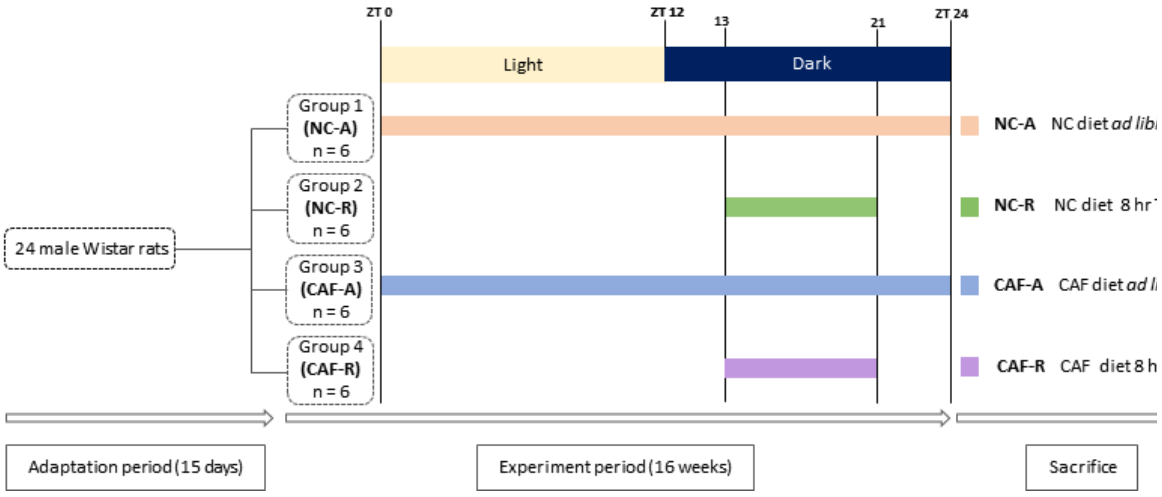

Figure 1. Schematic representation of the experimental feeding schedule. Male Wistar rats were allowed a 15-days *adaptation* period prior to beginning the experiment and were divided into four groups as follows: NC-A (normal chow *ad lib* feeding); NC-R (normal chow time-restricted feeding); CAF-A (Cafeteria diet *ad lib* feeding); and CAF-R (cafeteria diet time-restricted feeding). Time-restricted feeding groups were allowed to food for 8 h per day during the dark phase between ZT 13 and ZT 21. After sixteen weeks of experiment, animals were sacrificed. CAF: cafeteria diet; NC: normal chow diet; TRF: time-restricted feeding; ZT: Zeitgeber.

2.5. Measurement of Lipid Profiles and Atherogenic Indices

Serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were measured by using commercial kits in an automatic analyzer (Roche-Hitani molecular system 902, Branchburg, NJ).

To predict atherosclerosis and cardiovascular problems, the atherogenic indices [atherogenic index of plasma (AIP); atherogenic coefficient (AC); coronary risk index (CRI)] were calculated by using the following:

$$AIP = Log_{10} [TG/HDL\text{-}c] \text{ [37]}$$

$$AC = [TC - HDL\text{-}c/HDL\text{-}c] \text{ [38]}$$

$$CRI = [TC/HDL\text{-}c] \text{ [39]}$$

2.6. Microscopic Analysis

Following anesthesia, excised inguinal fat was fixed by immersion in 4% formaldehyde in 0.1 M phosphate buffer overnight at 4 °C, washed in PBS, dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin. The paraffin blocks from each group were cut with a microtome into 5µm thick sections, stained with hematoxylin and eosin (H&E), and inspected under a light microscope (×200 magnification; Leica Store Miami, Coral Gables, FL) equipped with a digital camera system (Premiere camera, model MA88-500. Microscopes America Inc., Cumming, GA). Image of H&E-stained tissue sections were digitized and adipocytes size and percentage of multilocularity were determined using Axio Vision software (Carl Zeiss Imaging Solutions). The average adipocyte size was expressed as the average cross-sectional area per cell (µm²/cell) of tissue sample, which was calculated based on the values of at least 20 adipocytes in 10 random fields per section. The percentage of total adipocytes population showing multilocularity was determined by quantifying the area of multilocular adipocytes in relation to the entire area of the section.

2.7. Western Blotting Analysis

About 100 - 200 mg of iWAT was homogenized in lysis buffer (150 mmol NaCl, 5 mmol EDTA, 50 mmol Tris-HCl, and pH 7.4) without Triton X-100 and homogenized with a Teflon pestle. Homogenates were centrifuged (3000 g for 15 min, 4°C), and the fat cake was removed from the top of the tube. Then, Triton X-100 was added to a final concentration of 1%. After incubating at 4°C for 30 min, extracts were cleared by centrifugation at 15,000 g for 15 min at 4°C. One hundred micrograms of total protein was analyzed by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The gels for immunoblot analyses were transferred to a nitrocellulose membrane (Bio-Rad Trans-Blot SD; Bio-Rad Laboratories, Hercules, CA, USA). The membranes were cut at UCP1 and PGC1α molecular weight level (33 kDa), and blots were reacted with a 1:2000 dilution of anti-UCP1 produced in rabbit (Sigma Aldrich, U6382, St. Louis, MO, USA), in blocking solution (PBS, 5% nonfat milk) and anti-PGC1α produced in rabbit. β-actin antibody generated in mouse (Santa Cruz Biotechnology, SC-81178, Santa Cruz, CA, USA) was used as a control. Horseradish peroxidase labeled secondary antibodies were goat anti-mouse IgG and goat anti-rabbit IgG (1:1000, Sigma Aldrich). Proteins were visualized by enhanced chemiluminescence (ECL kit, GE Healthcare Life Sciences, Pittsburgh, PA, USA).

2.8. Statistical Analysis

Statistical Package of Social Science (IBM SPSS Software, version 22) was used for statistical analysis. All results are expressed as mean ± standard error of the mean (S.E.M) values. Two-way ANOVA was used to examine two variables (diet and schedule feeding). When a significant F score on two-way ANOVA was recorded, pair-wise comparisons were conducted with Mann-Whitney U-test. Repeated-measures two-way ANOVA was also used to analysis change in body weight over time. Following a significant result, single time point comparisons were made using unpaired Mann-Whitney U-test. Differences between group means were considered statistically significant if $p < 0.05$.

3. Results

3.1. *Body Weight, Calorie Intake, Adipose Weight, and Locomotor Activity*

To evaluate the effectiveness of TRF regimen against the adverse effect of CAF diet, we first tested its effect on body weight. As expected, rats fed a CAF-diet on *ad lib* access exhibited a significant increase in body weight from the 2 nd week of the experiment until the end (16 wks), as compared to rats fed a NC-diet on *ad lib* access ($p < 0.01$; Figure 2a). The final body weight gain in CAF–A group was significantly higher compared with that in NC–A group (219.7 ± 7.7 g vs. 126.5 ± 5.3 g; $p < 0.01$; Figure 2b). TRF regimen significantly reduced body weight in rats fed with both NC- and CAF-diets, from respectively the 5 th and 3 rd week until the end of the experiment ($p < 0.05$;

Figure 2a). The final body weight gain in NC–R (75.0 ± 4.5 g) and CAF–R (138.1 ± 5.0 g) groups was significantly lower compared with that in their diet-matched *ad lib* groups ($p < 0.01$; Figure 2b). Interestingly, the final body weight gain in CAF–R group was not significantly different from NC–A group ($p > 0.05$; Figure 2b).

