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The effect of sugar and artificial sweetener on molecular markers of metabolic syndrome: a mice study

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Abstract

The usage of aspartame, as one of the most widely used sweetener, has been approved in many types of food products. Moreover, many studies have proven that replacing sugar with aspartame would contribute favorable effects on several health parameters; such as, body weight, blood glucose level, and inflammatory status. In this experiment, we examined the effects of aspartame consumption on some biomarkers; which potentially acted as early signals for a personal metabolic status. This study was aimed to investigate the effect of aspartame on the expression of a number of molecular markers related with appetite regulation (fto), fat accumulation markers (fabp4 and alt2) and inflammation marker (tnf-α) in Sprague Dawley rats. The population of *Clostridium coccoides* was also observed to give an insight about the effect of sweetener consumption on gut microbiota profiles. 15 healthy, male, eight-weeks old Sprague-Dawley rats were fed a standard diet and divided into 3 groups (n=5 for each): water only, sucrose (30% b/v), and aspartame (0.15% b/v). Body weight was measured weekly and blood glucose measurement was carried out on day 1 and 40. At the end of the experiment, all rats were euthanized and blood was collected from the vein. The liver, brain, and visceral adipose tissue were excised, weighed, and grinded with liquid nitrogen. Feces samples were collected on day 0 and 40. At the end of our experimental period; the body weight, liver weight, and blood glucose level of sucrose-treated rats were significantly higher ($p < 0.05$) than aspartame and control group. Sucrose showed the lowest level of fto gene expression; yet, the fto gene expression in aspartame group was still lower than the control group. Expression of several genes considered as metabolic syndrome-related biomarkers were measured (fabp4, alt2, and tnf-α); and our data demonstrated that sucrose treatment gave the highest increase in expression level of those genes; while aspartame treatment showed much lower values. Furthermore, sucrose also caused a significant reduction in *C. coccoides* population; while, the *C. coccoides* population in aspartame group did not differ significantly compared to the control group.

1. Introduction

Artificial sweeteners have been used for years as a sugar substitute for their "less calorie" property which leads to a healthier lifestyle and reduced metabolic syndrome rates. Aspartame, as one of the most widely used artificial sweetener, was claimed as a safe ingredient and would cause no harm as long as the consumption did not exceed the acceptable daily intake (ADI) based on United States Food and Drug Administration (USFDA) and European Food Safety Authority (EFSA). However, several recent studies indicated some unfavorable effects of consuming aspartame.

A research demonstrated that aspartame could decrease some inflammation parameters, such as tumor necrosis alpha (TNF-α), interleukin-2 (IL-2), and interferon gamma (IFN-γ), which were also used as markers of type 2 diabetes

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mellitus development. In contrary, this research also mentioned that aspartame acted as a chemical stressor and raised oxidative stress leading to inflammation (Choudhary and Devi 2015). Other studies also demonstrated that aspartame consumption could cause an increment of fat and body mass (Collison *et al*., 2012; Feijo *et al*., 2013), liver damage, and non-alcoholic fatty liver disease (NAFLD) (Abhilash *et al*., 2011). A study by Yang (2010) proposed that the sweet response produced after consuming artificial sweetener could increase the appetite and reduce satiety, which finally led to obesity development. One cohort study with a very large number of subjects also showed the increment of waist circumference after consuming diet coke containing artificial sweetener (Fowler *et al*., 2015).

To our knowledge, there was only very limited studies which investigate the effect of sweeteners on molecular markers related to appetite regulation, adiposity, and inflammation. Research were usually focused on parameters such as body weight, waist circumference, blood glucose and such; yet the influence on other surrogate biomarkers was not often explored. Thus, we would like to assess several promising markers, which potentially act as earlier signals.

