THE EFFECT OF STATIC MAGNETIC FIELD EXPOSURE ON BODY WEIGHT AND ADIPOSE CELLS DENSITY OF OBESE MICE

Puji Sari[1*], Luluk Yunaini[1], Widia Bela Oktaviani[2], Umiatin[3], Anita Dwi Suryandari[1]

[1]Department of Medical Biology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

[2]Master's Programme in Biomedical Science, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

[3]Department of Physics, Faculty of Mathematical and Natural Science, State University of Jakarta, Jakarta, Indonesia

Received: 6th June 2022; Revised: 4th August 2022; Accepted: 26th August 2022

ABSTRACT

Obesity is a mojar public health problem in worldwide, especially in Indonesia. Obesity in addition to affecting productivity, is also trigger for other chronic disease such as diabetes and cardiac disease. Body mass index is an assessment tool used to assess degree of individual adiposity to define overweight, obesity, and severe obesity.The determination of obesity is based on the calculation of Body Mass Index (BMI), which devide body weight (kg) by height (cm^2). In addition to the method of regulating diet, exercise, and bariatric surgery for weight loss, it was reported that the biophysical therapy tool, that is static magnetic field (SMF) became a modality for weight loss. Based on research reports, it proves that the static magnetic field affects weight loss in the group of obese mice after 30 days of exposure. Therefore, in this study, we carried out static magnetic field exposure to obese mice with a field intensity of 2 mT for 1 hour/day. Mice were exposed gradually to SMF on 2, 7, 14, and 21 days to determine the effectiveness of SMF to obesity in mice in terms of weight loss and cellular adipose cell density. The results showed that the weight of mice decreased significantly on 2nd and 7th days of exposure, the trend showed a decrease in body weight until the 14th day. The density of adipose tissue is increased after exposure to SMF on the 14th and 21st days of exposure. It showed that early exposure to SMF (2 and 7 days) could induce weight loss in mice, while cellularly SMF increased adipose cell density on late exposure (14 and 21 days).

Keywords: Static Magnetic Field; Obesity; Body Weight; Adipose Cell Density.

Introduction

Obesity is an excess accumulation of body fat stored in adipose tissue. Fat stored in adipose tissue in the form of triglycerides is a source of energy and is used when the body needs energy.[1] Normally, fat will be stored in white adipose tissue, but when the high intake of fat or energy is not matched by energy expenditure, the fat will be stored ectopically in other metabolic organs such as the liver, kidneys, and pancreas. This is what causes obesity to trigger other chronic diseases such as hypertension, diabetes, and hyperlipidemia through metabolic syndrome, which in turn leads to atherosclerotic diseases including cerebrovascular disease, coronary heart disease, chronic renal disease, and heart failure.[2] Body mass index is an assessment tool used to assess degree of individual adiposity to define overweight, obesity, and severe obesity.[3]

Indonesia as a developing country which is the 4th largest populated nation in the world also has a fairly high obesity rate. Based on a survey report by the Basic Health Research and Development Unit (Riskesdas) in 2007, 10.3% of the total number of adults (over 15

*Corresponding author.
E-Mail: puji_sari@yahoo.com

years) spread across 33 provinces of Indonesia were obese and 8.8% of them were overweight.[4] As a step to study the pathophysiology and molecular aspects of obesity in humans, previous studies have used many experimental animal models of obese mice induced by high-fat diets.

Determination of obesity in mice based on the calculation of index Lee. This index can identify obese mice with divide weight ($g^{0.33}$) by height (cm). Mice were categorized as obese if score of index Lee is $\geqslant$ 310.[5] In normal mice that were fed a high-fat diet, there was an increase in body weight and an increase in body fat leading to obesity.[6] Feeding high-fat diet to C57BL6/7 mice could induce weight gain faster than other strains. C57BL6/J mice were a mice strain that responded well to weight gain after being induced by high-fat diet.[7] Obesity in mice refers to the Lee index 310.

Static magnetic field is a type of magnetic field with a frequency of 0 Hz. The cell responds to a static magnetic field due to the influence of the field intensity generated by the Helmholtz coil when multiplied by direct current (DC). SMF field intensity is a very important parameter. The field intensity recommended by the International Commission on Non-Ionizing Reduction Protection (ICNIRP) in medical research is not to exceed 400 mT. In addition, the effect of the magnetic field is also influenced by the frequency and duration of exposure. Research on the molecular mechanism of SMF in inducing various cell biological functions is still very limited, however, SMF is reported to affect cell proliferation and differentiation by increasing the concentration of cytosolic Ca^{2+} which functions as a second messenger to influence various signaling pathways.