Although the final body weight gain was lower in time-restricted rats, the daily total calorie intake showed no statistically significant difference between time-restricted and *ad lib* rats that were fed with either NC- or CAF-diet ($p > 0.05$; Figure 2c). Notably, the total daily calorie intakes per individual rat were 52.1 ± 2.0, 52.8 ± 2.6, 72.4 ± 3.0, and 75.3 ± 2.4 Kcal in the NC–A, NC–R, CAF–A, and CAF–R groups, respectively.

To test whether reduced body weight gain in TRF rats was due to a physical condition, animals were tested in open-field arena to evaluate the spontaneous locomotor activity. As shown in Figure 2, CAF–A group had reduced locomotor activity than NC–A group, since the CAF–A group showed an overall decrease in the number of crossing squares (53.1 ± 2.8 vr. 62.3 ± 2.0; $p < 0.05$; Figure 2d) and rears (23.1 ± 3.0 vr. 29.3 ± 1.9; $p < 0.05$; Figure 2e). Importantly, TRF schedule had no significant effect on locomotor activity. Indeed, the number of crossing squares and rears in NC–R (62.0 ± 2.4 and 29.4 ± 1.4, respectively; Figure 2 d, e) and CAF–R (57.2 ± 2.5 and 26.1 ± 2.3, respectively; Figure 2 d, e) groups had no significant difference ($p > 0.05$) with those in their *ad lib* counterparts, although they tended to be lower in CAF–A group, as compared to CAF–R.

To identify whether TRF paradigm have a specific effect towards some adipose tissues, we weighted adipose tissues from different anatomical localization. As expected from their body weights, CAF–A group showed significantly higher total adiposity than NC–A group ($p < 0.01$; Table 1). The TRF paradigm significantly reduced the weight of the iWAT and all the visceral adipose tissue depots (mesenteric, epididymal, and retroperitoneal) in both NC- and CAF-fed rats ($p < 0.05$; Table 1). The total adiposity is significantly lower in rats on TRF compared to that in rats on *ad lib* ($p < 0.01$; Table 1).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 June 2020 doi:10.20944/preprints202006.0134.v1

Peer-reviewed version available at Nutrients 2020, 12, 2185; doi:10.3390/nu12082185

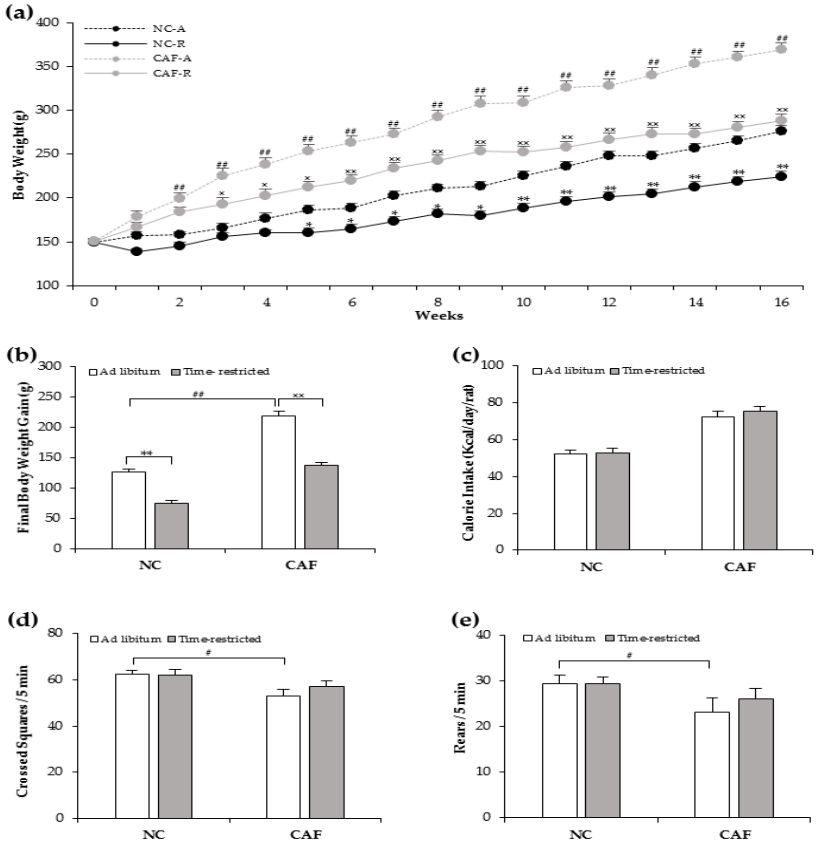

Figure 2. Effects of time-restricted feeding on body weight change, calorie intake, and locomotor activity in normal chow (NC) and cafeteria (CAF) fed rats: (**a**) Weekly body weight change over the experiment; (**b**) Final body weight gain; **(c)** Total daily calorie intake; **(d, e)** Locomotor activity on the 15 th week of the experiment: **(d)** Number of crossing squares; **(e)** Number of rears. NC–A: normal chow *ad lib* feeding; NC–R: normal chow time-restricted feeding; CAF–A: cafeteria diet *ad lib* feeding; CAF–R: cafeteria time-restricted feeding. Values are means ± S.E.M (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ CAF–A vs. NC–A rats; $^{*}p < 0.05$, $^{**}p < 0.01$ NC–R vs. NC–A rats; $^{\times}p < 0.05$, $^{\times\times}p < 0.01$ CAF–R vs. CAF–A rats.

Table 1. Effects of time-restricted feeding on white fat pads weights and total adiposity.

Parameters	NC–A	NC–R	CAF–A	CAF–R
iWAT (g)	2.9 ± 0.2	2.1 ± 0.2 *	8.9 ± 0.4 $^{\#\#}$	3.3 ± 0.1 $^{\times\times}$
mWAT (g)	2.7 ± 0.2	1.0 ± 0.1 **	11.0 ± 0.3 $^{\#\#}$	3.3 ± 0.2 $^{\times\times}$
eWAT (g)	3.4 ± 0.2	1.1 ± 0.2 **	10.5 ± 0.3 $^{\#\#}$	3.5 ± 0.3 $^{\times\times}$
rWAT (g)	2.8 ± 0.1	1.4 ± 0.1 *	8.8 ± 0.4 $^{\#\#}$	3.2 ± 0.2 $^{\times\times}$
Total VAT (g)	8.9 ± 0.4	3.4 ± 0.3 **	30.3 ± 0.4 $^{\#\#}$	10.1 ± 0.4 $^{\times\times}$
Total Adiposity (%)	4.2 ± 0.3	2.5 ± 0.2 **	10.6 ± 0.4 $^{\#\#}$	4.7 ± 0.4 $^{\times\times}$

NC–A: normal chow *ad lib* feeding; NC–R: normal chow time-restricted feeding; CAF–A: cafeteria diet *ad lib* feeding; CAF–R: cafeteria time-restricted feeding; WAT: white adipose tissue; iWAT: inguinal WAT; mWAT: mesenteric WAT; eWAT: epididymal WAT; rWAT: retroperitoneal WAT; VAT: visceral adipose tissue. Values are means ± S.E.M (n = 6). $^{\#\#}p < 0.01$ CAF–A vs. NC–A rats; $^{*}p < 0.05$, $^{**}p < 0.01$ NC–R vs. NC–A rats; $^{\times\times}p < 0.01$ CAF–R vs. CAF–A rats.

