The Effects of High Fat High Carbohydrate (HFHC) Diet on Body Weight in *Overweight* Sprague Dawley Rats

Novia Zuriatun Solehah[1][a], Adi Prayitno[2,3][b] and Eti Poncorini Pamungkasari[2,4][c]
[1]*Postgraduated Student of Human Nutrition Science, Universitas Sebelas Maret, Surakarta, Indonesia*
[2]*Postgraduated Program of Nutrition Science, Universitas Sebelas Maret, Surakarta, Indonesia*
[3]*Departement of Pathology Anatomic, Faculty of Medicine,Universitas Sebelas Maret, Surakarta, Indonesia*
[4]*Departement of Public Health, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*

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Abstract: Excessive body weight is caused by an inappropriate diet. Foods that are high in fat and carbohydrates may lead to *overweight.* When *overweight* condition is followed by an increase in the body fat level, it also increases the production of *Reactive Oxygen Species* (ROS) in the blood circulation and adipose cells. This study aims to determine the effect of High Fat High Carbohydrate (HFHC) diet on body weight of *overweight* Sprague dawley rats. This study used an experimental design with a pre-post-test control group design. Male Sprague dawley rats (n=10) were randomized into two groups. The normal group was given standard rats and the *overweight* group was given diet HFHC for 14 days. Their body weights were measured before and after treatment. After 14 days of treatment, the body weight mean was (210 ±1,5) in the treatment group increased significantly, compared with body weight mean (193±1,3) and before treatment (183,2±1,6) in the control group. In conclusion, those induced to be overweight used HFHC diet for 14 days and increased body weight. This finding can be used to induce overweight rats for a short time.

1 INTRODUCTION

Global prevalence of overweight rose trifold (1.9 billion) of world population and around 13.6% in Indonesia by 2018 (Khadaee and Saidi, 2016; Rebello *et.al.*, 2020; Riskesdas, 2018). Overweight and obesity is the fifth leading cause of mortality globally (Sudargo *et.al.*, 2018). Overweight is a starting point for obesity which then will lead to various metabolic syndromes including diabetes mellitus, hypertension, stroke, coronary heart disease, dyslipidaemia and cancer (Muhammad *et.al.*, 2019). Results of a study stated that the increase of Body Mass Index (BMI) within five years between the age of 25-74 years significantly increased the risk of hypertension up to 30% compared to people without an increase of BMI (Hall *et.al.*, 2019). In addition, a rise in body weight may trigger insulin resistance of peripheral tissue through disfunction or adipose tissue lipotoxicity, inflammation, dysfunction of mitochondria, hyperinsulinemia and endoplasmic reticulum stress mechanisms (ER) (Longo *et.al.*, 2019).

The accumulation of excess fat causes the release of high amount of free fatty acid to various organs thus generating metabolic syndrome. The prevalence of metabolic syndrome grows as the prevalence of obesity rises. One of the factors that sets overweight off is excessive fat and carbohydrate intake in addition to a sedentary lifestyle. In recent days, the diet pattern of Indonesians shifts as it receives heavy influence from western diet pattern where people consume more high fat and high carbohydrate food (Fernandez *et.al.*, 2018). Food with high content of carbohydrate, protein, and fat may contribute to the increase of oxidative stress and cause inflammation by forming white adipose tissue which secretes proinflammation factor (Tan *et.al.*, 2018; Tan and Norhaizan, 2019). Increased oxidative stress is the main cause of numerous metabolic diseases.

[a] https://orcid.org/0000-0003-0827-2695
[b] https://orcid.org/0000-0001-5548-4848
[c] https://orcid.org/0000-0002-4197-3226

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Imbalanced energy intake and outtake give rise to excessive fat accumulation not only in adipose tissue but also in ectopic tissue like the liver. If prolonged, it will cause excessive fat in liver thus lead to impaired liver function, fatty liver and liver failure (Rahman *et.al.*, 2017). The sign of accumulated fat in ectopic tissue is the increased percentage of visceral fat in intra-abdominal linked to abdominal obesity (Tchernof *et.al.*, 2013). Also, excessive fat and carbohydrate intake may change oxygen metabolism which then triggers oxidation reaction. Excessive intake may also induce enlargement of adipose tissue through adipocyte hypertrophy and hyperplasia. The imbalance between ROS and antioxidants causes an increase in oxidative stress, causing systemic inflammation.

Observing overweight model mice with diet induction can give illustration of the effect of poor eating habits among human. Several studies in animal used high fat high carbohydrate feed in inducing overweight to animals. This diet is believed to be the best method in following the pathogenesis of the development of metabolic syndrome, one of which is obesity (Panchal *et.al.*, 2011). Although, several studies conducting the induction of overweight animal models require quite a long time. Therefore, this study aimed to examine the effect of the high fat high carbohydrate (HFHC) diet on overweight rats for 14 days.

