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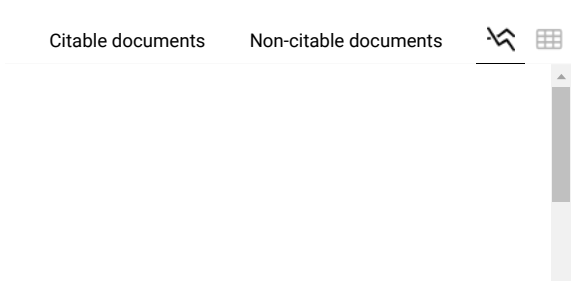
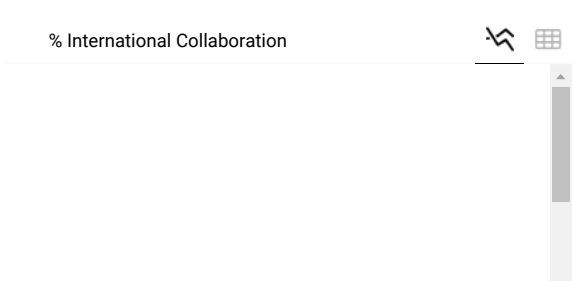
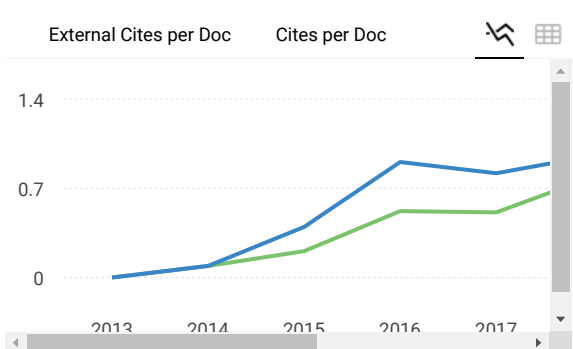
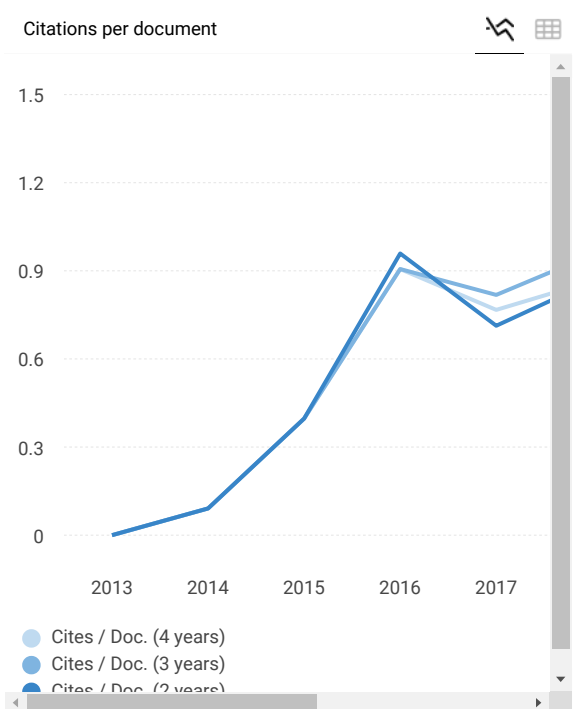
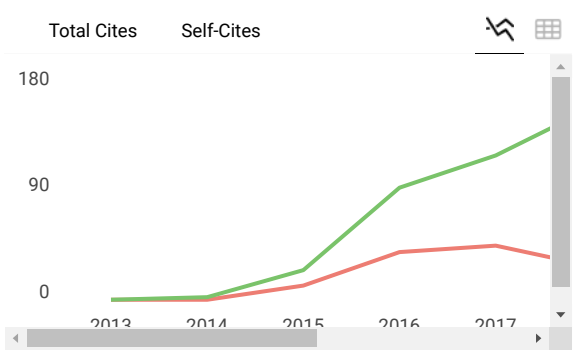
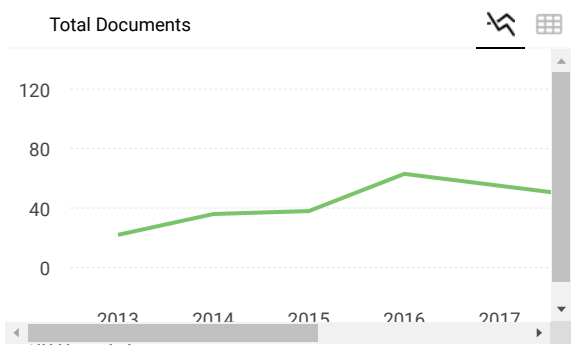
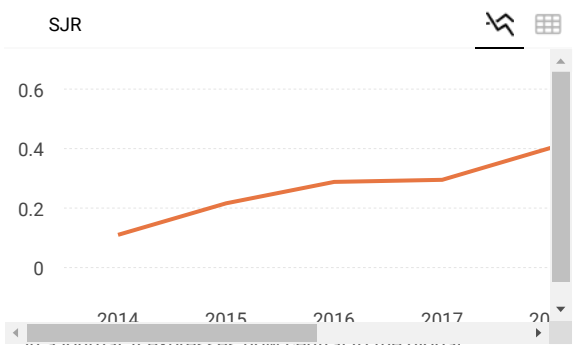
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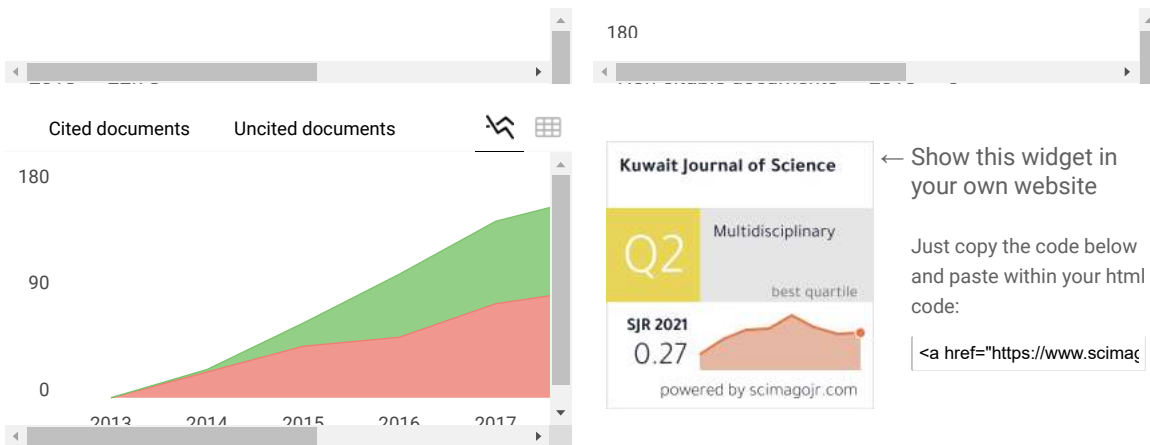
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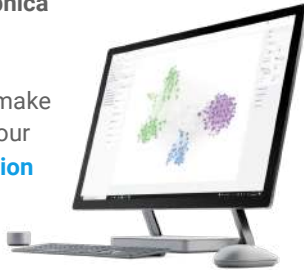
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Abstract

