Original Research Report

THE EFFECTS OF INTERMITTENT FASTING ON THE SIZE AND NUMBER OF SUBCUTANEOUS ADIPOCYTES IN OBESE MOUSE MODELS

Veny Larasati[1*], Riana Sari Puspita Rasyid[1], Soilia Fertilita[1] , Tri Suciati[2] , Muhammad Farhan[3]

[1]Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]Department of Anatomy, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[3]Medical Education Program, Universitas Sriwijaya, Palembang, Indonesia

ABSTRACT

The accumulation of adipose tissue can have deleterious effects and lead to obesity. Intermittent fasting (IF), an approach that involves time-restricted eating, has gained popularity as a treatment option for obesity because it enhances insulin sensitivity and promotes beneficial changes in glucose metabolism. This study used a time-restricted meal intake (TRM) approach to assess the effects of IF on the histological characteristics of subcutaneous inguinal adipose tissue in obese mouse models. This study used an in vivo experimental post-test only control group design. Twenty male mice were divided into four groups: a normal control group, an obese control group, a TRM group with a high-fat diet (TRM-HF), and a TRM group with a standard diet (TRM-S). The TRM treatment was administered for 14 days, with a fasting window from 4 p.m. to 8 a.m. The pre- and post-treatment weight analyses were conducted using the paired t-test for normally distributed data and the Wilcoxon test for non-normally distributed data ($p < 0.05$). One-way analysis of variance (ANOVA) was employed for unpaired data on the post-treatment weight. Per field of view, there were an average of 120,500 cells (49,700 ± 136,200) in the normal control group, 68,380 ± 9,194 cells in the obese control group, 70,860 ± 11,029 cells in the TRM-HF group, and 79,360 ± 5,112 cells in the TRM-S group. The average cell sizes (μm^3) were 56,730.142 ± 19,273.257 in the normal control group, 138,934.331 ± 27,670.558 in the obese control group, 106,827.767 ± 20,580.501 in the TRM-HF group, and 68,689.114 ± 8,219.727 in the TRM-S group. The number of cells in each group did not differ significantly, but there were significant differences in cell size. The mice receiving TRM treatment did not show significant changes in body weight, whereas the obese control group showed a significant increase in body weight. In conclusion, TRM had an effect on cell size but did not affect the number of adipocytes in subcutaneous inguinal fat tissue.

Keywords: Intermittent fasting; obesity; adipocyte; good health

***Correspondence:** Veny Larasati, Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia. Email: venylarasati@fk.unsri.ac.id

Article history
●Submitted 23/01/2024 ● Revised 18/04/2024 ● Accepted 13/05/2024 ● Published 10/06/2024

How to cite: Larasati V, Rasyid RSP, Fertilita S, et al (2024). The Effects of Intermittent Fasting on the Size and Number of Subcutaneous Adipocytes in Obese Mouse Models. Folia Medica Indonesiana 60 (2), 111-118. doi: https://doi.org/10.20473/fmi.v60i2.54389

pISSN:2355-8393, eISSN: 2599-056x

Highlights:
1. This study helps bridge the gap between systemic effects and tissue-level changes, providing a deeper understanding of how histological analysis can be used to explore the effects of intermittent fasting on adipocytes and body weight regulation.
2. This study contributes to obesity management through lifestyle modification, specifically intermittent fasting, by focusing on histological changes in adipose tissue.

INTRODUCTION

The accumulation of fat tissue is the hallmark of a multifactorial condition known as obesity, which can have detrimental effects on health. Obesity can be categorized into two types according to its histological features: hypertrophic obesity and hypercellular obesity (Setiati 2017, Gartner, 2017). The excessive accumulation of fat within fat cell droplets (adipocytes), namely unilocular white adipose cells, leads to hypertrophic obesity by causing these cells to grow up to four times their normal size. On the other hand, an excessive rise in

the number of adipocytes leads to hypercellular obesity. The term "central obesity" describes the accumulation of fat primarily in the abdomen (Ministry of Health of the Republic of Indonesia 2018, Jameson et al. 2018). Obesity can lead to the development of many diseases, including type 2 diabetes. It also increases the likelihood of developing cancer, musculoskeletal disorders, skin issues, hepatic and biliary disorders, respiratory problems, cardiovascular diseases, and reproductive disorders. Central obesity is a crucial element of the group of illnesses known as the metabolic syndrome (Jameson et al. 2018).

