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The Analysis of Tempe Consumption Effects to Obesity in Adolescents through DNA Methylation

Sukojo Suwandani
Environmental Science Program, Brawijaya University, Jalan Veteran, Malang 65145, Indonesia

Edi Widjajanto, M. Rasjad Indra, Sri Andarini,
Medical Science Program, University of Brawijaya

Abstract
Obesity is a health problem worldwide, even WHO states that obesity is already a global epidemic, so obesity is a health problem that must be addressed immediately. Obesity is a nutritional problem with an increasing prevalence and sufferers not only individuals aged but also school-age children and children aged under five. This study has the objectives of 1) Knowing the level of consumption of tempe in adolescents, 2) Knowing the influence of tempe consumption on obesity in adolescents through DNA methylation.This obesity study was taken from obese population in the 2nd grade of Kediri Junior High School, while the normal weight sample was equal to the number of Obesity samples and taken from the same grade students by Simple Random Sampling. The results of the laboratory analysis of this study used primary data to amplify the leptin promoter gene region. Based on the analysis results, there is no change in the methylation area. There are samples that have mutations or even large amounts of nucleotide changes in areas that are not predictive sites of mutilation. This study has the following conclusions: 1) The samples that have mutations or even large amounts of nucleotide changes in areas that are not methylated prediction sites, 2) Changes in this promoter area are likely to affect gene expression, 3) In the sample Obesity 100% mutation, while the sample Non Obesitas 50% have mutations, 4) After re-checking, these mutations or nucleotide changes in some samples occur in areas that are transcription factors associated with leptin production and obesity conditions, 5) The teens of Junior High School in Kediri is not found methylation in genes related to leptin production, but mutation is found, 6) In children, obesity has 2x mutation is greater than non obesity child.
Keywords: Obesity, DNA methylation, mutation, Simple Random Sampling.

1. Introduction

Obesity is a weight condition that exceeds the normal value of body fat accumulation. Obesity handling failure occurs globally, although medical and non-medical efforts are increasingly advanced and intensive, as evidenced by the prevalence that does not decrease even increasing from year to year. Obesity is a more nutritional problem with an increasing prevalence and sufferers not only individuals aged but also school-age children and children aged under five. Current lifestyles that tend to be unbalanced between food intake and physical activity lead to the occurrence of this incident (Hertoghe.T, 2008).

The occurrence of obesity is caused by many factors, including diet, lifestyle, culture, genetics, and the environment. Fast food, equipment that simplifies work, and inactive work habits can make the body become fat. The trend of modern society with a lifestyle of stress, competition, good food and excessive, lack of exercise time, triggers the buildup of body fat. In the emerging development hypothesis that obesity could be due to nutrients of growth, development of exposure to environmental chemicals or nutritional interactions and exposure to environmental chemistry during growth. Obesity is one of the many diseases that prove to be of growth (Heindel, 2009). Basic Health Research Indonesia in 2007 metioned there are 19.1 percent of obese cases in people aged over 15 years. This figure exceeds the number of malnutrition in children under five years of 18.4 percent.

Health problems in childhood and adolescence have a major impact on later life, including obesity. Obesity is classified as a chronic disease with all the consequences caused by chronic inflammation. Improvements during childhood and adolescence will provide more benefits than similar efforts in adulthood.

2. Research Question

The problem of this research is focused on several questions, namely: 1. Does tempe cause DNA methylation? 2. What is the level of obesogen Phthalate in urine in adolescents? 3. Does obesogen phthalate cause DNA demethylation? 4. Does obesogen phthalate affect increasing obesity in adolescents? 5. Does tempe reduce levels of obesogen phthalate?