This study aims to analyze the effect of the combination of intermittent calorie restriction and moderate intensity interval training (MIIT) on serum c-reactive protein (CRP) levels as one of the inflammatory mediators and blood glucose levels in female mice exposed to a-high glycemic index diet. The sample of this study was female mice Balb/c strain, divided into four groups; the control group (con), the intermittent calorie restriction group (restrict), the MIIT group (interv), and the combine group of intermittent calorie restriction and moderate-intensity interval training (restrict+interv). A high glycemic index diet was a high calorie and was provided by standard feed and oral gavage of 0.013 g/gBW glucosesolution. Intermittent calorie restriction was 50% reduction of the standard feed 3x/week. The MIIT was five-minute intervals of swimming and 30 seconds of passive rest consecutively for 30 minutes and 3x/week. Meanwhile, the treatment duration was four weeks. The oral glucose tolerance test consisting of fasting blood glucose (FBG) and a two-hour post -prandial (2HPP) blood glucose was measured before and after the treatment. CRP was examined only after the treatment. The restrict + interv group had a significant reduction in CRP levels compared to the restricted group ($p = 0.005$) and the interv group ($p = 0.044$). The FBG levels the restrict + interv group and restricted group had a significant decrease ($p = 0.026$). Moreover, 2HPP of the restrict + interv group and restricted group had a significant decline ($p = 0.000$). This study concludes that the combination of intermittent calorie restriction and moderate -intensity interval training can attenuate elevated serum CRP and blood glucose levels.