The overall prevalence of obesity is relatively high and continues to rise in various regions. Data from the National Health and Nutrition Examination Survey (NHNES) indicated that the percentage of the American population with obesity, as determined by a body mass index (BMI) was above 30, increased from 14.5% (1976–1980) to 36.5% (2011–2014) (Jameson et al. 2018). According to a systematic review conducted by Chooi et al. (2019), 1.9 billion out of 6.09 billion adults were obese in 2015. This number represents around 39% of the global population. The 2018 Basic Health Research (Riskesdas) reported by the Ministry of Health of the Republic of Indonesia (2018) showed an overall obesity prevalence of 21.8% among individuals aged >18 years in Indonesia. The highest prevalence was observed in the 40–44 age group (29.6%), with a higher prevalence of obesity in women (29.3%) compared to men (14.5%). Additionally, 31.0% of adults aged ≥15 years in Indonesia were found to have central obesity. Globally, obesity rates are higher among people of African, American, Hispanic, and Caucasian descent, while they tend to be lower among Asians. Furthermore, there is a higher proportion of obese women compared to men (Jameson et al. 2018).

Adipocyte size and quantity are significant markers of obesity, with an increase in either marker indicating two processes that fuel the expansion of adipose tissue. It is now recognized that adult obesity is associated with an increase in adipocyte size. Obesity is also linked to an increase in the number of adipocytes, although the underlying mechanisms remain unclear. The expansion of adipocytes can be classified into two categories: hypertrophic, an increase in adipocyte size, or hyperplastic, an increase in adipocyte quantity (Gartner 2017, von Bank et al. 2021). According to Jameson et al. (2018), dietary and lifestyle interventions are considered primary approaches in obesity management. One evolving form of dietary intervention is intermittent fasting (IF). Time-restricted eating, a form of intermittent fasting, has been shown to effectively reduce fat mass. This approach can decrease the size and number of adipocytes within the fat tissue. Furthermore, the adipocyte reduction constitutes a significant proportion (79%) of the overall weight loss (Rynders et al. 2019, Welton et al. 2020).

As shown by Tsaban et al. (2021), weight loss interventions have been found to decrease the amount of accumulated subcutaneous adipose tissue in the neck and submandibular region. This provides evidence that time-restricted eating can result in reduced fat mass. Fat mass reduction in individuals with a genetic predisposition demonstrates a decrease in adipocyte size and number (MacLean et al. 2015). Hatori et al. (2012) conducted an animal study to compare ad libitum feeding and a dietary intervention approach in mice that were subjected to a normal chow or high-fat diet. The study showed that time-restricted meal intake can prevent hypertrophy (increase in cell size) of unilocular and multilocular adipose tissues, as opposed to ad libitum feeding. Time-restricted eating has also demonstrated beneficial effects on weight loss. Intermittent fasting continues to offer advantages beyond body mass management in mice, even when given an obesogenic and proinflammatory diet (Marinho et al. 2019, Rynders et al. 2019). Existing research has shed light on the metabolic effects of intermittent fasting, particularly regarding obesity-related adipose tissue. However, limited data is currently available on the impact of time-restricted eating, necessitating further investigation on this matter. This study employed mouse models of obesity to analyze the histological features of subcutaneous inguinal adipose tissue in relation to the use of intermittent fasting combined with a time-restricted meal intake (TRM).

MATERIALS AND METHODS

With reference to the work of Ranstam & Cook (2017), this study used a posttest-only control group design to conduct an in vivo experiment. The research was conducted at the Animal House of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia, from December 2022 to January 2023. The ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya, with protocol No. 004-2023 issued on 3/1/2023. The data were processed using Microsoft Excel for Windows, version 15.0 (Microsoft Inc., Redmont, WA, USA) and then analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA).

The subjects in this study were 20 male mice with an age range of 6–10 weeks and a weight between 15 and 40 g. The mice were housed in the Animal House of the Faculty of Medicine, Universitas

Sriwijaya, Palembang, Indonesia. They were divided into four cages, with each cage housing five male mice. The preparation for the mouse models began with acclimatization for seven days. The exclusion criteria for the mice were based on several factors, including inactivity, anatomical defects, previous involvement in research, and death during the acclimatization period. The mice were randomly divided into four groups: a normal control group, an obese control group, a TRM group with a high-fat diet (TRM-HF), and a TRM group with a standard diet (TRM-S) (Emerson 2015).