3.2. Lipid Profiles and Atherogenic Indices

To determine whether TRF regimen could prevent dyslipidemia in CAF diet-induced obesity, serum lipid profiles and atherogenic indices were evaluated. As shown in Table 2, CAF–A group showed a significant increase in the serum TG, TC, and LDL-c levels compared to NC–A group ($p < 0.01$). After 16 weeks of TRF schedule, the serum levels of TG, TC, and LDL-c in CAF fed rats were significantly decreased by approximately 19.8 %, 27.8 %, 43.5 %, respectively, compared to those of CAF–A group ($p < 0.01$; Table 2). The serum level of HDL-c in CAF–A group was significantly lower than that of NC–A group ($p < 0.01$; Table 2); however, TRF paradigm increased the serum level of HDL-c by approximately 29.6 %, compared to that of CAF–A group ($p < 0.01$; Table 2).

Atherogenic and cardioprotective properties of TRF are also presented in Table 2. Rats fed CAF diet *ad lib* showed significantly higher AIP, AC, and CRI ($p < 0.01$) than rats fed NC *ad lib*. It was interesting to note that TRF regimen ameliorated these atherogenic indices in CAF-fed rats. Indeed, the values of AIP, AC, and CRI were significantly reduced in CAF–R group to the recorded for their *ad lib* counterparts ($p < 0.01$; Table 2), and the values were similar to those of NC–A group. In rats fed with NC-diet, there was no significant difference on either lipid profiles (TG, TC, HDL-c, and LDL-c) or atherogenic indices (AIP, AC, and CRI) between *ad lib* and timed feeding rats ($p > 0.05$; Table 2).

Table 2. Effects of time-restricted feeding on serum lipid profiles and atherogenic indices.

Parameters	NC–A	NC–R	CAF–A	CAF–R
Serum lipid profiles				
TG (mg/dl)	75.1 ± 2.2	71.2 ± 2.1	101.1 ± 1.6 ##	81.0 ± 1.7 **
TC (mg/dl)	64.5 ± 2.8	63.7 ± 2.7	93.8 ± 1.8 ##	68.0 ± 2.3 **
HDL-c (mg/dl)	54.0 ± 3.0	55.2 ± 2.0	38.7 ± 2.3 ##	50.3 ± 2.1 **
LDL-c (mg/dl)	18.6 ± 1.9	15.9 ± 1.4	35.3 ± 1.5 ##	19.8 ± 1.9 **
Atherogenic indices				
AIP	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0 ##	0.2 ± 0.0 **
AC	0.2 ± 0.1	0.2 ± 0.1	1.5 ± 0.1 ##	0.4 ± 0.0 **
CRI	1.2 ± 0.2	1.1 ± 0.1	2.4 ± 0.3 ##	1.3 ± 0.2 **

NC–A: normal chow *ad lib* feeding; NC–R: normal chow time-restricted feeding; CAF–A: cafeteria diet *ad lib* feeding; CAF–R: cafeteria time-restricted feeding; TG: total triglyceride; TC: total cholesterol; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; AIP: atherogenic index of plasma; AC: atherogenic coefficient; CRI: coronary risk index. Values are means ± S.E.M (n = 6). ## $p < 0.01$ CAF–A vs. NC–A rats; ** $p < 0.01$ CAF–R vs. CAF–A rats.

3.3. Morphology of Inguinal Adipose Tissue

To study the potential morphological changes and browning induction in iWAT by the effect TRF regimen, histological analysis were performed in the inguinal depots. These depots were selected because they are those with a greater browning capacity, according to the literature [40]. Our results showed that adipocytes size were significantly higher in rats fed a CAF diet *ad lib* compared to NC diet *ad lib* (5.7 ± 0.4 $\mu m^2 \times 100$ vs. 3.9 ± 0.3 $\mu m^2 \times 100$; $p < 0.01$; Figure 3a) and that TRF paradigm decreased adipocytes size of both NC–R (2.8 ± 0.3 $\mu m^2 \times 100$) and CAF–R (4.0 ± 0.3 $\mu m^2 \times 100$) groups, as compared to their diet-matched *ad lib* groups ($p < 0.01$; Figure 3a). Interestingly, adipocytes size of CAF–R group was decreased nearly to that of NC–A group.

As shown in hematoxylin and eosin staining preparation (Figure 3b), the majority of cells in all groups showed an appearance of white unilocular adipocytes with single lipid droplet. TRF schedule induced the formation of multilocular brown-like adipocytes dispersed among unilocular adipocytes in both NC- and CAF-fed rats. The percentage of multilocularity represented around 31.1% and 15.2% of total area analyzed in the NC–R and CAF–R group, respectively.