Static magnetic field (SMF) has a positive effect as a biophysical modality against bone diseases such as accelerating bone fracture healing and bone regeneration (in the in vivo journal SMF). Furthermore, SMF also affected weight loss in the group of obese mice after 30 days of exposure.[8] A large number of reports on the positive effects of SMF stimulation is the basis for this research. To further study the effect of SMF on obese mice, observations were made on the weight of the mice and the density of *adipose* tissue. SMF exposure to mice with a field intensity of 2 mT for 1 hour/day in vivo on different day groups of mice, that is the exposure on 2, 7, 14, and 21 days.t *man*

Methods

Ethical Approval

Experimental procedures and animal welfare used in this study were approved by The ethical committee of the Universitas Indonesia with number KET-678/UN2.F1/ETIK/PPM.00.02/2020.

High-Fat Feeding

A total of 24 mice strain C57BL6/J, aged 6 weeks were used in this research. After acclimatized for approximately 7 days, mice were randomly grouped into mice that were fed a high-fat diet (as obese group) and a standard diet (as normal group). The composition of the high-fat diet is 55% energy from fat obtained from the iRATco laboratory. During the obesity induction stage, the parameter observed was the weight of the mice. Mice that have experienced significant weight gain were then grouped into 4 major groups based on the intensity of exposure to the static magnetic field. The groups included: the 2^{nd}, 7^{th}, 14^{th}, and 21^{st} day exposure groups, each cage containing 4 mice. In normal and obese groups, termination was carried out on day 21. Termination of obese

 ISSN:1979-6374/ EISSN:2460-5999

group 2 after 2 days of exposure, group 7 after 7 days of exposure, group 14 after 14 days of exposure, and group 21 after 21 days of exposure.

Static Magnetic Field Exposure

The static magnetic field exposure was carried out at the iRATco Animal Laboratory. The static magnetic field component used consists of a pair of Helmholtz coils with a diameter of 65 cm, a DC power supply, which is a device used to transmit electric current in one direction to the Helmholtz coil, and a Gauss meter was used to read the magnitude of the magnetic intensity generated by SMF. The obese group mice without exposure and the obese group with exposure were placed in cages made of plastic material. The exposure was carried out with the cage being placed between 2 Helmholtz coils. The intensity of magnetic exposure was 2 mT within 1 hour every day depends on the group of mice exposed to the 2, 7, 14, and 21 days.

Adipose Tissue Confirmation

Mice were euthanized using ketamine selasin intraperitoneally. After being euthanized, the mice were biopsied for inguinal adipose tissue. After the adipose tissue was isolated, it was immediately rinsed with sterile saline and the blood vessels around the adipose tissue were removed with sterile tweezers. Adipose tissue was then cut and weighed as much as ± 200 mg, then put into a tube that already contains RNA Later solution to be stored in a -20˚C freezer to avoid tissue damage until it was used for further analysis. The isolated white adipose tissue was further confirmed by analysis with 0.21% (w/v) oil red staining for 10 minutes at room temperature after being previously fixed with 10% paraformaldehyde. The stained adipose cells were observed using an inverted microscope. Adipose cells observed under a microscope would look shiny.

Inguinal Adipose Cell Density Analysis

Inguinal adipose tissue was fixed using a 10% neutral buffer solution of formalin for 24 hours at room temperature. Tissue that was made into paraffin blocks were cut using a microtome with a thickness of 5 μm. The cut slides were then deparaffinized, rehydrated, then stained with Hematoxylin and Eosin (H&E). The staining process was carried out by placing the slide into a metal staining rack, then stained with Harris Hematoxylin for 10 seconds and washed using running tap water. The slides were stained with eosin for 20 seconds and washed again using tap water. Furthermore, the slides were dehydrated using ascending alcohol and continued with mounting. The stained slides were then observed using a light microscope with 400x magnification, then the density of adipose cells was measured in 4 fields of view.

Statistics analysis

Parameters of weight and density of adipose tissue were analyzed using the International Business Machines Statistical Package for the Social Sciences (IBM SPSS) version 26. Statistical test stages began with the analysis of normality test and homogeneity test. The sample used in this study was <30 mice, so the Shapiro-Wilk test was used to test for normality. The data were then analyzed using the One-Way Annova parametric test followed by Post Hoc LSD.

