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Calorie Restriction and Moderate-Intensity Continuous Exercise Decrease Free Fatty Acid Levels and Visceral Fat Weight on High Calorie Diet Female Mice

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Abstract

Objective: This study aimed to analyze the effect of a combination of calorie restriction and moderate-intensity continuous exercise on FFA levels and visceral fat weight in female mice exposed to a high-calorie diet.

Material and Method: This research was randomized posttest used only control group design with female mice as the sample Balb/c mice (*Mus musculus*), 8 weeks old, 20-30 grams. It was randomly divided into four groups, namely C (control group), G₁ (moderate-intensity continuous exercise group), G₂ (calorie restriction group), and G₃ (combination group calorie restriction and moderate-intensity continuous exercise) where in every group consisted six female mice. Providing a high-calorie diet using ad libitum plus D40 as much as 3-5% of calories (0.0325 mL/gBB) for 4 weeks. The provision of a high-calorie diet was done using the sonde technique. Calorie restriction was done 3x/week in 4 weeks by limiting calorie intake to 50% of standard foods. The moderate-intensity continuous exercise was carried out by swimming for 15 minutes plus 6% gBB loading, it was done 3 times/week for 4 weeks. The measurement of cholesterol, visceral fat weight, and FFA level was done at the end of treatments.

Results: The results found that the mean of cholesterol of C C (171.00±47.74 mg/dL), G₁ (126.00±15.92 mg/dL), G₂ (131.50±22.37 mg/dL), G₃ (121.17±21.23 mg/dL) dan (p=0.032). The mean weight of visceral fat at C (0.68±0.23 grams), G₁ (0.25±0.08 grams), G₂ (0.28±0.06 grams), G₃ (0.20±0.03 grams) and (p=0.000). The mean FFA levels were at C (1511.34±6.19 µmol/L), G₁ (1419.31±53.61 µmol/L), G₂ (1458.20±28.89 µmol/L), G₃ (1451.55±43.77 µmol/L) and (p=0.004).

Conclusion: Based on the results of the study, it could be concluded that the provision of calorie restriction and moderate-intensity continuous exercise significantly reduced cholesterol levels, visceral fat weight, FFA levels and in female mice exposed to a high-calorie diet.

Keywords: Calorie restriction, moderate-intensity continuous exercise, free fatty acids, visceral fat, cholesterol

Introduction

The increasing prevalence of overweight and obesity is a global problem, both in developed and developing

countries¹. The prevalence of overweight and obesity in women is greater than in men^{2,3}. In 2016, 39% of the population aged over 18 years or more than 1.9 billion people were overweight and 13% of the population over 18 years of age or 650 million people were obese⁴. In Indonesia, based on the results of Basic Health Research⁵ the prevalence rate of overweight increases every year with a prevalence rate of 8.6% in 2007, 11.5% in 2013, and 13.6% in 2018, while the obesity prevalence rate is 10.5% in 2007, 14.8% in 2013, and in 2018 to 21.8%.

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This is a serious problem that will threaten the quality of human resources⁶.

Overweight and obesity are conditions of abnormal or excessive fat accumulation in adipose tissue and become one of the main public health problems^{7,8,9}. Moreover, overweight and obese can increase the risk of chronic diseases, such as type 2 diabetes mellitus^{7,10,11}, coronary heart disease^{7,12}, several types of cancer^{13,14,15,16,17}, osteoarthritis and musculoskeletal disorders¹⁸. Overweight and obesity are often associated with multifactorial diseases resulting from lifestyle, unhealthy eating patterns, and lack of physical activity¹⁹. Poor diet and an inactive lifestyle are contributors to increased fat accumulation²⁰. Poor diet such as high-calorie consumption in normal subjects is considered to be a major contributor to the increase in body fat accumulation, especially visceral fat, and an impact on weight gain²¹. Body fat is stored in the form of triglycerides (TG) in adipose and liver tissue which are stimulated by blood glucose²². When glucose as the main energy source is unable to meet energy needs, body fat deposits, especially TG, will be hydrolyzed into free fatty acids (FFA) and glycerol as an energy source through fat metabolism²³. FFA is released from adipocytes into the circulation through lipolysis and circulating FFA concentrations can increase in obese subjects due to an increase in adipose tissue mass²⁴. The level of FFA concentrations can affect the increase and decrease in insulin resistance²⁵. Lifestyle modification is recommended to be one of the foundations in the management of overweight and obesity²⁶. Controlling energy intake and energy expenditure is one of the main mechanisms in regulating energy balance²⁶.

