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Original research

Serum mineral (Mg, Mn, and K) levels are associated with increasing the body mass index (BMI) and abdominal circumference

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ABSTRACT

Aim: The study aimed to investigate the effect of serum mineral (Mg, Mn, and K) levels to obesity in rat model.
Methods: A posttest-only control group design was used in this research. This research tested the feed type in Sprague-Dawley rats. A total 36 rats were divided into two groups (18 rats for normal feeding intervention (N) and 18 rats for high fat-high fructose modified standard diet (HFFD) feeding intervention). The treatments were conducted for 12 weeks. Energy, nutrients and mineral content of modified feed were measured. Moreover, calorie intake, weight, body length, abdominal circumference, lee index, and serum mineral content of rat samples were measured.
Results: The results showed that weight gain was 11.94% higher in the HFFD group, and its caloric intake was significantly ($p = 0.000$) higher than N group. The abdominal ratios of the HFFD group were significantly higher than N group ($p = 0.009$) and the Mg, Mn and K serum levels of HFFD group were significantly lower than N group. There was a significant and negative correlation between increased BMI and abdominal circumference with decreased serum mineral levels.
Conlusions: This study showed that HFFD causes a decrease in serum mineral (Mg, Mn, and K) levels which is associated with the incidence of obesity. Therefore, HFFD food consumption should be avoided.

1. Introduction

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health. About 13% of the world's adult population were obese in 2016 (Baqai and Wilding, 2014; WHO, 2019). It is estimated that up to 57.8% of world's adult population will be overweight or obese by 2030. In Indonesia, the prevalence of obesity in age population > 18 years old were 28.9% in 2013 (Indonesian Statistical Center, 2019). Riskesdas of Indonesia study in 2007, 2010 and 2013 showed a significant increase in the obesity prevalence. Similarly, the results of IFLS study in 1993, 1997, 2000 and 2007, showed that the number of overweight people increased dramatically, although number of underweight people was also decreasing both in men and women. Obesity is a predisposing factor for non-communicable diseases (NCD), a term for chronic diseases including diabetes mellitus, hypertension, heart disease, stroke, musculoskeletal disease and some cancers, and also has a significant economic impact.

Diet is an environmental factor that greatly contributes to the onset of obesity, for which the main cause is excessively eating calorie-dense foods. High fat diets will increase total energy intake and increase the likelihood of obesity as well as various systemic disorders associated with obesity. Increasing consumption of low-quality foods such as sweets and junk food will also significantly increase the risk of obesity. Similarly, research also shows that with an increasing consumption of foods and beverages containing high fructose corn syrup (HFCS), the prevalence of obesity also increases. HFCS is widely used in a variety of food and beverage products such as soft drinks, pastries, cookies, gums, jelly, and desserts. The effects of fructose on the brain cause addiction effects and leptin resistance, so long-term consumption of fructose can increase caloric intake due to loss of satiety signals in the brain, in turn resulting in increase incidence of being overweight and obesity.

Thus, high fat–high fructose diets (HFFD) not only lead to weight

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gain and obesity, but some studies also show that low intake of minerals is associated with low serum mineral levels. High fat-high sugar foodstuffs that contain carbohydrates from grains and rice generally have a reduced mineral content (Albertson et al., 2016). In other side, several studies showed a significantly negative relationship between BMI and serum mineral content (Jose et al., 2012; Murakami et al., 2012; Shin et al., 2013; Suliburska et al., 2013; Zaakouk et al., 2016) but the mechanism of this still unclear. The purpose of this study was to investigate the effect of serum mineral (Mg, Mn, and K) levels to obesity in rat model.

2. Materials and methods

This research was conducted after obtaining ethical approval from the Medical Faculty, Universitas Brawijaya Malang, Indonesia, Number 368/EC/KEPK/10/2017. The research was conducted from October 2017 to February 2018.

2.1. Chemicals and feed ingredients

The chemicals used in this research were obtained from the Chemistry Mathematics and Natural Sciences Laboratory of Universitas Brawijaya Malang, and the Life Sciences Central Laboratory of Universitas Brawijaya Malang. Feed ingredients consisted of cornstarch, dextrin cornstarch, sucrose, soybean oil, casein, egg white, gelatin, white butter, cow kidney fat, minerals (AIN-93 M-MX-Mineral Mix), vitamins (AIN-93 -VX-Vitamin Mix), L-cystine, and choline bitartrate. The normal diet followed by AIN standart (Reeves et al., 1993).

Tools used in this study included feed-making equipment, experimental animal care, metleen, digital analytic scales, energy content measuring instrument with Bomb Calorimetry method, a fat content measuring instrument with gravimetric method, a protein content measuring instrument with an acid–base titration method, a mineral content measuring instrument with spectrophotometric (Genesys 10S, USA) and atomic absorption spectrophotometry AA600 (Shimadzu, Japan).

