

## A SIMPLE METHOD FOR HIGH-FAT DIET (HFD)-INDUCED OBESE MICE MODEL AND ITS DETERMINATION OF HYPERPHAGIA RESPONSE

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### ABSTRACT

Many methods for induce obesity on the rodent model such as lesion of ventromedial hypothalamic (VMH), chronic acid injection, monosodium glutamate, goldthioglucose, and food induced obesity previously have been studied. Based on some studies, food induced obesity is easy method to induce in many species. Overconsumption of high-fat diet (HFD) to the rodent model leads to increases of body weight and adipose tissue mass. In addition, not only increases of body weight and visceral adipose tissue mass but also increases of some organs weight involving liver, spleen, kidney, and testicle as target of fat accumulation.

In this study were used mice model. Both obese mice group was fed high-fat diet and normal mice group was fed standard laboratory diet for 21 days. From the experimental data, occur increases body weight of obese mice group which compared to the normal mice group. Some organs like liver, spleen, kidney, and testicle also exhibit increases of size on the obese mice group.

In conclusion, high-fat diet-induced obese mice with simple ingredients which developed in our laboratory be able to increase of body weight in a short time and can draw a correlation about increase of body weight and hyperphagia response to the mice.

**Keywords:** Simple method, High-fat diet, Obesity, Hyperphagia.

### INTRODUCTION

According to the *World Health Organization* (WHO), obesity defined that abnormal or excessive fat accumulation and influence to the human health [1,2]. In addition, obesity has dramatically increased throughout in the world [3-5]. Obesity is a metabolic syndrome and has an important role in the pathogenesis of some disorders, such as diabetes mellitus, hypertension, dyslipidemia, insulin resistance, some cancers, atherosclerosis, and arthritis disorder [6].

Obesity also become first contributor to the metabolic dysfunction involving dysregulation of lipid and glucose metabolism. Dysregulation of lipid and glucose metabolism, give influence to organ functions involving cardiac, gastrointestinal, liver, and reproductive functions [7]. Many methods for induce obesity on the rodent model such as lesion of ventromedial hypothalamic (VMH) on the brain, chronic acid injection into suprasellar region, monosodium glutamate injection into subcutaneous tissue, administration of goldthioglucose, and food induced obesity previously have been studied [8]. Food induced obesity were known as easy method to induce in many species.

One of the most common factor which contributed to the obesity are high-fat diet (HFD) consumption. Excessive calorie intake above energy requirements has potential for development of obesity [9]. In rodent studies, high-fat diet consumption shown higher degree of obesity than carbohydrate diet. So, several reason appear that increased dietary fat give result obesity because fat stored more effectively than carbohydrate [10].

Furthermore, increases of body weight caused high-fat diet consumption might be correlated with hyperphagia response. Hyperphagia is a condition which exhibited through food overconsumption and often occur to the obese animal model [11]. Rodent has great ability to feed. Overconsumption of high-fat diet leads to increases of body weight, adipose tissue mass, and organ weight.

On the some studies about anti-obesity, high-fat diet method widely used to induce rodent model [12-15]. Generally, to induce obese condition in the mice through high fat diet takes more than 1-2 months. The ingredients of most common method generally contains fat, protein, and carbohydrate. Nowadays, in our laboratory, method development were performed with change diet components and its

ratio from the previous studies and enter new important components to accelerate increases of mice body weight. In addition, in this study also determine hyperphagia response to the mice.

### MATERIAL AND METHODS

#### Animal

Animal study were performed in the Laboratory of Pharmacology, Sekolah Tinggi Farmasi Bandung, West Java, Indonesia. Fourty Swiss-Webster mice of 2-3 month age, weighed about 25-30 g, were acclimatized on the room temperature ( $28 \pm 5^\circ \text{C}$ ) and humidity-controlled with appropriate light/dark cycle. Mice were given access to get food and drink *ad libitum* for acclimatization before enter to induction period.

### METHODS

#### Experimental design

Following acclimatization, mice were divided into two groups. Both normal mice group and obese mice group were placed in the different cage. The normal mice group was fed standard laboratory diet and the obese mice group was fed high-fat diet for 21 days.

The ingredients of the normal mice group diet is contains approximately 0% saturated fat mixture, 41% protein mixture, 55% carbohydrate mixture, and 4% polyunsaturated fat.

While, the high-fat diet contains 32% saturated fat mixture, 41% protein mixture, 27% carbohydrate mixture, and didn't used polyunsaturated fat.

The food was cut into smaller pieces (pellets) before being given to the mice. By the end of 21 days, the body weight of the obese mice group compared with the normal mice group.