Calorie restriction occurs when the intake of food as an energy source is reduced and it causes the utilization of energy sources in the body to increase, especially body fat deposits. When the calorie restriction is in the form of fasting, there is a decrease in blood glucose levels which causes a decrease in insulin secretion and an increase in lipolysis²⁷. The high lipolysis due to calorie restriction can increase the hydrolysis of TG to FFA and glycerol²⁸. Increasing FFA as the main energy source can result in a decrease in body fat accumulation. According to Maughan et al.²⁹ weight loss can come from oxidation of the substrate in the body, oxidation of water and loss of water due to the release of glycogen. Besides,

physical exercise also has a positive effect in reducing the increased risk of overweight, and obesity by reducing body fat deposits. During physical exercise, there is a decrease in blood glucose which results in a decrease in insulin and stimulates an increase in the hydrolysis of TG to glycerol and FFA as an energy source³⁰. Exercise will stimulate an increase in growth hormone (GH) which has a role in metabolism to stimulate fat to become a more dominant source of energy inactivity than glucose³¹. 30 minutes of moderate-intensity exercise can increase the production of FFA to be used as an energy source³² and have more potential to reduce visceral fat accumulation³³.

The combination of calorie restriction and moderate-intensity continuous exercise can be used as a non-pharmacological approach to reducing levels of FFA and body fat deposits. However, the effects of calorie restriction combined with moderate-intensity continuous exercise exposed to a high-calorie diet are not well known. Therefore, this study aimed to analyze the effect of a combination of calorie restriction with moderate-intensity continuous exercise on FFA levels and visceral fat weight in female mice exposed to a high-calorie diet. The hypothesis in this study was that there is an effect of a combination of calorie restriction with moderate-intensity continuous exercise on reducing FFA levels and visceral fat weight in female mice exposed to a high-calorie diet.

Materials and Methods

This research was a true experimental research design with the randomized control group posttest-only design using 24 Balb/c female mice (*Mus musculus*), 8 weeks, 20-30 grams and randomly divided into four groups, namely C (n=6, control group) G₁, (n=6, moderate-intensity continuous exercise group), G₂ (n=6, calorie restriction group), and G₃ (n=6, combination group calorie restriction and moderate-intensity continuous exercise). The research was conducted at the Embryology Laboratory, Faculty of Veterinary Medicine, Airlangga University. The experimental animals were placed in a room with a temperature of 26±2 °C and humidity of 50-60%, moreover the lighting was set to a light-dark cycle with a regulation of 12 hours of light and 12 hours of darkness. Before the treatment begins, the sample was given a swab test to determine the ovulation period and

the estrous phase as a starting point for providing calorie restriction and moderate-intensity continuous exercise. All of these research procedures had been approved by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya Number 11/EC/KEPK/FKUA/2020.

