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Effects of Functional Breakfast Product on Oxidative Stress in Overweight/Obese Students

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Abstract

Adolescent is an aged group which was prone to the development of obesity. Recent evidence approved that obesity is associated with oxidative stress. Several researches showed that high-in-antioxidant diet could control the oxidative stress. This study was intended to analyze the effect of functional breakfast product (FBP) on the markers of oxidative stress in overweight/obese students. This experimental research used quasi-experimental design and involved 40 subjects, consisting of overweight/obese (BMI ≥ 25 kg /m^2) and normal (BMI=18.5-24.9 kg/m^2) subjects (20 subjects in each group). All subjects obtained FBP (160 g) every day for 21 days. FBP was made from orange potato sweet, red bean, tempeh, carrot, and pumpkin. It contained 429 kcal energy, 13.47 g protein, 38.54 mg/100 g ascorbic acid equivalent antioxidant capacity (AEAC), and 4.02 mg β-carotene per serving size. The results showed that the intervention using a FBP significantly reduced serum malondialdehyde (MDA) levels while superoxide dismutase (SOD) activity was significantly increased in overweight/obese subjects ($p<0.05$). After the intervention period of FBP, serum β-carotene levels tended to increase. In conclusion, the intervention using a FBP could improve the markers of oxidative stress in overweight/obese adolescent subjects.

Keywords: functional breakfast product; overweight/obese; oxidative stress.

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1. Introduction

Adolescent is one of the most important stages in human life cycle. It is a transition period from childhood to adulthood, the final stage of growth process. Common nutritional problems in this period are obesity, chronic malnutrition, anemia, and other micronutrient deficiencies [1]. Obesity is one of the main problems and its prevalence is increasing in developed and developing countries. It is characterized by an increase in body weight due to excessive fat accumulation [2]. This problem is not only increased in adults but also in children and adolescents [3]. Adolescence is a susceptible age group for the development of obesity [4]. Obesity is defined as body mass index (BMI) $\geq$ 27 kg/m^2 while overweight is defined as having BMI between $\geq$ 25 and < 27 kg/m^2 [5]. Based on the data from Basic Health Research (Riskesdas) in 2013, obesity prevalence in adolescents aged 16-18 years was 7.3%. This prevalence has increased than Riskesdas' data in 2007 (1.4%). On the other hand, the prevalence in age > 18 years was 28.7% [5].

Obesity is a complex problem, which among others is related to the quality of the food consumed, unhealthy dietary pattern, lack of physical activity, as well as genetic, hormonal and environmental factors. Frequent breakfast skipping is an example of unhealthy dietary pattern. Thirty percent of all obesity cases occurs in adolescence will continue into adulthood and become a persistent obesity. The condition in adulthood is determined mostly by nutrition and health conditions in adolescence [6].

Obesity is usually followed by an increase in fat metabolism which then leads to an increase in the production of reactive oxygen species (ROS) in blood circulation and adipose cells. Increased ROS in adipose cells can lead to disturbances in the balance of reduction-oxidation reaction, resulting in a decrease in antioxidant enzyme; that is, superoxide dismutase (SOD) enzyme, in blood circulation. The imbalance between oxidants and antioxidants is referred to as oxidative stress. Indicator that can be used to identify the presence of lipid peroxidation and oxidative stress is malondialdehyde (MDA) levels. MDA, a substance with light molecular weight, is produced as the end-product of lipid peroxidation in the body due to the presence of free radical reactions [7,8].

Oxidative stress condition which continues over time can lead to systemic oxidative stress. Systemic oxidative stress may become an entrance or risk factor for the onset of degenerative diseases, such as coronary heart disease, cancer, hypertension, diabetes mellitus, and other metabolic disorders; thus, this condition needs to be addressed. Efforts that can be done to reduce the negative effect of obesity and oxidative stress are regular breakfast and consumption of food rich in antioxidants. Based on several studies, it has been confirmed that consumption of foods which contain antioxidants may inhibit lipid peroxidation and increase antioxidant enzyme levels [9]. Carotenoid is one of antioxidant components commonly found in plant foods, such as pumpkin, carrot, sweet potatoes, tomatoes, broccoli, spinach, apricots and other plant foods [6,8].