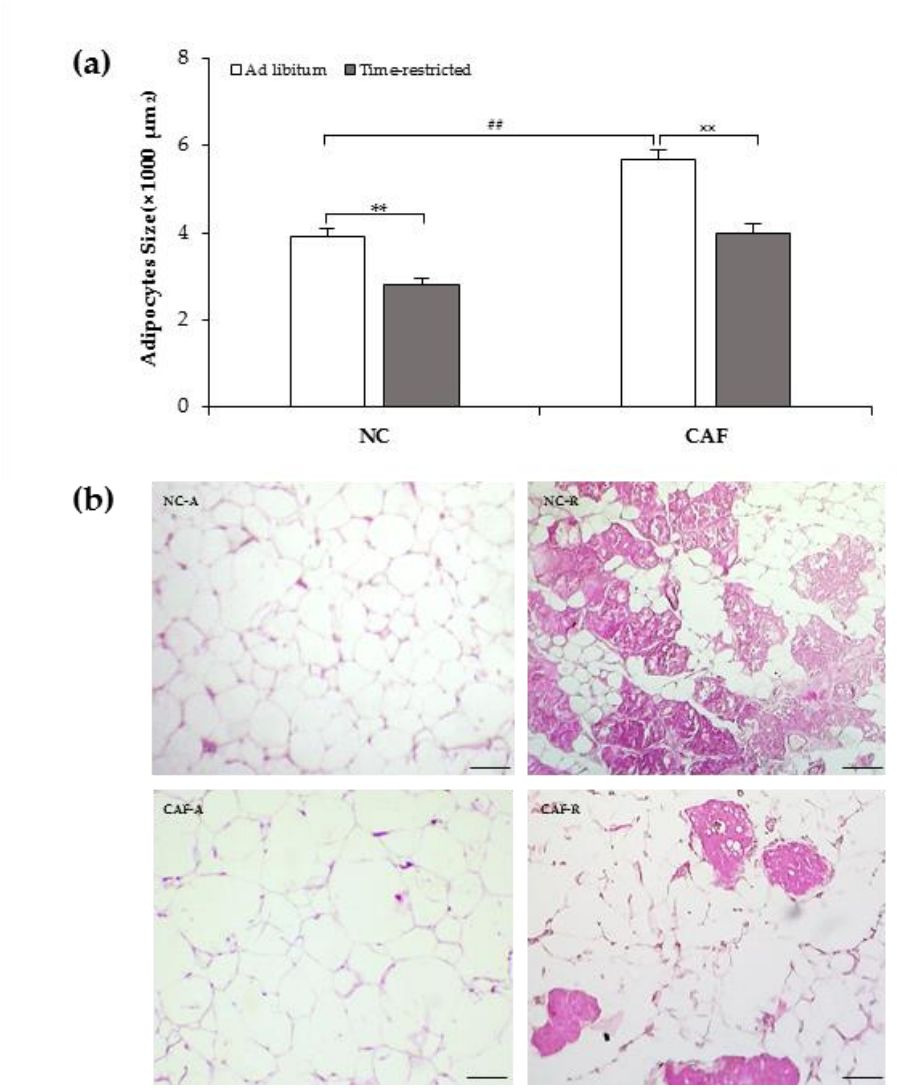

Figure 3. Effect of time-restricted feeding on inguinal WAT morphology of normal chow (NC) and cafeteria (CAF) fed rats: (**a**) Mean adipocyte area was measured using a morphometric quantitative method at ×200 magnification with Axio Vision software; (**b**) Representative hematoxylin and eosin staining of inguinal WAT sections (original magnification ×200): time-restricted feeding regimen induced the appearance of clusters of multilocular brown-like fat cells in normal chow (NC) and cafeteria (CAF) fed rats. NC–A: normal chow *ad lib* feeding; NC–R: normal chow time-restricted feeding; CAF–A: cafeteria diet *ad lib* feeding; CAF–R: cafeteria time-restricted feeding. Values are means ± S.E.M (n = 200 adipocytes/group). $^{\#\#}p < 0.01$ CAF–A vs. NC–A rats; ** $p < 0.01$ NC–R vs. NC–A rats; $^{xx}p < 0.01$ CAF–R vs. CAF–A rats. Scale bar: 50 μm.

3.4. Western Blot Analysis in Inguinal Adipose Tissue

The molecular signature that identifies brown and brown-like adipocytes is UCP1. Therefore, we examined UCP1 protein level in inguinal fat depots of both NC- and CAF-diet fed rats on TRF access using Western blot. As expected from their microscopic aspect, UCP1 protein expression was significantly enhanced in the inguinal fat of NC–R and CAF–R groups by ~ 3 and 2 fold, respectively, as compared to their diet-matched *ad lib* groups ($p < 0.01$; Figure 4b). Importantly, a weak signal of UCP1 was observed in inguinal fat from *ad lib*-fed rats, whereas in TRF-fed rats, they becomes much more pronounced (Figure 4a).

PCG1α is a master nuclear transcription factor that controls the expression of the thermogenic gene program, including the expression of UCP1 gene. Its expression level significantly enhanced in inguinal fat depots of NC–R and CAF–R rats by ~ 1.5 fold, as compared to their *ad lib* counterparts ($p < 0.01$; Figure 4c).

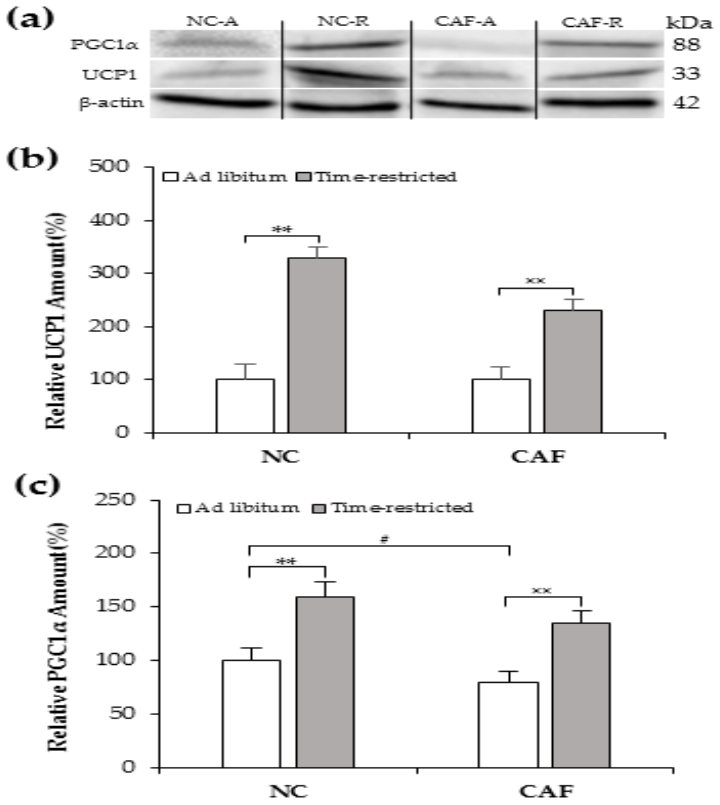

Figure 4. Effect of time-restricted feeding on thermogenic protein levels in the inguinal WAT of normal chow (NC) and cafeteria (CAF) fed rats as measured by Western blot: (**a**) Representative blot of UCP1 and PGC1α; (**b, c)** Densitometry quantification of UCP1 and PGC1α protein levels. NC–A: normal chow *ad lib* feeding; NC–R: normal chow time-restricted feeding; CAF–A: cafeteria diet *ad lib* feeding; CAF–R: cafeteria time-restricted feeding. Values are means ± S.E.M (n = 3) of ratios of specific protein levels to β-actin (Loading protein). Data of the NC–A group was set to 100 % and the rest of the values are referred to this. $^{\#}p < 0.05$ CAF–A vs. NC–A rats; $^{**}p < 0.01$ NC–R vs. NC–A rats; $^{\times\times}p < 0.01$ CAF–R vs. CAF–A rats.

4. Discussion

Here, we report, for the first time, that TRF regimen prevents obesity and lipid metabolic disarrangements in CAF diet-induced obesity models. This effect occurred via browning of the inguinal WAT without changing the calorie intake or locomotor activity.

