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Cardio protective effect of intermittent fasting on the lumen area ratio of the coronary artery in rats with a high-calorie diet

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Abstract

Atherosclerosis is a principal cause of coronary artery disease (CAD), one of the major causes of global premature mortality and morbidity. The glycolysis pathway will process excess carbohydrates in a high-calorie diet (HCD) into triglycerides, which, if accumulated, will cause an increase in the concentration of atherogenic triglyceride-rich lipoproteins. Therefore, intermittent fasting (IF) is a recommended lifestyle to prevent CAD through metabolic reprogramming pathways. This study was designed to evaluate the cardioprotective effect of eight weeks of IF 5:2 on the lumen area ratio of the coronary artery in rats with four weeks of HCD. A total of 24 Wistar male albino rats were divided into SD (standard diet), IF (IF 5:2), HCD (daily injection 0.013 g/gBW glucose), and IF-HCD (IF 5:2 and HCD). At the end of the experiment, all the rats were sacrificed. The coronary artery preparations were stained with H&E stain, then evaluated with the OLYMPUS cellSens Standard to measure the arterial lumen area. The statistical analysis was done using Brown Forsythe test. In this study, no significant differences between all groups, and the effect of IF will only be seen if an HCD intervention has a significant effect. However, the IF group achieved the highest mean in the arterial lumen area ratio (SD=0.526±0.097; IF=0.631±0.021; HCD=0.611±0.064; IF-HCD=0.594±0.060). In conclusion, IF 5:2 may have a cardioprotective effect in healthy individuals, but the effect is unknown in individuals with HCD.

Keywords: Intermittent fasting; High-calorie diet; Lumen area ratio; Coronary artery disease; Healthy Lifestyle

1. Introduction

Atherosclerotic is known to be common pathophysiology underlying coronary artery disease (CAD). Oxygen supply to the myocardium is reduced due to the formation of plaque in the walls of intima blood vessels and the narrowing of the lumen of coronary arteries. As a result, the myocardium is hypoxic and can lead to myocardial ischemia [1].

The global prevalence of CAD in 2017 was 1,655 per 100,000 population, which is approximately 1.72% of the world's population. The prevalence in Asia and Australasia is 1,440 per 100,000 population. CAD claimed the lives of nine million people worldwide. This figure is predicted to continue to rise from year to year [2]. In Indonesia, the high mortality from CAD can be indirectly attributed to a high-calorie diet (HCD) because most Indonesian are known to consume foods with a much higher proportion of carbohydrates than proportions of protein and fat [3].

HCD is a risk factor for many types of heart disease. Excess carbohydrates will be converted into triglycerides by the glycolysis pathway. The process of triglyceride accumulation causes an increase in the concentration of triglyceride-rich lipoproteins, which are atherogenic [4]. The earliest inflammatory response by the endothelium that precedes

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atherosclerosis is an increase in ICAM-1 (intercellular adhesion molecule-1) which binds to a wide variety of leukocytes. Vascular cell adhesion molecule-1 (VCAM-1) plays a role by recruiting monocytes and T lymphocytes from circulation. After monocytes attach to endothelial cells, monocytes will migrate through the gap between endothelial cells into the tunica intima of blood vessels. In the tunica intima, monocytes transform into macrophages after being stimulated by chemokines. The macrophages then digest the oxidized LDL lipoproteins to form foam cells. Through scavenger receptors, LDL particles enter macrophages on the cell surface, forming fat peroxidase, and facilitating the accumulation of cholesterol esters, forming foam cells and fatty streaks [4, 5].

Lifestyle changes are recommended as a method of preventing CAD. Intermittent fasting (IF) can potentially prevent CAD through several different pathways. IF can reduce insulin resistance through reduced caloric intake due to metabolic reprogramming. Energy/nutrient depletion (as achieved through reduced caloric intake) has been shown to increase the activation of AMP-activated protein kinase (AMPK), AMPK increasing the AMP/ADP: ATP ratio with endocrine signaling hunger and satiety [6]. In another pathway, IF can induce a ketogenic state. There was an increase in β-hydroxybutyrate levels in overweight individuals who fasted. After 6–8 hours of fasting, ketone levels become detectable, signaling a transition from fat storage to fat utilization with decreased low-density lipoprotein (LDL) levels and increased high-density lipoprotein (HDL) levels. This condition indicates a decrease in cardiovascular disease risk factors [7].

HDL is anti-atherogenic, which means it plays a role in the reverse pathway of cholesterol transport. HDL also provides anti-inflammatory and antioxidant effects in blood vessel walls. HDL can inhibit monocyte migration into the gap between endothelial cells by producing the enzyme paraoxonase 1 (PON1). This enzyme protects against LDL oxidation in the walls of blood vessels. On the other hand, HDL binds to the antioxidant α-Tocopherol from vitamin E, which prevents endothelial dysfunction when in contact with blood vessel walls [8].