Therefore, functional breakfast product (FBP) has been developed. It was made from local plant foods which contained nutrients required by the body and substances that had the potential as an antioxidant. This functional food intervention was expected to decrease the MDA levels, increase SOD activity and β-carotene levels in overweight/obese students. The aim of this study was to analyze the effect of FBP intervention on oxidative stress markers (MDA, SOD, and β-carotene) in overweight/ obese students.

2. Methods

2.1. Design, Location, and Time of Study

This research was an experimental study; that was, giving FBP to the subjects. It used quasi-experimental design and involved two groups of subjects as follows: (1) subjects with BMI ≥ 25 kg/m^2 (overweight/obese), referred to as overweight/obese group; (2) subjects with normal BMI (18.5-24.9 kg/m^2), referred to as normal group. FBP was made in Chemistry and Food Analysis Laboratory, Department of Community Nutrition, Bogor Agricultural University. Analysis of oxidative stress markers was performed in Laboratory of Biochemistry and Molecular Biology in Faculty of Medicine, University of Indonesia and SEAMEO-REFCON Laboratory. Intervention was conducted in the dormitory of joint preparation level (TPB) students in Bogor Agricultural University from November 2014 to March 2015.

2.2. Materials and Instruments of Study

Material used in this study was FBP prepared by the researchers based on the results of previous studies regarding FBP development based on local food to control oxidative stress overweight/obese adolescents. The composition of this FBP was as follows: orange sweet potato (30.36%), red bean (28.57%), tempeh (8.93%), carrot (14.29%), pumpkin (12.50%), white sugar (3.57%), and cornstarch (1.79%). This product had brownish yellow color, distinctive aroma of a blend of sweet potato and pumpkin, sweet flavor, and soft texture in the mouth.

FBP had 35.80% w/w water content. Energy content in one portion of FBP was 429 kcal, estimated from the sum of 4 (protein content) + 9 (fat content) + 4 (carbohydrate content) while the protein content was 13.47 g. Energy and protein requirements for adolescents (aged 17-19 years) are 2125-2675 kcal and 59-66 g a day, respectively [10]. The contribution of breakfast is 20% of total requirement, namely 425-535 kcal energy and 11-13 g protein [11].

Beta-carotene contained in one serving size was 4.02 mg, which was consistent with the previous study [12], while the antioxidant activity was 38.54 mg/100 g ascorbic acid equivalent antioxidant capacity (AEAC). Total number of microbes (total plate count) in the product was $9.6x10^2$ colonies/g, still below the threshold ($1x10^4$ colonies/g) for similar products [13].

Instruments used during intervention were microtoise, weight scale, FBP distribution tools, and tools for blood sample collection and analysis of serum MDA, SOD, and β-carotene levels of the subjects.

2.3. Research Subjects

The research subjects were overweight/obese male/female TPB students of Bogor Agricultural University and male/female students with normal nutritional status for control group. The inclusion criteria were as follows: not taking supplements and not suffered from chronic diseases, heart disease, liver disease, or sleep apnea (pauses in breathing).

The number of subjects was determined by the following formula: $\mathbf{n \geq 2\ (\sigma\ /\delta)^2\ (Z_{1-\alpha} + Z_{1-\beta})^2,}$ statistical power was 90%, σ and δ values were assumed based on the previous study [12]). Based on the calculations, minimum number of subjects for each treatment group was 18 people. Two people were added to anticipate the possibility of failure (loss to follow up). Total subjects in this study were 40 people.

