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A Static Magnetic Field Exposure in Obese Mice Induced by High Fat Diet: Its Effect on T-Box15 Gene and Uncoupling Protein 1 Expression

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Abstract: Increased Ca^{2+} cytosolic concentration caused by Static Magnetic Field (SMF) exposure modulates Tbx15 protein and Ucp1 gene interaction that is involved in the thermogenesis browning process of white adipose tissue. Activation of Tbx15 as a transcription factor that regulates the expression of the Uncoupling protein 1 (Ucp1) gene induced by SMF exposure in adipose tissue converts excess accumulated fat into heat. This discovery has led to a novelty in preventing obesity. Experimental studies to determine the effect of SMF on the browning process have not been widely reported. Hence, we investigated its effect on lee index, Tbx15, and Ucp1 expression, as well as adipose cell size in obese mice inguinal adipose tissue. This study used two control groups, namely normal and obese mice. We generated C57BL/6J obese mice only by inducing a High-Fat Diet (HFD). Mice were exposed to SMF at a 2 mT intensity for 1 h per day for 21 days of adipocyte differentiation. Lee index, Tbx15 protein, Ucp1 gene, and histological inguinal adipose histology were all investigated. Tbx15 expression increased after 2-7 days of SMF exposure and Lee index decreased significantly ($p<0,05$) for 2-21 days of SMF exposure. Ucp1 gene expression increased after SMF exposure, however, there was no significant change following SMF exposure. After 14-21 days of exposure, adipose cellsize was slightly reduced. Therefore, we can conclude that the SMF exposureat 2 mT intensity for 1 h per day could improve the browning process by increasing Tbx15 and Ucp1 expression after 2-7 days and adipose cell size phenotypically reduced at 14-21 days of SMF exposure. This study adhered to ethical guidelines and received the necessary approval from the ethical committee of universitas Indonesia no. KET-678/UN2.F1/ETIK/PPM.00.02/2020.

Keywords: Tbx15, Ucp1, SMF, Browning, HFD

Introduction

Obesity is defined as an accumulation of excess body fat caused by an imbalance in energy intake and expenditure. Excessive calorie intake causes lipogenesis, adipocyte formation via adipogenesis, adipocyte enlargement, and increased lipid storage (Marquez *et al*., 2017). Excess fat accumulation ectopically in metabolic organs such as the liver, skeletal muscles, kidney, and pancreas (Marquez *et al*., 2017). In 2016, 11% of men and 15% of women in the world's adult population were obese according to the world health organization. The category of obesity in humans is determined by calculation of Body Mass Index (BMI). The world health organization divides the normal BMI range for adults as 18,5-24,9 kg/m^2, overweight has a BMI of 25 kg/m^2, over 30 kg/m^2 is considered obese and 40 kg/m^2 is categorized as severe obesity. Obese mice were used to study the mechanism of obesity and the drug efficacy mostly used obese mice induced by high-fat diet. Obesity in mice refers to the Lee index 310 (Rogers and Webb, 1980).

Excess energy accumulates in White Adipose Tissue (WAT) as a result of an imbalance between high energy intake and low energy expenditure (Nascimento *et al*., 2018). There are two types of adipose tissue in mammals.

White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). WAT is responsible for fat storage in the form of triglycerides, whereas BAT is responsible for body temperature regulation via thermogenesis (Alcalá *et al*., 2019). Another type of adipose tissue discovered recently

in mice and humans is beige Adipose Tissue (bAT). Adipogenesis is regulated by a complex mechanism that includes several transcription factors such as Peroxisome Proliferator-Activated Receptor gamma (PPARγ) and several members of the CCAAT/Enhancer Binding Protein family (EBPs) (Park, 2014). Beige adipose tissue is white adipose tissue that can phenotypically transdifferentiate into BAT-like tissue with thermogenic activity, a processknown as browning.

Electromagnetic fields have been widely used as medical therapy tools, including Transcutaneous Electrical Nerve Stimulation (TENS), Neuromuscular Electrical Stimulation (NMES), High Voltage Pulsed Galvanic (HVPG), and Pulsed Electromagnetic Field (PEMF), which uses electricity to generate electric and magnetic fields (Wade, 2013). SMF is a type of biophysical stimulus whose waves do not change over time (static). The magnetic field produced by SMF is generated by a helmholtz coil with Direct Current (DC) (Miyakoshi, 2005). SMF with a moderate field intensity (1 mT-1 T) is widely used in various studies. SMF exposure of 2 mT field intensity affects the density of adipose tissue, and after 14 days of SMF exposure showed an increasing trend of adipose tissue density in obese mice (Sari *et al*., 2022a). Further research, 2 mT field intensity of SMF reduced apolipoprotein B and troponin T as markers of coronary heart disease in C57BL/6J obese mice (Sari *et al*., 2022b). According to the International Commission on non-ionizing radiation protection, the maximum safe limit of intensity for use in health is 400 mT (ICNIRP, 2009).

