Journal of Applied Molecular Biology (JAMB)
Molecular biology Research & Studies Institute, Assiut University, Egypt
ISSN 2974 – 4008
Volume 3 (2): July 2025

https://jamb.journals.ekb.eg

Comparative Effects of Time-Restricted Feeding and Alternate-Day Fasting on Weight Gain, Metabolism, and Adipose Tissue Gene Expression in Mice

Salma M. Abdelsamea[1,2], Sara A. Abouelmagd[3,4*], Asmaa A.A. Hussein[5], Mahmoud Abd-Elkareem[6], Sara A. Atta[7], Ikramy A. Khalil[3]

[1]Department of Biotechnology, Molecular Biology Research and Studies Institute, Assiut University, Assiut, 71526, Egypt.

[2]Faculty of Biotechnology, Badr University in Assiut, Assiut, 71526, Egypt.

[3]Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, 71526, Egypt.

[4]Institute of Drug Research, Innovation and Development Research, Assiut University, Assiut, 71526, Egypt.

[5] Department of Animal Hygiene & Zoonoses Department, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt.

[6]Department of Cell and Tissues, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt

[7]Medical Biochemistry Department, Faculty of Medicine, Assiut University, Assiut71526, Egypt.

*Corresponding Author: sabouelm@aun.edu.eg

ARTICLE INFO

Article History:
Received: 30-5-2025
Accepted: 07-07-2025
Online: 27-07-2025

Keywords:
Intermittent fasting, UCP-1, adipose tissue, restricted feeding, metabolic syndrome, obesity.

ABSTRACT

Intermittent fasting (IF) is an eating pattern that restricts food and energy intake for a specific time. Research has demonstrated that various IF eating patterns offer health advantages, such as weight reduction, enhanced metabolism, and decreased inflammation. The aim is to further our understanding of how IF patterns affect weight gain, regulate metabolism, and change fat deposition and gene expression in adipose tissue depots in animal models. We examined the effect of two 15-week IF regimens (time-restricted feeding (TRF), 6 hr of feeding and 18 hr of fasting every day, and alternate-day fasting (ADF), alternating between 24 hr of feeding and 24 hr of fasting). A normal-chow diet (ND) or high-fat diet (HFD) was used. Weight, caloric consumption, and blood glucose level in fasted mice were monitored. Cumulative blood glucose, lipid profile, liver enzymes, histology, and gene expression in adipose tissue were determined at the end. The level of gene expression of Uncoupling Protein 1 (UCP-1) was examined in adipose tissues to determine effects of IF patterns on energy expenditure. Our results indicate that during the tested time, the ADF pattern of IF was more efficient compared to the TRF pattern in terms of weight control and can reverse the effects of HDF. IF

significantly reduced food consumption and increased the level of the UCP-1 gene in adipocytes. IF is an effective method of controlling weight gain and reversing the HFD effects. A long-term genetic change may be responsible for controlling obesity and improving energy expenditure.

1. INTRODUCTION

Obesity is a chronic disease marked by increased fat accumulation in the body. It is mostly attributed to a sedentary lifestyle, decreased physical activity, and high-energy diet consumption [1]. It is acknowledged as a contributing risk factor for cardiovascular diseases, insulin resistance, type II diabetes mellitus, fatty liver, and some types of cancer [1]. Since the obesity rate is increasing, examining various dietary regimens and understanding how they affect calorie intake, fat accumulation, and energy expenditure, while avoiding drugs' adverse reactions and unpredictable therapeutic effects, is essential [2, 3].

Adipose tissue (AT) is a fundamental component of weight gain and has a vital role in obesity emergence and progression [4]. It is a metabolically active organ that regulates metabolism and energy storage in response to diet variations [5]. The two main kinds of mammalian adipose tissue are white (WAT) and brown (BAT) [2]. Additionally, a unique type of adipose tissue—referred to as beige or "brite"—was identified within the past decade [4]. White adipocytes act to store lipids, which is linked to the development of obesity. Conversely, brown adipocytes are smaller cells rich in mitochondria and have a specific function of expending energy and regulating core body temperature by producing heat through thermogenesis [2]. Thermogenesis is controlled by a specific protein known as uncoupling protein 1 (UCP-1), which is mainly located in brown adipocytes. UCP-1 acts via uncoupling of oxidative phosphorylation from ATP production [6].

Beige adipose tissue forms within WAT in response to appropriate stimuli such as exposure to cold, exercise, and activation of the beta 3-adrenergic receptor (β3-AR) [7]. It has traits common to both WAT and BAT [6]. Beige adipocytes have higher UCP-1 expression than WAT and share BAT's energy-expending traits, including small lipid droplets and dense mitochondria. The "browning" of WAT into beige adipocytes is a potential strategy for obesity treatment. Prolonged BAT activation may aid in weight management and metabolic homeostasis, making the conversion of WAT to BAT a promising and safe approach, especially for individuals with low BAT levels [8].

Intermittent fasting (IF) is a pattern of diet that restricts food and energy intake for a specific time [9]. IF has different facets but shares a fundamental premise, which is taking periodic breaks from eating [10]. IF eating patterns, which involve consuming food within shorter time intervals (usually <12 hr daily), were found to have many health benefits [11]. These benefits were reported for aging, neurodegenerative disorders, metabolic syndrome, and cancer [12-14]. Nevertheless, it is uncertain whether IF's metabolic advantages are due to the specific eating patterns or the actual meal composition and whether these benefits are associated with physiological and molecular alterations in WAT and other tissues.

In rodents, a high-fat diet (HFD) simulates diet-related obesity and metabolic disturbances [15]. A study on a rat model demonstrated that 20 weeks of HFD resulted in an increase in adiposity and glucose intolerance [16]. The C57BL/6 mouse model exhibited elevations in serum cholesterol and impairment of glucose tolerance within 12 weeks of an HFD [17].

The impact of different IF patterns on weight gain, metabolism, fat deposition, and gene expression in adipose tissue remains poorly understood. Most studies have focused on a single IF type, primarily alternate-day fasting (ADF), in HFD-fed rodents. A direct comparison of IF patterns is needed to assess their effectiveness and metabolic effects. It is also unclear whether IF influences WAT browning. This study evaluates various IF patterns in mice on a normal chow diet (ND) or HFD as a non-pharmacological strategy to mitigate HFD-induced metabolic disturbances and regulate weight gain. Our goal is to determine the most effective IF regimen for obesity control and explore its role in WAT-to-beige adipocyte transformation.