Diet conditioning with ed libitium plus sonde D40. Sonde D40 was offered at a dose of 3-5% of calories (0.013 gBB) or 0.0325 ml/gBB³⁴. The D40 solution was given at 10.00 a.m. Calorie restriction was done by limiting calorie intake by 50% of standard food or by 5 grams of feed/day. Calorie restriction is done 3x/week at night and a high-calorie ed libitium diet and Sonde D40 at noon 7 times/week for 4 weeks. The moderate-intensity continuous exercise was carried out by immersing the mice with a load of 6% of the bodyweight of the mice tied to the tail rank³⁵ for 15 minutes³⁶. Swimming training interventions were given 3 times/week for 4 weeks. The intervention was given at 15.00 p.m. Swimming intervention was carried out in a tub with a diameter of 55 cm and a height of 60 cm with a water temperature of 25±1°C and a water depth of 50±1 cm. In groups C and G₂, they were still given time to play with water as far as their feet on the same day and time to minimize the influence of water. Diet restrictions and physical exercises were given on different days.

One ml of blood was taken from the left ventricle of the mice. The visceral part of the mice was collected 24 hours after the last intervention. Blood was centrifuged for 10 minutes at 3000 rpm. The serum was separated, and stored at -80 °C for analysis of FFA levels the

following day. Measurement of serum FFA levels using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Catalog No: MBS026749; MyBioSource; San Diego, California, USA) with a concentration unit of µmol/L. Measurement of body weight, and visceral fat weight using a digital scale with a scale of 1 x 10⁻³ mg.

Cholesterol levels were measured using tail blood which was dripped on the Easy Touch GCU system with Easy Touch II Blood Test Strips by Biotek Technology, Inc. based on³⁷.

Visceral fat weight is fat tissue taken in the visceral area of mice and measured using a digital scale with a scale of 1 x 10⁻³ m.

Statistical analysis used the Statistical Package for Social Science (SPSS) software version 21 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test, while the homogeneity test used the Levene test. The difference test used One way-ANOVA, and continued with the Least Significant Difference (LSD) post hoc test. The linear correlation was used Pearson product-moment model. The data were presented as mean±SD and, the statistical analyzes used a significant level (*p*<0.05).

Results

The results of the statistical analysis of the mean body weight in the first week to the final week after giving a combination of calorie restriction, and moderate-intensity continuous exercise could be seen in Figure 1.

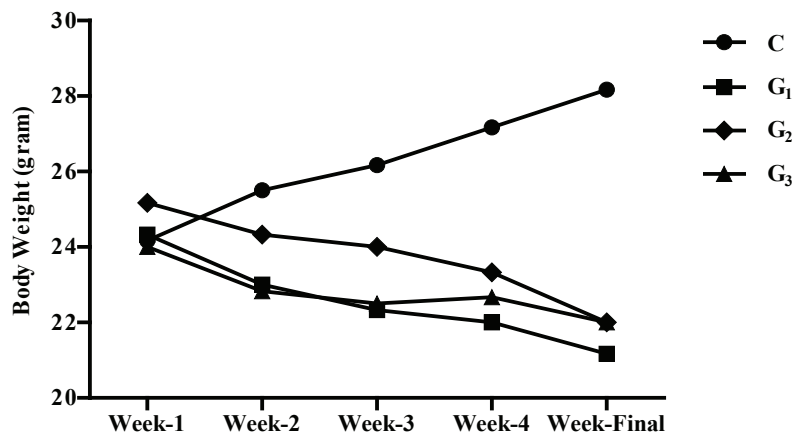


Figure 1. The Mean Body Weight of Mice in Each Group.

Note: C (control group), G₁ (moderate-intensity continuous exercise group), G₂ (calorie restriction group), and G₃ (combination calorie restriction and moderate-intensity continuous exercise).

Figure 1 showed that there was an increase in mean body weight at the initial week (week-1), and

body weight. For the end of the week (week-final) in C, while in G₁, G₂, and G₃ group, there was a decrease in mean body weight at the end of the week (week-final) compared to body weight in the initial week (week-1). The results of the analysis of the mean visceral fat weight could be seen in Table 1.