SMF's molecular mechanism of action on biological function is still being investigated. In theory, SMF exposure can activate voltage-gated ion channels, influencing ion movement across the plasma membrane and causing changes in ion concentrations in the cell environment (Zhai *et al*., 2020; Zhang *et al*., 2018). The main effect of the magnetic field is inducing the orientation of the membrane lipid molecules which affects the conformational changes of the ion channels. SMF exposure affects the α1 subunit of the voltage-gated calcium channels through the diamagnetic anisotropy and reorientation of membrane phospholipids. It caused a conformational change in the ion channel and facilitates ion transport across the membrane (Lu *et al*., 2015). Magnetic fields influence many cellular processes, including mitochondrial membrane potential (Wang *et al*., 2018), adipogenesis inhibition and osteogenesis induction (Maredziak *et al*., 2016), neuronal cellular activity, and cell proliferation and differentiation via increased cytosolic Ca^{2+} concentrations. Higher concentrations of Ca^{2+} ions in the intracellular compared to the extracellular environment can induce gene transcription by various signaling pathways. Ca^{2+} ions can phosphorylate various transcription factors such as (Tbx15) that are involved in the browning of white adipocytes (Sun *et al*., 2019). Tbx is a gene that encodes a transcription factor involved in various developmental processes and regulates mitochondrial activity. The Tbx15 family is highly expressed in brown tissue and Inguinal White Adipose Tissue (IngWAT), but only weakly expressed in visceral white adipose tissue, such as Epididymal White Adipose Tissue (EpiWAT). According to an experimental study, Tbx15 regulates the thermogenesis and browning of adipocytes via interaction with prdm16 at the prdm 16-2 kb promoter, which in turn includes the expression of the Ucp1 gene (Sun *et al*., 2019). Thermogenesis is the production of heat, which occurs in certain tissues such as BAT and bAT. The thermogenic function of bAT is mediated by Ucp1. Uncoupling protein 1 is found in the inner mitochondrial membrane and is involved in oxidative phosphorylation processes that result in energy dissipation as heat (Contreras *et al*., 2015). Increased Ca^{2+} cytosolic concentration caused by Static Magnetic Field (SMF) exposure modulates the Tbx15 protein and Ucp1 gene interaction that is involved in the thermogenesis and browning process of white adipose tissue. Activation of Tbx15 and Ucp1 gene by SMF exposure in adipose tissue converts excess accumulated fat into heat. This discovery has led to a novelty in preventing obesity. This study was conducted to examine the effect of 2 mT intensity of SMF exposure duration 1 h day on obese mice *in vivo* for 21 days during the adipose differentiation period beginning on days 2, 7, 14, and 21. The effects of SMFs on the preadipocyte stage have been studied mainly *in vitro*. Exposure duration and intensity of SMF used in this research based on *in vitro* study, the preadipocyte stage begins on days 0-7, while 14-21 days is adipocyte differentiation stage (Billon *et al*., 2010). 2 mT intensity of SMF resulted in inhibiting adipogenesis *in vitro* (Sari *et al*., 2020c). Duration 1 h/day affects lipid metabolism in rats *in vitro* (Lahbib *et al*., 2010). Until recently, the stage of preadipocyte formation and adipocyte differentiation *in vivo* is still unknown, therefore, this research refers to preadipocyte and adipocyte differentiation stages *in vitro*. The effect of SMF exposure on inguinal WAT was observed through differences in Lee index, adipose cell size, Tbx15 protein, and Ucp1 gene expression.

Materials and Methods

Induction of High Fat Diet

Twenty-four male C57BL/6J mice aged 6 weeks were obtained from iRATco. The number of mice in this study was obtained from the calculation of the minimum sample size using the Federer formula as follows:

$$(t-1)\,(n-1) \geq 15$$

Annotation:

t = Number of treatment
n = Number of samples

Four mice were taken for each normal (group of normal weight mice without SMF exposure), obese (group of obese mice without SMF exposure), obese 2 (group of obese mice exposed to SMF during 2 days), obese 7 (group of obese mice exposed to SMF during 7 days), obese 14 (group of obese mice exposed to SMF during 14 days) and obese 21 (group of obese mice exposed to SMF during 21 days).

A total of 24 mice used were kept at room temperature after they arrived. After one week of adaptation, mice were randomly assigned to two groups, kept on a 12 h light/dark cycle, and givenad libitum access to a standard diet (containing 3, 5% lipid) for the control group (n = 4) and a High Fat Diet (HFD) for the obese group (n = 20) (contained <55% lipid). Mice diets were designed and manufactured by indofeed, iRATco, Bogor, Indonesia. After being confirmed obese by index Lee (Rogers and Webb, 1980), 20 HFD-fed mice were randomly divided into the obese group (without SMF exposure) and four groups of obese mice with SMF exposure distinguished by day of SMF exposure (2, 7, 14 and obese 21).The body weight was measured on a weekly basis.

