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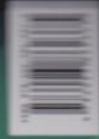
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# SURVIVAL RATE OF ANIMAL SUBJECT TO INTERVAL AND CONTINUOUS ADDITION OF HIGH CALORIE DIET

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## Abstract

High calorie diet increases the risk of diabetes mellitus. Nowadays, teens tend to consume high calorie diet. However, mechanisms of the effect of high calorie diet are still poorly understood, therefor animal model are needed to help to resolve it. This research was aimed to develop animal model that able to survive from continuous and interval administration of high calorie diet. Eight week of age of Balb/c mice were used as the subject. High calorie diet was dietary standard and an additional 8gram/kgBW and 14gram/kgBW of glucose solution via oral galvage, which were given continuously (7 times/week) and by interval of once, twice, and 3 times a week. Ad libitum 0.05gram/cc of glucose solution was given for all groups based on their continous or interval treatment. The subjects' survival rate were observed for 8 weeks. The survival rate of all the interval groups showed no significant difference with the control group. The group with continuous feeding of 14gram/kgBW showed significant difference with the control group ( $p=0.008$ ) and the survival rate was 16.7%. The group with continuous feeding of 8gr/kgBW showed no significant difference with the control group ( $p=0.468$ ), but it was prone to have worse survival rate (36.4%). This study revealed that survival rate of animal subjects with the interval addition of high calorie diet can be used as the animal model. As for continuous feeding, further investigation is needed to establish the appropriate dose for better survival rate.

Keywords: mice, glucose, survival

## Introduction

There are several factors, include internal (as an individual) and external, may become the predisposing factors of the diseases, for example, metabolic diseases such as diabetes mellitus (DM), heart disease, and obesity. One of the factor is the amount of the calorie intake of a person. Basu et al., (2013) and The American Heart Ascociation (2014) stated that 100-150 calories addition per person per day showed the increasing risk of diabetes and heart disease<sup>1,2</sup>. Another effect of high calorie intake is obesity. Other effect of high calorie intake is obesity. The incidence of obesity and diabetes mellitus, is increasing each year. In 2015, the incidence of overweight was estimated at 2.3 billion and obesity was 700 million<sup>3</sup>. Furthermore, the incidence of DM in 2013 was 382 million and it is estimated to be 592 million in 2035<sup>4</sup>.

Previously, ALFaris et al. (2015) mentioned that there was an increased evidence on the high consumption of fast food among Saudi girls<sup>5</sup>. A report released by WHO (2016) states the average consumption of the calorie in the world per person per day is 2940 Calories in 2015 and it is estimated in 2030 will rise to 3050 Calories<sup>6</sup>. In some countries, such as the developing countries, 2850 Calories will increase to 2980 Calories; East Asia 3190 Calories become 3060 Calories; South Asia 2700 Calories become 2900 Calories. In addition, according to the U.N.'s Food and Agriculture Organization (FAO), which was also released on the Cargill (2016) stated that during the past 50 years, daily per capita caloric intake globally has risen 27 percent in that timeframe<sup>7</sup>. This means the average of calorie intake increase two times fold globally.

Based on the phenomenon, it is important to understand the mechanism of the effect of high-calorie diet that can lead to several diseases. If the mechanism has established, the effort to prevent or to diminish the incidence of disease can be established. Concerning to that, the best way is doing researches on humans and observing their everyday dietary patterns. Unfortunately, this such a researches need a huge effort, because it will take a long time, and is expensive. In addition, the risk of the subject to drop out from such study is quite high. Therefore, it is proposed to use the animal model to conduct the experiment that relatively similar to human and much easier to control or change the dietary pattern.

The animal model or animal studies which are commonly used in Indonesia are white rat (Wistar or Spraguy) and white mice (Balb/C). However, there are some laboratory centers (but very rare) have C57BL mice. In developed countries, the variety

of the rats or mice have been more diverse but in a tight controlled. Unfortunately, it needs facilities and big funds.

This is a preliminary study for the advancement of animal models, using the material and method that is visible to be applied in Indonesia, to establish an animal model for high-calorie diet study.

## Material and Methode

The study was conducted in embryology laboratory, Faculty of Veterinary Medicine Universitas Airlangga, Surabaya. The mice was put in an individual cage and cleaned every 2-3 days. The subject of this study was 8-week old male Balb/C mice, from Exo Grimm breeding centre Surabaya.

Two weeks acclimatization period was conducted, therefor when the treatment started, the mice's age were about 10 weeks old. The mean of the mice's body weight was 18 grams. Eighty one mice, which was randomly divided into the control group, group 1 (8 kg/kg BW of oral galvage of glucose solution) and group 2 (14kg/kgBW of oral galvage of glucose solution).

All group were given standard feeding and ad libitum mineral water orally. But in group 1 and 2 were also added a glucose solution via oral galvage and ad libitum. Group 1 and grup 2, are divided into sub-groups continuous (7 times/week) and interval (once, twice, and 3 times/week), were given 0.5 cc glucose solution via oral galvage and ad libitum. While the control group were also given 0.5 cc mineral water via oral galvage.

Standard feeding was pellet made by the Faculty of Veterinary Medicine, Universitas Airlangga. Standard feeding composition was derived from fish powder (23%), soybean powder (6%), rice bran (10%), rice (31.5%), corn (20%), wheat flour (5%), mineral (2 %), sugar cane molasses (2%), and multivitamins (0.5%). The Calorie amount is 2732.61 Calories/kg.

The glucose solution was made from 100% dextrose monohydrate which was manufactured by Xingmao (China) and mineral water was used as the solvent. The concentration of glucose solution via oral galvage for group 1 (8gram/kgBW) was 0.3gram/cc and for group 2 (14gram/kgBW) was 0.5gram/cc. The volume for oral galvage was 0.5cc per mouse in both group. The concentration of ad libitum glucose solution was 0.05gram /cc.