2.2. Experimental animals and treatment

This research used a post-test–only control group design. The number of replications in this study was determined based on the Federer formula, which for the experimental test was: (t-1) (n-1) ≥15, where t is the number of intervention groups and n is the number of repetitions or number of samples on each group. The number of interventions (t) is 2, namely normal diet (N) and high fat diet high fructose diet (HFFD). Thus, (2-1) (n-1) ≥15, n-1 ≥15, n ≥ 16, so the number of replications should at least be 16 animals. Then a backup of experimental unit was added to each group to anticipate any undesirable possibilities such as death. Correction of the number of replications was based on Higgins formula which is 1/(1-f), with an estimated experimental unit that is droup out (f) as much as 10%, then the number of replications needed was 1/(1–0.10) x 16 18 rats per group. Thus the number of animals needed in this study was 2 × 18 = 36 rats.

A total of 36 male Sprague-Dawley strain rats (*Rattus norvegicus*) were obtained from IPB (Bogor Agricultural Institute) Animal Laboratory under inclusion criteria, namely 200–250 g weight, aged 70–90 days, male, and healthy. The exclusion criteria were abnormal motor movements, rats did not want to eat and drink, or experienced a weight loss > 10% during the adaptation period.

Clean individual enclosures with husk pads with a thickness of about 2 cm were provided and the pads replaced every 3 days. Temperature was maintained at 25 °C with a 12 h light and dark cycle. Rats were adapted for 7 days and fed ad libitum with normal feed and distilled water. After the 7thday, 18 rats in the intervention group were given the HFFD and drank a 30% fructose solution for 12 weeks (Marquest et al., 2015). Maintenance and euthanasia of rats was in accordance with the fixed procedure at Bioscience Institute Universitas Brawijaya Malang.

2.3. Feed preparation of N and HFFD intervention

Modified feed was made in the Food Science Laboratory of Malang State Health Polytechnic. The ingredients listed above were weighed using an analytic scale, then mixed using a dough mixer for 30 min to which was added 150 cc of water per formula recipe, and further mixed for 10 min. The dough was then molded and placed on a baking sheet and dried in a drying oven at 60–70 °C for 12 h. Nutrient content of feed was determined as outlined below.

2.4. Analysis of energy, nutrients and mineral content of modified feed

Energy content analysis was conducted in the Central Laboratory of Biological Sciences, Universitas Brawijaya, Malang, based on the bomb calorimetry method. All energy and nutrient analysis methods were based on AOAC 2005.

About 1 g of raw rice and cooked rice were placed into a nickel cup and then placed in a terminal knot. A 10 cm long chromed wire was cut and then attached to the terminal knot. The wire was kept in contact with the sample to be burned. The cylinder bomb was prepared and 2 ml distilled water was added. Then, a sample of rice with a nickel plate was placed into the bomb cylinder and covered. O_2 gas was flowed into the bomb, up to a level of 30 atm. The bucket was filled with distilled water, and the water temperature was maintained at less than 1.5 °C below room temperature. After that, the bomb cylinder was placed into the bucket and the power cord was connected to the terminal knot line. The calorie bomb was closed and then the dynamo button (stirrer) was turned on for ± 2 min. At this point, the thermometer was checked and the temperature recorded (initial temperature or T_o). After this, the bomb button was pressed until the indicator light came on; the combustion process was allowed to continue until the temperature was constant, which was marked by the sound of the alarm. This process took 5 min. The dynamo button (stirrer) was then turned off, the bomb cover opened, and the cable removed from the terminal knot. The bomb cylinder was removed from the bucket, along with the combustion gas side.

The remaining ash from combustion was collected in a glass spray of distilled water, and then 2 drops of methyl orange were added (as an indicator). Titration was performed with standard Na_2CO_3 (0.0725 N) solution. The titration was positive if the color changes (yellow). The volume color (ml) of the standard solution used was recorded, and the Gross Energy of the sample was calculated with the following formula:

$$GE = \frac{\text{delta t x 2470} - \text{titration volume (ml)+wire heat (cal)}}{\text{Sample weight (g)}}$$

Macronutrient content, including fat, protein, carbohydrate, magnesium, manganese, and potassium, was analyzed in the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Indonesia. Fat content was analyzed by the gravimetric extraction method, proteins by acid-base titration, carbohydrates and manganese by spectrophotometric methods, and magnesium and potassium by atomic absorption spectrophotometry.

Protein analysis consisted of three stages: destruction, distillation, and titration. In total, 2 g of sample was put into a 50 ml Kjeldahl flask and then 7 g of K_2SO_4, 0.005 g of HgO, 15 mL of concentrated H_2SO_4, and 10 mL H_2O_2 were added slowly into the flask; the solutions were allowed to stand for 10 min in the acid chamber. The samples were destructed at 410 °C for about 2 h or until the liquid had changed to clear green. The Kjeldahl flask was washed with 50–75 mL of distilled water and then put into the distillation apparatus. The distillation product was accommodated in a 125 mL Erlenmeyer glass containing 25 ml of 4% boric acid (H_3BO_3) containing 0.1% bromocresol green and 0.1% methyl red at a 2:1 ratio. The distillation was carried out by

adding 50 ml of NaOH/$Na_2S_2O_3$ solution to the distillation apparatus, up to 100–150 mL of distillate in the Erlenmeyer glass, resulting in a green distillate. The distillate was titrated with 0.2 N HCl until the first pink change occurred. The titrated volume was read and recorded. The blank solution was analyzed in the same way as the sample.