Table 1. Result of Statistical Analysis of Mean Visceral Fat Weight

Group	n	Mean±SD (gram)	ANOVA (p-values)
C	6	0.68±0.23	0.000
G1	6	0.25±0.08*	
G2	6	0.28±0.06*	
G3	6	0.20±0.03*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. The data were presented as mean ± SD. * Significant vs control group (C) (*p*<0.05). C (control group), G₁ (moderate-intensity continuous exercise group), G₂ (calorie restriction group), and G₃ (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 1, it proved that the mean visceral fat weight at C is higher than G₁, G₂, and G₃. The

results of the One way-ANOVA analysis exhibited that there was a significant difference in the mean weight of visceral fat in each group (*p*=0.000). The results of the LSD post hoc test provided the data that there was a significant difference in the mean weight of visceral fat between G₁, and C (*p*=0.000), G₂ with C (*p*=0.000), G₃, and C (*p*=0.000), while G₁, and G₂ (*p*=0.671), G₁ with G₃ (*p*=0.539), and G₂ with G₃ (*p*=0.303) did not show a significant difference in mean visceral fat weight (*p*>0.05). The results of the analysis of the mean cholesterol levels were apparent in Table 2.

Table 2. Results of Statistical Analysis of Mean Cholesterol Levels.

Group	n	Mean±SD (mg/dL)	ANOVA (p-values)
C	6	171.00±47.74	0.032
G1	6	126.00±15.92*	
G2	6	131.50±22.37*	
G3	6	121.17±21.23*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. Data were presented as mean ± SD. * Significant vs control group (C) ($p < 0.05$). C (control group), G₁ (moderate-intensity continuous exercise group), G₂ (calorie restriction group), and G₃ (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 2, it showed that the mean cholesterol levels in C are higher than those of G₁, G₂,

and G₃. The results of the One way-ANOVA analysis proved that there were significant differences in the mean cholesterol levels in each group ($p = 0.032$). The results of the LSD post hoc test offered the data that there was a significant difference in the mean cholesterol levels between G₁ and C ($p = 0.016$), G₂ and C ($p = 0.031$), G₃ and C ($p = 0.008$), while G₁ and G₂ ($p = 0.750$), G₁ with G₃ ($p = 0.780$) and G₂ with G₃ ($p = 0.551$) did not show a significant difference in the mean of FFA levels ($p > 0.05$). The results of the analysis of the mean FFA levels were observable in Table 3.

Table 3. Statistical Analysis Results of Mean FFA Levels.

Group	n	Mean±SD (µmol/L)	ANOVA (p-values)
C	6	1511.34±6.19	0.004
G1	6	1419.31±53.61*	
G2	6	1458.20±28.89*	
G3	6	1451.55±43.77*	

Note: One way-ANOVA, followed by LSD post hoc test, was used to compare the differences among groups. Data were presented as mean ± SD. * Significant vs control group (C) ($p < 0.05$). C (control group), G₁ (moderate-intensity continuous exercise group), G₂ (calorie restriction group), and G₃ (combination calorie restriction and moderate-intensity continuous exercise group).

Based on Table 3 showed that the mean FFA levels in C were higher than those of G₁, G₂ and G₃. The results

of the One way-ANOVA analysis showed that there were significant differences in the mean FFA levels in each group ($p = 0.004$). The results of the LSD post hoc test exhibited that there was a significant difference in the mean FFA levels between G₁ and C ($p = 0.000$), G₂ and C ($p = 0.024$), G₃ and C ($p = 0.012$), while G₁ and G₂ ($p = 0.089$), G₁ with G₃ ($p = 0.153$) and G₂ with G₃ ($p = 0.763$) did not show a significant difference in the mean of FFA levels ($p > 0.05$). The results of the correlation analysis of FFA levels with final body weight and visceral fat weight were shown in Figure 2.