2. MATERIALS and METHODS

2.1. Animals

The study was performed using eight-week-old female BALB/c mice, with an average body weight of 27 grams. All animals were weighed and housed in well-ventilated cages with sawdust bedding with 3-5 mice in each cage and supplied with water and chow ad libitum for one week as an acclimation period before the start of the experiment. All experimental procedures were performed and recorded in accordance with the ARRIVE guidelines. After the acclimation period, 30 mice were assigned randomly into two separate groups, 1 and 2 (n=15), and subjected to different diet content and regimens for 15 weeks (**Figure 1**).

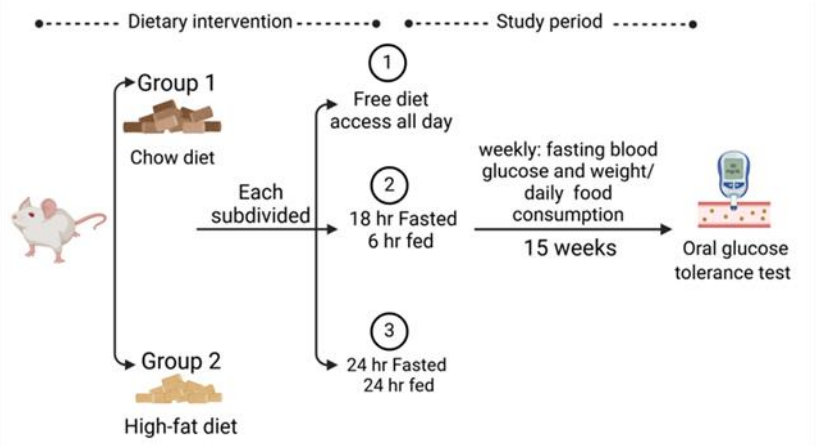

Figure 1. Schematic diagram showing the design of the study.

Group 1 was provided with a normal chow diet (ND) in the feeding period and subdivided into three subgroups as follows: *ad libitum* as a control (ND-C), time-restricted feeding (ND-TRF), where they were fed for only 6 hr (in the daytime/light phase) per day, and ADF (ND-ADF), where mice alternated between a day of fasting and a day of *ad libitum* feeding. Group 2 was subdivided, the same as group 1, but with HFD feeding as follows: HFD-C, HFD-TRF, and HFD-ADF. Details of diet composition are listed in **supplementary information**.

2.2. Body weight, food, and energy intake measurement

Body weight was recorded weekly before food introduction. Daily food intake was assessed by measuring the difference between food amounts supplied to the animals and remaining food in the cage, then dividing the result by the number of mice housed in each

cage to estimate the average food consumption per mouse. Energy intake was assessed by multiplying the quantity of food consumed by its corresponding calorie content.

2.3. Blood glucose measurements

Weekly measurements of fasting blood glucose levels were conducted using a glucometer (OKmeter Match II Blood Glucose Monitoring System, OK Biotech Co., Ltd, Taiwan). To collect blood for fasting blood glucose measurements, soft tissue amputation of a small portion of the distal tail tip was done.

2.4. Oral blood glucose tolerance test (OGTT)

OGTT was carried out one day prior to the conclusion of each experiment (15^{th} week). Mice were fasted from the night before, followed by the administration of glucose through intragastric gavage at a dosage of 2 grams for each kilogram of body weight. Samples of the blood were obtained from the mice's tails prior to administering glucose gavage at time 0 (baseline) and at 20, 40, 60, 90, and 120 minutes after gavage to measure glucose levels. The two intermittent diet groups implemented overnight fasting following the interval of HFD feeding.

2.5. Animal sacrifice and collection of samples

Upon completion of the experiment period (15th week), all mice belonging to group 2 and the control group in group 1 (ND-C) were deprived of food, but not water, overnight. Blood and tissue samples were obtained on the day of sacrifice. Briefly, mice were anesthetized to prevent pain and distress. Then, blood samples were drawn from the retro-orbital sinus utilizing a capillary tube. Following cervical dislocation as the method of euthanasia, tissue specimens were promptly extracted. White fat samples were collected from the abdominal cavity (visceral fat), while brown fat samples were collected from the fat pad in the back of the neck (interscapular fat).

For blood samples, ~0.5 mL was collected in blood collection tubes that were capped and centrifuged (5000 rpm, 15 min) for serum separation. The serum was preserved for further analysis at -20°C. For adipose tissue, samples were taken rapidly, rinsed with saline, dissected, and fixed in 10% formalin solution for histological analysis. Also, separate interscapular brown and visceral white adipose tissue samples were collected and stored at -80°C for gene expression analysis.

2.6. Biochemical analysis

Samples of serum were tested to determine alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), and glucose using kits with catalog no. 265-001, 230-003, 314-001, and 255-004, respectively. The biochemical tests were conducted using the standard procedures of Spectrum Diagnostics commercial kits, MDSS, GmbH, Hannover, Germany.

2.7. Histological analysis

2.7.1. Preparation of samples

Fat samples were fixed in neutral buffered formalin and processed for paraffin embedding. Briefly, samples were fixed in neutral buffered formalin (pH=7.2) fixative. It was composed of 100 ml of 40% formaldehyde, 900 ml of distilled water, 4 g of sodium

dihydrogen phosphate monohydrate, and 6.5 g of disodium hydrogen phosphate anhydrous. Then, fixed tissue samples were dehydrated in ascending grades of alcohol (70 to 100%), cleared in methyl benzoate, and embedded in paraffin wax [18]. Serial sections of 4-5 µm thickness were cut by a Reichert Microtome (LEICA 2155rm automatic microtome, Germany) and mounted on glass slides. Finally, sections were kept in an incubator at 40°C overnight to dry.

2.7.2. Staining and examination

Sections were deparaffinized in xylene. Then, sections were rehydrated in descending grades of ethanol (100 to 70%) and distilled water. Hematoxylin and eosin (H&E) staining was conducted as reported [19]. Tissue sections were dehydrated again in an ascending grade of ethanol (70 to 100%), cleared in xylene (2 x 10 min), and paraffin sections were covered and examined by the light microscope.

2.7.3. Image morphometric analysis

Analysis was performed to assess the complex color micrographs that were obtained and to examine the distribution of fat [20]; [21]. To estimate the relative fat content of different samples analyzed, image analysis was carried out. Tissue images, captured at x500, x200, x100, and x50 magnification, were first adjusted for contrast using Digimizer image analysis software, Digimizer version 6.4.0, 2005-2024 MedCalc Software LTD (www.Digimizer.com), where the contents of fat cells were colorized distinctively from the background and surrounding connective tissue (Supplementary Figures 1 and 2). Further analysis was conducted for these images through the Image Color Summarizer program (version: 1.0.3.0, 2024, Leizersoft.com) to quantify the area of fat cells in different samples. The image field % was used to quantify the areas (area % in pixels) by applying the color clusters at high precision (150 px) for sections per tissue sample. Average area percentages for different samples were plotted.