Exposure to SMF in Obese Mice

The Static Magnetic Field (SMF) used in this research consists of 2 main components, namely the DC power supply and the helmholtz coil. The helmholtz coil is a series of coils consisting of several coils of copper wire through an electric current flow. The helmholtz's diameter is 65 cm. DC power supply allows current to flow in one constant direction in the coil according to the amount of power needed (Rogers and Webb, 1980).

Obese mice were housed in plastic cages and placed 20-21 cm apart between two helmholtz coils. The cage measures 30 cm long, 20 cm wide and 15 cm high. The magnetic field intensity was 2 mT and mice were exposed for an hour per day. The magnetic intensity was measured using a Gauss meter, while the duration of SMF exposure was monitored using a timer.

Adipocyte Samples Collection

At the end of the experiment, mice were sacrificed intraperitoneally with ketamine selasin. Adipocyte tissue was extracted from subcutaneous inguinal adipose tissue made by an incision in the abdominal muscles of mice using clean forceps and surgical scissors. Inguinal adipose tissue was immediately sectioned and weighted. Later, 200 mg of inguinal adipose was transferred into 200 µL of RNA before being stored at -20°C.

Histopathological Examinations

Inguinal adipose tissue samples were fixed for 24 h in 10% buffer neutral formalin at room temperature. Following that, samples were dehydrated using alcohol. Xylol was used in the clearing process. The samples were then embedded in paraffin and cut into 5 µm thickness slices. After that, the slides were deparaffinized, rehydrated, and stained with hematoxylin and eosin. The adipose tissue morphology (Fig. 2) was examined under a microscope and thelength of the adipocytes was measured in 5 fields of viewusing the imageJ program.

Immunohistochemistry

The following slides were deparaffinized in xylol, rehydrated in alcohol, and then washed with distilled water. Slides were then placed in a buffered citrate solution with a pH of 6.0 and a concentration of 10 mm and antigen retrieval was accomplished by autoclaving at 120°C for 15 min. The activity of endogenous peroxidase was blocked with 0.3% H_2O_2 methanol at room temperature for 15 min. The slides were then incubated overnight at room temperature with rat anti-Tbox15 antibody (dilution 1:250). After washing with PBS/Brij, the slides were incubated for 30 min with biotinylated rabbit anti-rat secondary antibody and 30 min with peroxidase-conjugated streptavidin. After washing with PBS, the slides were treated with the DAB substrate kit for 5 min at room temperature before being counterstained with hematoxylin. The slides were then dehydrated with alcohol, mounted, and examined under a microscope in 5 fields of view at 40x magnification. The immunohistochemistry procedure addressed to profiling Tbx15 protein for OD analysis (Fig. 3) using the IHC profiler for semiquantitative assessment (Seyed Jafari and Hunger, 2017).

RT-qPCR

TRIzol™ LS reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate RNA from inguinal white adipose tissue. The concentration and purity of the isolated RNA were assessed using a nanodrop spectrophotometer to calculate the quantity and quality of total RNA. The isolated mRNA was then reverse-transcribed into cDNA using the revertraace™ qPCR RT master mix kit with gDNA remover from Toyobo (FSQ-301). Following backward transcription into cDNA, Sensi fast SYBR lo-rox two-step kit, primer, and nuclease-free water were added to each cDNA sample. The primer for the Ucp1 gene was designed using the NCBI primer designing tool software. The primer sequences are shown in Table 1.

The β- actin gene was used as a housekeeping gene to calculate Ucp1 gene expression using the Livak formula (2-ΔΔ CT).

Livak formula:

ΔC_T sample (obese 2, 7, 14, 21) = C_T gene target-C_T gene reference

ΔC_T kalibrator (normal) = C_T gen target-C_T gen referensi

$\Delta\Delta C_T$ = ΔC_T-ΔC_T kalibrator ekspresi relatif gen

= $2^{-\Delta\Delta CT}$

Table 1: Primer sequences of target and reference gene

No.	Gene name	Access code	Primer code
1	Ucp1	NM_009463.3	F[a]: GAGGTCGTGAAG GTCAG AATG R[b]: AAGCTTTCTG TGGTGGCTATAA
2	β-actin	NM_007393.5	F: CTCCCTGGAGA AGAGCT ATGA R: CCAAGAAGGA AGGCTGGAAA

(a) Primer forward; (b) Primer reverse

Table 2: Running program of qRT-PCR

Cycles	Temperature °C	Time	Phase
1	95	2 min	Polymerase activation
40	95	5 sec	Denaturation
	60	10 sec	Annealing
	72	5 sec	Extention

This mixture was performed in duplicate and then placed in the applied biosystems® 7500 fast engine and the running program was as follows in Table 2.