The subjects in the obese control group and both TRM groups (i.e., TRM-HF and TRM-S) were induced to become obese through a high-fat diet. The high-fat diet consisted of Hi Gro standard feed (31.5%), solid fat in the form of shortening (31.5%), duck egg yolk (10.5%), wheat flour (5%), and corn flour (21.5%) (Li et al. 2020). Throughout the induction period, the subjects' weight was measured on a weekly basis. Obesity was determined using the Lee index formula, where an index value above 0.3 was considered obese (Nugroho 2018).

The normal control group was given a standard diet and allowed to drink without any calorie restrictions. The obese control group was provided with a high-fat diet and allowed to drink without any calorie restrictions. The TRM-HF and TRM-S groups were subjected to a treatment that involved fasting for 16 hours from 4 p.m. to 8 a.m. and a feeding window from 8 a.m. to 4 p.m. each day. During the feeding window, the TRM-HF group received a high-fat diet, while the TRM-S group was given a standard diet. Both TRM groups were allowed unrestricted access to drinking water during both fasting and feeding periods. This treatment was administered for 14 days (Hatori et al. 2012).

The weight of all subjects was measured before and after the treatment period. Once the treatment was complete, all subjects were euthanized. Subcutaneous tissue was collected from the inguinal area for histological preparation. Hematoxylin-eosin (HE) staining was used to prepare the histological slides, which were then observed under a microscope. Images of the slides were captured using a computer in a high-power field at 400X magnification. Ten images were randomly captured for each sample. The collected images were analyzed using ImageJ for Windows, version 1.54 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA) to count the number and measure the size of cells. Cell counting was performed using a cell counter, and the average number of cells was determined for each sample (Rifano et al. 2014, Halim 2019).

Histological sectioning of an entire tissue or isolated cells, along with assessments of cellular and systemic function, was used in numerous prior studies to determine the average size of fat cells. This kind of size assessment was mainly employed due to its technical simplicity and minimal requirement for tissue or cells. However, there were cases where this approach failed to show a causal relationship between the function and size of adipocytes. Since it could be challenging to determine whether the histological approach could detect the entire range of cell sizes, sectioning must be performed at the widest diameter of each cell to obtain an accurate measurement. The very small cell population with a diameter of 30 µm could possibly be missed or underestimated with the use of this approach. Therefore, in this study, the cell size was determined by measuring the diameter of the largest ten cells in each field of view (a total of 100 cells per sample) using the measurement function. Afterwards, the formula for calculating the volume of a sphere was applied, under the assumption that the adipocytes had a spherical shape. In order to prevent the impact of tissue polarization, measurements of the diameter lines were obtained from 50 cells that were perpendicular to the diameter lines acquired from another set of 50 cells. The average volume of each cell was then calculated for every sample (Stenkula & Erlanson-Albertsson 2018).

Univariate data that followed a normal distribution were presented as mean ± standard deviation (SD), whereas non-normally distributed data were reported as median (minimum-maximum). The normal distribution and homogeneity of the data were determined using the Shapiro-Wilk test and the Levene test, respectively. The statistical test used for the cell count data that did not follow a normal distribution was the Kruskal-Wallis test. A one-way analysis of variance (ANOVA) was used to assess the cell size. Post-hoc analysis for the significant results was conducted using the Bonferroni test. The statistical tests used for comparing paired data on pre- and post-treatment weight were the paired t-test for non-normally distributed data and the Wilcoxon test for non-normally distributed data. A one-way ANOVA was also used to analyze unpaired data on post-treatment weight (Mishra et al. 2019).

RESULTS

The normal control group had the highest number of adipocyte cells per field of view compared to the other groups, whereas the obese control group had the lowest number of cells (Table 1). The number of cells in both TRM groups, i.e., TRM-HF and TRM-S, was greater in comparison to the obese control group. The number of cells was not normally

distributed only in the normal control group (p=0.026). Comparisons after the TRM treatment demonstrated that the number of cells among all groups did not differ significantly (p=0.157).

