ORIGINAL ARTICLE

Effect of High-Calorie Diet and Moderate-Intensity Physical Exercise on Gastric Histopathological Features of Mice

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ABSTRACT

Introduction: Habit of consuming excess calories and sedentary lifestyle can cause oxidative stress which is known to be involved in the pathophysiological process of various diseases including diseases of the gastrointestinal mucosa. Increased Reactive Oxygen Species (ROS) due to a high-calorie diet can damage lipids, proteins, and DNA oxidation of the mucosa and lead to inflammation, apoptosis, damage of mucosal and its barrier. Moderate-intensity physical exercise is known to have health benefits, improve quality of life, reduce the risk of death, and prevent the occurrence of diseases related to oxidative stress. The aim of this study was to analyze the effect of high-calorie diet and moderate-intensity physical exercise on gastric histopathological features of mice.

Methods: This research was a true experimental study with posttest only control group design. Female Balb/C strain were randomly assigned into three groups, the control group/K1 (n=12), high-calorie diet group/K2 (n=12), and high-calorie diet and moderate-intensity physical exercise group/K3 (n=9). High-calorie diet in the form of dextrose (D40) was given ad libitum using sonde in a dose of 3-5% of calories or 0.0325 mL/gBW. The moderate-intensity physical exercise was carried out by swimming three times a week for 15 minutes using 6% gBW loading. After 4 weeks of treatment, the mice were sacrificed and their stomach were sampled and prepared for microscopic analysis.

Results: The histopathological examination of the gastric mucosa showed the average number of inflammatory cells was 11.60 for K1, 11.25 for K2, and 14.5222 for K3. The Shapiro-Wilk test revealed that the data on inflammatory cells infiltrations were not normally distributed ($p<0.05$), so a non-parametric difference test was conducted using the Kruskal-Wallis test. Based on the Kruskal-Wallis test results, it was obtained that $p>0.05$, which means no significant difference was found between the three study groups.

Conclusion: There was no effect of a high-calorie diet and moderate-intensity physical exercise on the gastric histopathological features of mice (*Mus musculus*) in this study

Keywords: High-calorie diet; moderate-intensity physical exercise; gastric histopathology

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INTRODUCTION

Habit of consuming excess calories and sedentary lifestyles can cause an imbalance between prooxidants and antioxidants in the body, which is commonly called oxidative stress, and is known to be involved in the pathophysiological process of various diseases, such as cardiovascular disease, diabetes, obesity, as well as diseases of the gastrointestinal mucosa, such as gastroduodenal inflammation, ulceration, and gastric cancer (Sies et al 2005; Bhattacharyya et al 2014). Many factors play role in the disruption of gastric mucosa, including NSAID, alcohol, aspirin, portal hypertensive gastropathy, heat shock protein, *Helicobacter pylori* infection, and heavy metals (Bhattacharyya et al 2014; Suzuki et al 2012). Dietary factors are also potential sources of increased Reactive Oxygen Species (ROS), one of the prooxidants, thus leading to inflammation, apoptosis, and mucosal damage (Suzuki et al 2012; Contreras-Zentella et al 2017). High-calorie diet, especially a high-carbohydrate diet, also contributes to the inflammatory process through Nuclear Factor-κB (NF-κB) mediated cell signaling pathways that can be activated by ROS, resulting in inflammation by inducing proinflammatory cytokines, chemokines, and adhesion molecules. In addition, NF-κB also plays a role in cell proliferation, apoptosis, and angiogenesis (Tan et al 2018; Lingappan 2018; Liu et al 2017). Nevertheless, there have been no studies that discussed the effects of oxidative stress due to a high-calorie diet on the gastric mucosa.

Physical activity and regular exercise are known to have advantageous effects in lowering the risk of various diseases and improving immune system against free radicals. Researchers agree that moderate-intensity physical exercise gives benefits to health, improve quality of life, reduce the risk of death, and prevent diseases related to oxidative stress (Sholikhah and Ridwan 2021; Stone et al. 2015). It can lower oxidative stress through several mechanisms, for instance, improving enzyme activity and DNA repair, reducing DNA binding to ROS-sensitive transcription factors (such as NF-κB), and increasing the activity of cell enzymatic antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) (Berawi and Agverianti 2017). Previous studies have shown that moderate-intensity physical exercise reduces the risk of gastric inflammation and increases antioxidant status in experimental animals (Stone et al 2015; Murwaningsih et al 2018). However, the effects of high-calorie diet and moderate-intensity

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physical exercise on gastric histopathological features are still unclear. The purpose of this study was to analyze the effect of high-calorie diet and moderate-intensity physical exercise on gastric histopathological features, focusing on the infiltration of inflammatory cells, of Balb/C mice (*Mus musculus*).