Statistical Analysis

The International Business Machines Statistical Package for the Social Sciences (IBM SPSS) version 26.0 was used for statistical analysis. The shapiro-wilk test was used to compare the normality of groups (normal, obese days 2, 7, 14, and 21). Simply body weight data was normally distributed then used t-test independent for statistical analysis. Adipocyte length, gene, and protein expression used the mann-whitney and kruskal-wallis test caused data were not normally distributed. $p<0.05$ was considered statistically significant.

Results

Weight Gain of Mice Induced by High Fat Diet

HFD induction was carried out in C57BL/6J mice for 17 weeks. HFD administration resulted in weight gain in the obese group when compared to the control group, as shown in the bar chart (Fig. 1a). The normal group's average body weight was 35,51±1.99 g, which was lower than the obese group's average body weight of 41,49±4,77 g. This data suggested that the HFD induced obesity in mice, resulting in a significant ($p<0.05$) increase in body weight. Figure 1b depicts the effect of weight gain on obesity, with normal and obese groups differing in size, particularly in the abdomen, indicating that mice fed the HFD had larger abdominal sizes than the normal group. Comparison of bar chart to normal body weight vs obese mice. Data are shown as mean ± SD $p<0.05$ (B) comparison of the body size of normal and obese mice.

Index Lee of Mice Before and After SMF Exposure

Measurements of body weight and naso-anal length of mice were carried out before and after SMF exposure to obtain Lee's index data. The dependent t-test was used to determine the difference between the initial and final Lee index values of the normal, obese, obese 2, 7, 14, and 21 groups. The initial and final Lee index was therefore significant ($p<0.05$) for 2, 7, 14, and obese 21, respectively. The average Lee index of the normal and obese groups increased compared to the initial value. However, Lee's index value increased in both normal and obese groups and did not show any significance (Table 3).

Adipose Tissue Morphology

Figure 2 shows the results of HE staining. The cell nucleus is stained with hematoxylin, which produces a dark blue nucleus, whereas the cytoplasm is stained with eosin, which produces a pink color. Differences in adipocyte size were observed under a microscope with a magnification of 40x in 5 fields of view. Adipose cells in the normal group were smaller than those in the, 2, 7, 14, and obese 21 groups.

Table 4 displays quantitative data derived from adipocyte size measurements. The length of adipose cells was measured manually using the ImageJ program. Adipose cell size between groups showed a significant difference $p = 0,012$ ($p<0.05$).

Following a post-hoc mann-whitney analysis, the length of adipose cells differed significantly between groups ($p<0.05$). Adipose cells in the, 2, 7, 14, and 21 groups were larger than those in the normal group, though only the normal and obese group, normal and obese 2, normal and obese 7, and normal and obese 21 showed significant differences. Furthermore, there was no significant difference between the SMF-exposed and non-exposed obese groups, whereas the size of adipose cells in the obese 2, 7, 14, and 21 groups was smaller than the obese group that did not receive SMF exposure. Obese 14 had smaller cells than obese 7 and obese 2, whereas obese 21 had smaller cells than obese 2 (Table 5).

Static Magnetic Field Upregulated Tbx15 Protein Expression in Mice-Induced Obesity

Tbx15 protein was stained using Diaminobenzidine (DAB) staining in this study. The brown color produced by DAB staining on the tissue preparation indicates a positive result. The IHC images of mouse adipose tissue revealed that the Tbx15 protein was expressed in the nucleus (Fig. 3). The IHC profiler feature, not only provides color deconvolution in the image but also the percentage of strong positive, positive, weak positive, and negative contributions. The four percentage contributions wereaccumulated to get the optical density score in each field of view, respectively.

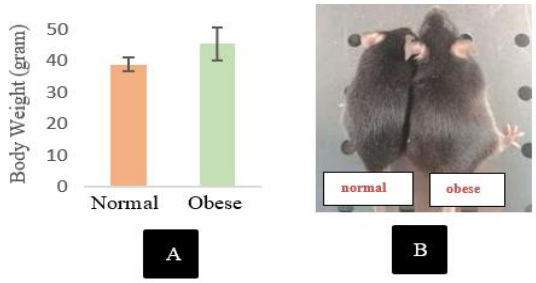

Fig. 1: High-fat diet induces weight gain in C5BL/6J mice; (a) Comparison of bar chart to normal body weight vs obese mice. Data is shown as mean ± SD. p<0.05; (b) Comparison of the body size of normal and obese mouse

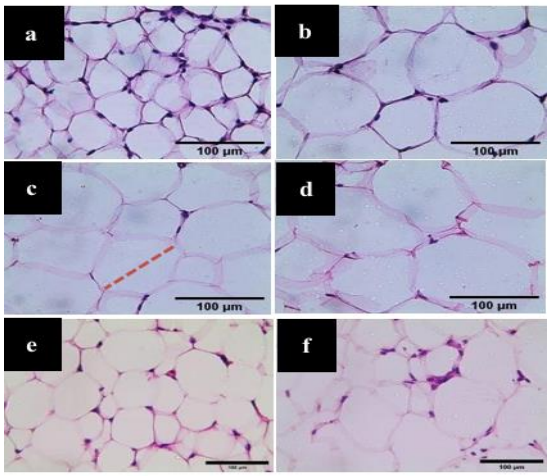

Fig. 2: H and E staining of inguinal white adipose tissue from the normal; (a) Obese; (b) Obese 2; (c) Obese 7; (d) Obese 14; (e) And obese 21; (f) Groups magnification: 40x