3. Literature Review

Basically the environment within the scope of human beings play a very important role in the human life aspect, it should be noted that a good environment means to indicate that the health is also good, and also indicate the good nutrition. Factors that affect it are also many of the most frequent is from food. Several studies have shown

that dietary factors may prevent agouti genes from activating, thus giving a protective effect. In particular, supplementing the diet of mothers with methyl substitute substances, such as folic acid, vitamin B12, betaine and choline showed a role against the decline in DNA methylation caused by bisphenol A. In addition, the constituents of soy products called genistein prevent the increase in the number of offspring that are not healthy. Whether the same diet might reverse the epigenetic effects after they appear, is unknown and await further experimental tests. However, the epigenetic mechanism clearly demonstrates how much environmental influence has on gene expression and phenotype.

Methylation is a common event occurring in the human genome, and is a very malleable effect that occurs during periods of growth but can also occur in childhood and even in adulthood. Because many infants receive soy milk, genistein in human should be assessed carefully. Pregnant women who are exposed to hundreds of compounds in food, prenatal and environmental vitamins that are potentially vulnerable to gene methylation. The effects of each compound can be beneficial or detrimental, depending on the exposure time, dose and tissue exposed.

A number of environmental triggers have been shown to affect the epigenome behavior of the organism, there is a change in the methylation balance thus affecting the gene in an "on" or "off" position. One of the triggers suspected of being a chemical found in many plastic bottles, including baby bottles, is bisphenol A. The hypothesis states that bisphenol A alters the effects of organisms' epigenomes by removing methyl groups from DNA (Dolinoy et al., 2007).

The nutritional composition of tempe in both protein, fat, and carbohydrate levels has not changed much compared to soybeans. However, due to the presence of digestive enzymes produced by tempe, the proteins, fats, and carbohydrates in tempe become more easily digested in the body than those found in soybeans. Therefore, tempe is very good to give to all age groups (from infants to the elderly), so it can be called as food of all ages.
The obesity is influenced by many factors, in the scheme is divided into 4 groups: 1) Food
2) Pattern activity
3) Environment
4) Genetics and Health

4. Methods

Food affects genetics through epigenetics, and health through illness and hormonal. The environment affects food primarily through quality, and affects genetics through epigenetics and health through disease and hormonal. The research design used was analytic observational research with case control approach. Target population is adolescent of obesity of junior high school age, age range of 15-18 years old.

In this study, the sample set for obesity which is taken from the obese population in the 2nd grade of all Junior High School Kediri (8 schools). The normal weight sample is equal to the number of obesity samples and taken from the same class students by Simple Random Sampling. Initial and final data processing is processed with SPSS 15.0 for Windows program, with statistical test (Sugiono, 2004). DNA methylation level was measured by T and Qi square test.

5. Findings

The Effect of Tempe Consumption on Obesity in Adolescents Through DNA Methylation

Kurniawati et al (2013) in her paper entitled "The Potential of Soybean Tempe As Diet Obesity Therapy Based on Local Wisdom" mentioned that some traditional food ingredients in Indonesia are known to have low glycemic index, such as tempe as the main product of soybean. Several studies had also known the use of soybean tempe as a low-glycemic index, low in saturated fat, cholesterol-free, digestible, a major source of minerals, antibiotic effects and growth stimulation, chemical-free toxic, and relatively affordable in terms of financing. The positive effect is mainly related to cardiovascular problems, as stated by the AHA (American Heart Association) that consuming soy protein containing isoflavones is recommended for high-risk populations, such as elevated total cholesterol and LDL cholesterol. The US Food and Drug Association (FDA) had agreed that adding 25 grams of soy protein a day to a diet low in saturated and low cholesterol reduces obesity. Medical nutrition therapy in the form of low-calorie glycemic index with the addition of traditional soybean meal can provide clinical benefit in order to prevent or delay the incidence of complications or risk factors in adult obesity.

The results of the laboratory analysis of this study used primary data to amplify the leptin promoter gene region consisting of:
- Primary Forward: GGA TTT CTC GCT CCT ACC AG
- Reverse primary: GCT CCC GGT AAC CTT CTA TC

Methylation checking had been done by using direct sequencing method. The distinction between methylated and non-methylated areas can be seen from the sequencing histogram produced after the bisulfite modification method.

