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Research Paper

Histopathological Evidence of Early Coronary Atherosclerosis in an Obese Rat Model Fed with High-Fat and Fructose Diet

Prima Adelin[1,2], Efrida[1,3], Rauza Sukma Rita[1,4], Eka Fithra Elfi[1,5]

[1]Doctoral Program in Biomedical, Faculty of Medicine, Universitas Andalas, Padang, Indonesia.
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Baiturrahmah, Padang, Indonesia.
[3]Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Universitas Andalas, Padang, Indonesia.
[4]Department of Biochemistry, Faculty of Medicine, Universitas Andalas, Padang, Indonesia.
[5]Department of Cardiology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia.

Corresponding Author: Efrida

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ABSTRACT

Background: Atherosclerosis is a major cardiovascular complication associated with obesity. Experimental models often require proinflammatory induction, while the direct effect of a high-fat and high-fructose diet (HFHF) on coronary artery pathology remains underexplored.
Objective: This study aimed to evaluate histopathological changes in coronary arteries of obese rats subjected to HFHF diet.
Methods: Male Sprague-Dawley rats were divided into control (AIN-93 diet) and HFHF groups for 12 weeks. Coronary arteries were processed for H&E staining. Lesions were assessed using histopathological atherosclerosis scoring (0–3) and measurement of intimal thickness with ImageJ. Data were analyzed using Mann–Whitney test, $p < 0.05$ considered significant.
Results: Rats receiving HFHF diet developed obesity (increased body weight and Lee's index, $p < 0.05$). Coronary arteries of HFHF group showed significant higher scores histopathological atherosclerosis compared to controls. Lesions included endothelial discontinuity, foam cells, and vascular smooth muscle cell disorientation.
Conclusion: HFHF diet for 12 weeks is sufficient to induce coronary artery lesions in obese rats without additional inflammatory induction.

Keywords: atherosclerosis, coronary arteries, high fat diet and fructose, histopathology, obesity

INTRODUCTION

Atherosclerosis is a major cause of cardiovascular morbidity and mortality worldwide, and its prevalence continues to rise in parallel with the global epidemic of obesity. Recent epidemiological reports estimate that more than one billion individuals are living with obesity, a condition strongly associated with the development of cardiovascular disease (CVD). Between 1990 and 2021, cardiovascular deaths attributable to high body mass index (BMI ≥25 kg/m²) more than doubled, reaching 1.9 million cases and accounting for nearly 10% of all CVD-related mortality. Excess adiposity contributes to cardiometabolic dysregulation through insulin resistance, endothelial dysfunction, systemic inflammation, and dyslipidemia, thereby accelerating the atherosclerotic process (1,2).

To investigate these mechanisms, various animal models have been developed and remain indispensable for preclinical studies. Classical models of atherosclerosis such as apolipoprotein E (ApoE) or low-density lipoprotein receptor (LDLR) knockout mice demonstrate robust plaque formation. However, most of these models require additional interventions, such as cholesterol enrichment, streptozotocin (STZ) injection, vitamin D3, or cholate administration to accelerate dyslipidemia and lesion development. While such approaches have provided valuable insights, their reliance on pharmacological or chemical induction limits their ability to reflect the natural course of diet-induced obesity and atherosclerosis in humans (3).

Moreover, the majority of rodent studies have focused on vascular changes in the aorta or carotid arteries, while direct exploration of coronary artery pathology remains limited. This is an important knowledge gap, as coronary atherosclerosis constitutes the main pathological substrate of ischemic heart disease and represents the leading cause of cardiovascular death globally.

Several experimental studies have attempted to induce vascular and metabolic injury through dislipidemic models. For instance, Sa'adah et al. (2017) induced hyperlipidemia in rats using a mixture of duck yolk and reused cooking oil to evaluate lipid profile and atherogenic index. Similarly, Othman et al. (2020) employed a high-fat diet supplemented with cholesterol, calcium, and vitamin D3 to exacerbate dyslipidemia, oxidative stress, and early aortic changes. While these studies successfully modeled hyperlipidemia and aortic involvement, they relied on exogenous inducers and did not specifically address coronary histopathology(4,5).

In contrast, dietary models using a combination of high-fat and high-fructose (HFHF) diets have emerged as more physiologically relevant approaches. Prolonged HFHF feeding has been shown to induce obesity, insulin resistance, dyslipidemia, hepatic steatosis, oxidative stress, and vascular dysfunction in rodents. Importantly, most HFHF models administer fructose in liquid form (e.g., syrup in drinking water), which may not fully replicate the integrated effects of dietary consumption(6,7). The present study employed a HFHF model in which fructose was incorporated directly into the pellet diet, without additional pharmacological inducers. This approach provides a closer parallel to human obesogenic dietary patterns and allows direct assessment of coronary artery lesions as the primary outcome.

