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Research Article

Analysis Protein APOB and TroponinT in Obese Mice (*Mus musculus*) Induced by Static Magnetic Field as a Marker of Coronary Heart Disease

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ABSTRACT

Obesity is closely related to cardiovascular disease. APOB protein was a better predictor of LDL particle count. Increased troponin T protein is also indicated as an indication of the risk of CHD disease. Static Magnetic Field (SMF) is an electromagnetic applied in therapy because only a simple magnetic disk is used to generate it both *in vitro* and *in vivo*. The use of SMF is considered to affect the biological state of cells so that it can affect weight loss in mice. The method used was male c57bl/6j mice with groups of Obes0, Obes2, Obes7, Obes 14, and Obes21 fed high-fat diet and then exposed to SMF with an intensity of 2mT for 1 hour. The results obtained were that there was a significant difference p=0.015 (p<0.05) in the protein concentration of APOB and there was no significant difference in the concentration of cTnT taken from the blood serum of mice. The increase in APOB protein levels in each control group was not found to have an increase in cTnT protein so that the two proteins did not have a linear correlation between the two proteins 0.598 (p>0.05). Most of the protein concentrations of APOB and cTnT decreased in concentration at longer days. It can be seen morphologically in the heart there is a decrease in the number of adipose cells.

Key words: Cardiovaskular, Electromagnetic, Obesity, Protein.

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INTRODUCTION

Obesity is a state of energy imbalance between the number of calories in is greater than the calories out which causes being overweight so that it becomes one of the centers of global attention.[1,2] The population in Indonesia according to riskedas data in 2015-2019 for ages 18 years and over has an average of 15.4% of obese people who have a risk of increasing every year.[3] Obese people generally have a BMI value of more than 30. A high BMI value will be associated with chronic diseases such as Cardio vascular Disease, Diabetes Mellitus Type II, and risk of other metabolic diseases.[4]

Cardiovascular disease is one of the risks of disease that can occur due to obesity. Patients with cardiovascular disease are most commonly found in obese patients.[5] The most common types of CVD are heart failure (HF) and coronary heart disease (CHD), although it does not rule out patients with hypertension and atrial fibrillation.[6]

Coronary heart disease (CHD) is caused by a state of dyslipidemia. Dyslipidemia is an abnormal condition in blood lipids such as an increase in LDL, triglycerides, and a decrease in HDL.[7] Increased retention of low density lipoprotein (LDL) is a key step in the pathophysiological process of atherogenesis that causes blockages that can cause atherosclerosis.[7,8] Increased LDL levels can be seen in the presence of an increase in the LDL carrier protein, Apolipoprotein B (APOB). ApoB is a better predictor of LDL particle count. In addition to the increase in protein APOB protein, an increase in troponin T protein is also an indication of the risk of CHD disease.[9] Troponins are divided into 3 types of cardiac troponins found in striated muscle cells, namely Troponin C, Troponin I, and troponin T (TnT). Troponin T functions to facilitate the binding of troponin C and I to tropomyosin.[10] An increase in troponin T levels in the blood can indicate the release of Troponin I and Troponin C binding which can be seen in patients with CHD.[11]

Electromagnetic Field (EMF) is a combination of electric and magnetic fields. The electric field is generated by a stationary charge while the magnetic field is produced by a moving charge or is called a current.[12] One type of electric field used is the Static Magnetic Field (SMF) which is an electromagnetic field that is more applied in therapy because only a simple magnetic disc is used to produce it both physically and mentally. *in vitro* and *in vivo*.[13] SMF exposure can activate voltage-gated ion channels, affecting the movement of ions which can increase the concentration of cytosic Ca^{2+} so that it affects the process of cell differentiation.[14] A good intensity to use is moderate intensity (1 mT-1 T).[15,16] *In vitro* studies at an intensity of 2 mT have been carried out and the results were found to be able to inhibit the adipogenesis process. Previously, it was investigated *in vitro* to inhibit adipogenesis. increased risk of CHD.

METHODS

Study approval

The Ethical Committee of the Universitas Indonesia approved animal welfare and experimental procedures with number KET-678/UN2.F1/ETIK/PPM.00.02/2020.

Induction high fat diet

Twenty-four male C57BL/6J mice aged 6 weeks were obtained from iRATco Divided into 6 groups per group consisting of 4 mice. 24 mice were acclimatized

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for 5-7 days, then the control group mice were given standard food and the obese group mice were given a high-fat diet (HFD) for ±17 weeks. The group consisted of for each group normal, obese0, obese 2, obese 7, obese14, obese 21. Food consumption and body weight of mice were monitored every week. Obesity was assessed based on the criteria for weight gain or Lee's obesity index with the formula weight (g0.33) naso-anal length (mm). If the index value is > 310 then the mice can be said to be obese.