#### Determination of organs and fat components weight

Body weight of each group was recorded at everyday. At the end of induction period, mice was sacrificed and the organs were collected and isolated including liver, spleen, testicle, and kidney.

Not only determination of organs weight but also determine fat components involving perirenal fat, perianal fat, epididymal fat, and retroperitoneal fat from visceral adipose region to estimate the organs and fat components weight.

**Determination of hyperphagia response**

Determination of hyperphagia response was recorded accurately at everyday during induction period and were conducted at the metabolic cage. This measurement involving observation toward weight of daily food remnants begin at first week until the last week induction period.

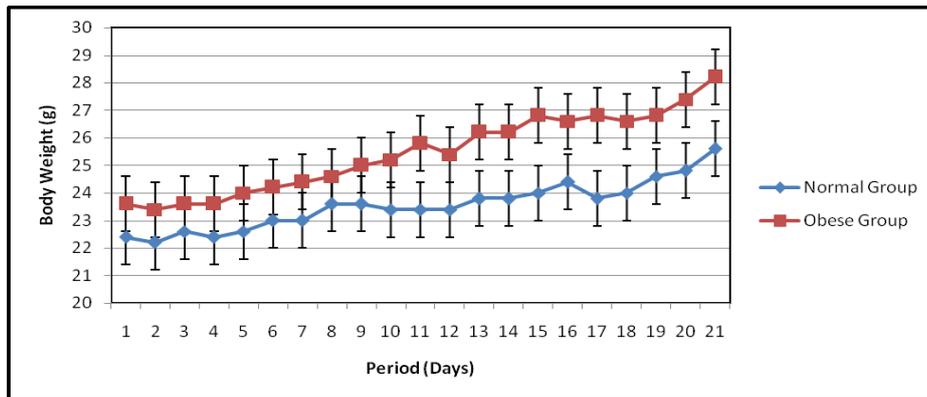
**RESULTS AND DISCUSSION**

**High-fat diet-induced obese mice model**

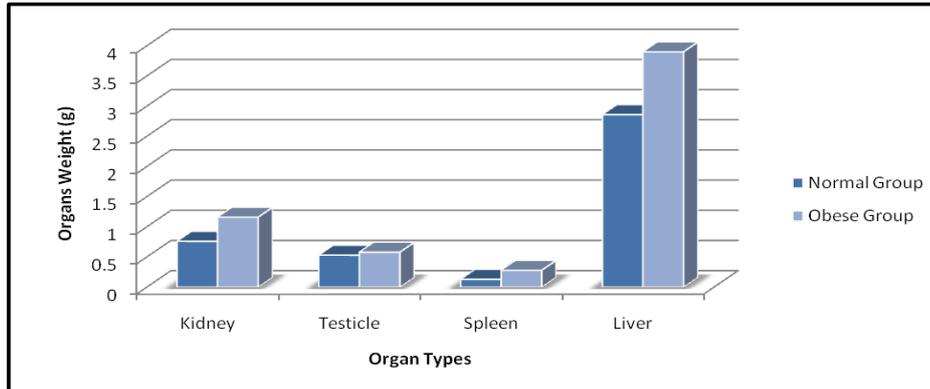
Based on the early studies of diet-induced obese rodent, researcher used cafeteria diet model. In this model, the type of food involving cookies, cheese products, and meat which has characterize strong palatable. These foods contains component sugar, salt, and fat. But, weakness of this model is didn't considering nutritive and non-

nutritive components. So, nowadays, approach to experimental animal model for obesity widely used is high-fat diet model.

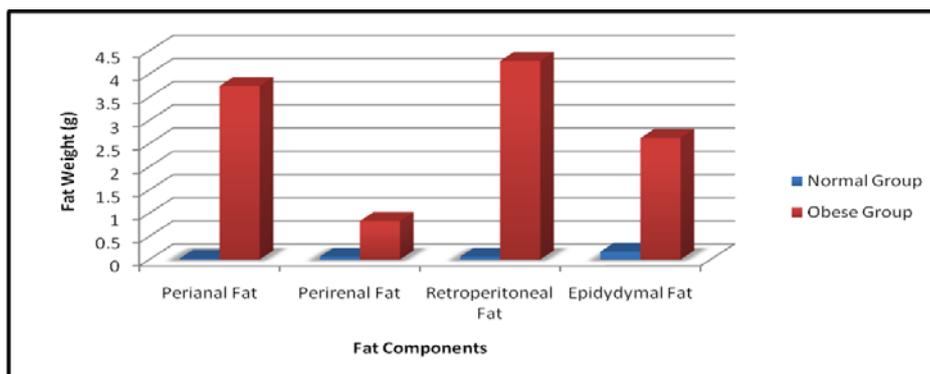
In this experiment, ingredients of the normal mice group diet is contains approximately 0% saturated fat mixture, 41% protein mixture, 55% carbohydrate mixture, and 4% polyunsaturated fat. While, the high-fat diet contains 32% saturated fat mixture, 41% protein mixture, 27% carbohydrate mixture, and didn't contain polyunsaturated fat. High-fat diet used in some laboratories generally contain about 32% to 60% of calories from fat component. Based on nutritional descriptive, a human diet about 60 kcal% fat is very risky. Its mean consumption of 60% kcal of fat can used to induce obesity in mice very quickly. In addition, researcher must be consider for choosing fat type. In high-fat diet induced obese mice, the type of fat were choiced is must contains more saturated fat such as lard or beef tallow [16].