Building upon this rationale, the present study was designed to evaluate histopathological changes in the coronary arteries of obese rats subjected solely to a HFHF diet. By focusing directly on coronary lesions rather than peripheral vessels, and by excluding chemical inducers, this study provides novel insights into the natural course of obesity-associated atherosclerosis. To our knowledge, this is among the few experimental studies that directly characterizes coronary artery lesions in diet-induced obese rats without pharmacological induction, thereby filling an important translational gap in preclinical cardiovascular research.

This study aimed to evaluate the histopathological changes of coronary arteries in a rat model of diet-induced obesity fed a high-fat high-fructose (HFHF) diet, without additional pharmacological or chemical inducers. Specifically, we sought to characterize early vascular lesions and endothelial alterations associated with diet-induced obesity.

We hypothesized that feeding rats a HFHF diet, in which fructose is incorporated into pellet feed, would induce obesity and metabolic disturbances sufficient to trigger early histopathological changes in coronary arteries, even in the absence of additional pro-atherogenic agents such as streptozotocin, cholate, or vitamin D3.

MATERIALS & METHODS

Study Design and Animals

This study used male Sprague–Dawley rats (8–10 weeks old, 200–250 g) obtained from the Animal Laboratory, Baiturrahmah University, Indonesia. All animals were housed under controlled conditions (temperature 22–25 °C, 12 h light/dark cycle) with ad libitum access to water and feed. After one week of acclimatization, rats were randomly assigned into two groups: (1) control group, fed with AIN93 diet, and (2) high-fat high-fructose (HFHF) diet group, fed with a modified diet for 12 weeks (8,9).

The HFHF diet was formulated by incorporating high-fat content and crystalline fructose directly into pellet feed, without supplementation of cholesterol, cholate, or vitamin D3. This design aimed to mimic an obesogenic dietary pattern without additional pharmacological or chemical inducers. The composition of the diets provided to the control and obesity model groups is presented in the table 1.

Table 1. Composition of Experimental Animal Diet Ingredients

Ingredients	AIN 93	HFHF
	1 kg	1 kg
Cornstarch	619,4	419,4
Casein	140	140
Sucrose	100	-
Corn oil	40	40
Alpha selulosa	50	50
Mineral mix	35	35
Vitamin mix	10	10
DL-methionine	3	3
Choline Chlorida	2,5	2,5
Fructose	-	100
Trans oil	-	200

Assessment of Obesity Parameters

Body weight and food intake were recorded weekly. At the end of the intervention, Lee's index was calculated as an indicator of obesity status using the following formula:

$$\text{Lee Index} = \frac{\sqrt[3]{Weight}}{NAL} \times 1000$$

Histopathological Examination of Coronary Arteries

At the end of the 12-week feeding period, rats were anesthetized and sacrificed. Hearts were excised, and coronary arteries were dissected and fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E).

Histopathological evaluation was performed using a light microscope at 400× magnification. Five fields per artery were examined, and the most severe lesion was recorded using an ordinal scoring system(10,11):

- Score 0: no evidence of atherosclerosis
- Score 1: foam cell accumulation and mild endothelial discontinuity
- Score 2: increased foam cells, vascular smooth muscle cell (VSMC) disorientation or proliferation, or collagen fibrosis
- Score 3: advanced lesion with plaque rupture, thrombus formation, or calcification

In addition, mean intima thickness was measured as the distance from the endothelial surface to the lamina elastica interna. Measurements were performed on nine microscopic fields of the most severely affected artery segment, using ImageJ software

Ethical Statement: All experimental procedures involving animals were reviewed and approved by the Research Ethics Committee of the Faculty of

Medicine, Universitas Andalas, Indonesia (Approval No: 435/UN.16.2/KEP-FK/2024), dated August 14, 2024. The procedures were conducted per institutional guidelines and international standards for the care and use of laboratory animals.