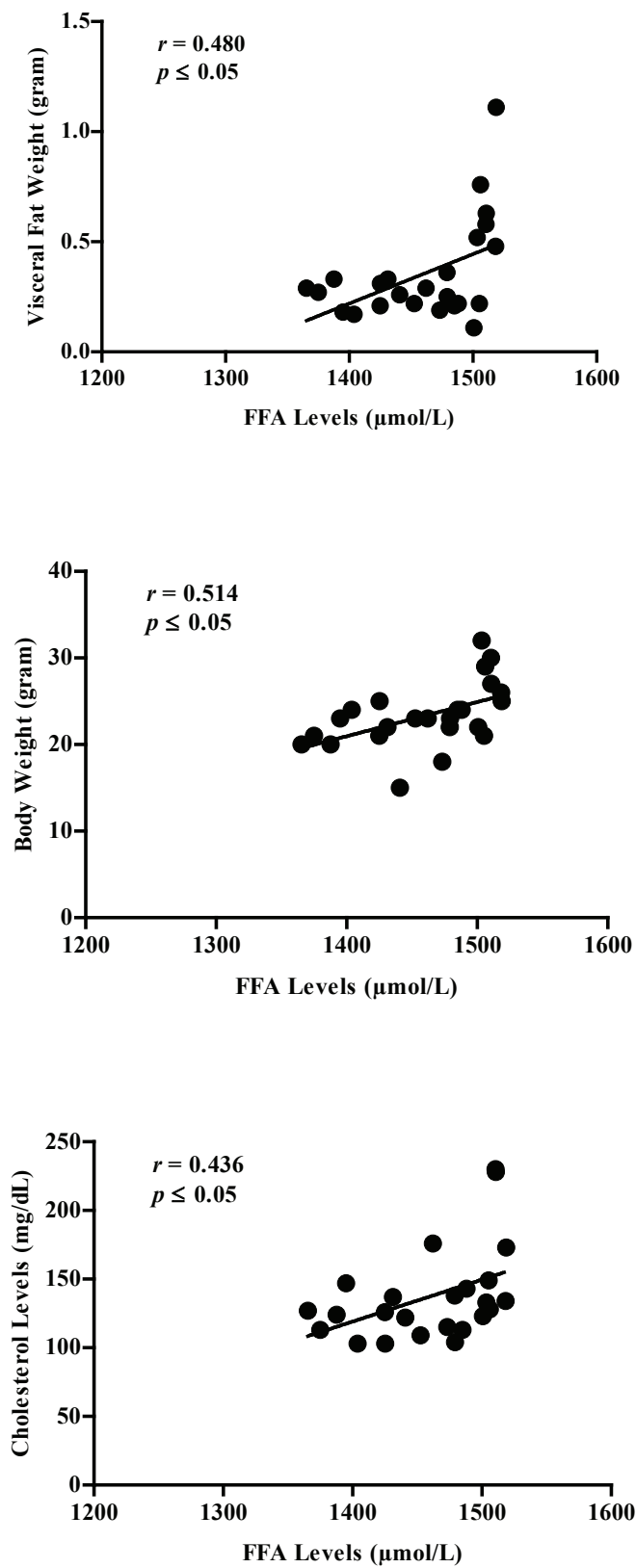


Figure 2. The correlations between FFA levels with visceral fat weight, body weight and cholesterol levels. The significant linear correlation among parameters was visualized in the plot model ($p < 0.05$). * Significant with $p < 0.05$ by Pearson's product-moment correlation test.

Based on the results of the study found a significant relationship between FFA levels and final body weight, visceral fat weight and cholesterol levels. The results of the Pearson product-moment linear correlation analysis found that FFA levels were positively correlated with final body weight ($r=0.514$, $p<0.05$), visceral fat weight ($r=0.480$, $p<0.05$) and cholesterol levels ($r=0.436$, $p<0.05$).