 ISSN:1979-6374/ EISSN:2460-5999

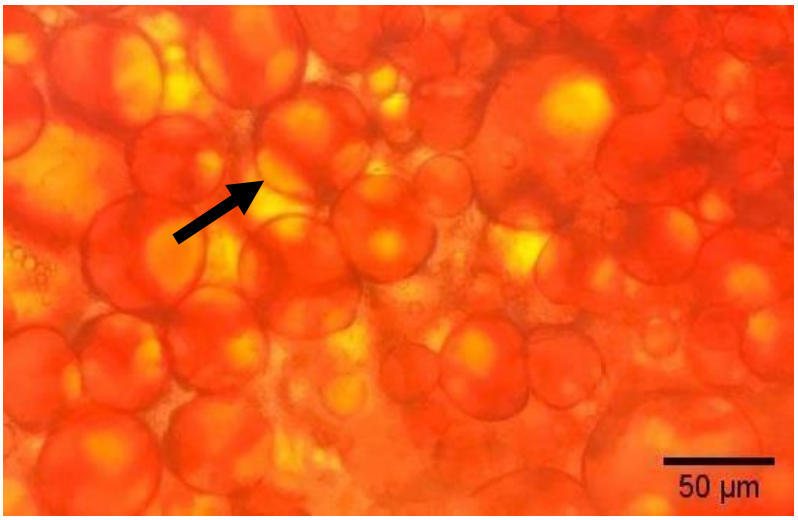

Figure 1. Oil red staining results on inguinal adipose under an inverted microscope. Arrows indicate fat droplet.

Table 1. The average initial and final weight

Group	Body Weight (gram) Mean ± SD		p-value
	Initial	Final	
Normal	35.51 ± 1.99	30.66 ± 1.93	0.005
Obese	35.87 ± 2.88	33.56 ± 2.15	0.160
Obese 2	46.44 ± 2.35	45.22 ± 2.10	0.021
Obese 7	44.51 ± 1.36	41.94 ± 2.06	0.033
Obese 14	40.53 ± 4.57	32.86 ± 4.96	0.169
Obese 21	40.11 ± 4.11	40.31 ± 2.93	0.927

Result and Discussion

Inguinal Adipose Tissue Confirmation

The observation results of inguinal adipose tissue under an inverted microscope can be seen in Figure 1.There are red fat droplets after being stained with *oil red o*. Oil red O dye is often used in research related to adipose tissue. Based on these results, the tissue isolated in the body of mice was fat tissue

Body weight data among groups

In each group of normal, obese, obese 2, obese 7, obese 14, and obese 21, the weight of the mice was measured. Body weight of the mice was weighed on the first day of SMF exposure to obtain initial data, while to obtain the final data, the body weight of the mice was adjusted on the last day the mice were exposed. The average initial and final weight can be seen in Table 1.

The initial and final weight data were then analyzed to see the effect of SMF exposure in each group. After confirming that the data are normally distributed, it was continued with *dependent t-test*. Based on *dependent T-test*, The weight of the mice before and after exposure showed significant ($p<0.05$), respectively in the obese group 2 $p=0.021$ and the obese group 7 $p=0.033$. Mice weight loss affected the intensity of the diet. The effect of SMF exposure on changes in body weight of mice had been reported by Tsuji et. al. Based on a study conducted using BALB/c strain mice that were exposed to 5T intensity SMF, there was a decrease in body weight of mice

after 24 hours of exposure and showed significance after 48 hours.[9] Weight loss of mice after exposure to SMF was also reported by Tian et al. 2019.[10] Observation of mice's body weight for 21 days showed a decrease in body weight compared to the control group, but the data showed significance on days 10-12 of exposure. The decrease in body weight was in line with the decrease in the graph of food consumption in mice exposed to 23.0 T.

The average weight of the obese and obese 14 group decreased, while the obese 21 group experienced an increase compared to before exposure. However, the difference did not show any significance. Based on Table 1, it can be seen that the average final body weight in each group experienced a decrease compared to the initial body weight, except for the obese group 21 which experienced weight gain but not significantly. The increased body weight of mice after 28 days of SMF exposure (2-4 T, 8-10 T, and 10-12 T) was also reported by Wang S., et al. The obesity parameter is weight gain above normal weight standard.[11] Research conducted by Yu B., et al (2021) reported that the weight gain of mice was lower in the group that was exposed to downward SMF and 0.6 T SMF than the group of diabetic mice induced by high fat diet without exposure to SMF.[12] b In this study, the average body weight of the normal, obese, and obese groups 2, 7, and 14 decreased with the length of exposure to SMF.