The results of the present showed that the effects of TRF regimen in CAF-diet-induced obesity rats. Our results showed that consumption of calorically dense, palatable human foods induced an excessive caloric intake resulting in rapid and drastic weight gain, abdominal adiposity, and dyslipidemic state in the *ad lib* fed group, suggesting that the CAF-diet used in the present study successfully induced an animal model of obesity. As shown in Table S1, CAF-diet contained higher caloric and fat and satured fatty acids contents; hence, the weight gain in the CAF- *ad lib* group could be due to the high rate of acylation of saturated fatty acids into triglycerides that is subsequently stored in the adipose tissue [41]. Besides, post-ingestive effects of high fat food contribute to weight gain through reduction of satiety signals and attenuation of fatty acids oxidation [42]. Interestingly, TRF significantly reduced the body weight gain in our rats fed a CAF-diet, suggesting the potential of TRF regimen to counteract the deleterious effect of such a robust obesogenic challenge. Similar findings have also been reported previously in different animal strain after exposure to TRF of other obesogenic diets [8,9,12,19–21,23]. This have also been observed among some overweight or obese humans undergoing time-restricted eating pattern [43,44]. It should be noticed that the body weight gain-lowering effect of TRF in the current study took place in the absence of changes in caloric

intake and locomotor activity, which is consistent with previous studies [8,9,12,19]. Therefore, the mechanism whereby TRF reduced body weight cannot simply be explained by changes in quantity of diet or physical activity, but rather by other mechanisms. One potential explanation to account for attenuated body weight gain without any overall change in calorie intake or physical activity upon TRF is through switching lipid metabolism from storage to oxidative as a strategy to enhance energy expenditure. Although energy metabolism was not measured in the current study, the study by Panda's group showed that 8h-TRF during the active phase attenuated the HFD-induced dampening of the daily rhythm of the respiratory exchange ratio (RER=volume CO2 / volume of O2) and led to an overall increase in VO2 and energy expenditure in mice, indicating lipid utilization for energy metabolism [8]. In another study by the same group, TRF of HFD or normocaloric diet increased PGC 1α gene expression in WAT of mice, leading to increased β-oxidation [9], which corroborate data of the current study.

Our finding of body weight gain was consistent with those of fat accumulation measurements. Notably, rats fed a CAF *ad lib* exhibited drastic increases in fat pad weights (inguinal, mesenteric, epididymal, retroperitoneal), as compared to rats fed a NC *ad lib*. These observations suggest that the exposure of rats to calorically dense diets facilitated fat accumulation in the abdominal regions due to the high effective energy content of high fat foods [45]. Previous studies has observed a marked reduction in adipose tissue mass in rodents under TRF regimen [8][9][23], which is in agreement with our finding showing that TRF of NC or CAF-diet reduced fat accumulation in subcutaneous and visceral fat (inguinal, mesenteric, epididymal, retroperitoneal), resulting in a significant decrease in total adiposity. The reduction of fat accumulation in WAT under TRF may be due to overall increase in fatty acid oxidation and decrease in free fatty acids synthesis [9], which corroborate the current data showing that adipocytes size of iWAT of TRF rats were smaller than those of diet-matched *ad lib* rats.

The excessive weight gain in CAF–A rats was associated with a dyslipidemic condition, as evidenced by abnormally elevated serum levels of TG, TC, and LDL-c and reduced serum level of HDL-c, as compared to NC–A rats, which is consistent with findings of previous study using the CAF-diet [46]. Dyslipidemia was probably evident due to the overall higher consumption of the CAF-diet which while being high in fat, is also high in saturated fatty acids, known to increase the production of TG and LDL-c by the liver [47], and in cholesterol, which can result in an hypercholesterolemia [48] and reduced HDL production or HDL clearance from plasma [49]. Interestingly, TRF regimen prevented CAF-diet-induced lipid profile disorders by decreasing TC, TG, and LDL-c and increasing HDL-c serum levels, suggesting its anti-hyperlipidemic effect; however, unexpectedly, TRF had no effect on dyslipidemic parameters when rats were fed with NC diet, in spite of marked effect on body weight. The lack of TRF effect on dyslipidemic parameters of normocaloric fed rats may indicate a corrective effect of TRF in dyslipidemia, or may indicate that the influence of TRF may be negligible when the overall content of diet is less caloric, which would require future investigation. To our knowledge, only three studies has been reported on the anti-hyperlipidemic potential of TRF in diet-induced obesity models (DIO) so far (Chaix et al. [9], Sherman et al. [23], and Sun et al. [12]). It is important to note that there are differences in obesogenic diets and species used as well as in the feeding window and the experimental duration between the current study and the aforementioned studies, which make direct comparison difficult. For instance, Chaix et al. [9] reported significantly reduced TG serum level in C57/BL6 mice subjected to 9 or 12 h-TRF of HFD for 12 wks when compared to their *ad lib* counterparts; however, when C57/BL6 mice were fed the same diet for 15 h-TRF or fed a less fatty diet, namely normal chow or high fat-high sucrose diet under 9h-TRF, the TG serum level was unchanged. It is worth nothing that this study reported decreased TC serum levels, regardless the obesogenic diet used or the feeding window duration applied. In the study by Sherman et al. [23], 4 h-TRF during 18 wks significantly reduced serum level of TC and increased that of HDL-c in C57/BL6 mice that were fed with HFD, whereas TG serum level was unchanged. Sun et al. [12] reported unchanged TC and reduced TG plasma levels in male Wistar rats that were fed with high-sucrose diet for 12 h-TRF during 4 wks. These dissimilarities between the present and previous studies may be explained by differences in species, obesogenic diet, feeding window, experimental duration, or other factors. The mechanism by which

TRF control lipid parameters is unclear. Typically, weight loss is expected to promote lipid improvements [50]; however, the outcomes of our study do not consistently support improvements in body weight, as despite reduced body weight gain, no change in lipid panel was recorded in rats subjected to NC-diet. Since liver is a central organ in lipid metabolism, we suggest that the beneficial effect of TRF regimen on lipid profiles in rats fed a CAF-diet may be due to the enhancement of hepatic lipid metabolism. This is supported by several studies in which TRF has been found to enhance lipid oxidation over lipogenesis [8,12,21,51]. For example, TRF has been shown to reduce hepatic expression of PPARγ, the key lipogenic gene, in HFD-fed rats [51]. In addition, TRF regimen reduced lipid droplet associated and lipolysis inhibitor gene Cidec expressions, TG storage-associated protein CD36, and plasma TG marker ApoA 4 in HFD-fed mice [8]. Decreased TG in the liver results in reduced serum level of LDL and VLDL, leading to loss of transported cholesterol and TG within them [50]. The TC-lowering effect of TRF may also be attributed to enhanced cholesterol catabolism to bile acids biosynthesis in the liver, as indicated by increased expression of two rate-limiting enzymes of bile acids biosynthesis, namely Hmgcs 2 and Cyp7α1, in the liver of time-restricted mice [9].