Discussion

Based on the results of the analysis, it had been found that the mean body weight of mice had a difference between before and after treatment (4 weeks) and there was a tendency to lose weight in G₁, G₂ and G₃, while in C the mean body weight increases (Figure 1). Weight loss in G₁, G₂ and G₃ is probably due to metabolic factors and the effects of calorie restriction treatment, exercise as well³⁸. Calorie restriction and exercise had a positive effect in reducing body fat stores. During calorie restriction and exercise, there was a reduction and decrease in food intake as an energy source which caused the utilization of energy sources in the body to increase, especially body fat deposits. When calorie restriction was in the form of fasting and exercise, there was a decrease in blood glucose levels which caused a decrease in insulin secretion and an increase in lipolysis²⁷. Increased lipolysis due to calorie restriction could increase the hydrolysis of triglycerides to FFA and glycerol²⁸. The increase in FFA as the main source of energy could result in a decrease in body fat stores, thus caused lower body weight of mice in G₁, G₂ and G₃ compared to C.

Another factor that determined the difference in weight gain and loss was due to appetite stimulation marked by the hormone Ghrelin³⁹. Ghrelin is divided into 2 types, namely Acylated Ghrelin (AG) which stimulates appetite and Desacyl Ghrelin (DG) which functions as a trigger for adipogenesis (Mackelvie, 2007). According to King⁴⁰, calorie restriction and exercise lead to appetite suppression that is mediated by AG which is inhibited by DG⁴¹. Emphasis on AG causes no binding on the Growth Hormone Secreting Receptor (GHSR) type 1a with AG, so that appetite stimuli cannot be sent to the hypothalamus and pituitary⁴². Inhibition of appetite stimulation and continuous use of energy during calorie restriction and exercise results in reduced

calorie intake and more calorie expenditure, resulting in disturbed energy balance, which causes weight gain in G₁, G₂ and G₃ to be lower than in C. Conversely, the increase in body weight in C is higher than that of G₁, G₂ and G₃ due to an imbalance between calories intake and expenditure. According to Restuastuti⁴³ in his research, it is concluded that an imbalance between calories included and calories expended causes weight gain⁴⁴. Likewise, according to Surabhi⁴⁵, the imbalance between calories in and calories out causes excessive accumulation of fat tissue in the body, leading to weight gain³⁸. Excess calories are converted into glucose and stored in the liver in the form of glycogen⁴⁶. Glycogen levels in the liver had a certain optimal limit so that if blood glucose levels are sufficient, while glucose intake continues, a mechanism for converting glycogen into fat will be stored in adipose tissue in the form of a fat pad⁴⁷. This was why the weight gain in C is higher than that of G₁, G₂ and G₃ (Figure 1).

The mean visceral fat weight in G₃ was lower than that of C, G₂ and G₃ then there was a significant difference in the mean visceral fat weight in each group. These results are in line with the results of previous studies conducted by Verheggen et al.⁴⁸ shows that exercise combined with calorie restriction is the right recommendation in reducing visceral fat weight. This is probably because the combination of calorie restriction and moderate-intensity continuous exercise is effective in reducing visceral fat weight. Exercise causes an increase in lean body mass and a decrease in fat mass⁴⁸. Therefore, the combination of calorie restriction and moderate-intensity continuous exercise was an appropriate method to use in reducing visceral fat weight. Calorie restriction could reduce the weight of visceral fat. Likewise, moderate-intensity continuous exercise could also cause a decrease in visceral fat weight, so that the combination of calorie restriction and moderate-intensity continuous exercise would be greater in reducing the weight of visceral fat. It was because of the combination of calorie restriction and moderate-intensity continuous exercise which could increase lipolysis of larger fat stores as an energy source. A greater increase in lipolysis could lead to a greater decrease in visceral fat weight.

