



The Comparative Effects of Branded and Local High Fat Foods on Body Mass Index and Vascular Wall Thickness in Male Wistar Rats for Development of Atherosclerosis Animal Model

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Abstract. Cardiovascular diseases are the leading cause of mortality over the world, which is approximately 17.9 million patients' death in 2019 or 32% of all deaths worldwide. Atherosclerosis formation due to accumulated plaques in the tunica intima of vascular walls underlies the pathogenesis of cardiovascular diseases. Inactive life style and bad diet are the major risk factors of these diseases. This study investigated the different effect of high-fat diets on body mass index (BMI), lipid profile and vascular wall thickness (VWT) in rats model atherosclerosis. A total of 10 male Wistar rats were used in this experimental study. The Wistar rats were randomly divided into two groups (A and B). Group A was fed by the DYETs food while group B were given local high fat foods. The BMI was calculated using body weight (BW) and body length. Internal carotid artery thickness was measured using a linear ultrasound. The t-tests were performed to compare within and between groups. There was a significant difference in carotid artery thickness between two groups with $p < 0.05$ but the mean BMI of group A did not differ from the mean BMI of group B with $p > 0.05$. In conclusion, A local high fat diet has a similar effect in obesity induction of male Wistar rats, compared to DYETs diet although a higher increased internal carotid artery thickness is detected in the later diet. Further research is required to identify histopathologically the plaque structure.

Keywords: Atherosclerosis · High fat diet · Vascular wall thickness (VWT) · BMI

1 Introduction

Atherosclerosis, which is an accumulation of plaques on arterial walls, becomes one of the main causes of high mortality and morbidity (approximately 17.9 million patients' death in 2019 or 32%) around the world and which leads to cardiovascular complications. In general, patients with atherosclerosis have minimal symptoms in the initial process [1]. Excessive cholesterol, TG, cellular waste products, calcium, and fibrin (blood clotting material) accumulate in the intima stratum of arterial wall, which result in reduction of diameter of the lumen arteries [2, 3]. Accumulation of those materials induces inflammation, endothelial dysfunction, mobilization of leukocytes and plain muscle cells into the arterial wall to generate foam cells and deposition of extracellular matrixes [9].

The aetiology of atherosclerosis remains unclear but specific conditions, nature, and habits can increase the risk of atherosclerosis. Most common risk factors include high levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (CHO), low levels of high-density lipoprotein cholesterol (HDL-C), smoking, hypertension, diabetes mellitus, obesity, sedentary lifestyle, and older age [4].

Early detection of atherosclerosis process is very important in order to reduce mortality and morbidity rates. Various techniques either invasive or non-invasive are available in the clinical setting but it has high cost and expertise [5]. One alternative for evaluating preclinical atherosclerosis uses an Ultrasonography (USG) device, which is able to identify plaques on the intima-medial layer (IMT) of artery, namely VWT [8].

So far, very limited studies have been reported animal model with atherosclerosis. Recently, some studies have used specific rat diets to generate obesity and/or dyslipidaemia. For instance, DYETs is one of rat foods which consists of 40% of calories as fat, lard and cholesterol. However, we have to wait for several months to get this product. Therefore, the aim of this study was to investigate the different effects of high-fat diets from branded and local foods on BMI, lipid profile and vascular wall thickness (VWT) in rats model atherosclerosis. In Indonesia, there are some materials that can be used to make a similar high fat diet to replace the imported high fat diet.

2 Materials and Methods

In this animal experiment, we used DYETs (Dyets, Inc. Bethlehem, USA) and local foods to induce obesity in male rats. The local food consisted of 2 kg Broiler-2 (BR-2) feed, 30 duck egg yolks, 30 dried organs of chicken liver, 500 ml lard.

This preliminary study of obesity, we used 10 male wistar rats, which aged 12 weeks and had 250–280 g BW. Before the research study began, all rats were adapted in their cages for 3 days with 12 h dark and light cycles, ambient temperature and controlled humidity. Selected rats were randomly divided in to 2 groups (DYETs and local) and placed in 4 different cages. The first group was given DYETs food while the other group was given local food (10% from total BW) in the morning for 14 days. The rats could freely access drinking water for 24 h. The protocol of this research study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital Surakarta with Number: 405/III/HREC/2021.

Before and after treatments, we measured BW and length using animal BW scale and stadiometer. The BMI was calculated using the Röhler index = {Body weight (g)/Naso-anal length (cm)³} × 10³ [7]. The VWT and lipid profile (CHO, HDL-C, LDL-C and TG) were determined using a portable Ultrasonography device (General Electric Logic p7, USA) and a routine laboratory method.

All collected data were analyzed using the SPSS (Statistical Program for Social Science) program, Version 26 and presented as mean ± standard deviation (SD). The Shapiro-Wilk test was used to determine normality data. The mean BW and BMI before and after treatment within groups were statistically tested paired student t-test while the mean VWT and lipid profile of DYETs group were compared to that of local group using the independent student t-test. The significant value was setup at $p < 0.05$.

3 Results and Discussion

3.1 Results

After 3 days adaptation, the mean BW rats in both groups were slightly different (Table 1). The rats in local group had BW (239.4 ± 13.56 g) lower than the rats in the DYETs group (258.4 ± 18.55 g) but it was not significant with $p = 0.102$. During 14 days treatment, the mean BW in DYETs and local groups increase significantly (289.2 ± 29.46 g with $p = 0.022$ and 271.4 ± 18.88 g with $p = 0.030$ respectively). However, the mean BW in DYETs group did not differ from the mean BW in local group ($p = 0.288$).