METHODS

Experimental Design and Treatments

This study was a true experimental study with posttest only control group design. Female Balb/C strain mice (*Mus musculus*) with the age of 8 weeks and weight of 20-25 grams were used in this present study. Before the intervention, the mice were acclimatized for seven days by being placed in the treatment environment at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, and they had full access of ad libitum feeding and water. A total of 33 experimental animals were randomly divided into 3 groups, the K1 (n=12, control group), K2 (n=12, high-calorie diet group), and K3 (n=9, high-calorie diet and moderate-intensity physical exercise). The control group received standard diet, while the high-calorie diet group was given ad libitum with dextrose (D40) using sonde in a dose of 3-5% of calories or 0.0325 mL/gBW every day (Herawati et al 2020). The combination group of high-calorie diet and moderate-intensity physical exercise received the same diet with K2 group plus swimming three times a week (Monday, Wednesday, Friday) for 15 minutes using 6% of the mice's body weight loading (Prasetya et al 2018; Zhao et al 2018). The swimming
intervention was carried out in the water with a temperature of 25±1°C inside a tub with a height of 60 cm and diameter of 50 cm. The K1 and K2 groups were also given time to play in the water with a depth of up to their feet on the same day as K3 to minimize the influence of the water. Each mouse was weighed every week to determine the dose of D40 sonde and the weight of the swimming loading. After four weeks of treatments, the mice were euthanized, and samples were collected from their stomach tissue.

Samples Preparation and Histological Evaluation

The samples were cleaned and fixed with 10% formalin buffer, and then it was processed into histological preparation through sliced paraffin blocks with Haematoxylin-Eosin (HE) staining. The evaluation of the gastric histopathological features was conducted by counting the inflammatory cells infiltration of the glandular stomach using a light microscope at 400x magnification, with 10 fields of view each. The average count of each stomach sample was calculated by dividing the total number of inflammatory cells seen by the number of the fields of view (Tavasoly et al 2012).

Statistical Analysis

The obtained data were analyzed statistically using Statistical Package for the Social Science (SPSS) Version 25.0 software. Statistical analysis was performed with Shapiro-Wilk normality test to determine whether the data were normally distributed and Kruskal-Wallis test to determine the difference between each group.

RESULTS

The results of the statistical analysis of the mean body weight in the first week to the fourth week after giving a high-calorie diet and moderate-intensity physical exercise could be seen in Figure 1. It shows that there is an increase in mean body weight in K1 (control group) and K2 (high-calorie diet group), while in K3 (high-calorie diet and moderate-intensity physical exercise group) there is decrease in mean body weight after four weeks of treatments.

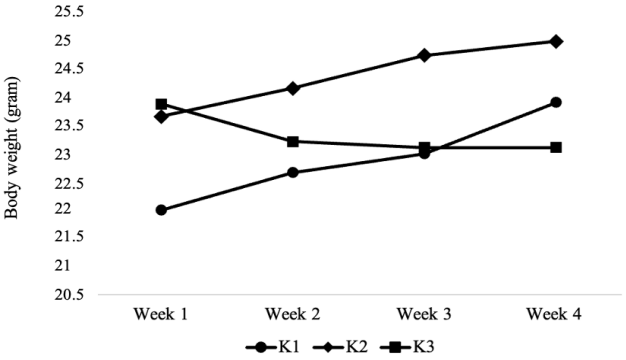

Figure 1. The mean body weight of mice in each group

Note: K1 (control group), K2 (high-calorie diet group), and K3 (high-calorie diet and moderate-intensity physical exercise group)

The histopathological examination of the gastric mucosa showed the average number of inflammatory cells was 11.60 for K1, 11.25 for K2, and 14.5222 for K3. The Shapiro-Wilk test revealed that the data on inflammatory cells infiltrations were not normally distributed ($p<0.05$), so a non-parametric difference test was conducted using the Kruskal-Wallis test. The Kruskal-Wallis test obtained $p>0.05$, which means there was no significant difference between the three study groups. The results of the statistical analysis are shown in Table 1.