**Fig. 1: Increased of body weight both normal mice group and obese mice group**



**Fig. 2: The difference of organs weight both the normal mice group and obese mice group**



**Fig. 3: The difference of fats weight both the normal mice group and obese mice group**

In addition, figure-2 was shown that occur increases of organs weight in obese mice group i.e liver, spleen, kidney, and testicle. Furthermore, not only increases of organs weight but also increases of fat components involving visceral adipose region like perirenal fat, retroperitoneal fat, and epididymal fat compared with normal mice group (figure-3). Based on figure-2 and figure-3, Fat accumulation might be not only occur on the visceral adipose region but also on the some important organs in mice. On the figure-2 exhibited that occur liver and spleen enlargement. Enlargement to liver and spleen was known as marker for pathologic condition on the animal body [18]. Moreover, fat accumulation on the visceral adipose region which involving perirenal fat, perianal fat, retroperitoneal fat, and epididymal fat can influence health level. Excessive fat accumulation stimulate released of adipocytokine from adipose tissue such tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL), resistin, and others, give effect on the dysregulation of lipid and glucose metabolism. Dysregulation of lipid and glucose

metabolism resulting lipotoxicity. The influence of lipotoxicity is change hormonal modulation, immune-system dysregulation, mitochondrial dysfunction, and oxidative stress. The final results in this mechanism was known as metabolic syndrome [7, 19].

#### Determination of hyperphagia response

Hyperphagia defined as condition which exhibited through food overconsumption and often occur to the obese animal model. In this study, determination of hyperphagia response was recorded accurately at everyday during induction period and were performed at the metabolic cage. Measurement focus is to observe weight of daily food remnants, begin at first week until the last week induction period. Based on figure-4 obese mice group show difference food pattern if compared with normal mice group. Obese mice group show ability to excessive feed than normal mice group. Its results, weight of food remnants were observed from day to day on the obese mice group more smaller than normal mice group.

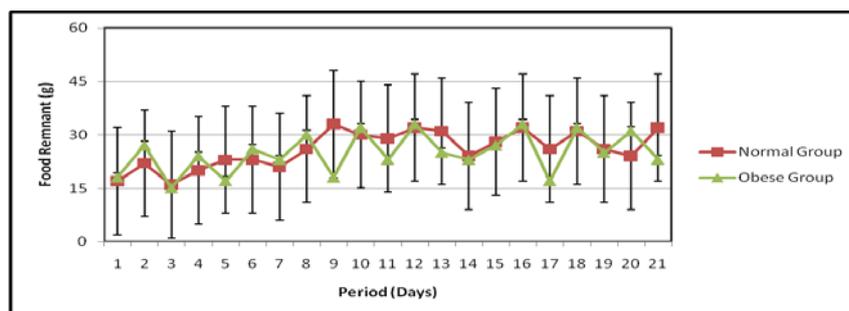


Fig. 4: The daily food remnants both the normal mice group and obese mice group

In addition, body weight is known as closely mechanism involving the balance of caloric intake and energy expenditure. Caloric intake were influenced by appetite factor. Its mean if occur increase of appetite would accompanied by increase of body weight. In the present study, over consumption of energy-rich diets (high-fat diet) cause increase of body weight and visceral fat mass. Recurrent high-fat diet administration and palatability of the high-fat diet will be affect "feeding pattern" to themice. In this case, obese mice have feeding pattern anomaly if compared with normal mice such have ability to excessive feed and uncontrolled was known as hyperphagia. Based on the experimental data, we concluded that high-fat diet (HFD) which developed in our laboratory can increase of body weight in a short time and we can explain that hyperphagia responderesulting increase of body weight and visceral fat mass.

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