It is commonly thought that unfavorable lipid profile, synonymous with intake of dietary saturated fat, is the hallmark for the progression of cardiovascular diseases (CVD) and coronary heart diseases [52]. Observations from epidemiological studies have confirmed that high TC, LDL-c, and TG and low HDL-c levels are associated with increased risk of CVD [52–55]. Together, these data corroborate our findings showing marked increases in AIP, AC, and CR in CAF–A rats relative to NC–A rats. These indices are strong indicators of the CVD risk in clinical practices by its expressions of imbalance between atherogenic and anti-atherogenic lipoproteins [56]. Findings from a recent human pilot study reported the effectiveness of 10-h time-restricted eating intervention for 12 wks to lower atherogenic lipids in obese subjects with metabolic syndrome, leading to improved cardiometabolic health [57]. Herein, TRF reduced values of AIP, AC, and CRI in rats subjected to CAF-diet, suggesting its very interesting cardioprotective potential. Since this is the first study to examine the effects of TRF on changes in atherogenic indices in DIO models, these results should be considered preliminary, therefore, further in-depth investigation is warranted to confirm the cardioprotective properties of TRF intervention under obesogenic challenges.

Since white fat browning is associated with body weight loss and metabolic improvements, we aimed to characterize the morphological and molecular features of iWAT. As predicted, we found that TRF intervention induced an obvious appearance of multilocular brown adipocytes and increased of UCP1 and PGC1α proteins expression in iWAT of rats fed with both NC and CAF-diets, indicating its potential to promote WAT browning. To the best of knowledge, this report is the first to show the potential of TRF regimen to promote WAT browning. Direct experimental evidences for the role of UCP1 in counteracting obesity has been reported previously in many studies. Of note, an animal model of genetic obesity using adipose tissue-targeted overexpression of UCP1 resulted in reduced level of obesity [58]. Also, UCP1 ablation resulted in increased obesity and metabolic deficiency in obesity-resistance mouse strain [59]. In human, UCP1 gene polymorphism was significantly associated with increased body weight index in obese subjects [60]. Therefore, the present data of TRF-induced iWAT browning and UCP1 expression may decipher the possible mechanism behind reduced body weight gain in rats under TRF paradigm. Although the current data support that TRF regimen could act as a WAT browning inducer, further in-depth studies are warranted to understand the effect of TRF regimen on other WAT depots and to investigate the expression of browning-related genes. Furthermore, the present study is limited by the fact that we did not measure whole-body energy expenditure due to practical reasons; hence, to what extent the effects of TRF regimen on WAT browning and UCP1 content could be translated into increased whole-body energy expenditure is unclear, and future studies will address this shortcoming.

5. Conclusions

This present study showed that TRF regimen improved body weight gain and dyslipidemia in CAF-diet model and acts as white fat browning inducer with thermogenic properties, which may explain the anti-obesity effect of TRF.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: normal chow and cafeteria food items compositions as given by the product manufacturers.

Author Contributions: The authors responsibilities are as follow: Research design "Conceptualization", A.A. and S.A.; animal experiment, S.A.; writing—original draft preparation, S.A.; writing—review and editing, A.A.; supervision, A.A. and S.B.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by SAF2016-79794-R grant from the "Ministerio de Economía y Competitividad (Spain)" and by Erasmus + Mobility Program Dimensión Internacional (grant number KA 107) from "European Commission".

Acknowledgments: We would like to express our appreciation to all stuff from "Diabesity-UGR group": (Ana Torrices, Diego Salagre, Juan Miguel Leiva, and Meriem chayah) for their technical supports and for being attentive to answer my research questions and to Asma Amalou form "Physiopathology-USTHB" group for the animal sacrifice assistance. We would also like to express our warmest thanks to Prof. Nemcha Lebaili and her stuff from "École Normale Supérieure (Kouba, Algiers)" for the use of their animal facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. WHO | Overweight and obesity. *WHO* **2018**.
2. Bouchard, C. Gene-environment interactions in the etiology of obesity: Defining the fundamentals. *Obesity* **2008**, *16*, S5-S10, doi:10.1038/oby.2008.528.
3. Brantley, P.; Myers, V.H.; Roy, H.J. Environmental and Lifestyle Influences on Obesity. *J La State Med Soc* **2005**, *157*, S19-27.
4. Procaccini, C.; Carbone, F.; Galgani, M.; La Rocca, C.; De Rosa, V.; Cassano, S.; Matarese, G. Obesity and susceptibility to autoimmune diseases. *Expert Rev. Clin. Immunol.* **2011**, *7*, 287–294, doi:10.1586/eci.11.18.
5. Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature* **2006**, *444*, 860–867.
6. Anderson, J.W.; Konz, E.C.; Frederich, R.C.; Wood, C.L. Long-term weight-loss maintenance : a meta-analysis of US studies. *Am J Clin Nutr*. **2001,** *74(5)*, 579-84.
7. Longo, V.D.; Panda, S. Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. *Cell Metab.* **2016**, *23*, 1048–1059.
8. Hatori, M.; Vollmers, C.; Zarrinpar, A.; DiTacchio, L.; Bushong, E.A.; Gill, S.; Leblanc, M.; Chaix, A.; Joens, M.; Fitzpatrick, J.A.J.; et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab.* **2012**, *15*, 848–860, doi:10.1016/j.cmet.2012.04.019.
9. Chaix, A.; Zarrinpar, A.; Miu, P.; Panda, S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab.* **2014**, *20*, 991–1005.
10. Olsen, M.K.; Choi, M.H.; Kulseng, B.; Zhao, C.M.; Chen, D. Time-restricted feeding on weekdays restricts weight gain: A study using rat models of high-fat diet-induced obesity. *Physiol. Behav.* **2017**, *173*, 298–304, doi:10.1016/j.physbeh.2017.02.032.
11. Cote, I.; Toklu, H.Z.; Green, S.M.; Morgan, D.; Carter, C.S.; Tümer, N.; Scarpace, P.J. Limiting feeding to the active phase reduces blood pressure without the necessity of caloric reduction or fat mass loss. *Am. J. Physiol. Integr. Comp. Physiol.* **2018**, *315*, R751–R758, doi:10.1152/ajpregu.00076.2018.
12. Sun, S.; Hanzawa, F.; Umeki, M.; Ikeda, S.; Mochizuki, S.; Oda, H. Time-restricted feeding suppresses excess sucrose-induced plasma and liver lipid accumulation in rats. *PLoS One* **2018**, *13*,1-18, doi:10.1371/journal.pone.0201261.
13. Sutton, E.F.; Beyl, R.; Early, K.S.; Cefalu, W.T.; Ravussin, E.; Peterson, C.M. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. *Cell Metab.* **2018**, *27*, 1212-1221, doi:10.1016/j.cmet.2018.04.010.