Table 1. Statistical analysis results of the average number of inflammatory cells of the stomach

					Saphiro -Wilk	Kruskal l-Walis
Group	Minimum	Maximum	N	Mean ± SD	p-value	p-value
K1	2.90	26.70	12	11,6000 ± 8.15598	0.025	
K2	3.30	35.00	12	11,2500 ± 9.68161	0.004	0.968
K3	3.30	59.60	9	14,5222 ± 17.96460	0.000	

Note: K1 (control group), K2 (high-calorie diet group), and K3 (high-calorie diet and moderate-intensity physical exercise group)

DISCUSSION

The data from this study showed that treatments of high-calorie diet and moderate-intensity physical exercise did not give significant difference to the histopathological features of inflammatory cells in the stomach of mice (Table 1), which could be due to several factors, such as durations and methods of treatments. The short durations and the use of simple carbohydrates (D40) as a high-calorie diet treatment in this study may cause the high-calorie diet group (K2) had the same inflammatory trend and showed no significant difference compared to the control group (K1). However, previous studies have revealed significant results because the experimental animals in those studies were provided with treatment in a longer duration and a high-calorie diet other than high-carbohydrate diet as used in this present study (Masi et al 2017; Li et al 2018; Trisnadi et al 2021; Auberval et al 2014). A study by Auberval et al (2014) indicated a significant increase in ROS and oxidative stress

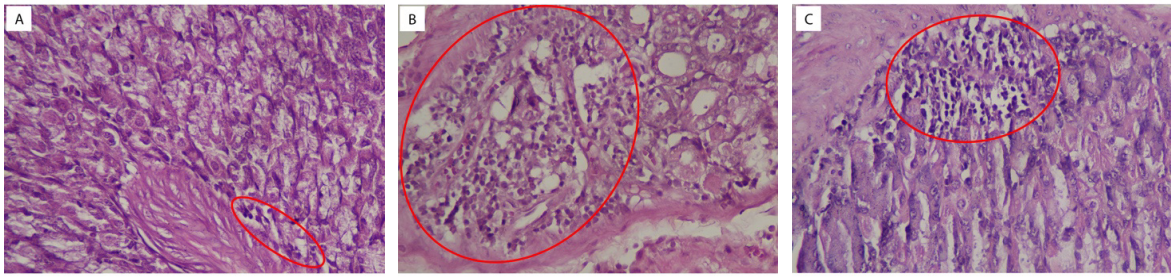

Figure 2. Gastric histopathological evaluation with Haematoxylin-Eosin (HE) staining, 400x magnification; (A) K1 (control group); (B) K2 (high-calorie diet group); (C) K3 (high-calorie diet and moderate-intensity physical exercise group). The red circle showed the inflammatory cells infiltration

levels on the pancreas, liver, and mesenteric arteries of male Wistar rats given with high-calorie diet in the form of a high-fat diet for 2 months. Another study showed an increase in inflammatory levels, especially the inflammatory cytokine of Tumor Necrosis Factor-alpha (TNF-α) in white Wistar rats fed with a high-cholesterol diet (Trisnadi et al 2021). Antonio et al found similar results after 26-38 weeks duration of high-calorie diet in Wistar rats (Antonio et al., 2023). TNF-α is one of the inflammatory cytokines whose production is activated by a cell signaling pathway mediated by Nuclear Factor-κB (NF-κB). The NF-κB signaling pathway can be activated by oxidative stress from the excessive ROS production due to a high-calorie diet, which then plays role in the inflammatory process (Tan et al 2018). Li et al (2018) conducted a 20 weeks study that proved an increasing NF-κB activity in the brains of mice treated with a high-fat diet. The study also proved an increase in levels of the proinflammatory cytokine TNF- α.