SMF exposure

SMF Exposure in Obese Mice Obese mice were housed in plastic cages and placed 20-21 cm apart between two Helmholtz coils. The cage is 30 cm long, 20 cm wide and 15 cm high. The magnetic field intensity is 2 mT, and it is exposed for one hour per day.

APOB and Troponin T protein levels testing

Protein levels were examined using the ELISA method. Samples were obtained from the blood serum of mice taken from the eyes and heart of mice. Furthermore, the examination was carried out using the KIT ELK Biotechnology Mouse APOB and KIT ELK Biotechnology Mouse TNNT2.

Cardiac morphology

Heart morphology examination was performed with Hematoxylin Eosin staining. The heart organs were isolated which were then fixed in 4% formaldehyde for 3 days at room temperature, then dehydrated using alcohol graded 70%, 96% (2x), 100% (2x) for 1 hour each. After that, a clearing process was carried out using xylol I, II, III for 1 hour each. The sample was then embedded in paraffin, and cut to a thickness of 5 m using a microtome. The preparations were then deparaffinized, rehydrated, then given hematoxylin and eosin. After that, observations were made under a microscope.

Statistical analysis

Statistical analysis was performed using SPSS 2.6 for Windows. Protein levels were tested for normality in each group (control, obese 0, obese 2, obese 7, obese 14, and obese 21) and a normally distributed one-way ANOVA test was performed. P < 0.05 was considered statistically significant.

RESULT

Protein content APOB

The following are the results obtained on the concentration of APOB protein levels in obese mice that have been exposed to SMF.

Based on the results obtained in table 1. the results obtained median concentrations of APOB protein levels. It can be seen that the highest value of APOB protein concentration was in the obese group 14, followed by obese 2, obese 0 and control groups. The protein concentration was then analyzed statistically using one-way ANOVA, the APOB protein concentration showed a significant difference with p value = 0.015 (p <0.05). In general, APOB protein levels decreased in each exposure group. This shows that 2 mT electromagnetic exposure for 1 hour has an effect on decreasing levels of APOB protein concentration which can be associated with a decrease in LDL-C concentration. Obes21 has the lowest protein content value, this determines that the length of time exposure affects the decrease in APOB concentrations.

Table 1: Protein content APOB *p <0.05.

Group	Median (ng/dl)	P<0.05
Kontrol	10.105	0.015*
Obes0	24.77	
Obes2	43.72	
Obes7	11	
Obes14	680	
Obes21	1.38	

To find out between groups that have differences, Post Hoc Mann-Whitney analysis was performed. Based on the test results, the results that have a significant difference are obese 14 and obese 21 (Table 2).

TNNT2 protein levels

The following are the results obtained on the concentration of TNNT2 protein levels in obese mice that have been exposed to SMF. Based on the results obtained in Table 3. The median concentration of cTnT protein was obtained. Based on the median value of these results, it can be seen that there was a decrease in cTnT protein levels from obese, obese 2, obese 7, obese 14 and obese 21. The control group had a smaller median value than the other obese groups. The protein concentration was then analyzed statistically using one-way ANOVA, the protein concentration of cTnT showed no significant difference with p value = 0.187 (p <0.05). In general, it can be seen that there are differences in cTnT protein levels, but these differences are not significantly different so it cannot be continued with post Hoc Mann-Whitney analysis to see differences in protein concentrations between groups.

Correlation between APOB and cTnT protein proteins

The results of the normality test on APOB and cTnT OD scores obtained data that were not normally distributed. Thus, the correlation test used is the Spearman correlation. It is assumed that the APOB protein (variable x) affects cTnT (variable y). The results of the Spearman correlation analysis test obtained a significance value of 0.598 (p>0.05) which indicates there is no linear relationship between the two variables (Figure 1). APOB protein is widely found in the blood circulation which functions as a carrier protein for LDL-C that is in the circulation for degradation. Meanwhile, the high levels of cTnT protein in the blood circulation can indicate damage to the structure of the heart muscle so that it can be used as a marker in detecting damage to the cardiovascular system. Research conducted by Yao *et al*., stated that patients with coronary artery syndrome (ACS) experienced an increase in cTnT levels accompanied by an increase in the concentration of APOB protein.[17]

Cardiac morphology

The following is a morphological result of the heart of mice induced by a high-fat diet. Based on this picture, it can be seen in Figure A. The control group shows little adipose tissue, while in Figure B, obese mice with no exposure show a lot of adipose tissue. in figure C. Obes2 it also clear that adipose cell collection is also visible, picture D. is obese 7, it can be seen that there is still little adipose tissue as well as in picture E. Picture F shows very little adipose tissue.