Table 1. The number of cells per field of view and the size of adipose tissue cells in the subcutaneous inguinal area.

Group	Cells/field of view		Cell size (μm³)	
	Mean±SD / median (min–max)	p	Mean±SD / median (min–max)	p
Normal control	120,500 (49,700–136,20)	0.03*	56,730.14±19,273.26	0.33
Obese control	68,380±9,194	0.45	138,934.33±27,670.56	0.09
TRM-HF	70,860±11,029	0.58	106,827.77±20,580.50	0.98
TRM-S	79,360±5,112	0.47	69,689.11±8,219.73	0.89

Legend: A value of p≥0.05 indicates normally distributed data.

The normal control group had the smallest average cell size compared to the other groups, while the obese control group had the largest average cell size (Figure 1). The average cell size in both TRM groups, i.e., TRM-HF and TRM-S, was smaller in comparison to the obese control group. However, the average cell size was larger in the TRM-HF group than the TRM-S group. The cell size data in all groups were normally distributed (p≥0.05). Table 2 shows that there is a statistically significant difference in average cell size among all groups (p=0.043). However, the post-hoc analysis conducted to compare two different groups did not reveal any statistically significant differences (p≥0.05).

Table 2. Comparisons of the size and number of cells.

Group	Cells/field of view		Cell size (μm³)	
	P*	Compared groups	Mean±SD / median (min-max)	p
Normal control	0.15	Obesity control	0.043*	0.07
		TRM-HF		0.59
		TRM-S		1.00
Obesity control		Normal control		0.07
		TRM-HF		1.00
		TRM-S		0.16
TRM-HF		Normal control		0.59
		Obesity control		1.00
		TRM-S		1.00
TRM-S		Normal control		1.00
		Obesity control		0.16
		TRM-HF		1.00

Legend: The symbol (▪) indicates the parametric comparison analysis results, while the symbol (⬩⬩) indicates the post-hoc analysis results. A value of p<0.05 is considered significant.

The weight of the mice was measured before and after the intervention period, resulting in paired data. The normal control group, which was not subjected to any intervention, had the smallest average body weight compared to the other groups. Prior to the TRM intervention, these other groups were in an obese state. Among them, the TRM-HF group had the highest average body weight (Table 3). After the intervention period, the average body weight in all groups did not decrease, except for the TRM-HF group, which only experienced a slight decrease. Before and after the intervention, the TRM-S group showed a lower average body weight than the TRM-HF group. Both of these groups revealed a lower average body weight than the obese control group after the intervention. The pre-intervention body weight data exhibited homogeneity (p=0.451 for all groups; p=0.477 for the obesity-induced group). The body weight data before the intervention was not normally distributed only in the obese control group (p=0.048).

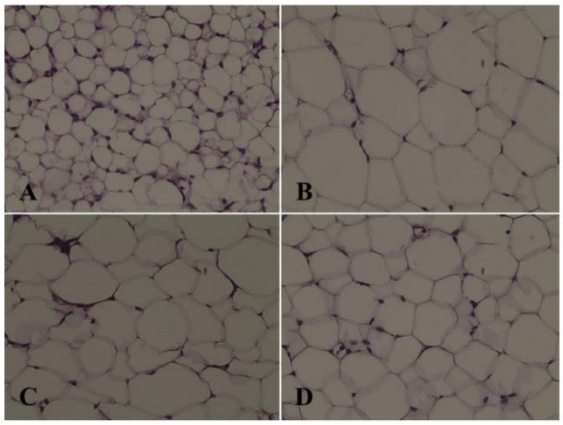

Figure 1. Subcutaneous inguinal adipose tissue of one mouse from each group, observed at 400X magnification through hematoxylin-eosin staining.

Table 3. Mean values of the mice's body weight before and after the treatment period.

Groups	Body weight					
	Pre-treatment (g)				Post-treatment (g)	
	Mean± SD / median (min– max)	p⬩	p⬩⬩	p⬩⬩⬩	Mean±SD / median (min– max)	p⬩
Normal control	28.200 ± 2.956	0.78			30.000±4.450	0.53
Obese control	35 (28-35)	0.04*	0.45	0.48	35.000±1.225	0.83
TRM-HF	35.000 ± 1.140	0.54			34.000±0.837	0.11
TRM-S	32.200 ± 2.289	0.31			32.600±2.400	0.50

Legend: The symbol (⬩) indicates the normality test results. The results of the homogeneity test of pre-treatment body weight data are represented by the symbols (⬩⬩) for all groups and (⬩⬩⬩) for obesity-induced groups. A value of p≥0.05 indicates a normal distribution or homogeneity of the data.