2.4. Stages of Study

The study began with the selection of subjects performed through the following stages. In the first stage, one male dormitory and one female dormitory buildings with the highest number of boarders were selected. In the second stage, weight and height measurements for male and female students living in the selected buildings were conducted. These measurements aimed to select candidates for research subjects who met the inclusion criteria. Based on these, there were 45 candidates for overweight/obese group and 75 candidates for normal group. In the third stage, the selection of subjects from the candidates was done randomly and then 20 subjects for each group were acquired. They were then given an explanation about the research and requested to sign the informed consent. Interview using questionnaires, weight and height measurements, blood sample collection, and FBP administration were then carried out on the subjects.

FBP used in this study was prepared by the researchers. In summary, the products were produced through the following stages: selection of raw ingredients, steaming, mixing, molding, baking, cooling, cutting, packaging, and distribution. The products were distributed every morning before breakfast and administered for 21 days.

2.5. Data Type and Collection Method

The data collected in this study included characteristics of the subjects which consisted of age, gender, weight, height, physical activity, and subjects' nutritional knowledge. Subject compliance data in consuming the product were collected during FBP administration. Data of oxidative stress markers included serum MDA, SOD, and β-carotene levels of the subjects.

The characteristics of the subjects were collected prior to the intervention. Age, gender, nutritional knowledge, and physical activity were collected through interview using questionnaires. Weight and height were collected through measurement. Weight was measured by digital scale with 0.1 kg accuracy and a capacity of 200 kg while height was measured by microtoise with 0.1 cm accuracy and a capacity of 200 cm.

Subjects' compliance in consuming FBP were obtained through observation and interview with monitoring form related to the compliance of food consumption. Blood samples of the subjects were collected to determine serum MDA levels, SOD activity, and serum β-carotene levels. Blood sample were taken after a 10-hour over night fast, collected two times, before and after the intervention. MDA were measure by thiobarbituric acid reactive substances assay (TBARS) method. The absorbance of the resultant pink product was measured by spectrophotometer at 530 nm [14]. SOD activity was measured by the degree of inhibition of reaction xanthine and xanthine oxidase were generated superoxide radicals with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye [15]. Beta-carotene was measured using high performance liquid chromatography (HPLC). The content of β-carotene in the serum determinated by HPLC

detector UV at wavelength of 450 nm [16].

2.6. Data Processing and Analysis

The data were processed and analyzed using data processing programs, namely 2007 Microsoft Excel and SPSS version 18. Physical activity were processed by converting them into physical activity level (PAL). Subjects' nutritional knowledge were processed by giving a score of 100 to the subjects who answered 15 questions correctly. Weight and height data were processed to acquire body mass index (BMI) using the ratio of weight (kg) by the square of height (m^2).

Independent sample t-test was performed on mean age, BMI, physical activity, and nutritional knowledge of the subjects (overweight/obese and normal groups). Mann-Whitney test was performed on mean reduction in serum MDA levels and mean increase of SOD and β-carotene between the two groups.

2.7. Ethical Considerations

Ethical clearance for this study was approved by Health Research Ethic Committee of the Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo General Hospital, Jakarta. It was obtained in the form of Ethical Approval Number: 795/UN2.F1/ETIK/2014 dated November 17, 2014.

3. Results and Discussion

3.1. Characteristics of Subjects and Their Families

Characteristics of the subjects were presented in Table 1. A total of 40 subjects participated in this study, 20 subjects in overweight/obese group consisted of 10 males and 10 females and 20 subjects in normal group consisted of 8 males and 12 females.