14. Gabel, K.; Hoddy, K.K.; Haggerty, N.; Song, J.; Kroeger, C.M.; Trepanowski, J.F.; Panda, S.; Varady, K.A. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study. *Nutr. Heal. Aging* **2018**, *4*, 345–353, doi:10.3233/NHA-170036.
15. Antoni, R.; Robertson, T.M.; Robertson, M.D.; Johnston, J.D. metabolic physiology in free-living human subjects. *J. Nutr. Sci.* **2018**, *7*,1-13, doi:10.1017/jns.2018.13.
16. Moro, T.; Tinsley, G.; Bianco, A.; Marcolin, G.; Pacelli, Q.F.; Battaglia, G.; Palma, A.; Gentil, P.; Neri, M.; Paoli, A. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *J. Transl. Med.* **2016**, *14*,2-10, doi:10.1186/s12967-016-1044-0.
17. Gill, S.; Panda, S. A Smartphone App Reveals Erratic Diurnal Eating Patterns in Humans that Can Be Modulated for Health Benefits. *Cell Metab.* **2015**, *22*, 789–798, doi:10.1016/j.cmet.2015.09.005.
18. Delahaye, L.B.; Bloomer, R.J.; Butawan, M.B.; Wyman, J.M.; Hill, J.L.; Lee, H.W.; Liu, A.C.; McAllan, L.; Han, J.C.; Van Der Merwe, M. Time-restricted feeding of a high-fat diet in male C57BL/6 mice reduces adiposity but does not protect against increased systemic inflammation. *Appl. Physiol. Nutr. Metab.* **2018**, *43*, 1033–1042, doi:10.1139/apnm-2017-0706.
19. Duncan, M.J.; Smith, J.T.; Narbaiza, J.; Mueez, F.; Bustle, L.B.; Qureshi, S.; Fieseler, C.; Legan, S.J. Restricting feeding to the active phase in middle-aged mice attenuates adverse metabolic effects of a high-fat diet. *Physiol. Behav.* **2016**, *167*, 1–9, doi:10.1016/j.physbeh.2016.08.027.
20. Sundaram, S.; Yan, L. Time-restricted feeding reduces adiposity in mice fed a high-fat diet. *Nutr. Res.* **2016**, *36*, 603–611, doi:10.1016/j.nutres.2016.02.005.
21. Chung, H.; Chou, W.; Sears, D.D.; Patterson, R.E.; Webster, N.J.G.; Ellies, L.G. Time-restricted feeding improves insulin resistance and hepatic steatosis in a mouse model of postmenopausal obesity. *Metabolism.* **2016**, *65*, 1743–1754, doi:10.1016/j.metabol.2016.09.006.
22. Park, S.; Yoo, K.M.; Hyun, J.S.; Kang, S. Intermittent fasting reduces body fat but exacerbates hepatic insulin resistance in young rats regardless of high protein and fat diets. *J. Nutr. Biochem.* **2017**, *40*, 14–22, doi:10.1016/j.jnutbio.2016.10.003.
23. Sherman, H.; Genzer, Y.; Cohen, R.; Chapnik, N.; Madar, Z.; Froy, O. Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB J.* **2012**, *26*, 3493–3502, doi:10.1096/fj.12-208868.
24. Chaix, A.; Lin, T.; Le, H.D.; Chang, M.W.; Panda, S. Time-Restricted Feeding Prevents Obesity and Metabolic Syndrome in Mice Lacking a Circadian Clock. *Cell Metab.* **2019**, *29*, 303-319, doi:10.1016/j.cmet.2018.08.004.
25. Sherman, H.; Frumin, I.; Gutman, R.; Chapnik, N.; Lorentz, A.; Meylan, J.; le Coutre, J.; Froy, O. Long-term restricted feeding alters circadian expression and reduces the level of inflammatory and disease markers. *J. Cell. Mol. Med.* **2011**, *15*, 2745–2759, doi:10.1111/j.1582-4934.2010.01160.x.
26. Buettner, R.; Schölmerich, J.; Bollheimer, L.C. High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity* **2007**, *15*, 798–808.
27. Nilsson, C.; Raun, K.; Yan, F.F.; Larsen, M.O.; Tang-Christensen, M. Laboratory animals as surrogate models of human obesity. *Acta Pharmacol. Sin.* **2012**, *33*, 173–181.
28. Sampey, B.P.; Vanhoose, A.M.; Winfield, H.M.; Freemerman, A.J.; Muehlbauer, M.J.; Fueger, P.T.; Newgard, C.B.; Makowski, L. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. *Obesity* **2011**, *19*, 1109–1117, doi:10.1038/oby.2011.18.
29. Gomez-Smith, M.; Karthikeyan, S.; Jeffers, M.S.; Janik, R.; Thomason, L.A.; Stefanovic, B.; Corbett, D. A physiological characterization of the Cafeteria diet model of metabolic syndrome in the rat. *Physiol. Behav.* **2016**, *167*, 382–391, doi:10.1016/j.physbeh.2016.09.029.
30. Lewis, A.R.; Singh, S.; Youssef, F.F. Cafeteria-diet induced obesity results in impaired cognitive functioning in a rodent model. *Heliyon* **2019**, *5*,1-23, doi:10.1016/j.heliyon.2019.e01412.
31. Shafat, A.; Murray, B.; Rumsey, D. Energy density in cafeteria diet induced hyperphagia in the rat. *Appetite* **2009**, *52*, 34–38, doi:10.1016/j.appet.2008.07.004.
32. Panda, S. Circadian physiology of metabolism. *Science* **2016**, *354*, 1008–1015.
33. Chaix, A.; Zarrinpar, A. The effects of time-restricted feeding on lipid metabolism and adiposity. *Adipocyte* **2015**, *4*, 319–324, doi:10.1080/21623945.2015.1025184.
34. Harms, M.; Seale, P. Brown and beige fat: Development, function and therapeutic potential. *Nat. Med.* **2013**, *19*, 1252–1263.