Keywords: Calorie restriction; CRP; glucose; Glycemic index; health lifestyle; intermittent; interval training; mice; moderate intensity.

1. Introduction

The prevalence of obesity and overweight in the world continuously increases. Male obesity cases have increased 3% and female obesity cases increased 6% in 2016 compared to 1975 ., World Health Organization (2016) reports that 11% of men and 15% of women in the world are

obese. Overweight cases increase of 20% in men and 23% in women compared to 1975 (World Health Organization 2016). This report shows that the increase in female obesity and overweight is higher than that in men. In Indonesia, 21.8% of the Indonesian population suffered from obesity and 13.6% suffered from overweight in 2018 (Risikesdas, 2018).

Obesity and overweight cases have increased, due to sedentary lifestyle and high-calorie intakes. Obese adolescents had a higher average energy intake than non-obese adolescents, and adolescents with active physical activity had a lower risk of obesity than adolescents with inactive physical activities (Kurdanti *et al.* 2015). The storage of energy alternatives in the shape of glycogen and fat escalates as a result of the high-calorie intakes and lack of physical activity. This condition is also directly proportional to the rise in inflammation, such as C-reactive protein (CRP).

The unhealthy lifestyle, lack of physical activities and high calories intake can lead to several non-communicable diseases, i.e. diabetes, heart disease, and also cancer (such as colorectal cancer) (Almulla 2021; Firat *et al.* 2016; Syamsudin *et al.* 2021).

C-reactive protein (CRP) is an indicator used to determine the level of inflammation. Overweight and obesity have a significant correlation with CRP because 75% of overweight individuals and 93.5% of obese individuals have high CRP levels (As'ad, Liben, and Herawati 2021; Lavanya *et al.* 2017). Meanwhile, giving a high-fat diet to mice can increase in CRP levels (Morrison *et al.* 2017). Blood glucose is one of the parameters to determine metabolic syndrome such as diabetes mellitus. A high-calorie diet and lack of physical activity also affect blood glucose levels. A high-calorie diet given to mice can increase levels of fasting blood glucose (Do *et al.* 2018; Ritze *et al.* 2014).

Interval training is training providing active exercises and rest periods intermittently. High-Intensity Interval Training (HIIT) and a low-fat diet can significantly decrease CRP levels (Homaee *et al.* 2014). Meanwhile, anaerobic interval training significantly decreases CRP levels in male rats (Alizadeh *et al.* 2015). Eight-week jumping rope interval training significantly decreases CRP levels and increases Interleukin-10 (IL-10) in obese and overweight adolescents (Zakavi *et al.* 2015).