Protein content was calculated using the following formula:

$$\% \text{ N} = \frac{(\text{mL HCl} - \text{mL blank}) \text{ x N HCl x } 14.\ 007 \text{ x } 100\%}{\text{mg sample}}$$

Fat content was analyzed by placing 5 g of sample (W1) in filter paper, with both ends of the wrapper covered with fat-free cotton, and was subsequently put into a fat cover, fed into a weighted fat gourd (W2) and connected to soxhlet tube. The fat cover was inserted into the soxhlet tube extractor chamber and doused with a hydrophobic solvent (benzene) and then refluxed for 6 h. The solvents present in the fat flask were distilled until all solvents had evaporated. At the time of distillation, the solvent was accommodated in the extractor chamber and removed so that it could not return to the fat flask. The fat flask was dried in an oven at 105 °C and then the flask was cooled in the desiccator until a constant weight was reached (W3). Fat content was calculated by:

$$\% \text{ fat content} = \frac{W_3 - W_2}{W_1} \times 100\%$$

where:

W1 = Sample weight (g)
W2 = The fat glass weight without fat (g)
W3 = The fat glass weight with fat (g).

For carbohydrate analysis, a total of 20–30 g of sample was added to 80% alcohol (1:1). The sample was blended to extract the sugar, before being removed into a glass beaker and filtered. The remaining solids were washed with 80% alcohol until all of the sugars had dissolved in the filtrate. The pH value of the sample was measured until it was neutral and then heated at 100 °C for 30 min. The solution after cooling to room temperature was then filtered with Whatman no. 2 filter paper. The alcohol was then removed by heating the filtrate in an 85 °C water bath or via a vacuum filter. When the resulting filtrate was clear, the volume of the solution was made up to a particular volume with water and then homogenized and prepared for carbohydrate content analysis using a spectrophotometric method at $\lambda = 630$ nm.

Fiber analysis was conducted at the Food Science and Nutrition Laboratory of Food Technology Faculty of Catholic University Widya Mandala Surabaya, and β-glucan analysis was conducted at the Center for Assessment and Application of Technology (BPPT) PUSPITEK Serpong, Indonesia, based on enzymatic methods. About 1 g of sample was placed into a 400 ml beaker before 50 ml of bufferospheric pH 6.0 was added. Next, 0.1 ml of termamyl was added, and the sample was covered with aluminum foil and placed in a boiling water bath at 95–100 °C for 15 min, with shaking every 5 min. A heating time was added if necessary (total time within water bath ± 30 min), after which the sample was cooled at room temperature and the pH was set to 7.5 ± 0.2 with the addition of 10 ml of 0.275 N NaOH solution. A total of 5 g of protease and 0.1 ml of enzyme solution were added. The solution was covered with aluminum foil and incubated for 30 min. Next, 10 ml of 0.325 M HCl solution was added and the pH was set to 4.0–4.6. Then, 0.3 mL of amyloglucosidase was added, and the solution was covered with aluminum foil and the sample incubated at 60 °C for 30 min with continuous agitation. Next, 280 ml 95% EtOH was added to the pan at 60 °C and the sample was precipitated at room temperature for 60 min. Solutions were strained with a device that had been treated with 0.1 mg Celite flattened with 78% EtOH. The residue was washed in a crucible containing 20 ml EtOH 78% (3×), 10 ml EtOH 95% (2×) and 10 ml acetone (1×). The residue was dried in a 105 °C oven to a constant weight. Fiber content was assessed by:

$$\% \text{ DF} = (\text{a} - \text{b})/\text{w x } 100\%$$

where:

DF = *dietary fiber*
a = constant weight of sample
b = ash weight
w = early sample weight.

For analyzing the feed mineral (magnesium, manganese and potassium, as well as silica) content, sample preparation was done by sample ashing. Dry ashing was done by weighing a number of pulverized samples, the weight noted, then heated the samples on a hot plate or burner on medium heat to evaporate as much organic matter as possible (until the sample was no longer smoky). Next the cup was moved into the furnace and heated to a temperature of 300 °C until all the carbon turned grey, then the temperature increased to 420 °C. Generally, ashing was carried out at 450 °C for 5–7 h. The ash produced was weighed, then the cup closed with a watch glass, to which slowly was added 40–50 ml of diluted HCl (1 + 1) using a pipette. Next, the cup was heated over a water bath for 30 min, the lid lifted and rinsed. Warming was continued for 30 min to dehydrate the silica. As much as 10 ml of HCl (1 + 1) and water was added to dissolve the salts and filtered using filter paper. The filtrate was then put into a 100 ml volumetric flask and diluted with distilled water until a 100 ml total volume was reached. The filter paper was returned to the cup, baked, and ashed in the furnace at 450 °C for 1 h, then cooled and weighed. This treatment provided an estimate of silica content in the sample. Magnesium, manganese, and potassium content in the ash solution was assessed using spectrophotometer or atomic absorption spectrophotometer at a wavelength of 285.2 nm, 515 nm, and 766.5 nm, respectively. Standard solutions for each analysis were also made to compare the absorbance results, to calculate the mineral content in the sample.