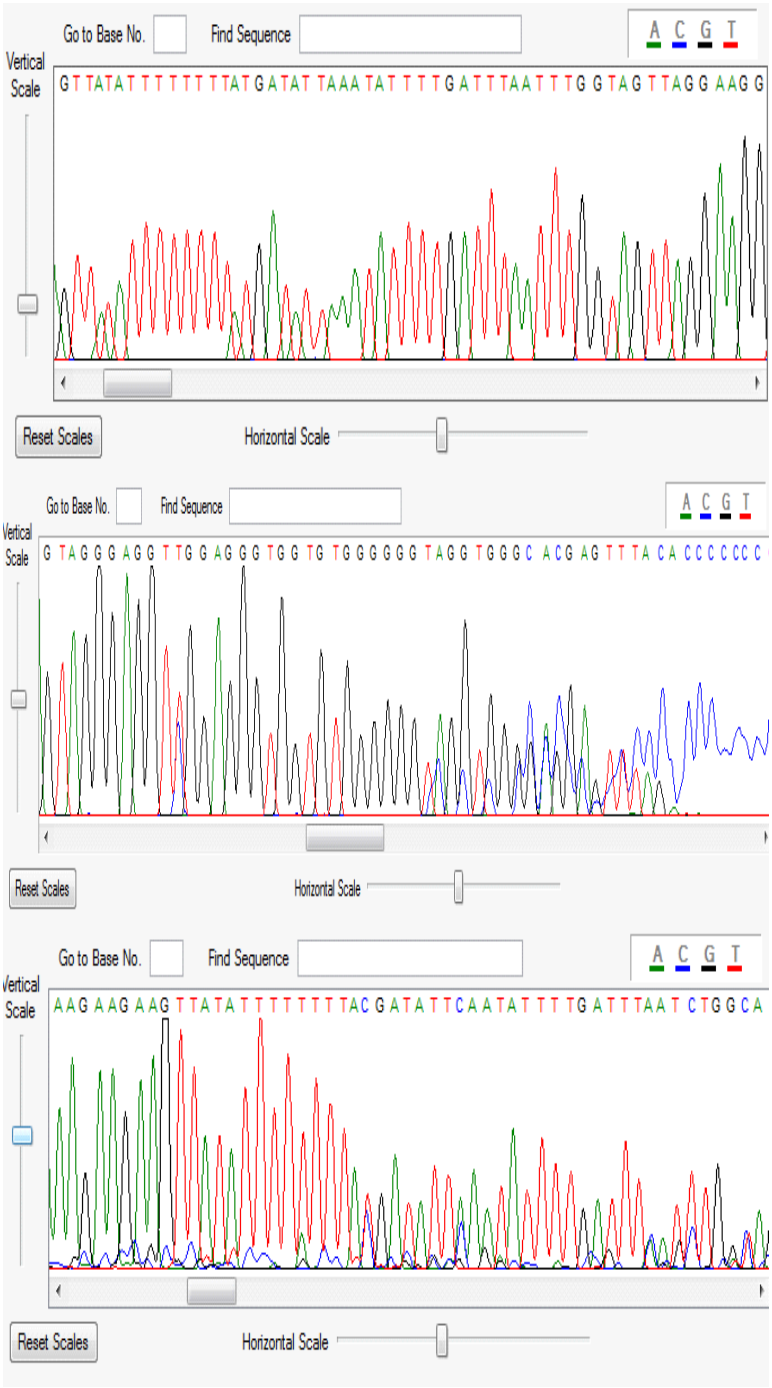

Figure 1.The area that experience methylation are indicated by C or T nucleotides which have double peaks (Figure 1 A and B), while the demethylated areas are indicated by nucleotides T (Figure 1C)

Based on the analysis results, there is no change in the methylation area. There are samples that have mutations or even large amounts of nucleotide changes in areas that are not predictive sites of mutilation. Changes in this area of the promoter are likely to affect gene expression.