2.8. Quantitative polymerase chain reaction (RT-qPCR)

The total RNA of adipose tissue samples was extracted with the RNA extraction kit (ABT total RNA Mini Extraction Kit (spin column), Applied Biotechnology, Egypt; Catalogue number: ABT002); the total amount of extracted RNA was evaluated using NanoDrop (Thermo Scientific, USA). The complementary DNA (cDNA) needed for qRT-PCR was obtained using the ABT H-minus MMLV cDNA synthesis kit (Cat. No.: ABT009) (Applied Biotechnology, Egypt). qPCR was carried out with the QuantStudio Real-Time PCR instrument and SYBR (HERAplus qPCR Master). The expression of the target gene, UCP-1, was normalized using GAPDH. The expression intensity of the gene was analyzed by qPCR. Primer and GAPDH sequences are shown in Supplementary Table 1. Relative mRNA expression ratio was calculated using the comparative cycle threshold method (ΔΔCT method) as the difference between the target gene's number of cycles and the endogenous control [22].

2.9. Statistical analysis

Unpaired Student’s t-test was used to perform statistical analysis, ordinary one-way ANOVA followed by Tukey’s multiple comparisons test, or two-way ANOVA followed by Šídák's multiple comparisons test [23]. Excel and GraphPad Prism (version 9.0, GraphPad Software Inc., CA, USA) were used to conduct data analysis. Results are

presented as mean ± SD, with a p-value less than 0.05 regarded as statistically significant (ns = $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

3. RESULTS and DISCUSSION

3.1. Effect of different patterns of IF on body weight

The effect of various IF patterns on the body weight of mice fed on ND was examined. Mice were divided into 3 groups; all fed on ND but differing in the time the feeding was restricted. The first group was exposed to the diet all the time and used as a control (ND-C). The second group was allowed to eat for a restricted time of day (6 hr) (ND-TRF). The third group was allowed to eat every alternate day (ND-ADF). Mice body weight was recorded weekly for a total of 15 weeks (Figure 2). Within week 1, there was an initial increase in body weight for all groups consuming ND (Figure 2a). From week 1 to week 9, some weight fluctuations (decrease followed by increase and vice versa) were observed until week 9, where the ND-ADF group demonstrated a transient reduction in body weight (-8.8%, p=0.20 for % weight change; weeks 0 - 9, Student's t-test) compared to the ND-C group (+31.3%, p=0.0004 for % weight change; weeks 0 - 9, Student's t-test) and the ND-TRF group (+19.88, p=0.15 for % weight change; weeks 0 - 9, Student's t-test). Then, the weight of the ND-ADF group increased again from week 9 until the end of the experiment.

When considering the initial vs. terminal body weight across all three groups (Figure 2b), there was a statistically significant increase for the group consuming a normal diet *ad libitum* (+30.49%, p=0.0017 for % weight change), while mice undergoing IF did not show a significant increase after 15 weeks (ND-TRF, +13.1%, p=0.3. ND-ADF, +4.81%, p=0.4 for % weight change). These results show that the two IF regimens provided protection against increased body weight, and the ND-ADF regimen probably had higher body weight control than the ND-TRF. Similar results were previously reported, where body weight remained statistically unchanged between the control and TRF groups after 38 weeks of fasting [24]. It should be taken into consideration regarding the effect of TRF that the time of feeding may affect weight gain and caloric intake because mice are nocturnal (mostly consume and metabolize food at night), so the food intake during the light phase can be a reason for greater weight increase compared to ADF [25, 26].

For groups consuming HFD (Figure 2c and d), in the first two weeks, there was a weight increase in the HFD-C group (+9.61%, p= 0.0208), but with a lesser degree than that of the ND-C group (+22.89%, p< 0.0001). The HFD-ADF group exhibited no significant change in weight in the first two weeks. In the HFD-TRF group, the weight increased in the first week, then decreased in the second week. In the remaining period, weight in the HFD-C group continued to increase gradually. The HFD-ADF group showed weight fluctuations until the last two weeks, when it showed a weight decrease. The HFD-TRF group showed a slight increase from week 2 to week 11, followed by fluctuations (Figure 2c).

Regarding the initial vs. terminal body weight, there was a statistically significant increase in the HFD-C group (+31.61%, p=0.0002), and there was no significant difference between the final weights in the ND-C and HFD-C groups.