2.5. Sample collection and preparation

Preparation of blood serum samples for mineral content analysis was carried by collecting as much as ± 3 cc blood through the heart of rat by using syringes, then it was directly inserted into vacutainer. The obtained blood samples were left to stand for 30 min at room temperature. Then they were centrifuged for 10 min at 3000 rpm. The formed serum was separated from the deposits of blood cells by using a pipette.

2.6. Experimental parameters

2.6.1. Calorie intake

Feed intake was calculated by subtracting the amount of feed given (g) and the remaining feed (g) for 24 h. The same was done for liquid in the HFFD group. Caloric intake was calculated based on the amount of feed intake (g) and 30% fructose (mL) beverage multiplied by N and HFFD energy density. The 30% fructose solution contained 1.125 calories per ml.

2.6.2. Weight, body length, abdominal circumference, and lee index

Body weight was measured using digital analytic scales. Weight measurement were done every week. The results of body weight measurements were the mean of the two weighing results. Body length was measured shortly after the rats was anesthetized using Ketamine-xylazine 75–100 mg/kg + 5–10 mg/kg. Rats were placed and stretched on a board on which there was placed a ruler marked in centimeters (cm) with an accuracy of 0.01 cm. The length of the rat body was measured from the tip of the nose to the anus/base of the tail (naso-anal).

The abdominal circumference was also measured using metleen at

the end of the study after anesthesia. The abdominal circumference was measured on the largest part of the abdomen in a cicular manner in cm. The degree of obesity (i.e., body mass index, BMI) was calculated by Lee Index:

$$\text{Lee index} = \frac{\text{Weight (g)}^{0,33} \text{ x } 1000}{\text{Body length (mm)}}$$

2.6.3. Serum mineral content

Mineral analysis was performed using a spectrophotometric method with a spectrophotometer and atomic absorption spectrophotometer at 285.2 nm, 515 nm, and 766.5 nm wavelengths for magnesium, manganese, and potassium analysis, respectively. Standard dilutions for each analysis were also made to compare the absorbance results, so the mineral contents in the sample could be calculated.

2.7. Statistical analysis

All observations and measurements were tabulated and mean values ± standard deviations calculated. Data of energy value and feed nutrients were analyzed descriptively. Data differences on experimental animal characteristics and dependent variables of HFFD and N groups were performed using independent t-tests at a 95% confidence level. The correlation among variables was tested using a bivariate test, and Pearson correlation test was used assess the relationship between variables. Data processing and analysis were done using Microsoft Excel and SPSS for Windows.

3. Results

The modifications of dietary feed used in the animal model of obesity in this study are presented in (Table 1).

Normal feeding intervention and the HFFD intervention were administered for 12 weeks. The characteristics of the initial subjects and intervention outcomes are presented in (Table 2). It shows that the weights of rats at the beginning of research were not significantly different (p = 0.945). After 12 weeks of HFFD intervention, there was an average increase in rat body weight, 11.94% higher than the body weight of rat with normal feed, although there was no statistically significant difference in weight between the two groups (p = 0.140). Body length and BMI between the two groups was also not significantly different (p = 0.325, p = 0.875, respectively). However, there were differences in abdominal circumference of HFFD group that was significantly higher than normal feeding group (p = 0.009).

Table 1
Composition of constituents, energy content and nutrients Normal (N) and High Fat High Fructose (HFFD) diet.

Component	Type of diet	
	Normal	HFFD
Cornstarch (%)	29.20	14.73
Dextrinnise Cornstarch (%)	9.71	3.29
Sucrose (%)	6.27	4.60
Fructose (%)		4.60
Copha + lard boiled (%)		27.59
Cow kidney fat (%)		4.91
Soybean oil (%)	12.09	1.46
Casein (%)	24.75	10.82
White egg (%)	8.25	18.25
Gelatin (%)	4.90	4.90
Minerals (%)	3.43	3.43
Vitamins (%)	0.98	0.98
L-cystine (%)	0.18	0.18
Coline bitartrate (%)	0.25	0.25
Calories	4298.06	5405.83
Carbohidrat (%)	42.87	20.51
Fat (%)	25.81	57.57
Protein (%)	31.32	21.90
Calories Density (Cal/g)	4.21	5.31
Magnesium (mg)	17.00	15.00
Manganese (*ppm*)	6.77	2.60
Potassium (mg)	16.00	3.00
Fiber (g)	28.42	24.25

Table 2
Characteristics of the initial subjects and intervention results of N and HFFD diet.