In the normal and obese groups, termination was carried out after 21 days. Significant weight loss occurred in the group of normal mice that were not exposed to SMF. This weight loss is thought to be related to decreased appetite and food intake caused by aging.[13-14] lden-Hanson T et al. have reported that fat mass and leptin levels increase with aging.[15] Leptin plays critical role in regulation of food intake and leptin deficiency is responsible for the hyperphagia in obese (ob/ob) mouse.[16]

Obesity occurs when adipose tissue stores excess triglycerides. When needed, the stored triglycerides are broken down into fatty acids and glycerol. The breakdown product of triglycerides in the form of fatty acids will be utilized by the muscles in the form of acetyl coenzyme A, the brain in the form of ketone bodies, and glycerol into glucose. Lipolysis of triglycerides involves the regulation of various hormones, enzymes, and signaling pathways.[17]

Adipose Tissue Density

The results of H&E staining can be seen in Figure 2. Hematoxylin stains the cell nucleus which was indicated by a dark blue nucleus, while the cytoplasm stained by eosin produced a pink color. Observations results under a microscope with 400x magnification in 5 fields of view. After obtaining pictures of H&E staining of adipose tissue results in each group, it was then analyzed to obtain quantitative density data. The results of the adipose tissue density analysis can be seen in Table 2.

Table 2. Average adipose tissue density among groups

Group	Adipose Tissue Density Mean ± SD	p-value
Normal	21.69 ± 5.88	0.001
Obese	7 ± 3.12	
Obese 2	5.31 ± 1.62	
Obese 7	6.12 ± 2.87	
Obese 14	12.62 ± 6.33	
Obese 21	10.75 ± 7.46	

 ISSN:1979-6374/ EISSN:2460-5999

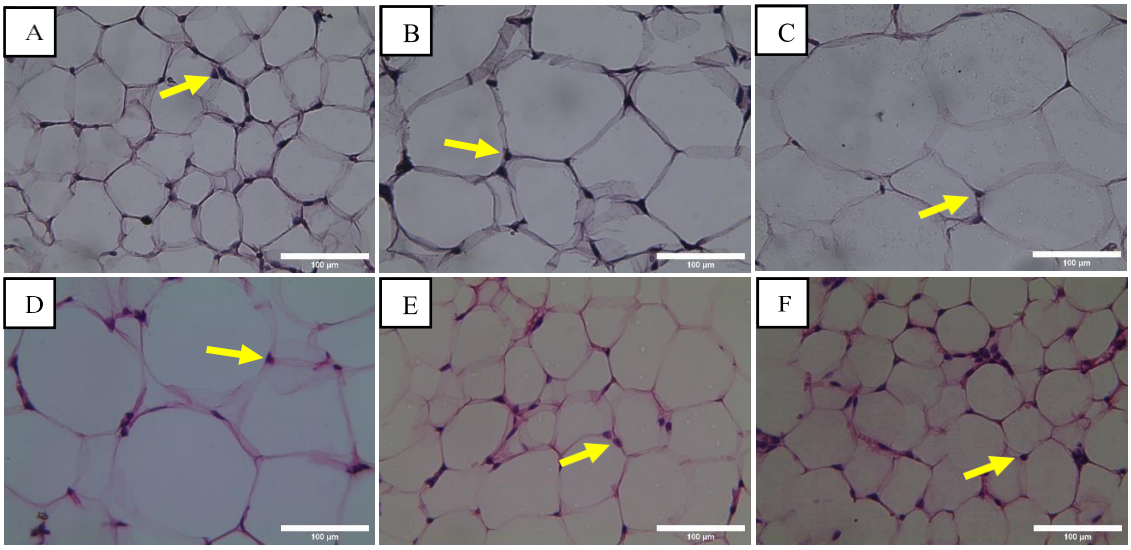

Figure 2. The results of H&E staining were observed under a microscope at 400x magnification. Groups (A) Normal, (B) Obese, (C) Obese 2, (D) Obese 7, (E) Obese 14, (F) Obese 21. The arrows indicate the nucleus of the cells.

In table 2, the average adipose tissue density among groups showed significance with a p-value = 0.001. The highest average adipose density value was in the normal group and the density value decreased after being induced with a high-fat diet which could be seen in the adipose cell density of the obese group. In this study, it was hypothesized that normal weight mice have high density values. Hence when obesity was induced by feeding high fat diet, adipose tissue density decreases, and the results of the study supported this hypothesis. In line with the research conducted by Murphy et al. 2014, the density of adipose tissue will increase when there is weight loss.[18] SMF exposure to the obese group showed an increasing trend of density, especially found in the obese group on 14th day. We suspected that the increased density value in the obese group exposed to SMF was due to the presence of signaling pathways that were affected by SMF exposure. SMF exposure had been reported to affect membrane lipids by modulating ion channel activity and increasing calcium levels.