35. Jiménez-Aranda, A.; Fernández-Vázquez, G.; Campos, D.; Tassi, M.; Velasco-Perez, L.; Tan, D.X.; Reiter, R.J.; Agil, A. Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. *J. Pineal Res.* **2013**, *55*, 416–423, doi:10.1111/jpi.12089.
36. Fan, S.J.; Jiang, H.; Yang, L.J.; Liu, X.; Song, J.; Pan, F. Effects of adrenergic agents on stress-induced brain microstructural and immunochemical changes in adult male Wistar rats. *Ann. Anat.* **2011**, *193*, 418–424, doi:10.1016/j.aanat.2011.06.001.
37. Althunibat, O.Y.; Al Hroob, A.M.; Abukhalil, M.H.; Germoush, M.O.; Bin-Jumah, M.; Mahmoud, A.M. Fisetin ameliorates oxidative stress, inflammation and apoptosis in diabetic cardiomyopathy. *Life Sci.* **2019**, *221*, 83–92, doi:10.1016/j.lfs.2019.02.017.
38. Adedokun, A.K.; Olisekodiaka.; Japhet, O.M.; Adetunji, A.D.; Musa, M.A.; Hammed, O.O.; Onifad, A.; Abdufatah, A.; Taofik, A.A.; Kabiru, A.A.; Roji, S.M.; Castelli Risk Index, Atherogenic Index of Plasma, and Atherogenic Coefficient: Emerging Risk Predictors of Cardiovascular Disease in HIV-Treated Patients. *Saudi J. Med. Pharm. Sci.* **2017**, *3*, 1101-1110.
39. Seki, M.; Ishiguro, T.; Gyohda, Y.; Ohsato, S.; Yokota, M. Evaluation of the Efficacy of Coronary Risk Index, a New Scoring System for Predicting Morbidity and Severity of Coronary Stenosis in Patients Undergoing Coronary Angiography. *J. JAPAN Soc. Clin. Anesth.* **1998**, *18*, 271–276, doi:10.2199/jjsca.18.271.
40. Zuriaga, M.A.; Fuster, J.J.; Gokce, N.; Walsh, K. Humans and Mice Display Opposing Patterns of "Browning" Gene Expression in Visceral and Subcutaneous White Adipose Tissue Depots. *Front. Cardiovasc. Med.* **2017**, *4*, doi:10.3389/fcvm.2017.00027.
41. Storlien, L.H.; Huang, X.F.; Lin, S.; Xin, X.; Wang, H.Q.; Else, P.L. Dietary Fat Subtypes and Obesity. In Fatty Acids and Lipids. *New Findings* **2000**, *88*, 148–154.
42. Little, T.J.; Horowitz, M.; Feinle-Bisset, C. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. *Am J Clin Nutr* **2007**, *86(3)*, 531-41, doi: 10.1093/ajcn/86.3.531.
43. Gabel, K.; Hoddy, K.K.; Haggerty, N.; Song, J.; Kroeger, C.M.; Trepanowski, J.F.; Panda, S.; Varady, K.A. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study. *Nutr. Heal. Aging* **2018**, *4*, 345–353, doi:10.3233/NHA-170036.
44. Antoni, R.; Robertson, T.M.; Robertson, M.D.; Johnston, J.D. A pilot feasibility study exploring the effects of a moderate time-restricted feeding intervention on energy intake, adiposity and metabolic physiology in free-living human subjects. *J. Nutr. Sci.* **2018**, *7*, doi:10.1017/jns.2018.13.
45. DeLany, J.P.; Windhauser, M.M.; Champagne, C.M.; Bray, G.A. Differential Oxidation of Individual Dietary Fatty Acids in Humans. *Am J Clin Nutr*. **2000**, 72, 905-11, doi: 10.1093/ajcn/72.4.905.
46. MacEdo, I.C.; Medeiros, L.F.; Oliveira, C.; Oliveira, C.M.; Rozisky, J.R.; Scarabelot, V.L.; Souza, A.; Silva, F.R.; Santos, V.S.; Cioato, S.G.; et al. Cafeteria diet-induced obesity plus chronic stress alter serum leptin levels. *Peptides* **2012**, *38*, 189–196, doi:10.1016/j.peptides.2012.08.007.
47. RJ, N. Dietary Fat Saturation Effects on Low-Density-Lipoprotein Concentrations and Metabolism in Various Animal Models. *Am. J. Clin. Nutr.* **1997**, *65*, doi:10.1093/AJCN/65.5.1617S.
48. Zulet, M.A.; Barber, A.; Martínez, J.A.; Garcin, H.; Higueret, P. Alterations in Carbohydrate and Lipid Metabolism Induced by a Diet Rich in Coconut Oil and Cholesterol in a Rat Model. *J. Am. Coll. Nutr.* **1999**, *18*, 36–42, doi:10.1080/07315724.1999.10718825.
49. Thomas, M.S.; Prack, M.M.; Dashti, N.; Johnson, F.; Rudel, L.L.; Williams, D.L. Differential Effects of Dietary Fat on the Tissue-Specific Expression of the Apolipoprotein A-I Gene: Relationship to Plasma Concentration of High Density Lipoproteins. J Lipid Res. **1989**, 30(9):139-403.
50. Santos, H.O.; Macedo, R.C.O. Impact of intermittent fasting on the lipid profile: Assessment associated with diet and weight loss. *Clin. Nutr. ESPEN* **2018**, *24*, 14–21.
51. Woodie, L.N.; Luo, Y.; Wayne, M.J.; Graff, E.C.; Ahmed, B.; O'Neill, A.M.; Greene, M.W. Restricted feeding for 9 h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice. *Metabolism* **2018**, *82*, 1–13, doi:10.1016/j.metabol.2017.12.004.
52. Jellinger, P.S.; Smith, D.A.; Mehta, A.E.; Ganda, O.; Handelsman, Y.; Rodbard, H.W.; Shepherd, M.D.; Seibel, J.A. American association of clinical endocrinologists' guidelines for management of dyslipidemia and prevention of atherosclerosis. *Endocr. Pract.* **2012**, *18*, 1–78.
53. Graham, I.; Cooney, M.T.; Bradley, D.; Dudina, A.; Reiner, Z. Dyslipidemias in the prevention of cardiovascular disease: Risks and causality. *Curr. Cardiol. Rep.* **2012**, *14*, 709–720, doi:10.1007/s11886-012-0313-7.

54. Wilson, P.W.F.; D'Agostino, R.B.; Levy, D.; Belanger, A.M.; Silbershatz, H.; Kannel, W.B. Prediction of Coronary Heart Disease Using Risk Factor Categories. *Circulation* **1998**, *97*, 1837–1847, doi:10.1161/01.
55. Boullart, A.C.I.; De Graaf, J.; Stalenhoef, A.F. Serum triglycerides and risk of cardiovascular disease. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2012**, *1821*, 867–875.
56. Millán, J.; Pintó, X.; Muñoz, A.; Zúñiga, M.; Rubiés-Prat, J.; Pallardo, L.F.; Masana, L.; Mangas, A.; Hernández-Mijares, A.; González-Santos, P.; et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc. Health Risk Manag.* **2009**, *5*, 757–765.
57. Wilkinson, M.J.; Manoogian, E.N.C.; Zadourian, A.; Lo, H.; Fakhouri, S.; Shoghi, A.; Wang, X.; Fleischer, J.G.; Navlakha, S.; Panda, S.; et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. *Cell Metab.* **2020**, *31*, 92-104.e5, doi:10.1016/j.cmet.2019.11.004.
58. Kopecky, J.; Clarke, G.; Enerbäck, S.; Spiegelman, B.; Kozak, L.P. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J. Clin. Invest.* **1995**, *96*, 2914–2923, doi:10.1172/JCI118363.
59. Luijten, I.H.N.; Feldmann, H.M.; von Essen, G.; Cannon, B.; Nedergaard, J. In the absence of UCP1-mediated diet-induced thermogenesis, obesity is augmented even in the obesity-resistant 129S mouse strain. *Am. J. Physiol. - Endocrinol. Metab.* **2019**, *316*, E729–E740, doi:10.1152/ajpendo.00020.2019.
60. Chathoth, S.; Ismail, M.H.; Vatte, C.; Cyrus, C.; Al Ali, Z.; Ahmed, K.A.; Acharya, S.; Al Barqi, A.M.; Al Ali, A. Association of Uncoupling Protein 1 (UCP1) gene polymorphism with obesity: A case-control study. *BMC Med. Genet.* **2018**, *19*, 203-2016, doi:10.1186/s12881-018-0715-5.