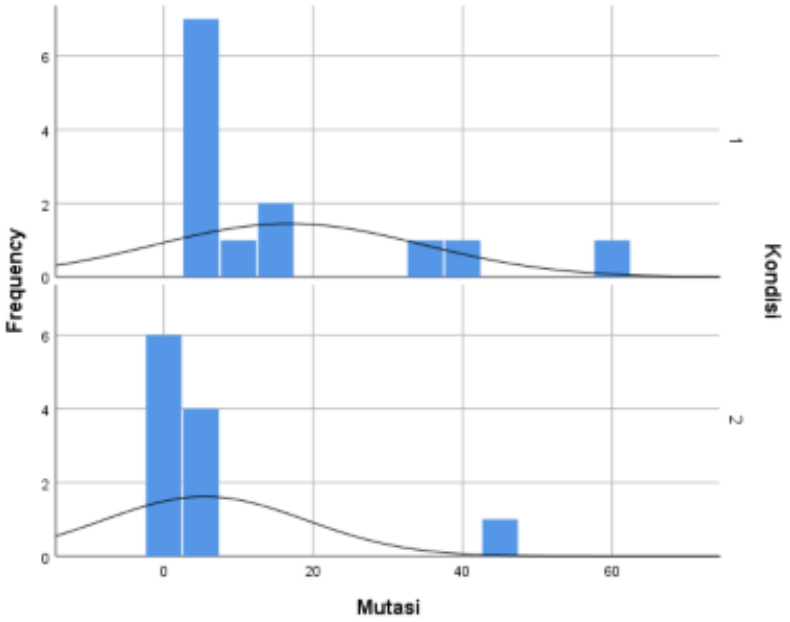

Figure 2. The curve show that there is an influence between the condition of obesity and non obesity with mutation. If the mutation is higher, the obesity is higher, and vice versa

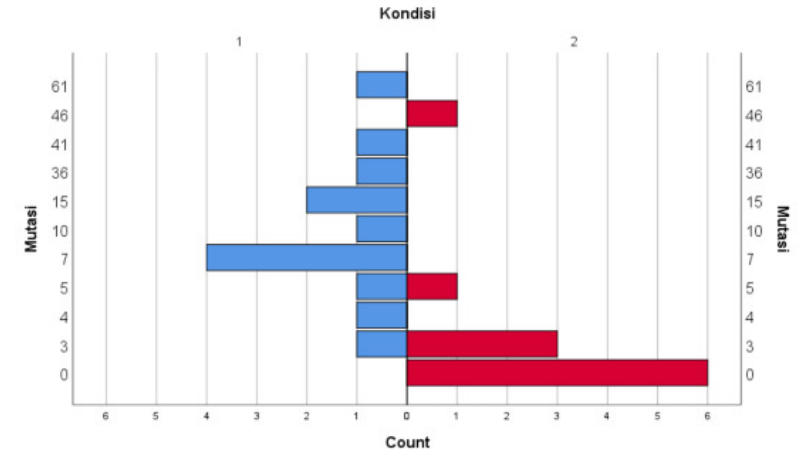

Figure 3. The graphs show that obese children are all mutations, namely as many as 13 children or 100%. Whereas for non obese children, there were 6 children who did not have mutations or as much as 55%, while the remaining mutations were as many as 5 children or 45%

6. Conclusion

This study has the following conclusions: 1) The samples that have mutations or even large amounts of nucleotide changes in areas that are not methylated prediction sites, 2) Changes in this promoter area are likely to affect gene expression, 3) In the sample Obesity 100% mutation, while the sample Non Obesitas 50% have mutations, 4) After re-checking, these mutations or nucleotide changes in some samples occur in areas that are transcription factors associated with leptin production and obesity conditions, 5) The teens of Junior High School in Kediri is not found methylation in genes related to leptin production, but mutation is found, 6) In children, obesity has 2x mutation is greater than non obesity child.

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