Characteristic (mean ± SD)	Normal	HFFD	*p value*
Body weight (g)	246 ± 19.11	245.58 ± 22.06	0.945
Feed intake (g)	12.23 ± 1.67	6.49 ± 1.52	0.000
Intake (Calories from drinks)	0	31.71 ± 7.08	0.000
Intake (Calories from the diet)	51.60 ± 7.04	34.59 ± 8.12	0.000
Total intake (Calories)	51.60 ± 7.04	66.30 ± 7.26	0.000
Magnesium intake (mg)	2.08 ± 0.28	0.97 ± 0.23	0.000
Manganese intake (ppm)	82.79 ± 11.30	16.87 ± 3.95	0.000
Potassium intake (mg)	1.95 ± 0.26	0.19 ± 0.05	0.000
Final weight (g)	261.93 ± 29.30	279.42 ± 33.22	0.140
Body length (cm)	22.29 ± 1.10	21.25 ± 0.65	0.325
Lee index	265.85 ± 10.15	287.65 ± 10.34	0.875
Abdominal circumference (cm)	14.64 ± 0.93	15.84 ± 0.41	0.009
Serum Magnesium levels (mg/dL)	43.18 ± 7.87	35.01 ± 12.39	0.038
Serum Manganese levels (ppm)	1.16 ± 0.38	0.87 ± 0.37	0.000
Serum Potassium levels (mg/dL)	314.40 ± 135.93	217 ± 76.99	0.000

The mean of feed intake (g) of the normal group was higher than that of the HFFD group (p = 0.000), but the total calories of feed and beverage intake in the HFFD group was significantly higher than the normal group (p = 0.000). Higher calorie intake obtained by HFFD group rats came from the beverage, made from 30% fructose solution that contained 31.71 ± 7.08 calories. The mean of calorie intake from the feed and the beverage is presented in (Fig. 1).

The results also show differences in mineral serum levels in terms of magnesium, manganese, and potassium between the two groups. Mineral serum levels in rats with normal diet were higher than those in HFFD (Fig. 2).

There was a relationship among magnesium, manganese, and potassium serum levels with BMI and abdominal circumference. Higher BMI means lower magnesium serum level (p = 0.000; R^2 = 0.536), lower manganese serum level (p = 0.000; R^2 = 0.367), and lower potassium serum level (p = 0.049; R^2 = 0.131). Likewise, higher abdominal circumference means lower magnesium serum level (p = 0.002; R2 = 0.285), lower manganese serum level (p = 0.003; R^2 = 0.267), and lower potassium serum level (p = 0.000; R^2 = 0.651). The graph of the relationship among BMI and abdominal circumference with mineral serum levels is presented in (Figs. 3–5).

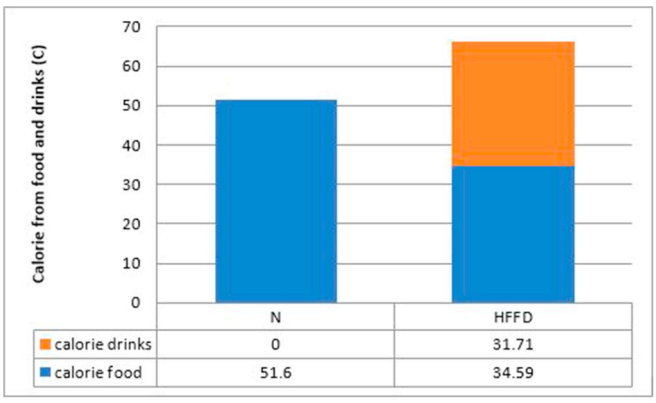

Fig. 1. Calorie intake from food and drinks by group.

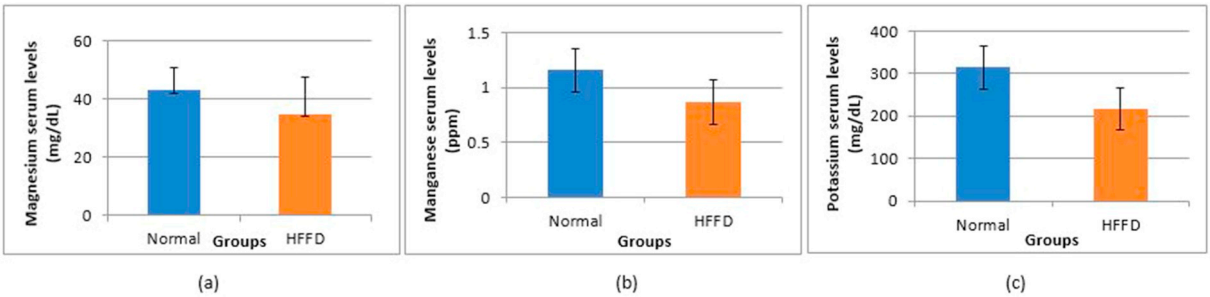

Fig. 2. Magnesium, manganese and potassium levels of rat serum in the group with normal feed and HFFD.

4. Discussion

The experimental animals, male white rats (*R. norvegicus*) Sprague-Dawley strains, were used as subjects to make animal model of obesity based on previous researches results using the same experimental animals (Imam et al., 2012; Lee et al., 2015; Marquest et al., 2015; Senaphan et al., 2015; Dong et al., 2016). The intervention food diet used was High Fat High Fructose Diet (HFFD). HFFD feed is recommended in making animal models of obesity.