The results of this study showed that there was no significant difference between the three groups based on the Kruskal-Wallis test, which means that moderate-intensity physical exercise did not reduce gastric inflammation in mice. This result is a contrast to the previous study because moderate-intensity physical exercise is known to have health benefits, including preventing diseases related to oxidative stress by lowering oxidative stress through several mechanisms, such as improving enzyme activity and DNA repair, reducing DNA binding to ROS-sensitive transcription factors (such as NF-κB), and increasing the activity of cell enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) (Stone et al 2015; Berawi and Agverianti 2017). Malaguti et al (2014) also obtained similar findings, that moderate-intensity physical exercise can act as an antioxidant because a light burst of ROS production formed by physical exercise acts as a signal that activates signaling pathways that induce antioxidant enzymes in tissues, with NF-κB as the predominant pathway. Earlier study proved improvement activity of antioxidant enzymes, SOD and GSH-px, in Wistar rats with moderate-intensity physical exercise treatment by swimming without loading five times a week for four weeks (Stone et al 2015). Shamsnia et al showed similar results that there was a significant increase of SOD enzyme activity and GSH-px after four weeks of treadmill running with moderate-intensity (Shamsnia et al., 2024). Antioxidant enzymes SOD and GSH-px are the main and fundamental key enzymes as the first line defense antioxidants. They are known to play a role in reducing oxidative stress that may cause inflammation due to increased ROS by converting ROS into harmless molecules (Berawi & Agverianti, 2017; Ighodaro & Akinloye, 2018).

Various factors could cause the insignificance of the results in this study, such as the short duration of treatment and different methods of moderate-intensity physical exercise compared to previous studies. To promote the adaptation of experimental animals at the physiological or molecular level, physical exercise protocols should be carried out with a duration of more than or equal to six weeks. It is to note that to be able to cause adaptation to oxidative stress, previous studies that involved physical exercise in experimental rats required duration of 60 minutes per day for six weeks (Veskoukis et al 2018), while in this study, the exercise was carried out only four weeks. A study conducted in 2018 revealed a reduced risk of gastric inflammation in Wistar rats that were given with mild to moderate-intensity physical exercise with a duration of 40 minutes, four times per week for four weeks. However, in that study, the moderate-intensity physical exercise was carried out using treadmill, which may have lead to different results from this present study (Murwaningsih et al 2018). Previous studies have indicated that swimming as a type of physical exercise offers greater advantages compared to other techniques, such as using a treadmill. This is mostly due to the fact that swimming corresponds to the normal behavior of rodents, preventing foot injuries and electric shocks that could potentially stimulate the production of ROS. Moreover, unlike rats, mice provide a greater challenge when it comes to running on a treadmill (Matsumoto et al 1996; Nakao et al 2000).

Despite the insignificant results, it had been found that the mean body weight of mice had a difference before and after treatments (four weeks) and there was a tendency of weight gain in K1 and K2, while K3 tended to a weight loss (Figure 1). High-calorie diet plays an important role in weight gain mostly because it increases energy intake. The tendency to a weight loss in K3 could be caused by the moderate-intensity physical exercise, which has direct impact on the energy expenditure and prevents energy imbalance (Riahi and Riahi, 2016). If body weight increases to the point of obesity because of a high-calorie diet, it has higher risk to trigger inflammation as a manifestation of oxidative stress that occurs in several mechanisms that have been described previously, such as prooxidant and antioxidant imbalance in the body that increases ROS and decreases the activity of antioxidant enzymes (SOD and GSH-px). Previous studies showed a significant decrease in SOD and GSH-px enzyme activity in obese individuals compared to healthy people (Susantiningsih et al., 2018). The tendency to lose weight is good, considering the lower risk to the occurrence of inflammation in the body. Thus, it is possible that experimental animals in this study had experienced physiological changes, but there has not been an

anatomically significant change caused by the inflammation.

Even so, research on the effect of physical exercise on gastric inflammation are still limited, and it is still not clear what the effects and their underlying mechanisms are. This study only focused on the changes in gastric histopathological features and did not assess the effect on inflammatory mediators, so it could not be determined whether inflammation had occurred or not molecularly. In addition, the duration of the intervention was only four weeks. Based on these limitations, further research is needed.

CONCLUSION

Four weeks of high-calorie diet and moderate-intensity physical exercise did not affect gastric histopathological features, in terms of inflammatory cells infiltration in mice. Thus, further research is needed to investigate the effects of high-calorie diet and moderate-intensity physical exercise on gastric histopathological features with longer duration and different method of treatments.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

ETHICS CONSIDERATION

The ethical eligibility of this study has been obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya (No: 3/EC/KEPK/FKUA/2021) on 18th January 2021.

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AUTHOR CONTRIBUTION

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting, and approval for publication of this manuscript.

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