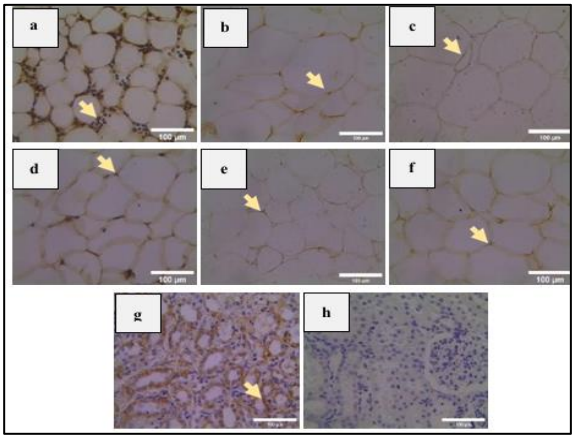

Fig. 3: Representative DAB staining for Tbx15 protein. Groups normal; (b) Obese; (c) Obese 2; (d) Obese 7; (e) Obese 14; (f) Obese 21. Mice kidney preparation was used asa positive control (g) and a negative control (h). Preparations were observed under a microscope at 40x magnification. The arrows indicate the Tbx15 expression in the nucleus

Table 3: Index lee of mice was reduced after SMF exposure

	Index Lee		
Groups	Initial	Final	p-value
Normal	0.288±0.014	0.289±0.012	0.300
Obese	0.318±0.010	0.319±0.005	0.391
Obese 2	0.329±0.016	0.326±0.015	0.035*
Obese 7	0.324±0.016	0.317±0.018	0.032*
Obese 14	0.314±0.003	0.304±0.004	0.029*
Obese 21	0.324±0.014	0.322±0.014	0.031

*p<0.05

Table 4: Inguinal adipose cell size

Group	Cell size µm median (min-max)	p-value
Normal	140.70 (130.31-175.90)	
Obese	255.05 (173.92-286.25)	
Obese 2	240.37 (231.62-260.96)	0.012*
Obese 7	241.33 (189.39-271.26)	
Obese 14	175.02 (153.41-203.23)	
Obese 21	214.74 (152.85-221.47)	

*p<0.05

Based on Table 6, there was a decrease in OD scores in the obese group without SMF exposure and all obese groups exposed to SMF to the normal group. Optical density score increased in all obese groups exposed to SMF compared to obese groups without exposure. Among obese groups exposed to SMF, the highest and lowest OD scores were found in the obese 7 and obese 14 groups, respectively. Following a post-hoc Mann-Whitney analysis, the optical density score of Tbx15 protein differed significantly (p<0.05) between normal and obese group, normal and obese 14, obese and obese 2, obese and obese 7, obese 7 and obese 14 group (Table 7) expression levels of the Ucp1 gene in the normal, obese, obese 2, obese 7, obese 14 and obese 21 groups. When compared to the normal and obese groups that did not receive SMF, the relative expression value of the Ucp1 gene increased in the obese 2, obese 7, obese 14, and obese 21 groups. Furthermore, Ucp1 gene expression was lower in the obese 7 groups than in the normal and obese groups, as well as between the obese groups exposed to SMF. Obese 2 and obese 7 had the highest and lowest relative expression values, respectively. Although there was an increase and decrease in Ucp1 gene expression, evidence suggests that there was no significant difference (Fig. 4).

Correlation Analysis of Tbx15 OD Score With Ucp1 Gene Expression

The Spearman correlation test is used to analyze the correlation of the Tbx15 OD score with Ucp1 gene expression. It was assumed that the Tbx15 protein (variable x) affected the expression of the Ucp1 gene (variable y). In the scatter plot, the correlation coefficient (r) of the Tbx15 protein and the Ucp1 gene is 0.290 and the results of the Spearman correlation analysis test obtained a significance value of 0.409 (p>0.05), which indicates there is no linear relationship between two variables (Fig. 5).