STATISTICAL ANALYSIS

All data were analyzed using SPSS software version 25. The image was created using the GraphPad Prism 10.6.0 (890) application. The normality of continuous variables, including body weight, Lee index, and intima–media thickness, was assessed using the Shapiro–Wilk test. Variables that followed a normal distribution were expressed as mean ± standard deviation (SD), whereas non-normally distributed variables were expressed as median with interquartile range (IQR). Comparisons between the control and obese (HFHF) groups were performed using the Mann–Whitney U test. This non-parametric test was selected because of the relatively small sample size (n < 30), which reduces the reliability of parametric analyses, and the ordinal nature of certain variables, particularly the histopathological scoring of coronary lesions (0–3), which does not fulfill parametric assumptions. The analysis focused on determining differences in initial and final body weight, Lee index, mean intima thickness, and coronary histopathological scores between groups. Results are presented in tabular form or representative figures. Statistical significance was defined at $p < 0.05$.

RESULT

Figure 1 shows the comparison of initial and final body weight between the control group (AIN93 diet) and the obese group (HFHF diet). The initial body weight of the obese group (261.71 ± 12.98 g) was significantly higher than that of the control group (221.57 ± 23.11 g, $p = 0.007$). After 12 weeks, the final body weight of the obese group (299.14 ± 17.66 g) remained significantly greater compared to the control group (249.29 ± 12.46 g, $p = 0.002$). Statistical analysis using the Mann–Whitney U test demonstrated that the final body weight of the HFHF group was significantly greater than that of the AIN93 group ($p < 0.05$). These findings confirm that the HFHF diet effectively induced obesity in the experimental rats.

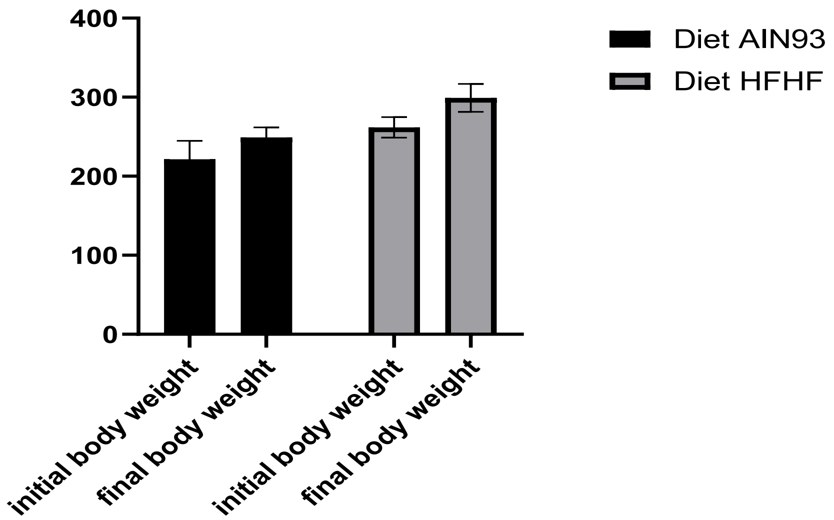

Figure 1. Comparison of initial and final body weight between control rats (AIN93 diet) and obese rats (HFHF diet).

The Lee index of rats in the obese group was markedly higher compared to the control group. As shown in Figure 2, the median Lee index in the HFHF group was shifted upward compared to the AIN93 group, with a clear separation between the two distributions. Statistical analysis confirmed that the mean Lee index of the HFHF group (339.22 ± 6.27) was significantly greater than that of the control group (295.67 ± 4.61, p = 0.002, Mann–Whitney U test). These findings demonstrate successful induction of obesity following 12 weeks of HFHF diet.

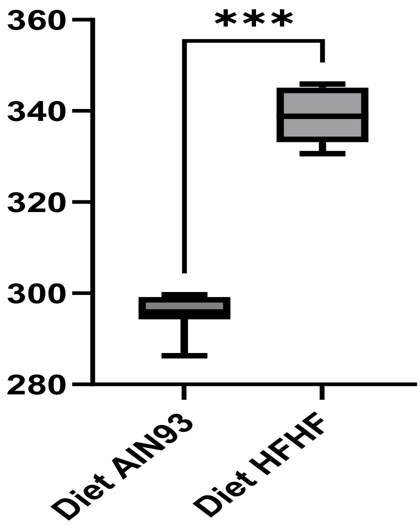

Figure 2. Comparison of Lee index between control (AIN93) and obese (HFHF) rat groups after 12 weeks of dietary intervention.
Data are presented as individual values with box-and-whisker plots (median, interquartile range).

Representative histopathological images of coronary arteries are presented in Figure 3. Control rats showed normal vascular structure with intact endothelium (Score 0), whereas obese rats fed with HFHF diet exhibited early lesions ranging from endothelial discontinuity with foam cell infiltration (Score 1) to more advanced changes with foam cell accumulation, lipid deposition, and vascular smooth muscle disorientation (Score 2).