Calorie restriction occurs in calorie intakes entering the body. Calorie restriction has several effects on body homeostasis, especially inflammation. Calorie restriction, such as intermittent caloric restriction in fasting, can increase fat oxidation and reduce blood glucose, triglycerides, and pro-inflammation mediators that cause inflammation (Azevedo *et al.* , 2013). Calorie restriction increases antioxidant and anti-inflammatory substance to lower oxidative stress and CRP. Moreover, 40% calorie restriction for eight weeks can reduce fasting blood glucose (FBG) levels in old mice (from 20 to 70 weeks) (Dommerholt *et al.* 2018). However, the effect of a combination of intermittent calorie restriction and moderate-intensity interval training (MIIT) on subjects with high-calorie habits remains unclear. This study aimed to analyze the effect of a combination of intermittent calorie restriction and moderate-intensity interval training in subjects with high-calorie consumption habits, such as a high glycemic index diet. The scopes of this research are physiology, health, and prevention of obesity.

2. Material and methods

This research received approval from the Research Ethics Commission, Faculty of Medicine, Universitas Airlangga (No. 12/EC/KEPK/FKUA/2020). The research was declared ethical for meeting seven WHO 2011 standards that refer to the 2016 CIOMS guidelines.

2.1 Subjects and interventions

This study conducted an experimental laboratory study for four weeks (Hoshino *et al.* , 2016; Prasetya *et al.* , 2018; Herawati *et al.* , 2020). The research subjects were 24 female mice (*Mus musculus*) Balb / C aged \pm 8 weeks with a bodyweight of 20-30 g. The mice were acclimatized to the laboratory environment for one week. They were divided into four groups: the control group (con), the intermittent calorie restriction group (restrict), the moderate- intensity interval training group (interv), and the combination of intermittent calorie restriction group and moderate-intensity interval training group (restrict+interv).

All groups received a high-calorie diet every day. The high-calorie was a high glycemic index of 0.0325 ml dextrose 40% per 1 g of mice weight by oral gavage (Herawati, Sari, and Irawan 2020). Intermittent calorie restriction was 50% of the volume of standard, mouse diet given three times/week (Pósa *et al.* 2015; Testa *et al.* 2014). The moderate- intensity interval training consisted of a five minute exercise and a 30-second passive rest intermittently and was performed for 30 minutes and three times/week. The combination group received intermittent calorie restriction treatment and moderate- intensity interval training.

2.2 Blood glucose measurement

Blood glucose measurements were conducted before and after the treatment. Blood glucose was obtained by taking the mice's blood that had been fasted for about 8 hours -(fasting blood glucose or FBG) from the tail tips. Next, the blood was given 20% dextrose as a procedure of oral glucose tolerance test (OGTT), and the blood was taken using two hours postprandial (2HPP). Blood glucose was measured using the Easy Touch GCU system with Easy Touch II Blood Glucose Test Strips by Biotik Technology, Inc. based on Abdelmotaleb *et al.* study (Abdelmotaleb *et al.* 2017). The Easy Touch GCU system is an accurate measurement of glucose levels. The results of blood glucose analysis were recorded in mg/dl units.

2.3 Measurement of C-reactive protein (CRP)

After four weeks of treatment, anesthesia and euthanasia were performed on the mice to collect blood. Surgery and blood draws were performed 48 hours after the last treatment. Then the blood was centrifuged and the serum was taken for the CRP analysis. CRP was analyzed using the ELISA method and reagents from Elabscience (E-EL-M0053) (Zhang *et al.* 2017). CRP levels were recorded in units of ng/ml.

2.4 Statistical analysis

This research analyzed the data using SPSS software. The data were presented in tables, numbers, and graphs. Numerical data indicated the mean and standard deviation (SD). Moreover, the data were analyzed using the one-way ANOVA test to determine its significance. The non-parametric Kruskal Wallis and Mann Whitney tests were performed if the data had not been normally distributed. The data stated a significant difference if p had been < 0.05 in the ANOVA, Kruskal Wallis, and Mann Whitney tests.

3. Results

This study involved twenty-four female mice aged ± 8 weeks and weighing 20-30g and they were divided into four groups. Each group was given a high glycemic index diet during the study.

The average bodyweight of mice was measured every week to determine the changes. The mice's body weight indicated an increment. However, there was no difference among the groups (Figure 1).