In addition to rats' BW, the same pattern was observed in the BMI before and after treatment (Table 2). A higher BMI was found in the DYETs group (64.11 ± 11.53 g/cm³) than the BMI in the local group (56.5 ± 5.41 g/cm³) with $p = 0.218$. The BMI in both groups increased significantly after 14 days treatment (71.8 ± 14.56 g/cm³ with $p = 0.034$ and 64.17 ± 7.84 g/cm³ with $p = 0.005$ respectively). In comparison to rats' BMI in the local group, the DYETs group had higher BMI but it was not significant ($p = 0.333$).

We further evaluated lipid profile in DYETs and local group in order to verify increased BW and BMI after 14 days treatment. Table 3 indicated that the mean lipid

Table 1. Comparison of rats' BW in both groups

	BW (g)		<i>p</i>
	Day 1 (Mean ± SD)	Day 14 (Mean ± SD)	
DYET	258.4 ± 18.55	289.2 ± 29.46	0.022*
LOCAL	239.4 ± 13.56	271.4 ± 18.88	0.030*
<i>p</i>	0.102	0.288	

* Significant ($p < 0.05$)

Table 2. Mean comparison of rats' BMI in both groups

	BMI (g/cm ³)		<i>p</i>
	Day 1 (Mean \pm SD)	Day 14 (Mean \pm SD)	
DYET	64.11 \pm 11.53	71.8 \pm 14.56	0.034*
LOCAL	56.5 \pm 5.41	64.17 \pm 7.84	0.005*
<i>p</i>	0.218	0.333	

* Significant ($p < 0.05$)

Table 3. Mean comparison of lipid profile in both groups

	Day 14 Treatment		<i>p</i>
	DYET (Mean \pm SD)	LOCAL (Mean \pm SD)	
CHO (mg/dL)	48.8 \pm 5.63	80.0 \pm 28.33	0.042*
HDL-C (mg/dL)	12.6 \pm 3.8	18.8 \pm 2.17	0.0113*
TG (mg/dL)	97.8 \pm 29.95	214.6 \pm 105.32	0.044*
LDL-C (mg/dL)	46.6 \pm 2.08	38 \pm 5.71	0.013*

* Significant ($p < 0.05$)

profiles in the local group were significantly higher than the mean lipid profiles in the DYETs group except the LDL-C levels. The mean total CHO level in the local group increased around 32 mg/dL while the mean HDL-C increased around 6 mg/dL. The mean TG levels increased more than double compared to the mean TG levels in the DYETs group (97.8 \pm 29.95 mg/dL). In contrast, a lower LDL-C was observed in the local group (38 \pm 5.71 mg/dL), compared to the DYETs group (46.6 \pm 2.08 mg/dL with $p = 0.013$).

From the USG measurement, the mean VWT of local group was a half higher thickness (0.84 \pm 0.015 mm) than the mean VWT of DYETs group (0.44 \pm 0.014 mm). The mean differences of VWT in both groups reached significantly with $p = 0.002$ (Table 4).

Table 4. Mean comparison of VWT in rats DYETs and local groups

	Day 14 Treatment		<i>p</i>
	DYET (Mean ± SD)	LOCAL (Mean ± SD)	
VWT (mm)	0.44 ± 0.014	0.84 ± 0.015	0.002*

* Significant ($p < 0.05$)

3.2 Discussion

Here in, we have demonstrated that a local rat food can significantly increase BW and BMI although it is not as high as DYETs food. However, the rat group which was given a local food, has higher lipid profile (except LDL-C) and VWT than the rat in the DYETs group. Thus, it suggests that the local food can be used as an alternative to generate rat model with atherosclerosis.

In contrast to increased BW and BMI in the DYETs group, another study used a Diet-induced Obesity (DIO) rodent which contained high fat and energy content (HE): 8% corn oil, 44% sweetened condensed milk, and 48% Purina rat chow to generate obese rats [15]. Furthermore, using DIO rats in anti-obesity compound toxicity testing may also help to better delineate findings related to pharmacological overuse associated with weight loss from actual drug-related toxicity [10, 11]. This is because the polygenic inheritance [15] that has a significant effect on BW in this DIO rat model is similar to humans [12].

Secondly, administration of local rat food increases CHO, HDL-C, and TG than that of the DYETs rat food, due to high amount of yolk egg, chicken liver and lard. A previous study showed that excessive consumption of yolk egg, containing very high cholesterol, induces obesity and dyslipidaemia [13]. Foods enriched with lard and sugar also result in induction obesity with high levels of CHO and TG [14].

In our study, high levels of total CHO and TG in blood circulation increase VWT. Our findings are in line with a study from Ianuzzi et al. (2021), which reported that lipid is an important to influence the formation of atherosclerosis in arterial wall [6]. However, we need a further investigation to confirm this result using a histopathological staining.

Administration of high fat diet from local food increases BMI as high as administration high fat diet from branded food. However, lipid profile and VWT in rats treated with high fat diet from local food are significantly higher than that of rats treated with high fat diet from branded food. A further research study is required to verify whether or not high fat diet from local food can induce atherosclerosis.

Acknowledgments. We gratefully acknowledge to the Institute of Research and Community Services, UNS and Dr. Moewardi General Hospital for providing research grants to do this research project.

Authors' Contributions. PSP, BP and DI conceived and developed the ideas. PSP and DI carried out research design and experiment. WS and SK statistically analyzed the experimental data. PSP prepared the draft article whiles the other authors reviewed and revised the full text article. All authors read and approved the final manuscript.

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