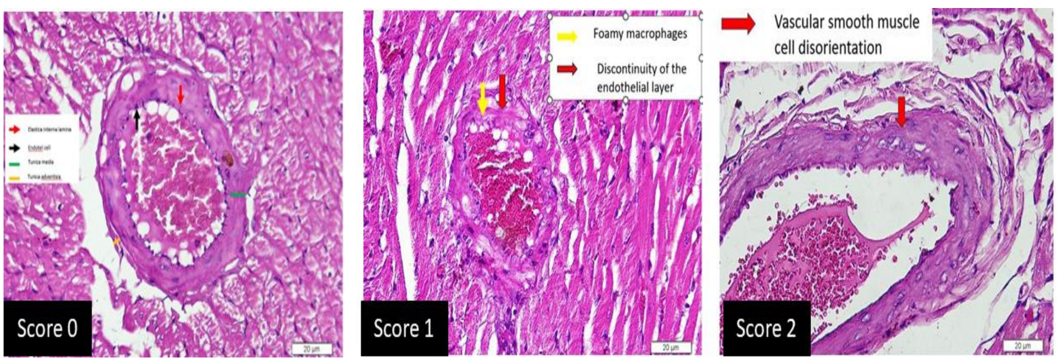

Figure 3. Representative histopathological images of coronary arteries showing pathological atherosclerosis (PA) scores in obese rats fed with HFHF diet
Score 0: Normal artery with intact endothelial lining, tunica media, and tunica adventitia, without foam cell infiltration. Score 1: Presence of foam cells (yellow arrow) and discontinuity of the endothelial layer (red arrow), indicating early intimal alteration. Score 2: Marked accumulation of foam cells, lipid deposition, and disorientation of vascular smooth muscle cells (red arrow), reflecting early atherogenic lesion development. (Hematoxylin & Eosin, 400× magnification, scale bar = 20 µm).

The distribution of coronary artery histopathological scores is summarized in Table 2. All rats in the control group exhibited normal histology (Score 0), while the obese group showed early atherosclerotic changes (Scores 1–2). Statistical analysis confirmed a significant difference in score distribution between the two groups ($p < 0.05$).

Table 2. Frequency Distribution Coronary Arteries Histopathological

Histopathological Score	Group of Rats		p value
	Diet AIN93 N (%)	Diet HFHF N (%)	
0	7 (100)	3 (42,8)	0.009
1	0 (0)	2 (28,6)	
2	0 (0)	2 (28,6)	
3	0 (0)	0 (0)	
Total	7 (100)	7 (100)	

As shown in Table 3, the mean intimal thickness of coronary arteries was higher in the obese group than in controls. However, the difference was not statistically significant ($p < 0.05$).

Table 3. Mean Intimal Thickness Coronary Arteries

Group	n	Intimal Thickness (µm)				p value
		Mean±SD	Median	Min	Max	
Diet AIN93 (Control)	7	8.23 ± 1.73	8.35	4.66	9.71	0.142
Diet HFHF (Obese Model)	7	11.34 ± 3.05	12.3	7.78	15.28	

DISCUSSION

This study demonstrated that a high-fat and high-fructose (HFHF) diet successfully induced obesity and early coronary atherosclerotic lesions in rats. Histopathological examination revealed endothelial discontinuity, foam cell infiltration, and disorientation of vascular smooth muscle cells (Figure 3). These alterations were accompanied by a significant shift in the distribution of coronary arteries histopathological scores, with all control rats remaining at Score 0 while obese rats developed Score 1–2 lesions (Table 2, $p = 0.009$). In addition, intimal thickness was increased in obese rats compared to controls, although the difference did not reach statistical significance when tested with Mann–Whitney analysis (Table 4, $p = 0.142$). These findings strengthen the evidence that an obesogenic diet alone is sufficient to initiate subclinical coronary atherosclerosis.

Several previous studies have reported similar observations. Ismawati (2016) and Othman (2020) described that chronic intake of obesogenic diets contributed to early vascular changes, consistent with the endothelial disruption and foam cell accumulation observed in our HFHF-fed rats (5,11). More recently, Handayani et al. (2021) reported that prolonged administration of dietary fat and fructose promoted obesity, hepatic steatosis, and cardiac histopathological alterations, further supporting our findings(12). A systematic review by Prabhu et al. (2025) also highlighted that high-fat diet models consistently produced dyslipidaemia, endothelial dysfunction, and arterial wall thickening, all of which are hallmarks of early atherogenesis (13).