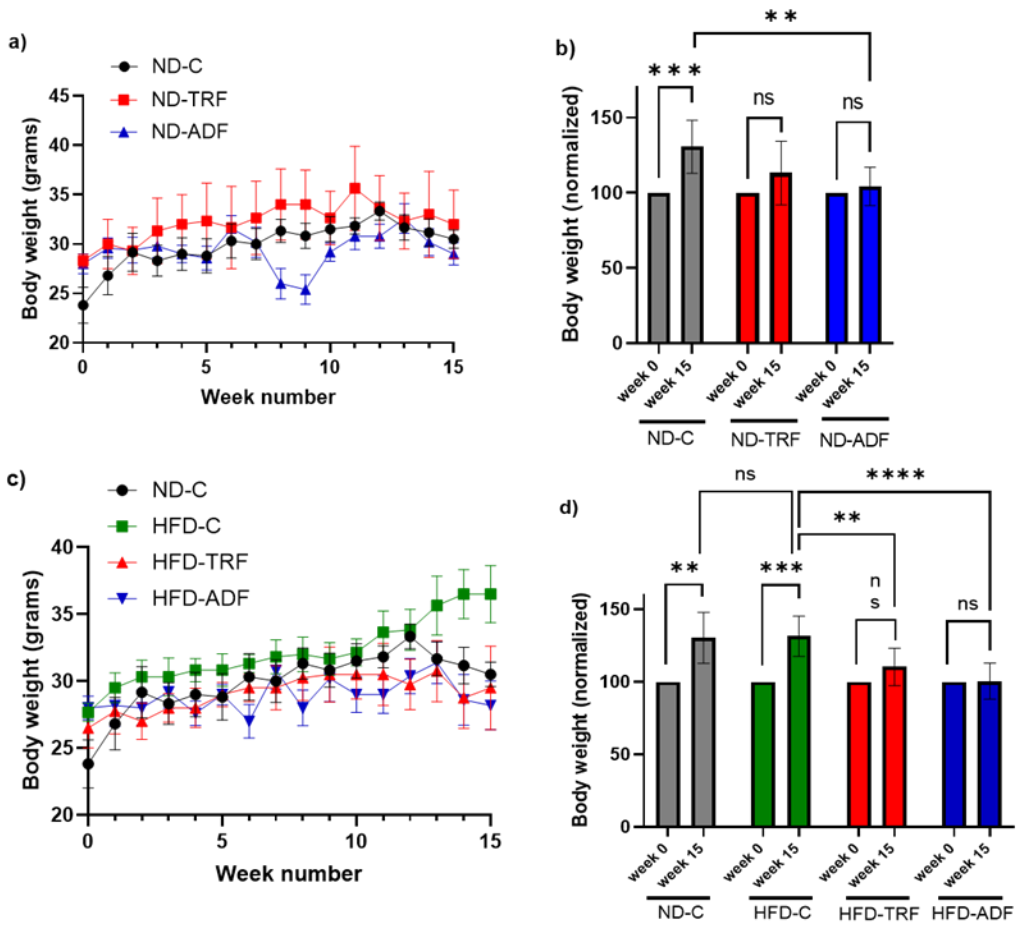

Figure 2. Effects of IF on mice observed at two regimens, ADF or TRF, in comparison to *ad libitum* feeding as a control (ND-C), tested using a normal chow diet (ND): (a) effect on body weight during the 15 weeks of the experiment, (b) initial (week 0) vs. final body weight (week 15), (c) effect on body weight for mice feeding on a high-fat diet (HFD), and (d) initial vs. final body weight for HFD-fed mice.

Meanwhile, mice undergoing IF did not show a significant change in body weight after 15 weeks (HFD-TRF, +10.51%, p = 0.156; HFD-ADF, +0.66%, p = 0.91). There was a significant difference between the final weight in the HFD-C group and both IF regimen groups (Figure 2d). When comparing body weight, weight gain was more effectively regulated in the IF groups than the HFD-C group. These results indicate that IF can control weight gain even with HFD and that the ADF regimen leads to better weight control. Similar results regarding the effect of TRF and ADF with HFD on body weight were previously reported [15 , 17] .

3.2. Energy intake

The calorie intake in each group was measured daily and calculated every 48 hr to avoid the impact of dietary switch in the ADF groups (Figure 3a and c). For the groups consuming ND, the ND-TRF group showed a lower level of energy intake than the ND-C group until the 10th week (from day 70), where the energy intake increased and reached that of the ND-C group. Regarding the ND-ADF group, energy intake was significantly lower than the ND-C and ND-TRF groups and continued at the same level until the end of the experiment (Figure 3a).

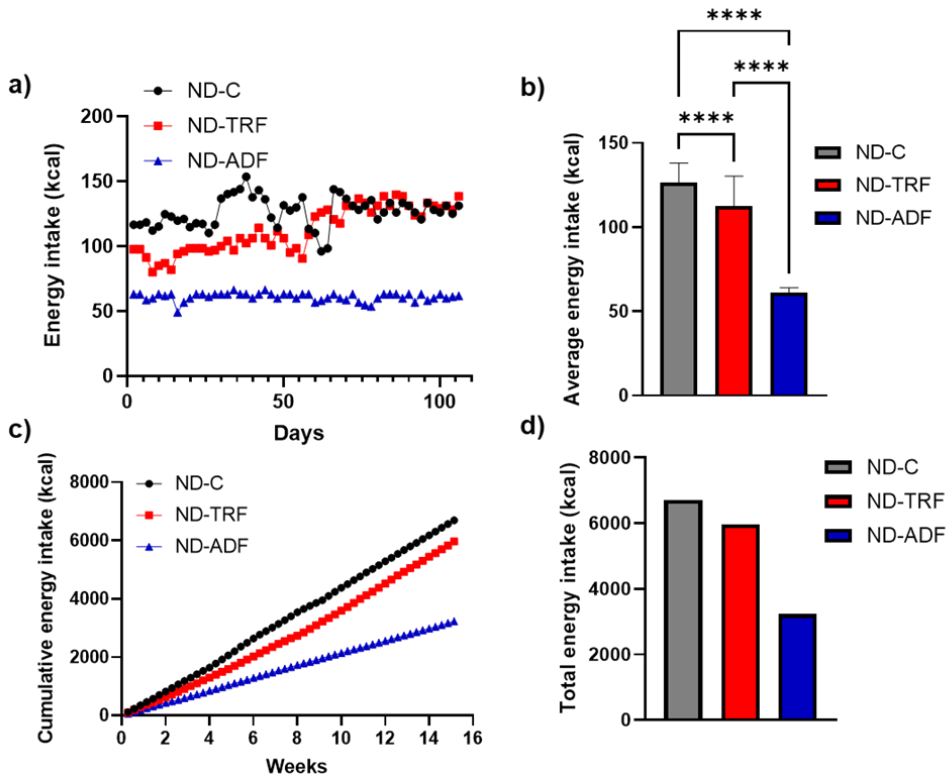

Figure 3. Effects of IF on mice observed at two regimens, ADF or TRF, in comparison to ND-C, tested using an ND: (a) effect on average energy intake (kcal) per mouse every 48 hr during the 15 weeks of the experiment, (b) average energy intake per mouse every 48 hr during the period of the experiment, (c) cumulative energy intake per mouse over the course of the experiment, (d) total energy intake during 15 weeks of IF.

Figures 3b and d show the average energy intake per mouse every 48 hr and the total energy intake over the 15 weeks. A statistically significant variation in caloric intake was observed among the three groups ($p < 0.0001$). The ADF group showed the lowest caloric intake, and the ND-C had the highest (Figure 3b). This indicates that the reduction in mouse body weight in the IF groups is attributed to a reduction in caloric intake. It seems that the ADF regimen results in a more pronounced caloric deficit over time (Figure 3c). The TRF regimen may allow mice to eat enough during the eating window to maintain normal caloric intake. Hence, TRF doesn't necessarily result in as pronounced a caloric deficit as ADF does [27]. In the groups consuming HFD, the HFD-C group showed the highest caloric intake, while the HFD-TRF group showed lower intake and did not show a significant difference when compared to the ND-C group. The HFD-ADF regimen showed the lowest intake of all groups (Figure 4). This is consistent with our body weight results.