Table 1: Characteristics of subjects by the subjects' group

Characteristics	Overweight/obese group (n=20)	Normal group (n=20)	p-value
Age (years)	17.90 ± 0.45 [a)]	18.10 ± 0.55 [a)]	0.461
Body mass index (kg/m^2)	30.30 ± 3.61 [a)]	21.43 ± 1.46 [b)]	0.000
Physical activity (PAL)	1.59 ± 0.10 [a)]	1.56 ± 0.12 [a)]	0.539
Nutritional knowledge (score)	94.67 ± 4.64 [a)]	91.33 ± 7.25 [a)]	0.100

Note: The numbers followed by different letters in the same row showed significant difference between groups ($p<0.05$)

The age, physical activity, and nutritional knowledge of the overweight/obese subjects were not significantly different ($p>0.05$) from the normal subjects while BMI of overweight/obese subjects was significantly higher ($p<0.01$) than the normal subjects (Table 1). Obesity occurs due to excessive fat accumulation as a consequence

of positive energy balance; that is, the amount of energy intake is greater than energy expenditure. This happens because of the interaction of several factors, including dietary pattern; that is, increased intake of energy-dense foods and decreased intake of foods rich in micronutrients, physical activity, as well as genetic, environmental, cultural, and economic factors [8].

3.2. Effects of Functional Breakfast Food Provision on Serum Malondialdehyde (MDA), Superoxide dismutase (SOD), and β-carotene Levels

Mean percentage of compliance rate in overweight group related to FBP consumption for 21 days was 90.0%±4.64, not significantly different ($p>0.05$) from the normal group (89.0%±4.17). The effect of FBP intervention on oxidative stress in overweight/obese TPB students was identified by its markers (serum MDA, SOD, and β-carotene levels).

Oxidative stress is an imbalance between free radical production and antioxidant defenses. MDA is a substance with light molecular weight produced as an end-product of lipid peroxidation in the body due to the presence of free radical reactions [17]. Oxidative stress is indicated by the low status of antioxidants, SOD is a marker for primary antioxidants while β-carotene is a marker for secondary antioxidants [18]. Mean values of serum MDA levels of the subjects was presented in Table 2.

Table 2: Mean values of MDA serum levels by the subjects' group

Subjects' group	MDA serum levels (nmol/mL)		
	Before intervention	After intervention	Difference
Overweight/obese group	2.70 ± 0.61	1.73 ± 0.50	-0.97 ± 0.52 [a)]
Normal group	1.41 ± 0.28	1.36 ± 0.31	-0.05 ± 0.17 [b)]

Note: The numbers followed by different letters in the same column showed significant difference between groups ($p<0.05$)

Based on Table 2, it could be seen that mean value of MDA serum levels before the intervention in the overweight/obese group was higher than the one in the normal group. This finding was consistent with the results from the previous study in which obese subjects had higher mean value of MDA levels (2.00±0.77) than non-obese subjects (1.63±0.14) [19]. A review from several studies on obesity-induced oxidative stress concluded that MDA was involved in systemic oxidative stress and impaired glucose metabolism in obese people [20]. Chronic hyperglycemia in obese people leads to a continuous increase in MDA formation.

The results of statistical analyses showed that the decreased serum MDA levels in overweight/obese group (-0.97 ± 0.52) was significantly higher ($p<0.05$) than the normal group. This result showed that FBP intervention had a significant effect in decreasing the serum MDA levels in overweight/obese students. Antioxidant content, especially β-carotene, found in FBP may have a role in counteracting free radicals by breaking the chain reaction of free radicals. β-carotene is a great free-radical trapper; therefore, it can provide protection against lipid peroxidation. Antioxidant is a molecule that can prevent the negative effects of oxidation and protect cells

and tissue damaging from free radicals [21]. Previous study revealed that a diet rich in antioxidants could decrease MDA levels [22].

Antioxidant status can represent the inhibition of free radicals by antioxidants in sample tissues, such as serum or plasma. Antioxidant defenses include enzymatic and non-enzymatic antioxidants. Mean values of serum antioxidant enzyme (SOD) levels by subjects' group were presented in Table 3.