The results showed that along with increasing consumption of food and beverage containing high fructose corn syrup (HFCS), there is also an increase in the prevalence of obesity. HFCS is widely used in a variety of food and beverage products such as soft drinks, pastries, cookies, gums, jelly, and dessert that are popular among people. The effects of fructose on the brain cause addiction effects and leptin resistance, so long-term consumption of fructose increases calorie intake due to loss of satiety signals in the brain resulting in overweight and obesity. The composition of the HFFD feed was 20.51% carbohydrate, 57.57% fat, and 21.90% protein; and the beverage was 30% fructose.

The results of this study show that the modified HFFD AIN 93 standard did not result in significant changes in body weight. However, there was a weight gain of 11.94% in the HFFD food group compared to N feed. This has met the criteria that in the study of diet feeding, rats are moderately obese if there is a change in body weight of 10–25% above the normal feed group (Hariri and Thibault, 2010). This is probably due to the high energy density of the N diet, so the caloric intake from N diet group is higher than the HFFD group. The modified feed formula produced in this study shows that the energy density of N diet feed is still relatively high at around 4.21 cal/g. The mean of energy density for N feed in making rats model of obesity is < 3.5 cal/g (Miras et al., 2014). This high energy density of N feed diet is caused by the use of a quite high carbohydrate source material that aims on making the formula to be easily molded as rat pellets. On the other hand, the HFFD contains < 60%. Fat that is also a contributing factor. Fat content of an HFFD is > 60%. Calorie intake from feed in the HFFD group was lower than the N group, but the total calories were significantly higher (Senaphan et al., 2015). The high caloric intake in HFFD group was caused by high caloric contribution from the beverage, which contained 30% fructose.

Besides the final weight, the results of BMI calculation based on Lee index also do not show significant differences between the HFFD and N groups, although the mean of BMI group with HFFD was higher than in the N group. However, the measurement of abdominal circumference shows significant differences; the abdominal circumference of HFFD group is higher than of the N group. Excess lipids derived from food will be stored in stomach, liver, and hips which will interfere with most of metabolic functions and contribute to an increase in metabolic syndrome. In rodents, the size of the total body weight does not provide relevant information about body composition, which is an important parameter in the study of metabolic syndrome. The size of the abdominal circumference has been shown to be significantly correlated with cardiovascular in humans. Among several anthropometric measurements, abdominal circumference is also the best predictor of intra-abdominal fat in central obesity. The size of the abdominal circumference is very important in experimental animal studies of overweight or obese rats (Gerbaix et al., 2010).

Mineral content in a high fat diet is lower than in a normal diet. This is due to in high fat diet, carbohydrate content is lower, and generally carbohydrates from grains, cereals, and rice are also sources of minerals. Some of the minerals contained in carbohydrate sources in rat feed include cornstarch and cornstarch dextrin. Minerals that are associated with energy metabolism and in high levels in whole grains and cereals, are among others, magnesium, manganese, and potassium. The magnesium serum level in HFFD group rats in this study was significantly lower compared to the level in the N group. This is consistent with the results of epidemiological studies in Europe and North America, in which the consumption of in Western diets is generally 30%–50% of the RDA for magnesium. The decline in dietary consumption of magnesium sources in the United States in the last 100 years is from about 500 mg/day to 175–225 mg/day. This is probably due to the increasing use of processed foods that are generally high in fat and sugar (Rude, 2012; Shah et al., 2017). The serum magnesium level is significantly lower in the overweight group compared with the group with normal weight.

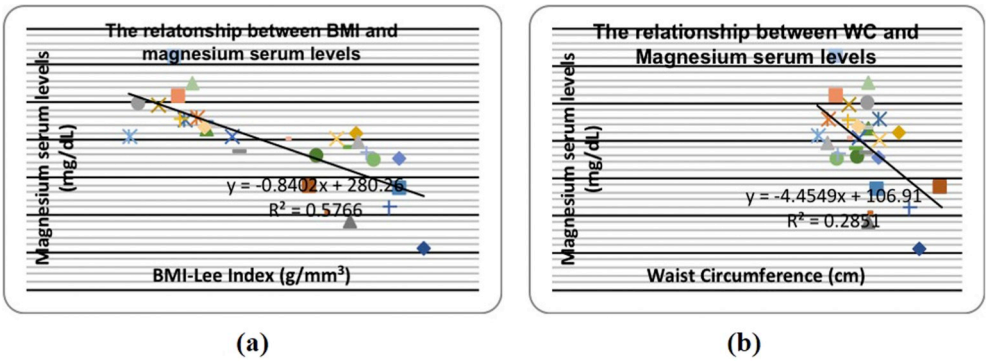

Fig. 3. The relationship between BMI (a) and WC (b) with Magnesium serum levels.