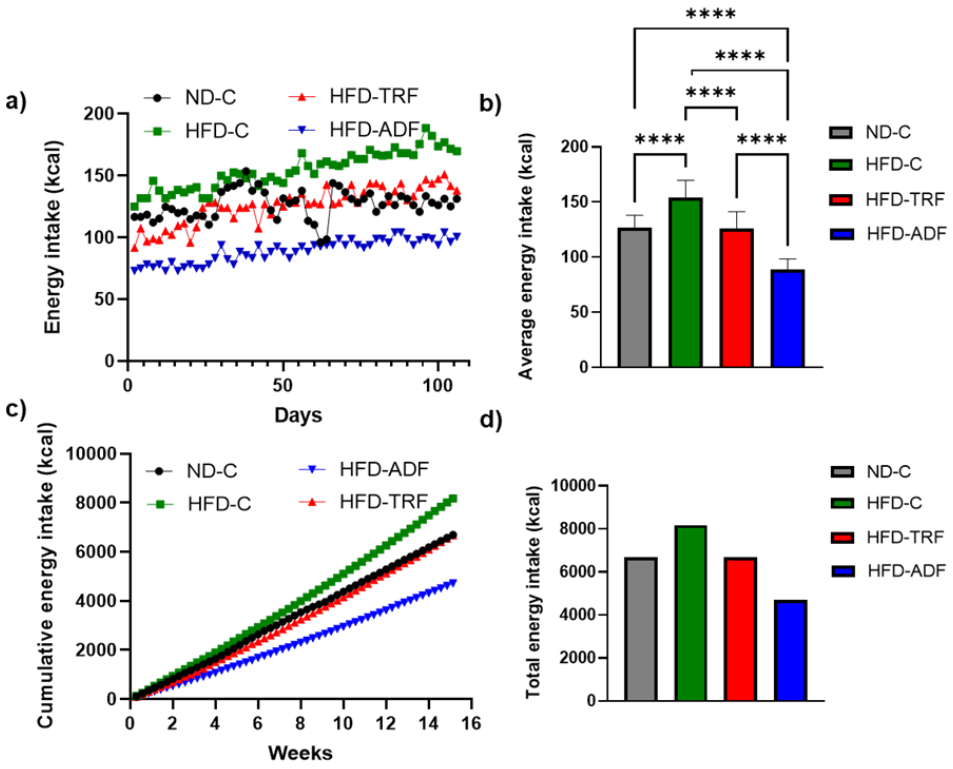

Figure 4. Different effects of IF on mice, observed using two regimens, ADF or TRF, in comparison to ND-C and HFD-C, were tested using a high-fat diet (HFD). (a) Effect on average energy intake (kcal) per mouse every 48 hr during the 15 weeks of the experiment, (b) average energy intake per mouse every 48 hr during the experiment period, (c) cumulative energy intake per mouse over the course of the experiment, (d) total energy intake during 15 weeks of IF.

3.3. Fasting blood glucose and OGTT

We next examined the effect of IF on blood glucose levels (Figure 5). Regarding fasting blood glucose (Figure 5a and d), for groups consuming the normal diet, prior to IF (week 0), the ND-C group had the lowest measurements, while the ND-TRF group showed the highest measurements, and there was no significant difference between the ND-ADF and ND-C groups (Figure 5a). Afterwards, fasting blood glucose levels in the ND-C group increased during weeks 13 and 14, reaching the highest level in the last week (15^{th}). In the ND-TRF and ND-ADF groups, fasting blood glucose levels started high, showed fluctuations within weeks 13 and 14, and then recorded a marked decrease in comparison to the control group (week 0 vs. 15, ND-C: +53%; $p < 0.01$, ND-TRF: -25%; $p < 0.05$, ND-ADF: -26%; $p < 0.05$, unpaired t-test, data not presented in the figure). These results suggest that IF impacts glucose metabolism, probably through improving insulin resistance.

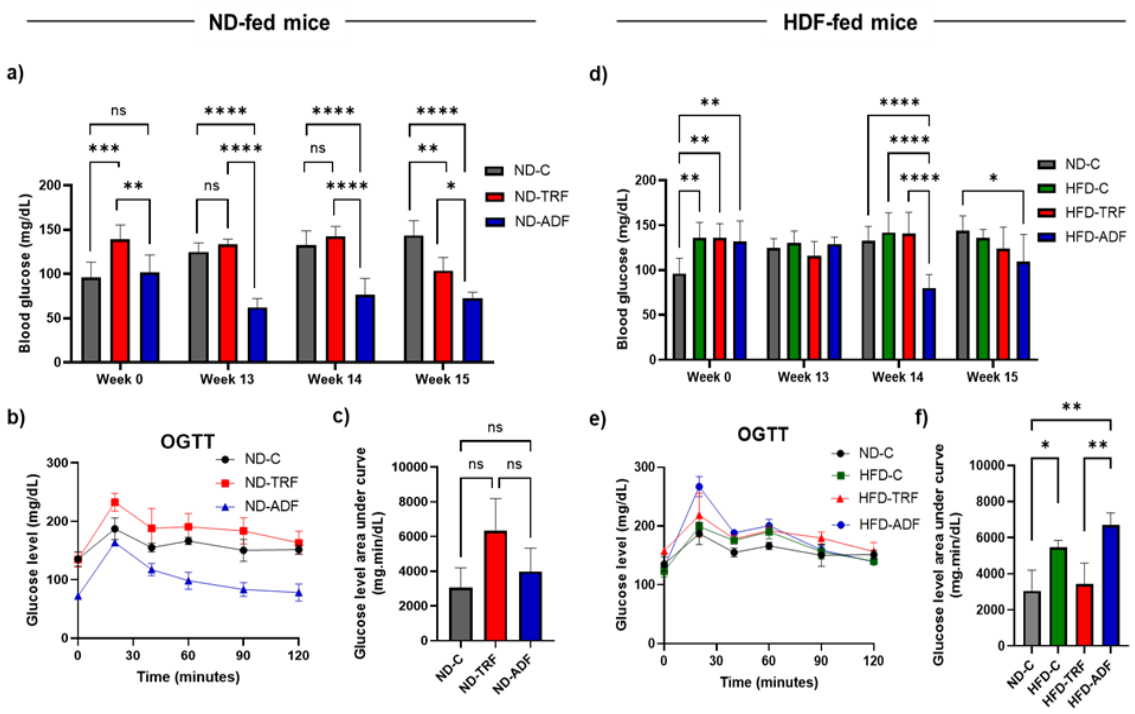

Figure 5. Effect of IF on fasting blood glucose levels at two regimens: ADF or TRF in comparison to *ad libitum* feeding as a control (C), tested using an ND (ND; a-c) or HFD (HFD; d-f). (a and d): Effect on fasting blood glucose level in the last three weeks of the experiment (weeks number 13, 14, and 15) in comparison to week 0 (before the start of the experiment). (b, c, e, and f): effect on glucose and area of glucose under the curve during oral glucose tolerance test (OGTT).