2 MANUSCRIPT PREPARATION

2.1 Animal Experimental Protocol

This study has received approval from the Health Research Ethics Commission, Faculty of Medicine, Sebelas Maret University Surakarta (KEPK UNS) No.23/UN27.06.6.1/KEP/EC/2021.

2.2 Samples

The samples in this study were *Rattus norvegicus* strain male Sprague dawley rats aged 8-12 week with the average of weight of 150-200 gram. The subjects are 10 rats divided into normal or control groups and HFHC diet group. The subjects were obtained from the Nutrition Laboratory of the Food and Nutrition Studies Centre PAU, Gadjah Mada University, Yogyakarta.

2.3 Study Design

This is an experimental study with pre posttest with control group design. 10 rats are divided into two groups, control group and HFHC diet group. Control group was given standard Comfeed which was AD II feed. All the rats were acclimatized for seven days in individual cages and were given standard Comfeed AD II with ad libitum drinking water. Every 100 gram of standard AD II feed contains 12% water, 7% ash, 15% crude protein, 3-7% crude fat, 0.9-11% calcium, 0.6-0.9% phosphor, antibiotic, and coccidiostat maximum 20 mg/h. The raw materials used include yellow corn, SBM, MBM, CGM Palm olein, essential amino acid, essential mineral, premix, and vitamin. The average feed intake was 5 g/100gBB/h. The rat rearing cage was a plastic cage sized 25 cm x 15 cm x 7 cm. Each cage is used by only one rat. The subjects were reared in a specific room with controlled temperature (27-29ºC) inside a hygienic polypropylene cage. The room is set with a 12 hours light and 12 hours dark cycle (lights were turned on at 07.00 pm, and 70-90% humidity. The rats were considered stable if the feed consumption and drink was sufficient. The HFHC group was given HFHC feed. The composition of the HFHC feed includes 5% cheese, 10% egg yolk, 15% cow fat, oil 15%, rice 45% and standard feed 20% (Ardiansyah *et.al.*, 2018). The HFHC diet was given for 14 days. All the feed was given ad libitum. In a study conducted by Adriansyah *et al*, the induction of obese rats was carried out for 8 weeks. And in this study, HFHC feed was added with 0.02 g of cholic acid wihch functions as a fat binder in the body and produce overweight rats in a shorter time.

2.4 Anthropometry Measurement

The body weight measurement was conducted using a digital scale. It was a digital scale with 0.1 accuracy. The measurement was done three times, after 7 days acclimatization period, after 7 days diet induced overweight, and after 14 days diet induced overweight right before termination.

2.5 Statistical Analysis

The statistical analysis was conducted using SPSS version 16.0. The data was presented with a mean value and standard deviation. To analyse the mean difference between groups a paired t test and *oneway anova* was performed. The result is considered significant if the p value is <0.05.

3 RESULT AND DISCUSSION

The development of overweight model rats was carried out by feeding HFHC. The difference in the composition of HFHC with the standard can be seen in Table 1.

Table 1: Composition of Standart and HFHC

Ingredients	Standard	HFHC
Carbohydrate	53-57 %	67.69%
Protein	16%	22%
Fat	3-7%	74%

Table 1 shows that the composition of HFHC feed contains higher carbohydrates and fats compared to standard feed. The body weight increased due to the administration of feed with 67.69% carbohydrate and 74% fat from the total calorie. Results displayed that consumption of fat >30% of total calories can cause obesity (Hariri and Tibault, 2010). In this study, the composition of the HFHC feed includes cheese, egg yolk, cow fat, oil and rice. In addition, the mice induced with HFHC feed tend to consume a lot of water. Water is essential in digestive system especially during the enzymatic hydrolysis and lipid absorption. Lipase enzyme plays a role to hydrolyse triglycerides into diglycerides, monoglycerides fatty acid and glycerol (Udomkasemsab and Prangthip, 2019). The animal response to the diet given to them can be used to evaluate the rate of success during the overweight induction. Thus, the composition of HFHC diet compared to control group's standard diet must have a similar basic nutritional content except for the types of the macronutrients, namely carbohydrate and fat. For this research, the composition of macronutrients in HFHC diet include cheese, cow fat, egg yolk, and rice.

On the other hand, the composition of macronutrients in control group's standard feed included yellow corn as a carbohydrate. The content of carbohydrate and fat in the HFHC diet is significantly higher compared to the standard feed which only contains 53-57% carbohydrate and 3-7% fat. Results of the systematic review conducted by Tibault and Hariri (2010) demonstrated that the best method to induce obesity into mice is by using high semi-pure fat which contains 40% animal fat with a low amount of amino acid omega 3 and pure vegetable oil and high in amino acid omega 6 and omega 9. The composition of selected raw ingredients will influence the amount consumed. This will also affect the increase of the body weight. The consumption of food high in fat, sugar, and salt contributes to the occurrence of overweight and obesity by changing the expression of gene and sending dopamine signal in the brain. Several researches mentioned that high fat diet is a source of fat which in it includes saturated fatty acid (de Moura e Dias *et.al.*, 2021). Results indicated that there is a positive association between body adiposity and HFD including saturated fatty acid (Norris *et.al.*, 2017).