DISCUSSION

After obtaining a significant difference in the concentration of APOB protein levels between groups, it was continued with post Hoc Mann-Whitney analysis. There was a difference in protein concentration levels between the normal group and the other groups. The Obes0, Obes2, Obes 7, and Obes 14 groups had lower protein levels than the normal group. Meanwhile, the obese group had significantly lower protein levels than the normal group. Thus, the results of significant differences between groups only occurred between the obese 14 and obese 21 groups. Furthermore, between the obese and non-exposed groups of SMF, there was no significant difference, although the protein content was lower between the exposed mice and the unexposed obese mice.

Table 2: POST HOC analysis of inter-group relationships *p <0.05.

Group	Control	Obese0	Obese2	Obese7	Obese14	Obese21
Kontrol	-					
Obes0	0.895	-				
Obes2	0.877	1.000	-			
Obes7	0.401	0.938	0.950	-		
Obes14	0.098	0.503	0.530	0.950	-	
Obes21	0.877	0.311	0.290	0.064	0.011*	-

Table 3: Protein content cTnT *p >0.05.

Group	Median	P > 0.05
Kontrol	0.3105	*0.187
Obes0	0.64	
Obes2	0.4695	
Obes7	0.315	
Obes14	0.3485	
Obes21	0.30525	

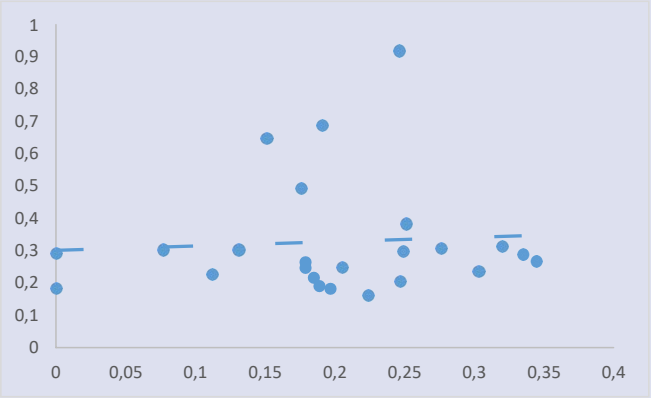

Figure 1: Graphic correlation of APOB and cTnT.

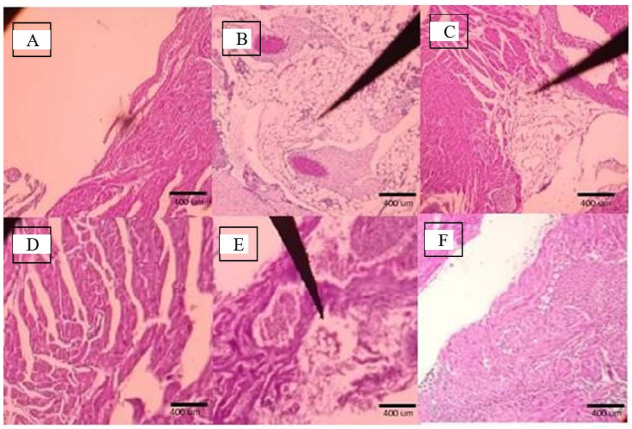

Figure 2: Cardiac morphology of mice. A. control, B. Obese0, C. Obese2, D. Obes7, E. Obese14, F. Obese21.

Another study that exposed SMF with an intensity of 0.5T was shown to reduce cholesterol and blood glucose levels, as well as reduce weight gain and lipid accumulation in the liver.[18] A decrease in cholesterol levels in the body will directly increase the expression of LDL receptors (LDLR) which will reduce LDL-C and APOB concentrations.[19]

In general, it can be seen that the cTnT protein levels in obese, obese, obese 7, and obese 14 have higher values than controls. However, in obese people the concentration levels were lower than controls. It can be seen that exposure time affects cTnT protein levels. The use of cTnT protein as a CHD marker is still uncertain. Testing of cTnT levels below conventional detection limits has recently been shown to predict cardiovascular events and mortality in people with congestive heart failure, CHD and in the general population.[20]

Coleration of APOB and cTnT

Based on the results obtained, the concentration of APOB and cTnT protein levels did not experience a correlation between the two. The concentration of APOB protein tended to increase in obese people, obese 2, obese 7, obese 14, and decreased in obese 21. Meanwhile, cTnT concentrations tended to decrease in concentration in each group. This could be due to the concentration of cardiac troponin (cTn) bound to myofilaments, and the remainder free in the cytosol. When the sarcolemma membrane is damaged from the continuous release of cTnT, the amount of this protein will increase in the peripheral blood. Then, cTnT begins to increase within three to four hours after the onset of myocardial injury and remains elevated for 10-14 days.[21] It is suspected that in this study despite the increase in APOB concentrations, the mice had not experienced cardiac injury that could cause the release of cTnT protein released into the circulation. blood.