The effect of intermittent fasting on patients with CAD has been demonstrated. However, whether intermittent fasting can prevent changes in the lumen area ratio of coronary arteries fed a high-calorie diet is unknown. Therefore, the present study has aimed to analyze the effect of intermittent fasting on changes in the lumen area ratio of coronary arteries in white male rats with a high-calorie diet. In addition, this study's findings may help make IF acceptable in the community for preventing CAD.

2. Material and methods

This experimental study was carried out in the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, in 2020.

2.1. Inclusion criteria, acclimatization, and animal protocol

Table 1 Experimental groups

Experimental Groups	Number of animals	Application
SD	6	The duration of the intervention is four weeks. Standard feed (75% carbohydrates, 20% protein, and 5% fat) is given with a dose of 15-20 g/head/day.
IF	6	The duration of the intervention is four weeks. Fasting is held on Mondays and Thursdays. The fasting period is carried out at night from 4.00 p.m. to 06.00 a.m. WIB. The free meal period is starting at 06.00 a.m. to 4.00 p.m. WIB [9].
HCD	6	The total duration of the intervention was eight weeks. During the first four weeks, only standard feed was given. For the past four weeks, standard feed plus oral glucose (0.013 g/g body weight/day) was administered every 06.00 a.m [10].
IF-HCD	6	The total duration of the intervention was eight weeks. The rats always underwent IF interventions on Mondays and Thursdays for eight weeks. However, for the last four weeks, oral glucose (0.013 g/g body weight/day) was administered every 06.00 a.m.

Twenty-four (24) male Wistar albino rats with good health and 150 to 200 grams body weight were selected for the study [11]. During the experiment, the animals were kept in the laboratory of the animal biochemical unit, Faculty of Medicine, Universitas Airlangga. Before conducting the study, they underwent a 14-day acclimatization period to adapt

the rats to the new environment. During this period, all rats were free from any intervention. After acclimatization, they were divided into four experimental groups (Table 1). After grouping, the initial body weight of all rats was weighed on the first day.

2.2. Preparation of coronary arteries

After undergoing the intervention according to their respective durations, all rats were anesthetized with 30% chloroform and sacrificed. Then, the heart sample was put into the collecting organ container and immersed in 10% formalin buffer to prevent organ degradation. Heart sample preparations that have been processed using formalin-fixation and paraffin-embedding (FFPE) techniques will be cut into 5 µm thick tissue and then stained with H&E staining to make the structure and histological changes clear.

2.3. Measurement of coronary artery lumen area

Coronary artery preparations were observed using a microscope with a magnification of 400x. Arteries measured for lumen area are arteries found to be more or less transversely cut. The lumen area ratio is calculated by comparing the area of the inner wall to the area of the outer wall of the artery [12]. The area of the artery's inner wall is calculated by tracing the bend of the internal elastic lamina, and the area of the outer wall of the artery is calculated by following the curve of the external elastic lamina. The calculations were carried out with the help of the OLYMPUS cellSens Standard software.

2.4. Statistical analysis

Data were analyzed by alternative analysis of variance Brown Forsythe test to compare all intervention groups because the data are not significant on the homogeneity of variance analysis Levene test. P value < 0.05 was accepted as the level of significance. Statistical analysis was performed using SPSS (Statistical package for the social sciences) Version 23.

3. Results

3.1. Effect on vascular endothelium inflammatory response

The results of a microscopic evaluation of the rat coronary arteries were not found inflammatory cell infiltration in all groups. This finding means that there is no inflammatory response of the vascular endothelium, which leads to no changes in the histological structure of the rat coronary arteries.

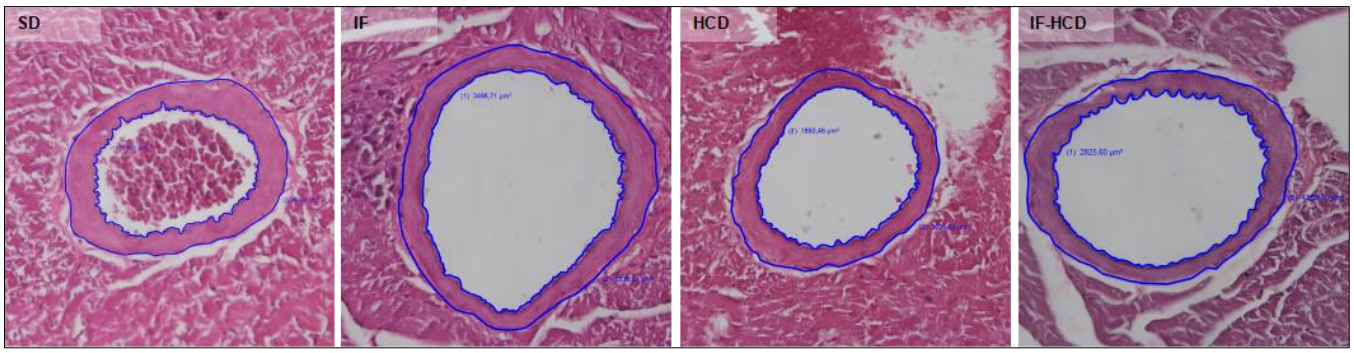

Figure 1 Photomicrograph histology representative of each group. LAR = lumen area ratio, SD = standard diet group, IF= IF 5:2 group, HCD= high-calorie diet group & IF-HCD= IF 5:2 and HCD group. (H&E stain; 400×)