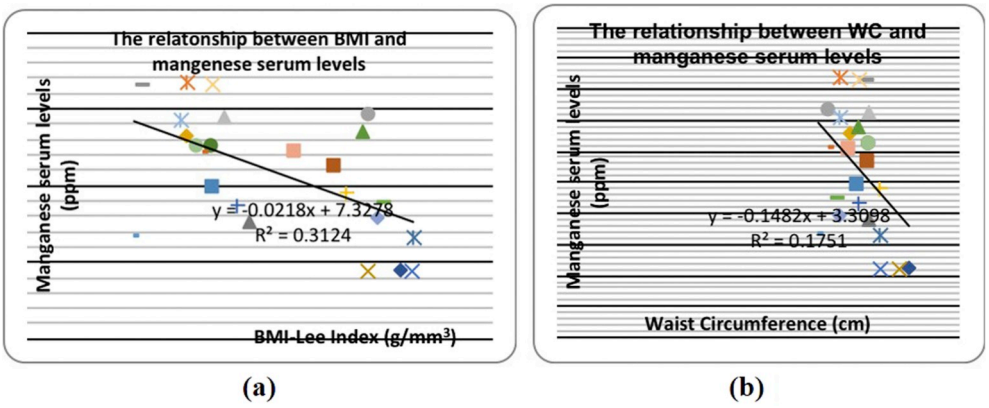

Fig. 4. The relationship between BMI (a) and WC (b) with Manganese serum levels.

Serum magnesium levels are significantly negatively correlated with BMI and abdominal circumference. The closeness of the correlation with BMI was higher than that of the abdominal circumference, but both showed a strong correlation. This is consistent with the results of several studies that showed serum magnesium levels are correlated and inversely proportional to body mass index (Jose et al., 2012; Suliburska et al., 2013; Zaakouk et al., 2016). Low serum magnesium levels in the obese group are possible due to decreased magnesium absorption in the intestine or increased excretion. Thus, increased fat intake in obesity cases may interfere the absorption of magnesium (Jose et al., 2012). The evidence that magnesium is involved in weight regulation is still controversial. Similarly, the mechanism of the relationship between magnesium and obesity remains unclear. The results of *in vitro* studies show the role of magnesium in cellular energy metabolism. Adenosine triphosphate (ATP) binds magnesium ions (Mg^{2+}) to form biologically functioning components. Most intracellular ATP and Mg^{2+} is assumed to form Mg-ATP complexes in the cytosol, which contributes to energy metabolism. Enzymatic activity depends on Mg^{2+}, and some enzymes in mitochondria rely on Mg^{2+} to maintain their cellular function. Enzyme activity in the tricarboxylic acid (TCA) cycle is regulated by Mg^{2+}, and this is shown by computer simulations that show a clear metabolic model of the role of Mg^{2+} in mitochondria in the TCA cycle (de Baaij et al., 2015). Mg^{2+} homeostasis is important for maintaining electron transport chain, and mitochondrial ATP-Mg/Pi is an important carrier export of ATP in mitochondria to the cytosol. Therefore, Mg^{2+} is an important regulating factor of metabolic status in mitochondria (Nasulewicz et al., 2004; Romani, 2011).

Rat cells with magnesium deficiency showed a decrease in TCA cycle substrate, thus reducing mitochondrial access to Mg^{2+} and suppressing ATP efflux from mitochondria that is likely mediated by ATP-Mg/Pi. Mitochondrial ATP accumulation inhibits many enzymatic processes in TCA cycle and activities of the electron transport chain by means of negative feedback. Suppression of TCA electron transport chain and activity cycle will result in reduced ATP production in mitochondria. Mitochondrial morphological changes are also observed in rat cells with magnesium deficiency. The condition of mitochondria that becomes abnormally large and round inhibits synthesis (Youle and van der Bliek, 2012). This is consistent with the opinion of the experts that mitochondrial morphology is controlled by energy metabolism and abnormal mitochondrial morphology associated with the incidence of cancer, obesity, type-2 diabetes, and neurodegenerative disorders (Roy et al., 2015). Besides magnesium, the research results also show that serum manganese level in HFFD group is significantly lower than in the N group. The serum manganese level significantly negatively correlates with BMI and abdominal circumference with a lower correlation coefficient than magnesium. The same condition is also shown from the results of research on humans that obese individuals have lower plasma Mn level than individuals with normal weight and inversely related to abdominal circumference (Zhou et al., 2016; Lee et al., 2013). It can be explained that increasing BMI and abdominal circumference is one indicator of increased fat reserves in the body. Increasing lipogenesis will also increase insulin amounts, decrease insulin sensitivity resulting in insulin resistance, triglyceride accumulation, and increasing adipocyte apoptosis. Finally, there will be an increase in the release of ROS (Reactive Oxygen Species) causing oxidative stress conditions. The main source of ROS is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and uncoupled eNOS. NADPH oxidase is a potent source of O_2 and can be activated by high LDL and free fatty acids (Griendling and Ushio-Fukai, 1997; Huang et al., 2015). Manganese functions to activate many enzymes. Enzyme groups that have Mn cofactors are oxidoreductase, transferase, hydrolase, liases, isomerase, ligases, lectin, and integrins. Mn activates the enzymes associated with fatty acid metabolism and protein synthesis and is involved in neurological function. Mn is present in the metaloenzyme