Cardiac morphology

Figure 2. The following is an interpretation of the morphology of the heart of mice. Based on the picture, it can be seen that in the obese group, which is the group without exposure, there is a lot of adipose tissue as well as in the obese group. This was very different from the control which had few adipose cells. This is related to the high concentration of APOB protein. An increase in the concentration of APOB indicates that a lot of LDL-C is in the circulation.[22] As a result, the LDL-C will then remain in the lumen of the heart which if left unchecked for a long time will cause atherosclerosis.[22] Visually, the results of SMF exposure carried out can reduce the accumulation of adipose cells in parts of the heart that can trigger CHD for living things

CONCLUSION

Based on the results of the study, it can be concluded that most of the protein concentrations of APOB and cTnT decreased in concentration at longer days. It can be seen morphologically in the heart there is a decrease in the number of adipose cells. Thus, exposure to SMF with an intensity of 2 mT with a duration of 1 hour can be used as an alternative to obesity reduction therapy in order to reduce the risk of CHD. It is necessary to do research with the intensity and amount of time used in SMF therapy in order to find the effectiveness in SMF therapy.

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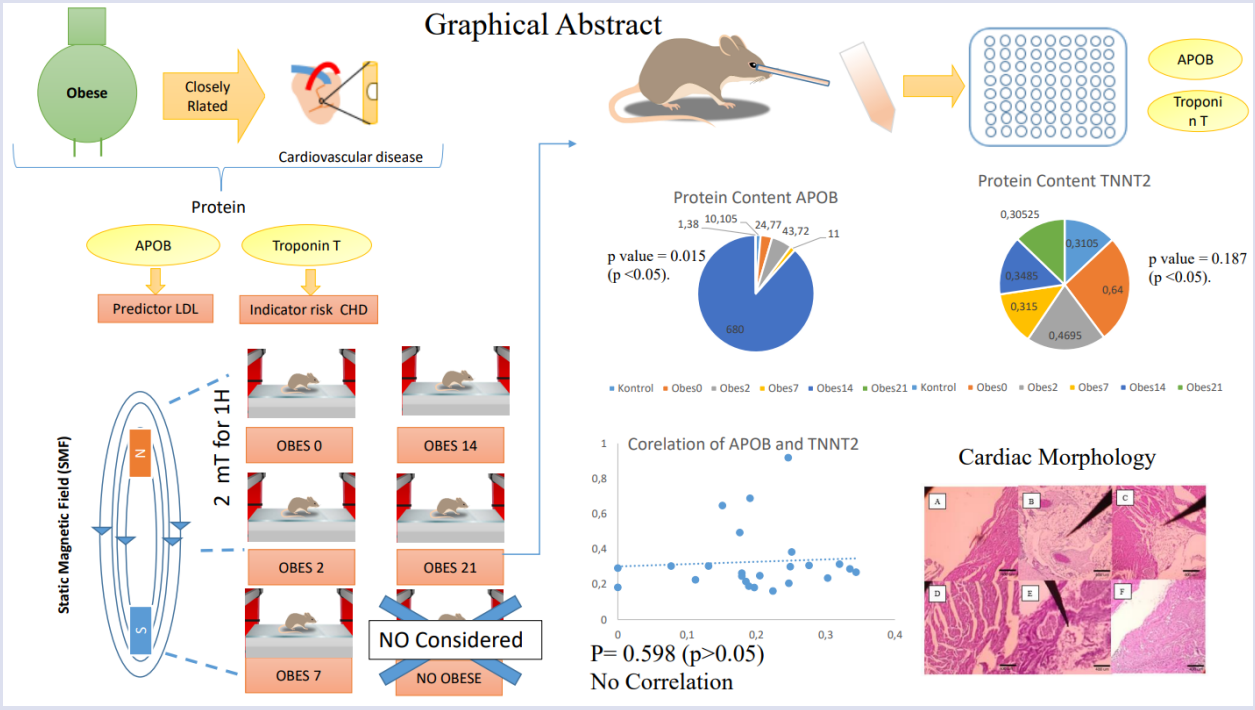
GRAPHICAL ABSTRACT



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