The highest CRP level was in the control group while the lowest was the restrict+interv group (Figure 2). The intermittent calorie restriction, moderate-intensity interval training, and a combination of intermittent calorie restriction and moderate-intensity interval training had a significant effect on reducing CRP levels compared to the con group ($p = 0.000$).

The combination of Intermittent calorie restriction and moderate-intensity interval training had a significant difference in reducing CRP levels compared to intermittent calorie restriction ($p = 0.005$) and moderate intensity interval training ($p = 0.044$). However, there was no significant difference in the decreasing CRP levels of intermittent calorie restriction and moderate-intensity interval training ($p = 0.332$).

The timeline pattern of blood glucose hinged on OGTT before and after treatment was shown in Figure 3.

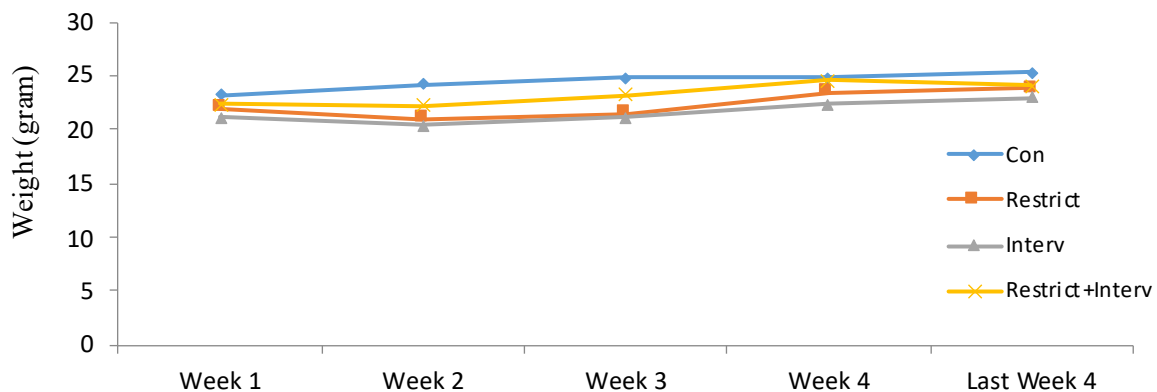


Fig. 1. The average body weight of mice during the study.

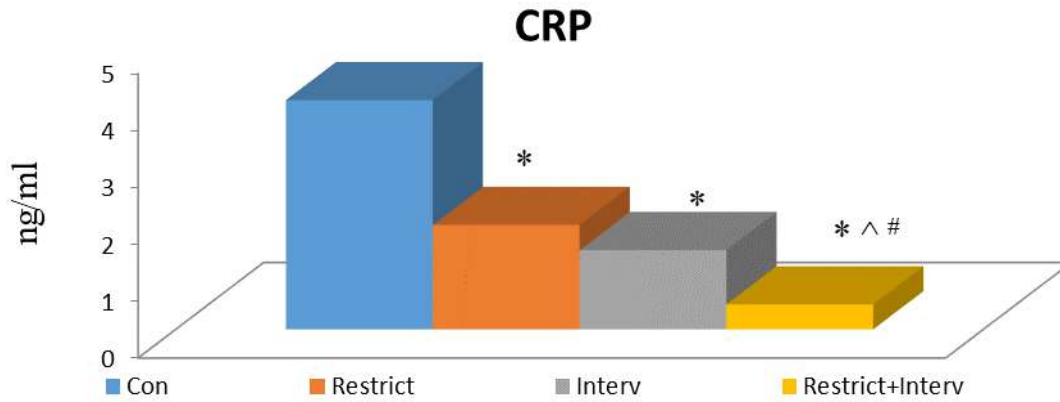


Fig. 2. The serum CRP levels (Statistical analysis using ANOVA test).

* Significant difference with the con group.

^ Significant difference with the restricted group.

Significant differences with the moderate-intensity interval training group.