To evaluate glucose metabolism in mice undergoing different regimens, OGTT was performed before the end of the experiment (Figure 5b, c, e, and f). Glucose levels showed an increase after glucose administration via gastric gavage, in all groups, reaching the peak at 20 minutes. For mice consuming the normal diet (Figure 5b and c), the ND-ADF group exhibited a fast decline in glucose levels toward baseline and almost returned to fasting level by 120 min, as indicated by the glucose level area under the curve (AUC), suggesting average insulin sensitivity and glucose tolerance (Figure 5c). However, contrary to expectations, the ND-TRF group had the highest peak and 120-min glucose levels, which reveals slower recovery. All three groups did not exceed 200 mg/dL at the 60 min timepoint. The AUC was lower for the ND-ADF group than that of the ND-TRF. The ND-TRF had the highest AUC and thus did not confer metabolic advantages over *ad libitum* feeding. This further confirms the value of the ADF pattern, which includes both less weight gain and improved glucose metabolism. This pattern seems to be an interesting approach to reverse type-2 DM.

For groups consuming HFD (Figure 5d). Within weeks 13 and 14, fasting blood glucose levels in the ND-C group increased, fluctuated in the HFD-C and HFD-TRF groups (similar pattern to the ND-TRF group; decreased then increased, Figure 5a), and decreased significantly in the HFD-ADF group. At the end of the experiment (week 15), fasting blood glucose levels in the HFD-ADF group were the lowest of all groups and showed a significant difference compared to the ND-C group. This is consistent with the data seen with the ND-fed mice.

Examining the OGTT results for HFD-fed mice, the fasting blood glucose measurements were within the normal levels at baseline (0 min) (Figure 5e). The HFD-ADF group showed the highest peak glucose level at 20 min, but almost returned to the fasting level after 120 min. Likewise, the HFD-C and the HFD-TRF groups' measurements also returned to the fasting level and did not exceed 200 mg/dL at 60 min (Figure 5e). Comparing the control groups, mice that were fed on HFD showed a marked increase in the AUC in comparison to those receiving ND. Regarding the AUC and contrary to expectations, the HFD-ADF group had the highest AUC. The HFD-ADF group showed a significant AUC increase in comparison to the ND-C and HFD-TRF groups, with no significant difference from the HFD-C group. Moreover, the HFD-TRF exhibited no significant difference compared to the ND-C group (Figure 5f). These results indicate that improved glucose homeostasis was observed with the ADF regimen for ND only. This is different from previous studies where IF did not show improvement in glucose homeostasis [28].

3.4. Serum analysis

The serum analysis at the end of the experiment showed that the HFD-ADF group exhibited a lower level of blood lipids (cholesterol and triglycerides) and terminal blood glucose compared to other HFD groups, which were more similar to the levels of the ND-C group. Oppositely, the ALT level in the HFD-ADF group was the highest, although not statistically significant. The HFD-TRF group showed higher levels of blood lipids (cholesterol and triglycerides) and terminal blood glucose than those of ND-C and HFD-ADF, and similar to those of the HFD-C group. The ALT level in the HFD-TRF group was the lowest. However, the variations among the groups were not significant ($p > 0.05$, one-way ANOVA; Figure 6).

3.5. Histological analysis

Consistent with previous results regarding body weights and caloric consumption in our study, when white fat cells in the HFD-C are compared to monolocular fat cells (FC) of white adipose tissue in the ND-C group, they showed a prominent and conspicuous size increase and lipid accumulation, forming fused fat cells (FFC) (Figure 7a, Supplementary Figure 3).

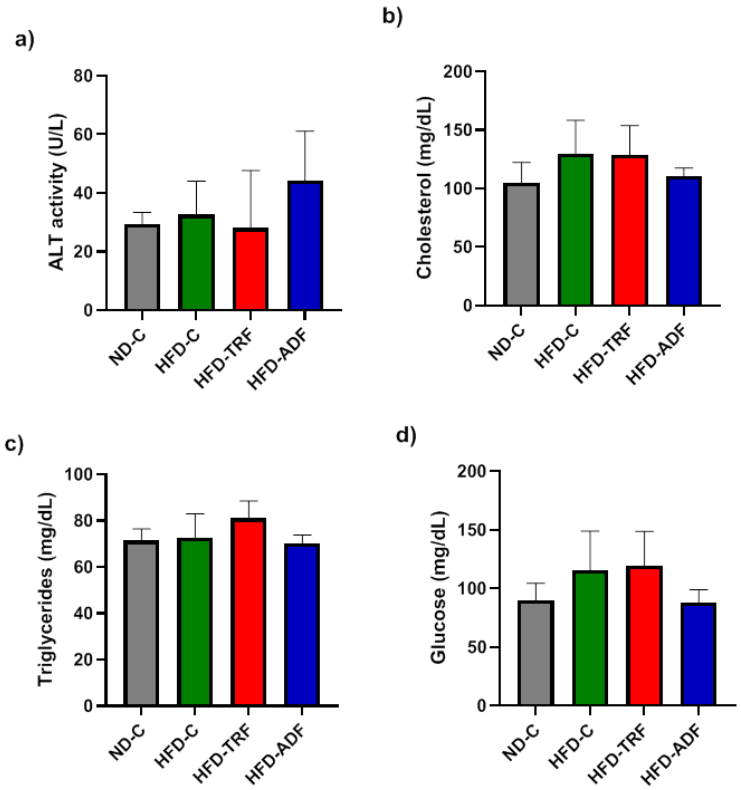

Figure 6. Effects of IF on mice, observed at two regimens, ADF or TRF, on serum samples at the end of the experiment, in comparison to ND and HFD *ad libitum* feeding as a control (C), tested using a high-fat diet (HFD), (a) activity of glutamic-pyruvic transaminase (ALT), (b) and (c), blood lipids (cholesterol and triglycerides), (d) terminal glucose level (differences between groups were found to be statistically nonsignificant).

Figures 7a and b show the effect of the two IF regimens on white fat cells, where cells in the HFD-TRF group are mostly monolocular adipocytes with some cells still appearing fused, while cells in the HFD-ADF group are more clearly affected, composed of monolocular adipocytes of white fat (WF), and beside them developed multilocular adipocytes of brown fat (BF), with blood vessels (BV) and the beige fat cells (BeF); they have features intermediate between white and brown fat cells. As white fat cells are affected by dietary intervention, brown adipocytes in the HFD-C group increased in size and accumulated lipid droplets, indicating a possible transformation into white fat cells (WF) with an increased amount of beige fat cells (BeF) (Figure 7a; panel J). Brown adipose tissue in the HFD-TRF group showed the appearance of only a few white fat cells (Figure 7a; panel K). Most fat cells are of the brown type, multilocular fat cells (MF) in the HFD-ADF group (Figure 7a; panel L).