Table 3: Mean values of SOD activity by subjects' group

Subjects' group	Serum superoxide dismutase levels (U/L)		
	Before intervention	After intervention	Difference
Overweight/obese group	0.43 ± 0.14	1.09 ± 0.79	0.67 ± 0.77 [a)]
Normal group	0.61 ± 0.48	1.10 ± 0.55	0.49 ± 0.79 [b)]

Note: The numbers followed by different letters in the same column showed significant difference between groups ($p<0.05$)

The mean value of SOD enzyme activity in the serums of the subjects in overweight/obese group before intervention was lower (0.43±0.14) than the normal group (0.61±0.48) (Table 3). The low activity of SOD proved that oxidative stress occurred in the body in which antioxidant enzymes were not able to eliminate the amount of oxidants (free radicals). Previous study found that obese animal models appeared to have lower SOD activity by 29-42% than the controls [23]. Another study found that SOD activity in obese people was significantly lower than those with ideal body weight [24]. In this study, SOD activity in overweight group was 29% lower than the normal group. Previous study revealed that initial response of the antioxidant on free radicals in obesity was a decrease in SOD activity [25].

Statistically showed that the increased SOD activity in overweight/obese group (0.67±0.77) was significantly higher ($p<0.05$) than the normal group (0.49±0.79). This result showed that FBP intervention affected the increased SOD activity in overweight/obese students. Obesity-induced oxidative stress is a systemic problem that should be corrected by improving antioxidant defenses, including by diet modification i.e. diet rich in antioxidants [8,20,26].

Excessive production of free radicals in the body occured under the oxidative stress conditions; thus, decreasing the activity of antioxidant enzymes. Therefore, exogenous antioxidant intake is very important to help the activity of antioxidant enzymes [27]. Mean values of serum β-carotene levels by the subjects' group were presented in Table 4.

The mean value of serum β-carotene levels in overweight/obese group before intervention was lower than the normal group (Table 4). Previous review found that serum β-carotene in obese children was lower (0.22) than those with normal nutritional status (0.29). In obese adults, β-carotene levels were lower by 18-37% than those with normal weight. In this study, it was found that serum β-carotene in overweight/obese group was lower by 30% than the normal group.

Table 4: Mean values of serum β-carotene levels by the subjects' group

Subjects' group	β-carotene serum levels (µmol/L)		
	Before intervention	After intervention	Difference
Overweight/obese group	0.21 ± 0.13	0.32 ± 0.23	0.11 ± 0.15 [a)]
Normal group	0.30 ± 0.21	0.38 ± 0.25	0.08 ± 0.26 [a)]

Note: The numbers followed by different letters in the same column showed significant difference between groups ($p<0.05$)

Statistically showed that increased β-carotene levels in the overweight/obese group was not significantly different ($p>0.05$) than the normal group. However, the data in Table 4 showed that the increase in serum β-carotene levels in the overweight/obese group was likely to be higher than the normal group. Previous study found that serum β-carotene of the subjects increased after given soup with high β-carotene content [12].

A diet rich in antioxidants is one of the efforts to improve oxidative stress conditions in overweight/obese subjects. FBP which was content 4.02 mg β-carotene, given to the subjects has been proven to improve oxidative stress status in overweight/obese subjects through the significant decrease in MDA levels and an increase in SOD activity, despite the insignificant increase in β-carotene levels. It happened because β-carotene, as a component that had a capacity as an antioxidant contained in FBP, had fought against the free radicals so that it could reduce lipid peroxidation, resulting in the decreased levels of MDA. On the other hand, β-carotene acted as free-radical trapper; thus, helping the work of antioxidant enzyme, i.e. SOD [22]. However, this study has limitations, namely indeterminate include control group that did not get breakfast and control group got breakfast that does not contain antioxidant. In this study, used agroup of overweight/obese, due to the limited number of obese subjects, would be more appropriate in this group are all obese.

4. Conclusions and Recommendations

FBP intervention significantly decreased serum MDA levels in overweight/obese students. SOD activity increased significantly after FBP intervention on overweight/obese students. After the FBP intervention, serum β-carotene levels of the students tended to increase. Utilization of local food to produce functional food products should be encouraged so that it can contribute to the countermeasure of oxidative stress in overweight/obese adolescents in order to prevent the onset of degenerative diseases.

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