Conclusion

SMF exposure to obese mice on days 2, 7, 14, and 21 showed that SMF was effective in reducing body weight in mice at early stage of exposure (2 and 7 days), evidenced by a decrease in body weight after exposure compared to before exposure on the 2nd and 7th day groups. Meanwhile, cellularly SMF increased adipose cell density on late exposure (14 and 21 days).

Acknowledgment

We would like to gratefully acknowledge to Hibah Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) grant number NKB-128/UN2.RST/HKP.05.00/2021,which provide the funding to conduct this research.

References

1. Marquez MP, Alencastro F, Madrigal A, Jimenez JL, Blanco G, Gureghian A, Keagy L, Lee C, Liu R, Tan L, Deignan K. The role of cellular proliferation in adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells. Stem cells and development. 2017 Nov 1;26(21):1578-95..
2. Corrales P, Vidal-Puig A, Medina-Gómez G. PPARs and metabolic disorders associated with challenged adipose tissue plasticity. International Journal of Molecular Sciences. 2018 Jul 21;19(7):2124.
3. Heimburger DC, Weinsier RL. Handbook of clinical nutrition. St.

Louis, MO. Mosby-Year Book.1997.
4. Harbuwono DS, Pramono LA, Yunir E, Subekti I. Obesity and central obesity in Indonesia: evidence from a national health survey. Medical Journal of Indonesia. 2018 Sep 9;27(2):114-20.
5. Rogers P, Webb GP. Estimation of body fat in normal and obese mice. British Journal of Nutrition. 1980 Jan;43(1):83-6.
6. Tschöp M, Heiman ML. Rodent obesity models: an overview. Experimental and clinical endocrinology & diabetes. 2001;109(06):307-19.
7. Wang CY, Liao JK. A mouse model of diet-induced obesity and insulin resistance. InmTOR 2012 (pp. 421-433). Humana Press.
8. Abou-Saleh RH. Assessment of biological changes of continuous whole body exposure to static magnetic field and extremely low frequency electromagnetic fields in mice. Ecotoxicology and environmental safety. 2008 Nov 1;71(3):895-902.
9. TSUJI Y, NAKAGAWA M, SUZUKI Y. Five-tesla static magnetic fields suppress food and water consumption and weight gain in mice. Industrial health. 1996;34(4):347-57.
10. Tian X, Wang D, Feng S, Zhang L, Ji X, Wang Z, Lu Q, Xi C, Pi L, Zhang X. Effects of 3.5–23.0 T static magnetic fields on mice: a safety study. Neuroimage. 2019 Oct 1;199:273-80.
11. Wang S, Luo J, Lv H, Zhang Z, Yang J, Dong D, Fang Y, Hu L, Liu M, Liao Z, Li J. Safety of exposure to high static magnetic fields (2 T–12 T): a study on mice. European Radiology. 2019 Nov;29(11):6029-37.
12. Yu B, Liu J, Cheng J, Zhang L, Song C, Tian X, Fan Y, Lv Y, Zhang X. A static magnetic field improves iron metabolism and prevents high-fat-diet/streptozocin-induced diabetes. The Innovation. 2021 Feb 28;2(1):100077.
13. Akimoto S, Miyasaka K. Age-associated changes of appetite-regulating peptides. Geriatrics & gerontology international. 2010 Jul;10:S107-19.
14. Morley JE. Decreased food intake with aging. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2001 Oct 1;56(suppl_2):81-8.
15. Wolden-Hanson T, Marck BT, Smith L, Matsumoto AM. Cross-sectional and longitudinal analysis of age-associated changes in body composition of male Brown Norway rats: association of serum leptin levels with peripheral adiposity. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 1999 Mar 1;54(3):B99-107.
16. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998 Oct;395(6704):763-70.
17. Chaves VE, Frasson D, Kawashita NH. Several agents and pathways regulate lipolysis in adipocytes. Biochimie. 2011 Oct 1;93(10):1631-40.
18. Murphy RA, Register TC, Shively CA, Carr JJ, Ge Y, Heilbrun ME, Cummings SR, Koster A, Nevitt MC, Satterfield S, Tylvasky FA. Adipose tissue density, a novel biomarker predicting mortality risk in older adults. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2014 Jan 1;69(1):109-17.

 ISSN:1979-6374/ EISSN:2460-5999