Group 1 and group 2 was given glucose solution via oral galvage based on its sub-group. For the continuous sub-group, the oral galvage was given every day; for the once a week sub-group were given every Tuesday; for twice a week, every Tuesday and Friday; and for three times a week, every Tuesday, Friday, Sunday. The glucose solution is given about 0.5cc once a day in accordance with the days of sub-group continuous or interval. The ad libitum glucose solution, were set in similar days based on the continuous or interval sub-groups.

The survival rate of mice was observed for 8 weeks. Dead mice were observed and noted each week. At the end of the experiment, the data was collected in percentage number and then analyzed with the chi-square. The duration of this study was similar to Adeyi et al. (2012) which used the white bread diet as high glycaemic index diet in experimental animals (rats) to increase blood glucose levels above 200 mg/dl.

## Result

The worst survival rate in group 1 was the continuous sub-group, where there was only 36.4% mice were still alive and the best survival rate was twice a day intervals sub-group. In group 2, the survival rate of mice on continuous sub-group was poorer compared to the same sub-group in group 1, and the highest survival rate of mice in group 2 was three times a week interval sub-group. The data can be seen in the table 1.

From the chi square analysis, the life expectancy of group 1 and group 2 for all interval sub-groups showed no significant difference with the control group. Compared to the control group, the continuous sub-group from the group 1 showed no significant difference, but in group 2 showed a significant difference ( $p = 0.008$ ) and had the lowest life expectancy when compared to others from both group 1 group 2.

After 8 weeks of observation, the results showed that 33.3% mice of the control were dead after being treated for about two weeks. The mice were dead mostly in the first week, especially in group 1 which is continuous sub-group (71%) and the rest was died on the 2nd week. However, the dead mice also found the next week.

Tabel 1. The survival rate of subject

	Status	
	Life	Dead
Control group	66.7%	33.3%
Group 1 (8gram/kgBW)		
a. continue	36.4%	63.6%
b. interval 1x	50.0%	50.0%
c. interval 2x	75.0%	25.0%
d. interval 3x	62.5%	37.5%
Group 2 (14gram/kgBW)		
a. continue	16.7%	83.3%
b. interval 1x	37.5%	62.5%
c. interval 2x	77.8%	22.2%
d. interval 3x	87.5%	12.5%

## Discussion

In this study, the estimation of the calory amount of standard diet was 25 Calories per day. It is the same amount as the research conducted in Johns Hopkins University (2016)<sup>8</sup>.

The calculation of calory addition from glucose solution, are listed below:

$$\begin{aligned}
 &8 \text{ gram/kgBW (oral galvage)} \hat{=} 32 \text{ Calories/kgBW} \hat{=} 0.032 \text{ Calories/gBW} \\
 &14 \text{ gram/kgBW (oral galvage)} \hat{=} 56 \text{ Calories/kgBW} \hat{=} 0.056 \text{ Calories/gBW} \\
 &0.05 \text{ gram/cc} \hat{=} 0.2 \text{ Calories/cc}
 \end{aligned}$$

If it is assumed that the mice's body weight was 20 grams (0.02 kg), so the addition calories via oral galvage of group 1 was 0.64 Calories and group 2 was 1.12 Calories. It was also assumed that the mouse needs 6 cc for everyday ad libitum drinking<sup>9</sup>. So via ad libitum the addition of calories was 1.2 Calories.

The addition of calories of group 1 was 1.84 calories per day according to the day of its sub-group and the group 2 was 2.32 calori per day according to the day of its sub-group. So the total intake calories of group 1 was 26.84 Calories and group 2 was 27.32. For easiest calculation, the percentage of calories addition were 7.4% for group 1 and 9.3% for group 2.

The previous research by Andrikopoulos et al. (2008) which was conducted to achieve glucose intolerance model, the amount of total calories needed was 13,11-14,81 Calories per day from the CHO diet and fat diet<sup>10</sup>. Compared to our study, the number of calories is 2-fold higher. In another earlier study by Basu et al., (2013) mentioned that an additional of 100-150 Calories per day of sugar in humans can increase the risk of diabetes mellitus<sup>1</sup>. If the daily calories intake in human is 2000 Calories, therefore the percentage of the calory addition is approximately 5-7.5%.

Based on the comparison of the calorie addition, the dose of glucose to gain calory addition in this study is alike. The possibility of the dead animal can be caused by multiple organ failure that is likely due to hyperglycemia, especially in the sub-group which were given daily high calory of glucose solution (continuous sub-group). This condition also can be caused by, for example the heart disease. However, more calory addition (especially from sugar) is still needed to cause heart disease. AHA (2014) stated that the risk of heart disease will increase if the sugar addition is 17-21%.

In contrast to the continuous sub-group, the interval sub-groups is relatively have better survival rate. The interval calory addition is better be given 2-3 times per week. This interval administration of additional calory may trigger the mechanisms of animal adaptation to the stressor which is high glucose level. However, further investigation are still needed to understand the mechanism of the adaptation. Futhermore, animal model for continuous high-calorie diet, should be more explored to get a better survival rate, i.e by lowering the dose of the glucose, therefor it can be used for the next research.

## Conclusions

Our finding showed that the survival rate of animal subjects with the interval addition of high calory diet of 8 gram/kgBW and 14 gram/kgBW glucose solution via oral galvage and 0.05gram/cc ad libitum drinking can be used as the animal model for high calory diet. However, for continuous feeding purposes, further investigation is needed to establish the appropriate dose for better survival rate.

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