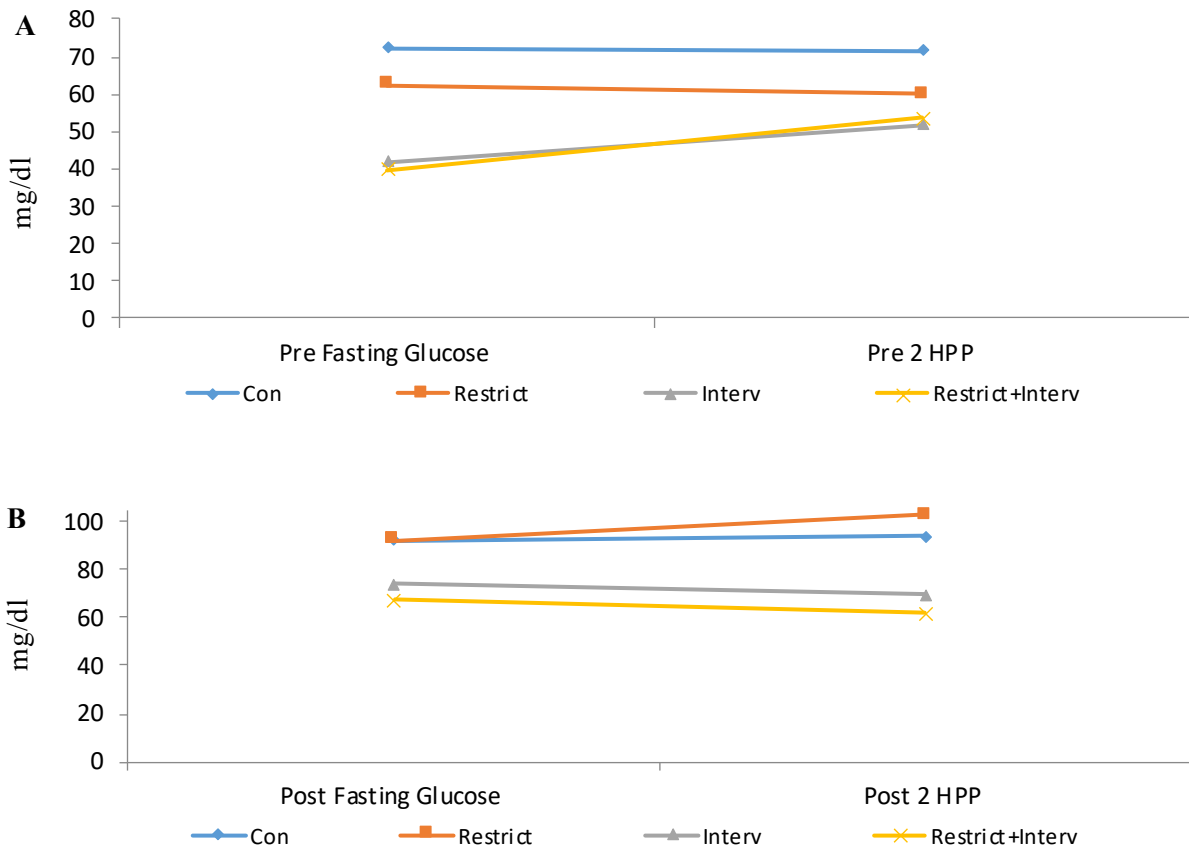


Fig. 3. The average blood glucose before (post: A) and after (post: B) the treatment of FBG levels and 2HPP.