In humans, the detrimental role of fructose on cardiovascular health has been increasingly recognized. Busnatu et al. (2022) emphasized that fructose-rich diets contribute to metabolic syndrome, insulin resistance, and endothelial injury. These data support the translational relevance of our findings, where coronary lesions developed in obese rats exposed solely to an HFHF diet (14).

In our study, the mean intimal thickness of coronary arteries was higher in obese rats compared to controls (Table 3), although the difference did not reach statistical significance ($p = 0.142$). This trend suggests that diet-induced obesity may contribute to early intimal remodeling before the development of advanced atherosclerotic lesions.

Comparable findings have been reported in other experimental models. A recent study from Kellet at al (2024) demonstrated that feeding Wistar rats with an atherogenic diet for 28 days significantly increased the thickness of the tunica intima of the abdominal aorta compared with controls, while the combined intima–media and total wall thickness did not differ significantly (15). This supports the notion that the intima layer is the earliest vascular compartment affected by hyperlipidemia and dietary insults.

Earlier histological and imaging studies also emphasize the importance of selective intimal measurement. Choi et al. (2009) showed that intimal thickness measured histologically in rat carotid arteries was significantly increased in hypertensive and high-fat diet groups compared with controls, and correlated well with high-frequency ultrasound biomicroscopy findings. Similarly, Razuvaev et al. (2008) reported that ultrasound biomicroscopy allowed reliable non-invasive monitoring of intimal hyperplasia in rats after carotid balloon injury, with strong agreement between histology and imaging ($r = 0.97$, $p < 0.0001$) (16,17).

Interestingly, not all conditions that predispose to vascular disease are associated with measurable intimal thickening. A study in diabetic rats by Cüce et al. (2015) found no significant differences in tunica intima–media thickness between control and diabetic groups, despite clear evidence of increased Bax and Caspase-3 expression indicating apoptosis of smooth muscle cells in the tunica media. This highlights that functional and cellular alterations may precede structural changes in vascular thickness (18).

Taken together, these findings suggest that intimal thickness is a sensitive but sometimes variable marker of early atherosclerosis. Our observation of a non-significant increase in coronary intimal thickness in obese rats is in line with literature showing that changes may appear subtle and require longer diet exposure or additional risk factors to reach statistical significance. Nevertheless, the trend underscores the importance of tunica intima as an early site of vascular injury in diet-induced obesity.

A key novelty of this study is the induction of coronary lesions without the use of additional inflammatory stimulants such as lipopolysaccharide (LPS) or streptozotocin (STZ). The presence of early coronary atherosclerosis, evidenced by histopathological scoring (Figure 3, Table 3) and increased intimal thickness (Table 4), demonstrates that an obesogenic diet itself can provide sufficient metabolic and oxidative stress to impair vascular integrity. This HFHF model may therefore serve as a valuable platform for studying the mechanisms of obesity-related coronary atherosclerosis and testing preventive or therapeutic interventions.

Nevertheless, some limitations should be acknowledged. The sample size was relatively small, and only a single time point was analyzed, which may have limited the statistical power of the Mann–Whitney analysis for intimal thickness (Table 4). Furthermore, inflammatory and oxidative stress markers were not assessed in this study, restricting our ability to fully delineate the mechanisms underlying the observed vascular lesions.

Future research should address these limitations by including larger sample sizes, longer duration of diet exposure, and multiple time points to capture lesion progression. The use of immunohistochemical staining for inflammatory mediators (e.g., TNF-α, IL-6, CRP) and oxidative stress markers will provide deeper mechanistic insights. In addition, interventional studies testing dietary modification or pharmacological therapies would clarify the translational value of the HFHF diet-induced model for cardiovascular prevention and treatment.

CONCLUSION

This study demonstrated that a high-fat and high-fructose (HFHF) diet induced obesity and early coronary atherosclerotic lesions in rats. Histopathological alterations, including endothelial discontinuity, foam cell infiltration, and vascular smooth muscle disorientation, were observed in obese rats, as reflected in increased pathological atherosclerosis scores and greater intimal thickness compared to controls.

The novelty of this study lies in the development of coronary lesions without the use of additional inflammatory or chemical inducers, underscoring that obesogenic diet alone can trigger early coronary pathology. These findings highlight the HFHF diet model as a valuable platform for investigating the mechanisms of subclinical cardiovascular disease and for exploring preventive or therapeutic interventions.

Declaration by Authors

Ethical Approval: Research Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No: 435/UN.16.2/KEP-FK/2024), dated August 14, 2024

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Conflict of Interest: The authors declare no conflict of interest.

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