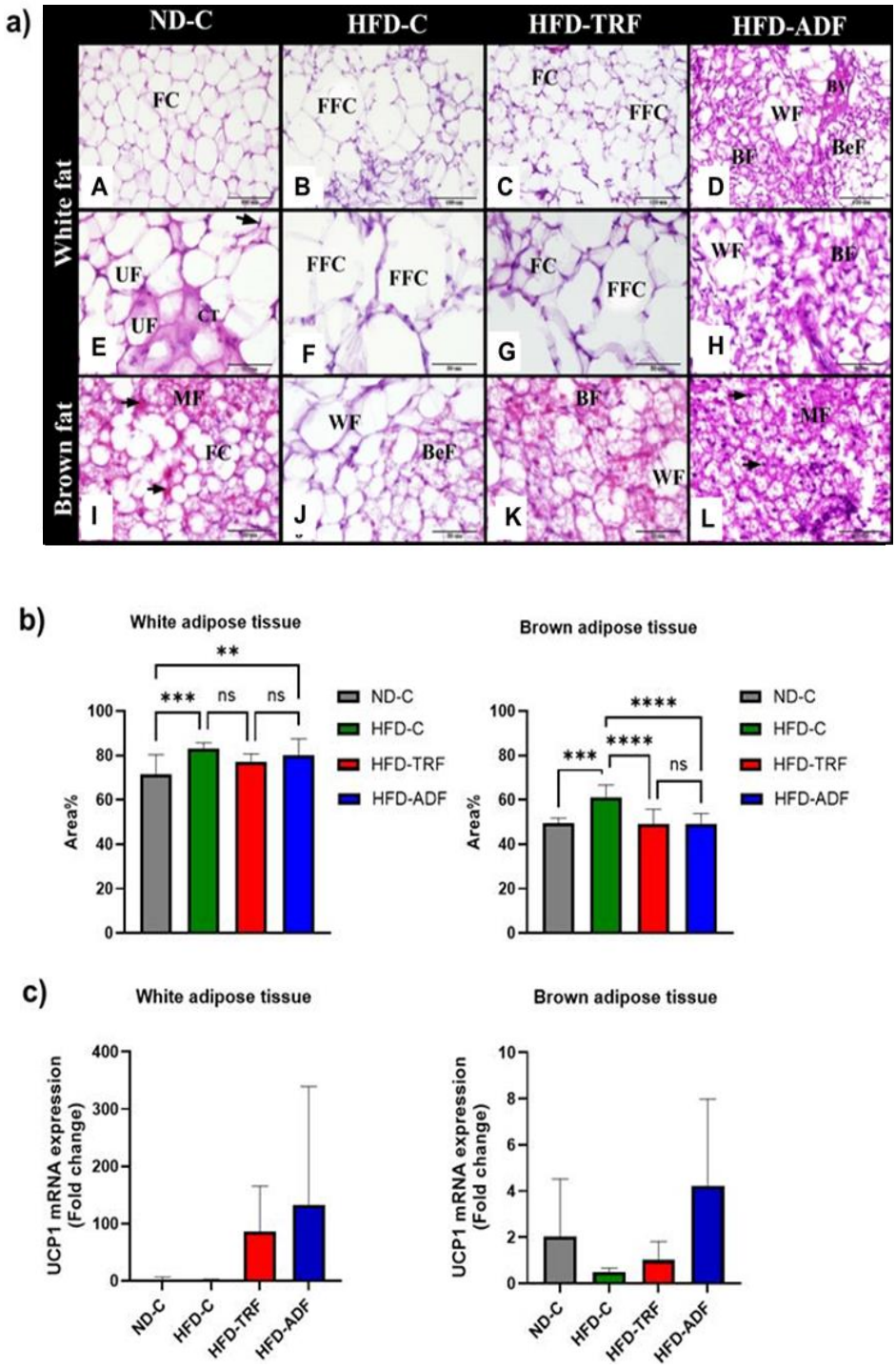

Figure 7. (a) Photomicrographs showing the effect of IF at two regimens, ADF or TRF, on an HFD in comparison to ND-C and HFD-C on (A-H) white adipose tissue (visceral) and (I-L) brown adipose tissue (interscapular) stained by H&E. Original magnification: A-D: X200, scale bar = 100 µm; E-H: X400, scale bar = 50 µm, (b) Histogram showing the differences between the area % of stored lipid in histological sections in both white and brown adipose tissue in female mice, (c) mRNA level of UCP1 in visceral (white) fat and in interscapular (brown) fat.

These results explain that HFD affected both white and brown fat cells, indicated by the formation of large, fused fat cells in WAT and the whitening of brown fat cells in BAT,

while at the same time showing the role of IF in reversing the effects of a high-fat diet, which appeared in the reduction of adipocyte size and lipid accumulation in WAT in the HFD-TRF and the induction of WAT browning in the HFD-ADF group. In BAT, HFD-TRF reduced lipid accumulation and whitening of brown fat cells, and in HFD-ADF, brown fat cells preserved their nature and morphology. The HFD-ADF regimen had a more pronounced effect on white and brown adipose tissue, which is consistent with the reduction in mouse body weight. The percentage area of stored lipid in the HFD-C group is the highest in both unilocular (WAT) and multilocular (BAT) tissues (Figure 7b). While HFD-ADF shows an obvious decrease in the % area of the stored lipid compared with the other HFD-C group within the BAT, it doesn't show the same effect in the experimental groups for WAT. HFD-C, HFD-TRF, and HFD-ADF nearly have the same % area of stored fat in the WAT, which is higher than ND-C, but in BAT, the % area of ND-C, HFD-TRF, and HFD-ADF nearly have the same effect on the % area of stored lipid within the multilocular adipocytes.

3.6. RT-qPCR

UCP-1 gene expression in WAT (Figure 7c) was higher in the HFD-ADF group in comparison to HFD-TRF, and both were higher than ND-C and HFD-C. This suggests that IF is a promising approach for the browning of WAT with possible higher energy expenditure, especially with the ADF regimen. In the case of BAT, higher UCP-1 gene expression was observed in ND-C and HFD-C groups compared to WAT. This is normal since it is well known that UCP-1 expression is higher in BAT compared to WAT. UCP-1 gene expression in BAT was higher in the HFD-ADF group in comparison to other groups, but the level was low in the case of the HFD-TRF group. The weight loss in the case of the ADF regimen seems to be a result of both reduced calorie intake and increased energy expenditure.

4. CONCLUSION

Our findings reveal that different IF regimens have variable effects on glucose metabolism, lipid profile, and gene expression. We directly compared two patterns of IF in mice fed with ND or HFD. Among the two, the ADF pattern of IF is a promising and efficient strategy for weight loss and blood glucose control. This could be explained by the decreased calorie intake as well as the increased energy expenditure, mediated by increased levels of UCP-1 gene expression in WAT.