Table 5: Post Hoc Mann-Whitney Inguinal adipose cell size between groups

Group	Normal	Obese	Obese 2	Obese 7	Obese 14	Obese 21
Normal	-					
Obese	0.043*	-				
Obese 2	0.021*	1.000	-			
Obese 7	0.021*	0.564	1.000	-		
Obese 14	0.083	0.083	0.021*	0.043*	-	
Obese 21	0.043*	0.149	0.021*	0.386	0.248	-

*p<0.05

Table 6: Value of optical density

Group	Optical density median (min-max)	p-value
Normal	2.19 (2.01-2.26)	
Obese	1.98 (1.97-1.99)	
Obese 2	2.01 (1.99-2.03)	0.036*
Obese 7	2.03 (1.99-2.04)	
Obese 14	1.99 (1.98-2.02)	
Obese 21	2.01 (1.96-2.09)	

*p <0.05

Table 7: Post Hoc Mann-Whitney optical density score of Tbx15 protein

Group	Normal	Obese	Obese 2	Obese 7	Obese 14	Obese 21
Normal	-					
Obese	0.019*	-				
Obese 2	0.076	0.019*	-			
Obese 7	0.146	0.019*	0.294	-		
Obese 14	0.042*	0.369	0.144	0.041*	-	
Obese 21	0.083	0.559	1.000	1.000	0.767	-

*p<0.05

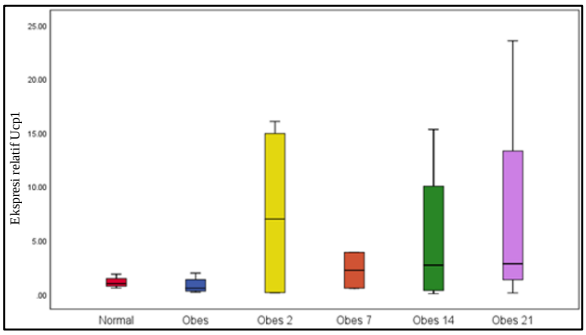

Fig. 4: Boxplots of the Ucp1 gene's relative expression. The box's bar represented the median, as well as the lower and upper hinges of the IQR. The calibrator used to calculate the relative expression of the Ucp1 gene was the normal group

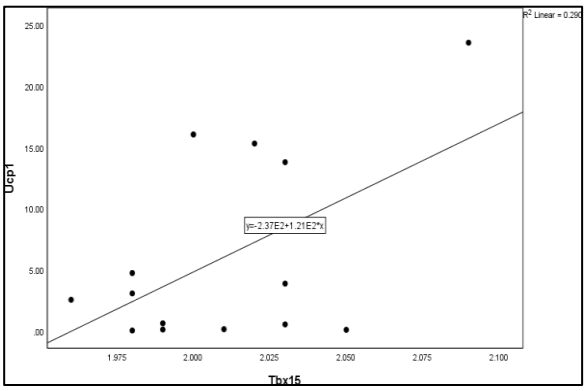

Fig. 5: Scatter plot. Tbx15 protein (variable x) and Ucp1 gene (variable y)

Discussion

Body Weight Gain in Mice Following Induction of High Fat Diet

The selection of animal species and strains in animal model studies of diet-induced obesity should be considered in addition to feed composition factors (Buettner *et al*., 2007). This study used C57BL/6J as obese mice because the C57BL/6J mice model gained more weight after being induced by HFD than the A/J strain, which was more resistant (Gallou-Kabani *et al*., 2007). The body weight of the obese group mice increased significantly during 17 weeks of high-fat feeding compared to the standard feeding group. This data is consistent with other studies in that the body weight of C57BL/6J mice increased after being fed HFD (Sun *et al*., 2019). After being fed HFD, not all animals can become easily obese. The HFD increased the body weight of the C57BL6/J mice and as shown in Fig. 1B, the difference in the size of the abdomen of the mice fed the HFD was greater than that of the mice fed the standard diet. The enlargement of the abdomen of mice is caused by lipid accumulation in the visceral and subcutaneous adipose areas. Obese mice induced by HFD for 13 weeks had larger inguinal and visceral (epididymal and retroperitoneal) subcutaneous adipocytes than normal mice (Yu *et al*., 2019).

SMF Exposure Effect on Index Lee

Statistical analysis shows that SMF exposure significantly reduces the average Lee index value of the 2,

7, 14, and obese 21 groups comparedto before exposure. According to a study conducted by Yu *et al.* (2021), mice weight gain was lower in the groupthat was exposed to downward SMF and 0.6 T SMF compared to the diabetic mice group that was induced by HFD without SMF exposure. In this case, the average index level value of obese 2, 7, 14, and 21 groups decreased as the duration of SMF exposure increased. Research by Hashish *et al.* (2008) demonstrated that there was a relationship between time and gradual weight loss after 30 days of SMF exposure at -20 to +2, 9 T in mice. Body weight was significantly reduced beginning in week 2, specifically on days 12, 20 and 30 of SMF exposure. After 39 days, the SMF group's body weight decreased, whereas the control group's increased.

SMF exposure may have an impact on hormone regulation. In the SMF-exposed group compared to the control group, glucose levels decreased, followed by a significant decrease in total protein (Hashish *et al*., 2008). Magnetic field exposure induced norepinephrine levels in rat muscle. SMF significantly increased the catecholamine hormone and Norepinephrine (NE) levels in rat muscle after sub-acute 128 mT SMF exposure (Abdelmelek *et al*., 2006). Through adrenergic receptors, NE is involved in the process of lipolysis (Crichton *et al*., 2017). Thus, the decrease of index lee value in this study's mice could be attributed to hormonal changes caused by SMF exposure.