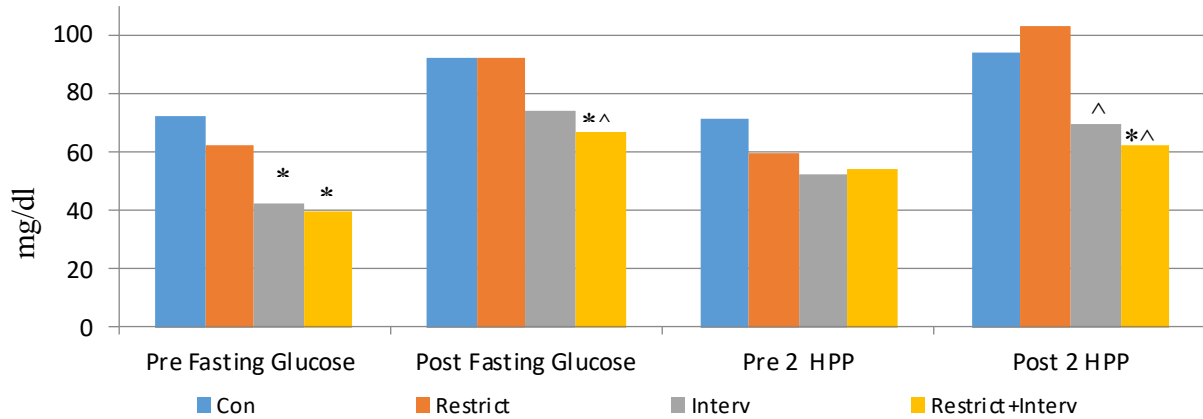


Fig. 4. Pre- and post-blood glucose levels (Statistical analysis using ANOVA and Mann Whitney tests).

* Significant difference with the con group.

^ Significant difference with the restricted group.

Significant differences with the moderate-intensity interval training group.

Table 1. CRP serum levels and blood glucose

	Con (n=6)	Restrict (n=6)	Interv (n=6)	Restrict+Interv (n=6)
CRP (ng/ml)	4.033±1.344	1.836±0.684*	1.394±0.190*	0.439±0.248*^#
Pre-FBG (mg/dl)	72.33±21.267	62.33±32.141	42.000±8.741*	39.667±2.658*
Post-FBG (mg/dl)	92.33±16.813	92.33±15.293	73.833±19.924	67.000±6.325*^
Pre-2HPP (mg/dl)	71.67±25.414	59.83±18.302	51.833±12.734	53.667±8.595
Post-2HPP (mg/dl)	93.83±17.893	102.67±8.311	69.333±19.408^	61.833±7.782*^

* Significant difference with the con group.

^ Significant difference with the restricted group.

Significant differences with the interval training group.

The combination of intermittent calorie restriction and moderate-intensity interval training had lower fasting and 2HPP blood glucose level. There was a significant difference in FBG levels between the combination of the restrict+interv group and the restricted group ($p = 0.026$). However, there was no significant difference in FBG levels between the combination of intermittent calorie restriction and moderate-intensity interval training ($p = 0.852$). This study revealed a significant difference in 2HPP blood glucose levels between the combination of restrict+interv group and the restricted group ($p = 0.000$). However, the combination of intermittent calorie restriction and moderate-intensity interval training did not have a significant difference in 2HPP blood glucose levels ($p=0.816$). The moderate-intensity interval training and calorie restriction had a significant difference in 2HPP blood glucose levels ($p = 0.026$).

Figure 4 showed that FBG and 2HPP blood glucose levels in all groups increased after the treatment (post). Albeit, the combined group of intermittent calorie restriction and exercise had the lowest levels of fasting and 2HPP blood glucose among all groups after the treatment (post).

The complete data of CRP and blood glucose (FBG and 2HPP), that consist of average and the standard deviation are presented in Table 1.

4. Discussion

The mean weight of all groups increased at the end of the study. The increase was triggered by the high-calorie diet given to all groups. Moreover, the moderate-intensity interval training affected the increase in muscle mass. The average final body weight of the interval and combination training group was lower than that of the control group (Chang *et al.* 2020).