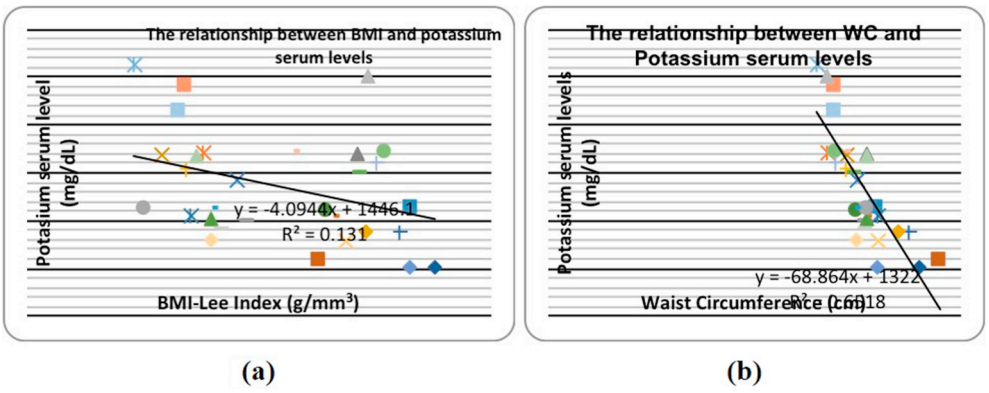

Fig. 5. The relationship between BMI (a) and WC (b) with Potassium serum levels.

superoxide dismutase (MnSOD), which has Mn metal cofactors and is found in all aerobic organisms from bacteria to humans, and even anaerobes. MnSOD is distributed evenly through cytoplasm inside prokaryotic cells. MnSOD acts as primary antioxidant enzyme that protects cells from oxidation by catalyzing dismutation superoxide ($O_2^{\bullet-}$) into hydrogen peroxide and oxygen in mitochondria of eukaryotic cells. In general, mitochondria is the main source in $O_2^{\bullet-}$-production. Several studies indicate that MnSOD plays an important role in protecting cells from oxidative stress (Whittaker, 2013).

Several epidemiological studies on the association among Mn intake, plasma Mn level, and metabolic syndrome have been controversial, but studies on experimental animals show that there is a link between Mn dietary intake and metabolism of amino acids, lipids, proteins, and carbohydrates and also that deficiency of dietary intake of Mn sources is associated with several components of metabolic syndrome. Mn is important for maintaining lipoprotein structure because of its high affinity to form complexes with polyps of phospholipids, thus it stabilizes lipoprotein particles. Oxidative stress induced by Mn deficiency accelerates vascular cell proliferation, leading to thickening blood vessels and constriction of internal diameter and also endothelial damage; thus, it increases vasoconstriction and hypertension risk. In addition, interference with superoxide dismutase also causes a significant decrease in β-cell function index (Kang et al., 2014; Lee et al., 2015).

Sufficient intake of Mn or Mn supplementation reduces visceral fat accumulation, decreases fatty acid synthesis and malic dehydrogenase activity in the liver and glycerol in adipose tissue, thus resulting in a decrease in total cholesterol and LDL cholesterol (Bae et al., 2011; Lee et al., 2015). Subjects with Mn intake in the lowest quartile (< 2.62 mg/day) have a higher risk of developing metabolic syndrome than subjects with the highest quartile (> 4.56 mg/day) Mn intake. Treatment with manganese tetrakis benzoic acid porphyrin (MnTAB) shows weight loss effects, adiposity reduction, and subsequently improves insulin sensitivity (Pires et al., 2014; Brestoff et al., 2015).

The other mineral that is also associated with energy metabolism is potassium. The results of this study indicate a significant difference in serum potassium levels in the HFFD group, lower than that of N group. Besides, potassium level is significantly and inversely associated with increasing BMI and abdominal circumference: the higher the BMI and the abdominal circumference, the lower the serum potassium level. The closeness of relationship between abdominal circumference and serum potassium levels is the highest compared to magnesium and manganese. The results of studies currently indicate that the relationship between potassium intake and obesity or the metabolic syndrome is controversial (Shin et al., 2013). Several studies have also shown that potassium intake is inversely associated with BMI and the risk of obesity. Subjects with higher potassium intakes have lower abdominal circumference and lower risk of obesity (Vergnaud et al., 2012; Jain et al., 2014). A higher potassium/sodium ratio in urine occurs in subjects with obesity compared to subjects with normal weight, and subjects with higher potassium/sodium ratios have higher abdominal fat percentages (Binia et al., 2015) fasting hyperglycemia (Vergnaud et al., 2012), incidence of kidney stones, and hypertension (Tabbaa et al., 2015; Cai et al., 2016).

A possible mechanism to explain the link between potassium and obesity or metabolic syndrome is that obesity is a component of metabolic syndrome and the obesity mechanism is linked to the occurrence of a homogeneous metabolic syndrome. Obesity has been shown to be linked with the function of potassium channel, which affects the carbohydrate accumulation and glucose homeostasis and also plays an important role in insulin secretion and carbohydrate metabolism (Shin et al., 2013).

5. Conclusion

Mineral content (Mg, Mn, and K) in the N diet is higher than in the HFFD. The rat serum mineral level in group N was significantly higher than in the HFFD group. This study shows that HFFD causes a decrease in serum mineral levels associated with the incidence of obesity.

Conflict of interest

None.

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