Moreover, the mean of FFA levels in G₁ were lower than those in C. These results are in line with the results of research conducted by Yu et al.⁴⁹ concluded

that swimming exercises performed for 8 weeks on Sprague-Dawley Rats significantly reduced FFA levels compared to controls. The decrease in FFA levels in G₁ is likely due to the influence of the intervention. During physical exercise, there is a decrease in blood glucose which results in a decrease in insulin and stimulates an increase in the hydrolysis of TG to glycerol and FFA as an energy source³⁰. Exercise increases growth hormone (GH), which has a role in metabolism to stimulate fat to become a source of energy used for muscle contraction during exercise³¹, causing the FFA concentration in blood circulation to decrease⁴⁹.

Exercise is an optimal way that can be used to lose weight⁴⁹, improve metabolic health and increase insulin sensitivity⁵⁰. During exercise, triacylglycerol will be hydrolyzed into FFA, then FFA is released into the blood circulation to provide a source of muscle energy during exercise⁵¹. During intense and intermittent exercise, muscle glycogen particles are broken down, releasing glucose molecules which are then oxidized by muscle cells through anaerobic and aerobic processes to produce adenosine triphosphate (ATP) which is required for muscle contraction during exercise⁵². Exercise performed acutely can increase non-esterified fatty acids in the circulation by increasing sympathetic nerve activity through the release of adrenaline by activating lipase sensitive hormone (HSL) and adipose triglyceride lipase (ATGL)³⁰. This lowers blood glucose which will stimulate a decrease in insulin and is associated with greater oxidation of fatty acids and reduced muscle glycogen degradation which can decrease circulating FFA in muscles³².

Long-duration exercise can increase fat oxidation as energy production⁵³. This is indicated by an increase in blood ketone concentrations, a decrease in insulin concentrations and an increase in lipolysis^{54,55,56}. The enhanced lipolysis state with reduced lipogenesis theoretically contributes to a decrease in body fat storage. Physical exercise helps reduce waist circumference and visceral fat, lowers blood pressure, increases high-density lipoprotein (HDL) cholesterol, lowers plasma triglyceride concentrations, and especially promotes an increase in glucose transporter type 4 (GLUT-4) in muscle fiber membranes, improves glucose transport from blood to muscles, which lowers blood glucose⁵⁷. Muscle as a whole is a major contributor to total body

glucose and FFA metabolism⁵⁸, individual muscles can contribute differently depending on their fiber composition. Type 1 red muscle fibers are thought to be more sensitive to insulin, with a greater oxidative capacity for glucose and FFA⁵³, whereas type II white muscle fibers contain less mitochondria, are considered less sensitive to insulin and contribute less on FFA oxidation⁵⁹.

While the mean of FFA levels in G₂ were lower than those in C. These results are in line with the results of research conducted by Kostogrys et al.⁶⁰ concluded that there was a significant difference in FFA levels after caloric restriction (CR) administration compared to controls in mice. The decrease in FFA levels in G₂ is likely due to the influence of the intervention. Throughout calorie restriction, the body will respond to energy use by taking fat deposits using the lipolysis method²⁷. The increased lipolysis state with reduced lipogenesis theoretically contributes to the decrease in body fat stores⁵¹. High lipolysis causes TG to be hydrolyzed into glycerol and FFA²⁸. FFA is mobilized into the blood circulation as a source of energy during calorie restriction, causing FFA levels to decrease⁵¹.

Calorie restriction is a calorie restriction that is carried out within a certain time which is useful for reducing body fat deposits⁶¹ and losing weight⁵⁰. According to Chaouachi et al.⁶², calorie restriction is avoiding eating, drinking and cravings for 11-13 hours. Calorie restriction in Islam is known as fasting which is divided into 2 types, namely compulsory fasting (Ramadan fasting) and sunnah fasting (eg fasting Monday and Thursday). Fasting does not eat or drink for 12-19 hours every day depending on geographic location⁶³. Calorie restriction can reduce body weight, cholesterol, low-density lipoprotein (LDL) and triglycerides and increase insulin sensitivity⁶⁴. Physiologically calorie restriction can reduce the function of the digestive organs for 12 hours⁶⁵, Calorie restriction can reduce blood pressure, glucose²⁷, leptin and c-reactive protein (CRP)⁶⁶. During caloric restriction, mitochondrial protein increases. In active tissue⁶⁷. Calorie restriction after a high-calorie diet can trigger an increase in mitochondrial sirtuin 3 (SIRT3) transcription, then stimulate antioxidant productivity (SOD and GPX1) and suppress oxidative stress⁶⁸. When in a calorie restriction condition, the body responds to energy use by taking fat deposits using the lipolysis