The combination of the intermittent calorie restriction group and the moderate- intensity interval training group had a significant difference in the reducing of CRP levels to the calorie restriction group and the interval group. Teich *et al.* (2017) has proved that the combination of calorie restriction and exercise significantly decreased CRP levels. The reduction in CRP levels was also supported by a greater decrease in malondialdehyde (MDA) levels and an increase in superoxide dismutase (SOD) in the combined calorie restriction and exercise group compared to the calorie restriction group (Macit *et al.* 2020). The combined calorie restriction and moderate-intensity interval training group had the lowest fat mass among the calorie restriction and moderate-intensity interval training group (Huang *et al.* 2010). This study revealed that the combination of intermittent calorie restriction and moderate- intensity interval training had the most significant effect in reducing CRP levels compared to the intermittent calorie restriction and moderate-intensity interval training.

Bodyweight, fat percentage, pro-inflammatory, anti-inflammatory, free radicals, and antioxidants affected the reduction level in CRP levels. The group with the lowest bodyweight, fat percentage, and pro-inflammatory cytokines also had lower CRP levels than the other groups (Huang *et al.* 2010; Suárez-García *et al.* 2017). Moderate- intensity physical exercise significantly decreased MDA levels and increased SOD levels compared to the calorie restriction group in male rats (Macit *et al.* 2020). This suggested that the combination of intermittent calorie restriction and moderate-intensity interval training had a better effect in reducing CRP levels.

FBG and 2HPP glucose levels of all groups increased after the treatment (post). This condition was the impact of a high-calorie diet given for four weeks. The study by (Huang *et al.* 2010) showed that the group receiving the high-fat diet had higher blood glucose levels. The combined group of intermittent calorie restriction and exercise had the lowest fasting and 2HPP blood glucose levels among all groups. The combined group of eight week calorie restriction of fat and physical exercise endurance had the lowest FBG levels among the calorie restriction and exercise groups in male mice (Huang *et al.* 2010).

There was a significant difference in 2HPP blood glucose levels (post) between the intermittent calorie restriction group and moderate-intensity interval training group and between the intermittent calorie restriction group and the combine group of intermittent calorie restriction

and moderate-intensity interval training group. This difference was due to the role of cortisol, insulin, and glucose transporter-4 (GLUT-4). Calorie restriction and moderate-intensity interval training could cause stress on the body. This stress can elevate cortisol levels (Tomiyama *et al.* 2010). The increased cortisol levels can also increase blood glucose levels (Tabrizi *et al.* 2012). The combination group (intermittent calorie restriction and moderate-intensity interval training) probably had the highest stress level among the calorie restriction group and moderate-intensity interval training group only. However, the group receiving the moderate-intensity interval training treatment was compensated with an increase in GLUT-4. Therefore, it maintained normal blood glucose levels of 2HPP.

Blood glucose levels of 2HPP indicated the role of insulin and glucose transporter (GLUT) in maintaining homeostasis of blood glucose levels (Röhling *et al.* 2016). Pósa *et al.* (2015) discovered that the calorie restriction group had the highest glucose levels 2HPP and insulin among the physical exercise group and the combined group of calorie restriction and exercise in rats treated with placebo surgery (sham operation). This finding implied that the calorie restriction group had the longest time of glucose absorption. Meanwhile, the moderate-intensity interval training group and the combined group of intermittent calorie restriction and moderate-intensity interval training had the fastest glucose absorption into cells and tissues due to an increase in GLUT 4. Physical exercise increased glucose uptake via the GLUT 4 activation (translocation) and decrease insulin and leptin levels (Afzalpour *et al.* 2016; Chang *et al.* 2020; Chavanelle *et al.* 2017).

5. Conclusion

The combination of intermittent calorie restriction and moderate-intensity interval training effectively reduced serum CRP levels and blood glucose levels in mice with high-calorie habits which is a high-glycemic index diet. The combination of intermittent calorie restriction and moderate-intensity interval training could maintain normal blood glucose levels and reduce inflammatory mediators. However, further research is still needed to uncover the underlying mechanism and provide alternative variations of preferred methods for the community, such as a combination of calorie restriction and HIIT and /or a combination of calorie restriction and moderate-intensity continuous exercise.

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