As presented in Table 4, the paired analysis comparing body weight before and after the TRM intervention revealed no statistically significant differences (p≥0.05). Furthermore, the body weight

comparison among all groups after the intervention did not demonstrate any statistically significant differences (p=0.578).

Table 4. Results of the paired and comparison analyses of the mice's post-treatment body weight.

Groups	p♦	p♦♦
Normal control	0.46	0.58
Obesity control	0.06	
TRM-HF	0.29	
TRM-S	0.18	

The symbol (♦) denotes the results of the paired parametric comparison between the pre- and post-treatment body weight. The symbol (♦♦) represents the results of the unpaired parametric comparison of the post-treatment body weight among all groups. A value of p<0.05 indicates a significant difference.

DISCUSSION

In this study, the morphological characteristics of subcutaneous inguinal adipose tissue in mice were described by the number and size of cells. These morphological characteristics reflect the level of adiposity in the fat tissue, which generally increases when there is a higher energy intake and limited energy expenditure (Fernández et al. 2021). The morphological characteristics of fat tissue correlate with cellular function and metabolic diseases. The results of this study showed that the TRM-treated groups had a greater number of cells than the obese control group, whereas the normal control group exhibited the highest average number of cells among all groups. Additionally, the TRM-treated groups had a smaller average cell size than the obese control group, while the normal control group showed the smallest average cell size among all groups. The number of cells in this study was calculated in 10 high-power fields (HPF) before determining the average values. A consistent magnification of the field of view was used during the observation. The number of cells appearing in one field of view is influenced by the size of the cells, demonstrating the importance of cell size analysis in this study. The higher number of cells in the normal control and TRM-treated groups suggests that, in general, the cell size is larger in the obese control group compared to the other groups, resulting in a reduced number of visible cells (Mao et al. 2021).

The results that showed a higher number of cells observed in the groups treated with intermittent fasting are consistent with previous research conducted by Mao et al. (2021), who investigated the effects of short-term fasting (acute fasting) on the morphology and metabolism of subcutaneous and epididymal white fat tissue in mice fed with high-fat and low-fat diets. Their study showed that 36-hour fasting increased the number of fat cells, accompanied by a rise in multilocular droplets. Similarly, Fernández et al. (2021) examined the effects of restrictive intermittent fasting on the function and morphology of subcutaneous and visceral adipocytes in diet-induced obese mice. In their study, subcutaneous adipocyte hyperplasia was observed in diet-induced obese mice as well as in standard diet mice that underwent calorie restriction with intermittent fasting. However, visceral fat hypoplasia was anticipated to occur after the intermittent fasting therapy. Both studies described that the compensatory mechanisms occurring in subcutaneous white fat tissue were a response to increased energy expenditure under caloric restriction conditions with intermittent fasting. Increased energy expenditure triggered the browning of white fat tissue, indicating lipid mobilization and heat production. The presence of hyperplasia suggests that it might be compensation for limited energy intake and food efficiency resulting from intermittent fasting. It has been found that intermittent fasting in mice boosts energy expenditure and encourages the browning of white adipose tissue. Furthermore, individuals with obesity are associated with an increase in the number and size of fat cells (Li et al. 2017, Liu et al. 2019). Therefore, the studies that assess morphology using hematoxylin-eosin staining may not fully reflect fat cell hyperplasia. In this present study, the comparison of the number of cells among all groups did not show statistically significant differences. This indicated that the 14-day intermittent fasting treatment did not have a notable effect on the number of visible cells in the microscopic field of view.