method²⁷. The increased lipolysis state with reduced lipogenesis theoretically contributes to a decrease in body fat stores⁵¹.

High lipolysis causes TG to be hydrolyzed into glycerol and FFA²⁸. FFA becomes a source of energy and is mobilized to the blood circulation, causing a decrease in FFA⁵¹. The provision of calorie restriction by fasting can improve the standard of life than the untreated restriction treatment in obese rats and an increase in FFA levels⁶⁹. According to Alvarez-Curto and Milligan²⁴ explained that the concentration of FFA circulating in the bloodstream increases in obese subjects due to an increase in the amount of adipose tissue. This is in line with the results of this study which showed that the FFA levels in the control group were higher than the FFA levels in the calorie restriction group.

Based on the results of the study, it showed that the mean FFA levels in G₃ were lower than those in C. These results differ from the results of research conducted by Bruss et al.⁷⁰ concluded that giving calorie restriction increased FFA synthesis in mice. The difference in the results of this study may be due to differences in the form of intervention given. In this study, a combination intervention between calorie restriction and exercise was provided, whereas previous studies only provided calorie restriction without exercise. The combination of calorie restriction and exercise causes an increase in energy demand, increasing in the lipolysis process to increase the hydrolysis of TG to FFA and glycerol to meet primary energy needs²⁸. Based on the research results of Gopalan et al.⁵⁰ concluded that the combination of calorie restriction and exercise reduces body weight, depletes adipose tissue and reduces TG in muscle. A decrease in TG can decrease FFA production. Decreased FFA production can reduce insulin resistance²⁵ and lose weight⁵⁰.

The combination of calorie restriction with exercise has been recommended to reduce body weight, improve metabolic health and increase insulin sensitivity and can be used as a non-pharmacological treatment method^{50,61}. This is because exercise triacylglycerol will be hydrolyzed into FFA, then FFA is released into the blood circulation to provide a source of muscle energy during exercise⁵¹. During the provision of calorie restriction with exercise, there is an increase in energy needs,

which causes a decrease in blood glucose levels, which causes a decrease in insulin secretion and an increase in lipolysis²⁷. Increased lipolysis as a result of calorie restriction intervention with exercise can increase the hydrolysis of TG to FFA and glycerol to meet primary energy needs²⁸. When the provision of calorie restriction with exercise is carried out continuously, it can reduce body fat deposits, especially visceral fat, thus causing FFA production to decrease^{32,33}.

The combination of calorie restriction with exercise could reduce food intake which affected the production of fatty acids in the body. During exercise, hypoglycemia occurred, which could increase glucagon. When providing calorie restriction with exercise, blood glucose levels decrease which caused a decrease in insulin secretion and an increase in lipolysis²⁷. The increased lipolysis state with reduced lipogenesis theoretically contributed to a decrease in body fat stores. High lipolysis causes TG to be hydrolyzed into glycerol and FFA²⁸. FFA was used as a source of energy and mobilized to the blood circulation, caused FFA levels to decrease.

Conclusion

Based on the results of the study, it can be concluded that the provision of calorie restriction and moderate-intensity continuous exercise 3x /week for 4 weeks was effective in reducing serum FFA levels and visceral fat weight in female mice exposed to a high-calorie diet. Further research is needed to reveal further about the mechanism that might be involved such as hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL).

Conflict of Interest: The authors declared that there is no conflict of interest.

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