Effect of SMF Exposure on Adipose Cell Size

A detailed histological examination revealed that administering HFD to the obese group for 17 weeks increased the body weight of mice significantly more than the normal group. Therefore, adipose tissue histology was examined in each group to see if there were differences in the size of inguinal adipose cells when body weight increased due to HFD. The size of adipose cells was larger in the obese, 2, 7, and obese 21 groups than in the normal group, according to the data. These findings are consistent with previous research indicating that HFD administration affects adipose cell size and causes adipose cell hypertrophy.

Because the adipose cell size in the obese group was larger than in the normal group, it is expected that SMF exposure treatment will affect adipose cell size slightly. Adipose cell size is one of the differences between white, brown, and beige adipose tissue. White adipose cells typically have a diameter of 20-150 µm, which is larger than brown adipose cells, which have a diameter of 10-25 µm (Stock and Cinti, 2015). According to Cedikova *et al.* (2016), the size of white and brown adipose cells is 25-200 µm and 15-60 µm, respectively. In this study, white adipose cell size was obviously found to be smaller in obese 2 (240, 37 µm), obese 7 (241, 33 µm), obese 14 (175, 02 µm), obese 21 (214, 74 µm) than obese group (255, 05). The size of beige adipose cells was not described in either study by Stock and Cinti (2015); or Cedikova *et al.* (2016). However, beige adipose cells are larger than brown adipose cells but smaller than white adipose cells (Cedikova *et al*., 2016).

Adipose cell measurements in the SMF-exposed obese groups (obese 2, 7, 14, and 21) revealed significant differences in obese 2 and 14, obese 2 and 21, and obese 7 and obese 14 group. This suggests that SMF exposure affected adipose cell size differences between these groups, as seen in the obese 14 group, which had smaller adipose cell size than the obese 2 and obese 7 groups, and also in the obese 21 group, which had smaller cell size than the obese 2 group.

In subcutaneous adipose tissue, Tbx15 expression is high which may influence adipogenesis, especially at the preadipocyte stage. Furthermore, Tbx15 is involved in the development of beige adipose. Research on Tbx15 gene knockout mice, there was a decrease in the expression of the thermogenic gene Ucp1, which proved that there was a relationship between Tbx15 expression and inguinal adipose tissue thermogenesis through Ucp1 activation. T-box15 is a transcription factor that can increase the transcription of the Ucp1 gene. As previously described, in this study the expression of Tbx15 protein was significantly increased in the obese group exposed to SMF at 2 and 7 days of exposure compared to the obese group without exposure.

Based on the Spearman correlation test, Tbx15 expression was not significantly correlated with Ucp1 gene expression but tended to be positively correlated. This is thought to be due to the influence of transcription factors and other co-regulators, such as PGC1α. there was no linear relationship between the Tbx15 protein and the Ucp1 gene. The transcription factor Tbx15 is involved in the regulation of thermogenesis and adipocyte browning by interacting with Prdm16, thereby inducing the expression of the Ucp1 gene. The transcription factor zinc finger protein 516 (Zfp516) and co-regulator PGC-1α have been reviewed by Villarroya *et al.* (2017) involved in the transcription of the gene Ucp1. The transcriptional activator Zfp516 binds directly to the promoter Ucp1. In this study, the increase or decrease in Tbx15 protein expression did not correlate with the Ucp1 gene, presumably due to the influence of transcription factors and other co-regulators that are also involved in the transcriptional regulation of the Ucp1 gene.

Tbx15 Protein Expression After SMF Exposure

According to the hypothesis in this study, the OD value of each SMF-exposed group (obese 2, 7, 14, and 21) was higher than the obese group without SMF exposure. Tbx15 expression was found to be significantlyhigher in the obese groups 2 and 7. According to the findings of this study, the expression of Tbx15 was higherin the normal

group than in the obese mice with and without SMF exposure. Significant differences were found between the normal and obese groups, as well as between the normal and obese 14 groups.

Tbx15 expression was very high in inguinal adipose tissue in the normal group, so it was used as a marker gene in beige adipose tissue (Gburcik *et al*., 2012; Waldén *et al*., 2012). Tbx15 expression was lower in the obese group than in the normal group. This is because of the HFD administered to the obese group. A previous study (Yamamoto *et al*., 2010) mentioned that the Tbx15 gene in interscapular subcutaneous WAT of ob/ob mice exhibits decreased gene expression compared to C57Bl/6 lean mice.