The average cell size was larger in the obese control group compared to the groups subjected to intermittent fasting in this study. This finding aligns with the research carried out by Mao et al. (2021). The 36-hour fasting experiment in their study demonstrated a decrease in the size of subcutaneous and epididymal adipocyte cells in mice that received high-fat and low-fat diets. Tang et al. (2017) assessed the effects of fasting and refeeding on mice's histomorphology of epididymal, inguinal, and visceral fat cells, as well as changes in gene expression. They found that fasting for 48 hours reduced the size of white fat cells in the inguinal area. Similarly, another study reported that mice subjected to intermittent fasting experienced a decrease in body fat while maintaining consistent muscle mass (Yoshii et al. 2023). This contradicts the findings of Fernández et al. (2021), who observed the presence of subcutaneous white fat cell hypertrophy after intermittent fasting therapy. However, it might be related to the sample selection criteria in the study, which included female mice. Various studies on both rodents and humans have shown that sex can influence differences in energy storage, leading to greater hypertrophy and

hyperplasia of subcutaneous white fat tissue in female rodents. These occurrences may arise as a protection mechanism against insulin resistance. The differences in fat deposition mainly stem from hormonal differences, such as estrogen-supporting lipolysis. Hormone receptor type β is more abundant in the abdominal area, while receptor type α has a larger proportion in the gluteofemoral area. This can result in higher enzyme concentrations and increased insulin sensitivity in female rodents. In relation to the findings of this present study, the comparative analysis revealed a statistically significant difference in the average cell size. Therefore, this study underscores the effect of 14-day TRM on changes in the morphological aspects of subcutaneous fat cells.

In addition to microscopic characteristics, the mice's body weight was also considered as an outcome for evaluation in this study. The average body weight was measured before and after the intervention. The measurement showed that all groups, with the exception of one, did not exhibit any weight loss. The TRM-HF group only saw a slight decrease in the average body weight. However, unlike the obese control group, neither of the TRM groups experienced an increase in their average body weight. The TRM-HF group, which continued to consume a high-fat diet throughout the treatment, was able to maintain their average body weight. There were no significant differences between the pre- and post-intervention body weights. Intermittent fasting in animals may lead to weight gain if they consume the same amount of food but with lower energy expenditure. Additionally, intermittent fasting can cause hyperphagic behavior on free-feeding days, leading to an increase in stomach dimensions. Intermittent fasting in rodents can induce elevated expression of appetite-stimulating neurotransmitters, such as agouti-related peptide (AGRP), neuropeptide Y (NPY), and orexin, even when the stomach is full. These neurotransmitters play a crucial role in appetite modulation and metabolic regulation. The increasing concentrations of these neurotransmitters in plasma may result in an increase in food intake. Prior human research has reported an increased subjective appetite sensation as a result of fasting cycles (Chausse et al. 2014). However, intermittent fasting has demonstrated its effects on weight loss in both animals and humans. Long-term intermittent fasting in female Wistar rats was found to reduce body weight gain, possibly due to a 35% lower average food intake. Additionally, intermittent fasting in 4-week-old rats significantly decreased their growth rate, resulting in smaller livers, kidneys, muscles, hearts, and bones (Munhoz et al. 2020).

One possible explanation for the consistent body weight of obese animal models after fasting is the physiological diurnal rhythm and growth patterns in rodents during their young age. Generally, the diurnal rhythm demonstrated metabolic flexibility in the TRM groups, allowing for increased use of lipids as fuel during the later light phase as well as carbohydrates during the dark phase (Woodie et al. 2018). Mice with normal growth are typically characterized by a weight gain of about 1 g for every 10 g of food intake per day. This growth pattern is especially common in young mice, as in the subjects of this study (Nugroho 2018). This might explain the differences between the current study and the study conducted by Chaix et al. (2014), who documented slight weight loss (from 40 g to 38 g and from 53.7 g to 47.5 g) in diet-induced obese mice fed with a high-fat diet and subjected to intermittent fasting plans of 13:12 and 26:12 weeks. A study with a longer duration, resulting in older research subjects, may demonstrate a greater effect compared to the current study, which only lasted for 14 days. However, it is worth noting that even if the earlier study also showed minimal weight loss, the potential influence of the longer experiment duration cannot be disregarded (Muñoz-Hernández et al. 2020).

Strength and limitations

This study presents additional data regarding the use of intermittent fasting as an option for managing obesity. The findings of this research provide a more comprehensive insight into the utilization of histological analysis to examine the effects of intermittent fasting on adipocytes and the regulation of body weight. Nevertheless, the results might be constrained by the short experiment duration of only 14 days.