Weighing was carried out to evaluate whether there was a difference in the weight of rats fed HFHC for 14 days with rats fed only standard feed. Rats were weighed 3 times before HFHC induction, after HFHC induction on the 7 days, and after HFHC induction on the 14 days. The average of body weight before and after induction are presented in Table 2.

Table 2: Average on Body Weight Before and After Induced HFHC

Group	**Pre test (Mean ± SD)**	**Post test (Mean ± SD)**	**p**
Normal	183,2 ± 3,70	193,0 ± 2,92	>0.005[a]
HFHC	184,2 ± 3,77	210,6 ± 3,36	>0.005[a]
p	>0.005[b]	>0.005[b]	

In table 2 shows that the administration of the HFHC diet for 14 days significantly increased body weight (p>0.005), the mean body weight rose from 184,2 ± 3,77 to 210,6 ± 3,36. Within control group which was given standard feed, the mean body weight was lower than the HFHC group. This supports the findings of a study conducted by Wong (2018) which concluded that HFHC diet for 6 weeks significantly increased rats body weight to obesity. Other study on Sprague dawley rats displayed that the administration of high fat diet of 61% for 8 weeks did develop obesity and increase visceral fat accumulation (Udomkasemsab and Prangthip, 2019).

Rats overweight determined based on the Lee index. The rats stated if overweight index value Lee > 300. Heavy rats soul uses scales and the height is measured using ruler. The average of Lee index can be seen in Tabel 3.

Table 3: Indicator of *Overweight*

Group	**Post test (Mean ± SD)**	**Lee Indekx**	**p**
Normal	193,0 ± 2,92	285,97 ±1,77	>0.005
HFHC	210,6 ± 3,36	324,76 ±4,62	>0.005
p	>0.005*	>0.005*	

Consumption of food high in fat can cause excessive fat accumulation in our body which then resulted in the increase of free fatty acid in adipose

tissue and release a high level of triglycoid (Marques *et.al.*, 2016). Also, excessive fat accumulation will also be stored in ectopic tissue like liver, muscle, and heart which will be indicated by hypertrophy and hypoxia thus lead to inflammation and insulin resistance (Longo *et.al.*, 2019).

The incidence of overweight and obesity occur due to the intake of fat that is way higher than the outtake thus the excess will be stored in the form of fat in our body, especially adipose tissue. The increase in adipose tissue mass causes changes in adipokine production where adipose tissue plays a role in energy storage which is an important endocrine. Furthermore, the enlargement of adipose tissue and the increase of adipocyte progressive may disturb the blood flow (McArdle *et.al.*, 2013; Exley MA *et.al.*, 2014). High fat diets have been proven to increase body weight, fat deposition, increase of oxidate stress biomarkers, increase of fasting plasma glucose and insulin. The occurrence of inflammation around the blood vessel is triggered by the accumulation of fat in cellular molecules by releasing inflammation adipocytes like *tumour necrosis factor* (TNF) and interleukin-6 (IL-6).

In *overweight,* the production of adipocytes is disrupted, including leptin, resistin, adiponectin, *Monocyte Chemoattractant Protein-1* (MCP-1), *Tumor Necrosis Factor-α* (TNF-α), and interleukin-6 (IL-6) (Mesquida *et.al.*, 2020). Animal research conducted by Feillet-Coundray *et.al.* (2019) showed that the administration of high fat/high fructose cause overweight, glucose intolerance, and increase of IL-6. Analysis results review done by Tan *et.al.* (2018) mentioned that high fat diet for 12 months increased plasma triglycerides and total cholesterol which led to increase of oxidative stress biomarker in blood. High fat diet not only worsen lipid profile but also increase the accumulation of ROS and trigger damage to mitochondria. The mechanism of inflammation in adipose tissue is caused by the activation of proinflammation line in this case being *nuclear factor-kappaB-* (NF-κB-). The occurrence of this inflammation can be detected by the increase inflammation biomarker and cytokines. In addition, the occurrence of inflammation is also linked to the increase of oxidative stress (de Melo *et.al.*, 2017). Overweight and obese people go through an increase in the production of raective oxygen species (ROS) due to imbalance between prooxidant and antioxidant (Bondia-Pons *et.al.*, 2012; Yosika *et.al.*, 2020). This condition contributes to the incidence of metabolic disordersnamely insulin resistance, type 2 diabetes mellitus, hyperlipidaemia, and atherosclerosis.

4 CONCLUSIONS

Feeding HFHC diet for 14 days can increase body weight in overweight induced mice. However, further studies are needed to further analyse the effect of HFHC to overall health. Furthermore, this study can be used as a reference to rear overweight model mice in shorter period of time.

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