Ethics statement:

The study protocol approved by the Research Ethics Committee of the Molecular Biology Research and Studies Institute, Assiut University, Egypt. The protocol was established according to the ARRIVE guidelines, ensuring proper practices for animals throughout the study. Mice obtained from the animal house, Faculty of Medicine, Assiut University.

Authors' contributions

Salma M. Abdelsamea: Methodology, Experimentation, Data Analysis, Resources, Validation, Writing – original draft; **Asmaa A.A. Hussein**: Supervision, Validation, Data Analysis; **Mahmoud Abd-Elkareem**: Methodology, Experimentation, Data Analysis; **Sara A. Atta**: Methodology, Experimentation, Data Analysis, **Ikramy A. Khalil:**

Methodology, Supervision, Conceptualization, Data Analysis, Validation, Writing – original draft; **Sara A. Abouelmagd**: Methodology, Supervision, Conceptualization, Data Analysis, Validation, Writing – original draft. **All authors** have read and approved the final manuscript and agree to its submission.

Acknowledgements:

The authors thank the late Prof. Ahmed AbouElmagd for his help with the histological analysis. Figure 1 was created with BioRender.com.

Finding:

The authors did not receive specific funding from any public or private funding agencies for this work.

Data Availability Statement:

All data and materials reported in this manuscript are available upon request from the corresponding author.

Conflicts of Interest:

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent to Participate: N/A

Consent to Publish: N/A

5. REFERENCES

[1] Khedkar, P.H., *Intermittent fasting-The new lifestyle?* 2020: Acta Physiol (Oxf). 2020 Aug;229(4):e13518. doi: 10.1111/apha.13518. Epub 2020 Jun 19.

[2] Cheng, L., et al., *Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus.* Adipocyte, 2021. **10**(1): p. 48-65.

[3] Heo, M.-G. and S.-Y. Choung, *Anti-obesity effects of Spirulina maxima in high fat diet induced obese rats via the activation of AMPK pathway and SIRT1.* Food & Function, 2018. **9**(9): p. 4906-4915.

[4] Kim, K.H., et al., *Intermittent fasting promotes adipose thermogenesis and metabolic homeostasis via VEGF-mediated alternative activation of macrophage.* Cell Res, 2017. **27**(11): p. 1309-1326.

[5] Moreno-Mendez, E., et al., *Early-life programming of adipose tissue.* Nutr Res Rev, 2020. **33**(2): p. 244-259.

[6] Ghesmati, Z., et al., *An update on the secretory functions of brown, white, and beige adipose tissue: Towards therapeutic applications.* Rev Endocr Metab Disord, 2023. **5**(10): p. 023-09850.

[7] Cheong, L.Y. and A. Xu, *Intercellular and inter-organ crosstalk in browning of white adipose tissue: molecular mechanism and therapeutic complications.* J Mol Cell Biol, 2021. **13**(7): p. 466-479.

[8] Genchi, V.A., et al., *Pharmacological modulation of adaptive thermogenesis: new clues for obesity management?* J Endocrinol Invest, 2023. **46**(11): p. 2213-2236.

[9] Kang, S.H., et al., *Intermittent Fasting: Current Evidence in Clinical Practice*. 2020: J Obes Metab Syndr. 2020 Jun 30;29(2):81-83. doi: 10.7570/jomes20022.

[10] James, D.L., et al., *Impact of Intermittent Fasting and/or Caloric Restriction on Aging-Related Outcomes in Adults: A Scoping Review of Randomized Controlled Trials.* Nutrients, 2024. **16**(2).

[11] Anton, S.D., et al., *Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting.* Obesity, 2018. **26**(2): p. 254-268.

[12] Elias, A., N. Padinjakara, and N.T. Lautenschlager, *Effects of intermittent fasting on cognitive health and Alzheimer's disease.* Nutr Rev, 2023. **81**(9): p. 1225-1233.

[13] Tiwari, S., N. Sapkota, and Z. Han, *Effect of fasting on cancer: A narrative review of scientific evidence.* Cancer Sci, 2022. **113**(10): p. 3291-3302.

[14] Wilkinson, M.J., et al., *Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome.* Cell Metab, 2020. **31**(1): p. 92-104.

[15] Duncan, M.J., et al., *Restricting feeding to the active phase in middle-aged mice attenuates adverse metabolic effects of a high-fat diet.* Physiol Behav, 2016. **167**: p. 1-9.

[16] Matias, A.M., et al., *Differential Effects of High Sugar, High Lard or a Combination of Both on Nutritional, Hormonal and Cardiovascular Metabolic Profiles of Rodents.* Nutrients, 2018. **10**(8): p. 1071.

[17] Ao, D.Z., et al., *Alternate-Day High Fat-Normal Chow Diet Ameliorates HFD-Induced Obesity and Restores Intestinal Immunity.* Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 2022: p. 3843-3853.

[18] Bancroft, J.D., et al., *Theory and practice of histological techniques.* Connective tissues and stains, 15thedn. Churchill Livingstone Publications, 2004: p. 139-200.

[19] Harris, H., *On the rapid conversion of haematoxylin into haematein in staining reactions.* Journal of Applied Microscopic Laboratory Methods, 1900. **3**(3): p. 777.

[20] Abd-Elkareem, M., *Cell-specific immuno-localization of progesterone receptor alpha in the rabbit ovary during pregnancy and after parturition.* Anim Reprod Sci, 2017. **180**: p. 100-120.

[21] Gross, C.A., C.K. Reddy, and F.B. Dazzo, *CMEIAS color segmentation: an improved computing technology to process color images for quantitative microbial ecology studies at single-cell resolution.* Microb Ecol, 2010. **59**(2): p. 400-14.

[22] Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.

[23] Lee, S. and D.K. Lee, *What is the proper way to apply the multiple comparison test?* Korean J Anesthesiol, 2018. **71**(5): p. 353-360.

[24] Chaix, A., et al., *Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges.* Cell Metab, 2014. **20**(6): p. 991-1005.

[25] Arble, D.M., et al., *Circadian timing of food intake contributes to weight gain.* Obesity, 2009. **17**(11): p. 2100-2.

[26] Oishi, K. and C. Hashimoto, *Short-term time-restricted feeding during the resting phase is sufficient to induce leptin resistance that contributes to development of obesity and metabolic disorders in mice.* Chronobiol Int, 2018. **35**(11): p. 1576-1594.

[27] Boutant, M., et al., *SIRT1 Gain of Function Does Not Mimic or Enhance the Adaptations to Intermittent Fasting.* Cell Rep, 2016. **14**(9): p. 2068-2075.

[28] Varady, K.A., *Impact of intermittent fasting on glucose homeostasis.* Curr Opin Clin Nutr Metab Care, 2016. **19**(4): p. 300-2.