CONCLUSION

This study suggested that time-restricted intermittent fasting did not significantly reduce body weight or the number of adipocyte cells. However, it resulted in a significant reduction in the size of adipocyte cells. Mice subjected to intermittent fasting had smaller cell sizes compared to obese mice that were not subjected to time-restricted meal intake. Additionally, the body weight of mice that undergo intermittent fasting remained stable even with the administration of a high-fat diet.

Acknowledgment

The authors acknowledge everyone who assisted us in course of this research, all staffs of Histology Department, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.

Conflict of interest

None.

Ethical consideration

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia, issued the ethical approval for this study under protocol No. 004-2023 on 3/1/2023.

Funding disclosure

None.

Author contribution

VL contributed to the conception and design, drafting of the article, obtainment of funding, and final approval of the article. MF carried out the collection, assembly, analysis, and interpretation of the data, as well as provided administrative and technical support. RSP contributed to the final approval and critical revision of the article for important intellectual content. SF contributed to the analysis and interpretation of the data as well as obtainment of funding. TS contributed to the final approval of the article.

REFERENCES

von Bank H, Kirsh C, Simcox J (2021). Aging adipose: Depot location dictates age-associated expansion and dysfunction. Ageing Research Reviews 67**,** 1-11. doi: 10.1016/j.arr.2021.101259.

Chaix A, Zarrinpar A, Miu P, et al (2014). Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metabolism 20**,** 991–1005. doi: 10.1016/j.cmet.2014.11.001.

Chausse B, Solon C, Caldeira da Silva CC, et al (2014). Intermittent fasting induces hypothalamic modifications resulting in low feeding efficiency, low body mass and overeating. Endocrinology 155**,** 2456–2466. doi: 10.1210/en.2013-2057.

Chooi YC, Ding C, Magkos F (2019) The epidemiology of obesity. Metabolism: Clinical and Experimental, 92. doi: 10.1016/j.metabol.2018.09.005.

Emerson RW (2015). Convenience sampling, random sampling, and snowball sampling: How does sampling affect the validity of research? Journal of Visual Impairment & Blindness 109**,** 164–168. doi: 10.1177/0145482X1510900215.

Fernández MR, Vilca CC, Batista LO, et al (2021). Fasting and refeeding cycles alter subcutaneous white depot growth dynamics and the morphology of brown adipose tissue in female rats. British Journal of Nutrition 126**,** 460–469. doi: 10.1017/S0007114520004055.

Gartner LP (2017) Textbook of histology. 4th edn. Philadelphia: Elsevier. Available at: https://www.asia.elsevierhealth.com/textbook-of-histology-e-book-9780323390682.html.

Halim M (2019). Image J. Laboratorium Anatomi, Departemen Anatomi Fakultas Kedokteran, Kesehatan Masyarakat, dan Keperawatan Universitas Gadjah Mada. Available at: https://lab-anat.fk.ugm.ac.id/2019/07/25/image-j/.

Hatori M, Vollmers C, Zarrinpar A, et al (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metabolism 15**,** 848–860. doi: 10.1016/j.cmet.2012.04.019.

IBM Corp. (2017). IBM SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp. Available at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25.

ImageJ (2023). ImageJ for Windows, version 1.54. USA. Available at: https://imagej.net/ij/.

Jameson JL, Fauci AS, Kasper DL, et al (2018). Harrison's principles of internal medicine, 20e. McGraw-Hill Education, New York, NY. Available at: http://accessmedicine.mhmedical.com/content.aspx?aid=1190788497.

Li G, Xie C, Lu S, et al (2017). Intermittent fasting promotes white adipose browning and decreases obesity by shaping the gut microbiota. Cell Metabolism 26**,** 672-685. doi: 10.1016/j.cmet.2017.08.019.

Li J, Wu H, Liu Y, et al (2020). High fat diet induced obesity model using four strains of mice: Kunming, C57BL/6, BALB/c and ICR. Experimental Animals 69**,** 326–335. doi: 10.1538/expanim.19-0148.

Liu B, Page AJ, Hutchison AT, et al (2019). Intermittent fasting increases energy expenditure and promotes adipose tissue browning in mice. Nutrition 66**,** 38–43. doi: 10.1016/j.nut.2019.03.015.