3.2. Effect on the arterial lumen area ratio

The arterial lumen area ratio of all the rats was almost similar and showed no statistically significant difference among the groups. (Table-2).

Table 2 Arterial lumen area ratio in different groups of rats (N=24)

SN.	Groups	Lumen area ratio (µm)
1	SD	0.526 ± 0.097
2	IF	0.631 ± 0.021
3	HCD	0.611 ± 0.064
4	IF-HCD	0.594 ± 0.060

Values are means ± SD. Statistical analysis was done by one-way ANOVA and then Brown Forsythe test. N = Number of rats. SD = standard diet group, IF= IF 5:2 group, HCD= high-calorie diet group & IF-HCD= IF 5:2 and HCD group

4. Discussion

The present study found no inflammatory cell infiltration in all groups. These findings indicate that there is no inflammatory response of the vascular endothelium, which leads to no changes in the histological structure of the rat coronary arteries. This may be because the intervention did not increase the triglyceride level in the blood. According to Syahbanu and Pawestri [13], the intervention of a high-carbohydrate diet will affect the increase in triglyceride levels in a slower time when compared to the treatment of a high-fat diet because experimental animals must reach the hyperglycemia process first.

Another possible cause is the inappropriate composition of the intervention and the long duration. The composition of the feed that was proven to increase blood cholesterol levels and induce the formation of foam cells significantly was the feed added with 2% cholesterol, 0.2% cholic acid, and 5% pork oil. With this composition, the time required is eight weeks [14]. In another study, changes in the histological structure of the coronary arteries were obtained with feed intervention containing 0.2% egg yolk, 2% cholic acid, 5% goat fat, and 92.8% standard feed, given 20 grams daily for eight weeks [15].

The effect of HCD should have a significant effect in finding the cardioprotective effect of IF in rats with HCD. Therefore, this study could not assess the effect of IF on coronary artery lumen area ratio. However, the IF group achieved the highest mean in arterial lumen area ratio (SD=0.526±0.097; IF=0.631±0.021; HCD=0.611±0.064; IF-HCD=0.594±0.060). Although the difference was insignificant, this finding may be related to the vasodilation resulting from short-term IF. A study found that short-term IF for four weeks in rats could improve endothelial function. In this study, there was an increase in nitric oxide production which caused a vasodilation mechanism and a decrease in blood pressure [16].

Meanwhile, the treatment of IF 5:2 for four weeks in humans showed no effect of IF 5:2 on the function of blood vessel endothelium [17]. IF intervention has a more rapid effect on endothelial function than arterial wall thickness. Arterial wall thickness decreased in calorie restriction treatment in spontaneously hypertensive rats for 15 weeks [18]. In addition, IF showed a positive effect in previous studies with subjects with confirmed T2DM [6], spontaneous hypertension [18], and coronary heart disease [7]. Thus, the effect of IF is likely to be more pronounced in individuals with confirmed metabolic disease.

Another reason that may cause insignificant results is the difference in IF regimen and calorie restriction levels. A full 24-hour IF 5:2 regimen is recommended to achieve weight loss and insulin sensitivity as risk factors for CAD. While limiting the hours of eating gives better results if done every day [6]. A caloric restriction of 800 kcal/day for two weeks in obese and hypertensive subjects can improve endothelial function by vasodilatation. In another study, the intervention was carried out with a low carbohydrate calorie restriction of 1,500 kcal/day for 12 weeks [21]. Therefore, a longer fasting time and duration of the fasting intervention and a higher level of calorie restriction may prevent the decrease in vascular endothelial function.

5. Conclusion

In conclusion, IF 5:2 may have a cardioprotective effect in healthy individuals, but the effect is unknown in individuals with HCD. It is possible that a longer duration of HCD intervention and a higher dose of calorie fed can have a positive inflammatory response of the vascular endothelium, and study designs with different fasting periods are recommended. Future experiments need to be conducted to elucidate how fasting can improve endothelial function in subjects receiving HCD.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby disclose no conflicts of interest regarding the publication of this paper.

Statement of ethical approval

Ethical clearance of this study was obtained from the Health Research Ethics Committee of the Faculty of Medicine Universitas Airlangga. (No. 21/EC/KEPK/FKUA/2022).

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