Tbx15 expression significantly increased after 2 and 7 days of SMF exposure, indicating that SMF exposure increased Tbx15 gene expression. Several studies have found that the magnitude of the magnetic field's intensity has different biological effects. The intensity was set to 2 mT in this study and moderate range intensity (1 mT-1 T) has previously been investigated *in vitro* to inhibit adipogenesis (Sari *et al*., 2020a). Mice exposed to 5 mT SMF for 12 weeks had lower angiotensin II levels (Okano *et al*., 2005). However, after SMF exposure, OCN protein levels in osteoblasts increased significantly (Zhao *et al*., 2016). In this study, Tbx15 expression after 21 days of exposure was not significantly different although it showed an increase in OD value compared to the obese group. This suggests that the effect of SMF exposure on cellular function is largely determined by the field's duration and intensity.

Effect of SMF Exposure on Ucp1 Gene Expression

Tbx15 expression is high in subcutaneous adipose tissue, which may influence adipogenesis, especially at the preadipocyte stage (Gesta *et al*., 2011). Tbx15 is also involved in the development of beige adipose tissue. The expression of the thermogenic gene Ucp1 was found to be lower in Tbx15 gene knockout mice (Sun *et al*., 2019), indicating a relationship between Tbx15 expression and inguinal adipose tissue thermogenesis via Ucp1 activation. Tbx15 is a transcription factor that can increase the transcription of the Ucp1 gene. As previously stated, Tbx15 protein expression was significantly higher in the obese group exposed to SMF compared to the obese group not exposed to SMF in this study. It is also expected in the Ucp1 gene's expression.

However, there was no significant difference in the relative expression of the Ucp1 gene between the SMF-exposed and non-exposed groups in this study, even though the relative expression of the Ucp1 gene was similar to Tbx15, which was highly expressed in the obese 2 group. The median value for each SMF exposure group (2, 7, 14, and 21) showed a fairly high increase compared to the obese group without exposure.

There has been little research on the effect of SMF exposure on the genes involved in the browning process. However, studies on the effect of SMF exposure on gene expression in various tissues have been conducted. It has been reported that SMF effects increase or decrease gene expression. Mice exposed to 4 mT SMF experienced an increase in BMP2 expression (Zhang *et al*., 2018). In contrast, after being exposed to SMF with moderate magnetic field intensity, the PPARγ gene, which is the master regulator in adipogenesis, decreased mRNA expression (Chen *et al*., 2020). The differentiation ofbeige adipocyte progenitors and transdifferentiation from mature white adipocytes can result in the formation of beige adipocytes. There are two critical stages in the adipose maturation process. According to *in vivo* studies the stage of adipose differentiation was not at the same stage, where the cell population was more heterogeneous than *in vitro* cell conditions (Ali *et al*., 2013). As a result, it is difficult to say whether SMF exposure causes browning by increasing Tbx15 expression at the start of progenitor differentiation or after white adipocyte maturation.

Correlation Analysis of Tbx15 Optical Density Value With Ucp1 Gene

In subcutaneous adipose tissue, Tbx15 expression is high which can influence adipogenesis, especially at the preadipocyte stage (Gesta *et al*., 2011). Furthermore, Tbx15 is involved in the beige adipose development. Research on Tbx15 gene knockout mice, there was a decrease in the expression of Ucp1 thermogenic gene (Sun *et al*., 2019), which proved that Tbx15 expression was related to thermogenesis through Ucp1 activation. Tbox15 is a transcription factor that can increase the transcription of the Ucp1 gene. As previously described, in this study Tbx15 protein expression was significantly increased in the obese group exposed to SMF at 2 and 7 days of exposure compared to the obese group without exposure.

Tbx15 expression tended to be positively correlated with Ucp1 gene expression. Limited studies report a direct correlation between how Tbx15 may induce Ucp1 gene expression. Sun *et al*. (2019) mentioned that Tbx15 is involved in the regulation of thermogenesis and adipocyte browning through Prdm16 interaction, thereby inducing the Ucp1 gene expression. The transcription factor zinc finger protein 516 (Zfp516) and co-regulator PGC-1α are involved in Ucp1 gene transcription (Villarroya *et al*., 2017). The transcriptional activator Zfp516 binds directly to the Ucp1 promoter (Dempersmier *et al*., 2015). In this study, the increase or decrease of Tbx15 protein expression did not correlate to the Ucp1 gene, presumably due to the influence of other transcription factors and co-regulators that were also involved in the transcriptional regulation of the Ucp1 gene.

Conclusion

SMF exposure has the potential to activate thermogenesis and prevent obesity. This is proven by SMF exposure decreasing the value of Lee's index from 2-21 days of exposure. SMF exposure increased the expression of the Tbx15 protein and Ucp1 gene at the preadipocyte stage, then phenotypically adipose cell size decreased on days 14-21.

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Author's Contributions

Widia Bela Oktaviani: Conducted the research and collected the data.

Puji Sari: Formulated and planned the research.

Luluk Yunaini: Oversaw and coordinated the data analysis.

Umiatin: Designed static magnetic field.

Dwi Anita Suryandari: Analyzed and interpreted the data.

Ethics

The ethical committee of the universitas Indonesia approved animal welfare and experimental procedures with number KET-678/UN2.F1/ETIK/PPM.00.02/2020.

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