MacLean PS, Higgins JA, Giles ED, et al (2015). The role for adipose tissue in weight regain after weight loss. Obesity Reviews 16**,** 45–54. doi: 10.1111/obr.12255.

Mao T, Wei Q, Zhao F, et al (2021). Short-term fasting reshapes fat tissue. Endocrine Journal 68**,** 387–398. doi: 10.1507/endocrj.EJ20-0405.

Marinho T de S, Ornellas F, Barbosa-da-Silva S, et al (2019). Beneficial effects of intermittent fasting on steatosis and inflammation of the liver in mice fed a high-fat or a high-fructose diet. Nutrition 65**,** 103–112. doi: 10.1016/j.nut.2019.02.020.

Microsoft Corporation (2015). Microsoft Excel. Available at: https://office.microsoft.com/excel.

Ministry of Health of the Republic of Indonesia (2018). Report on result of national basic health research (RISKESDAS) 2018. Minist Heal Repub Indones. Available at: https://kesmas.kemkes.go.

id/assets/upload/dir_519d41d8cd98f00/files/Hasil-riskesdas-2018_1274.pdf.

Mishra P, Singh U, Pandey C, et al (2019). Application of student's t-test, analysis of variance, and covariance. Annals of Cardiac Anaesthesia 22**,** 407. doi: 10.4103/aca.ACA_94_19.

Munhoz AC, Vilas-Boas EA, Panveloski-Costa AC, et al (2020). Intermittent fasting for twelve weeks leads to increases in fat mass and hyperinsulinemia in young female wistar rats. Nutrients 12**,** 1029. doi: 10.3390/nu12041029.

Muñoz-Hernández L, Márquez-López Z, Mehta R, et al (2020). Intermittent fasting as part of the management for T2DM: From animal models to human clinical studies. Current Diabetes Reports 20**,** 13. doi: 10.1007/s11892-020-1295-2.

Nugroho RA (2018). Get to know mice as laboratory animals. Mulawarman University Press, Samarinda. Available at: https://repository.unmul.ac.id/bitstream/handle/123456789/1305/file_10219000341.pdf?sequence=3&isAllowed=y.

Ranstam J, Cook JA (2017). Considerations for the design, analysis and presentation of in vivo studies. Osteoarthritis and Cartilage 25**,** 364–368. doi: 10.1016/j.joca.2016.06.023.

Rifano R, Fahrudin M, Santoso K (2014). ImageJ application to calculate changes in duck erythrocyte nuclear area due to hypotonic solutions. Sci Repos. Available at: https://repository.ipb.ac.id/handle/123456789/70661.

Rynders CA, Thomas EA, Zaman A, et al (2019). Effectiveness of intermittent fasting and time-restricted feeding compared to continuous energy restriction for weight loss. Nutrients 11**,** 2442. doi: 10.3390/nu11102442.

Setiati S (2017). Textbook of internal medicine. Interna Publishing, Jakarta. Available at: https://lib.ui.ac.id/detail?id=20510639&lokasi=lokal.

Stenkula KG, Erlanson-Albertsson C (2018). Adipose cell size: Importance in health and disease. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 315**,** 284–295. doi: 10.1152/ajpregu.00257.2017.

Tang H-N, Tang C-Y, Man X-F, et al (2017). Plasticity of adipose tissue in response to fasting and refeeding in male mice. Nutrition & Metabolism (Lond) 14**,** 3. doi: 10.1186/s12986-016-0159-x.

Tsaban G, Bilitzky-Kopit A, Yaskolka Meir A, et al (2021). The effect of weight-loss interventions on cervical and chin subcutaneous fat depots; The central randomized controlled trial. Nutrients 13**,** 3827. doi: 10.3390/nu13113827.

Welton S, Minty R, O'Driscoll T, et al (2020). Intermittent fasting and weight loss: Systematic review. Canadian Family Physician Medecin de Famille Canadien 66**,** 117–125. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC7021351.

Woodie LN, Luo Y, Wayne MJ, et al (2018). 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 82**,** 1–13. doi: 10.1016/j.metabol.2017.12.004.

Yoshii R, Higashida K, Nakai N (2023). Intermittent fasting reduces mouse body fat while maintaining muscle mass by regulating protein synthesis and autophagy. Nutrition 115**,** 112130. doi: 10.1016/j.nut.2023.112130.