Fto is a regulator protein which is produced in many organs, including hypothalamus; implying it plays a role in appetite regulation (Vujovic *et al*., 2013). Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced in blood, which initiates the immune response (Kempf *et al*., 2007). The high expression level of TNF-α is often found in subjects with metabolic disorder diseases like obesity and diabetes (Kempf *et al*., 2007; De Koning *et al*., 2012; Jung and Choi 2014; Choudhary and Devi 2015). Fabp4 is mainly expressed in adipose tissue and its expression is up-regulated during adipogenesis; hence it is often used as adipogenesis marker (Simon *et al*., 2013; Furuhashi *et al*., 2014). Alt2 is gluconeogenic enzymes which expressed mainly in the liver and frequently used as a marker for NAFLD development. A high expression of Alt2 also indicates a state of insulin resistance (Saltiel and Khan 2001).

Recently, the association of sweetener consumption with higher appetite was proposed to be mediated via shifting in gut microbiota population (Palmnas *et al*., 2014). Commensal Clostridia consist of *Clostridium* cluster XIVa and IV (*Clostridium coccoides*), which are known for years as fusiform-shaped bacteria; make up a substantial part (10-40%) of the total bacteria in the gut microbiota. They are strongly involved in the maintenance of overall gut function. This leads to important translational implications in regard to the prevention and treatment of dysbiosis, to drug efficacy and toxicity, and to the development of therapies modulating the composition of the microflora (Lopetuso *et al*., 2013). Thus, it would be interesting to investigate how the sweetener consumption affects the Clostridia population. However, this is a new area of research and more studies are needed to obtain more convincing data.

Therefore, this study was aimed to investigate the effect of aspartame on several molecular markers related to appetite regulation (*fto*), fat accumulation marker (*fabp4* and *alt2*) and inflammation marker (*tnf-α*) in Sprague-Dawley rats. The population of *Clostridium coccoides* was also observed to give an insight into the effect of sweetener consumption on gut microbiota profile.

2. Materials and methods

2.1. Materials

RNase Zap was purchased from *Ambion* (Huntingdon, Cambridgeshire, United Kingdom), RNAprotect Animal Blood Tubes was purchased from Qiagen (Hilden, Germany), RNeasy Protect Animal Blood Kit was purchased from Qiagen (Hilden, Germany), RNeasy Lipid Tissue Mini Kit was purchased from Qiagen (Valencia, California, USA), RNeasy Plus Mini Kit was purchased from Qiagen (Valencia, California, USA), Allprotect Tissue Reagent was purchased from Qiagen (Valencia, California, USA), Revertaid First Strand cDNA Synthesis Kit was purchased from Thermo Fisher Scientific (Waltham, Massachusetts, United States), GoTaq Green Master Mix was purchased from Promega (Madison, Wisconsin, USA), SYBR Green PCR Master Mix was purchased from Applied Biosystem (Foster City, California,USA), *tnf-α* primer, *fabp4* primer, *alt* primer, *fto* primer and *β-actin* primer were purchased from IDT (Iowa, USA).

2.2. Animal experiment

This experiment was carried out by veterinarian from PT Bimana Indomedical (Bogor, Indonesia) after all protocols were approved by the Animal Welfare Supervision Commission and Use of Research Animals (ACUC number: R.04-14-IR). 15 healthy, male, eight-weeks-old Sprague-Dawley rats weighing approximately 56.5–129.3 grams were fed a standard diet calculated based on rats' body weight and divided into 3 groups (n=5 for each group) based on the administered treatment: water only, sucrose 30% b/v, and aspartame 0.15% b/v. The amount of diet and water consumed were

observed every day. Body weight was measured once a week for 40 days treatment and blood glucose measurement was carried out on day 1 and 40.

2.3. Sample collection

At the end of the experiment, all rats were euthanized and blood was collected from the vein. Blood samples were transported to the analytical laboratory using RNAprotect Animal Blood Tubes and the other tissues were transported using Allprotect Tissue Reagent. Feces samples were collected on day 0 and day 40 during the intervention period. The liver, brain, and visceral adipose tissue were excised, weighed and grinded with liquid nitrogen, followed with homogenization using the buffer from RNA isolation kit.

2.4. Blood glucose measurement

Blood glucose was measured using GlucoDR (Hasuco Korea, Dongjakgu, Seoul, Korea). The tail's end of the rat was wounded to get the drop of blood for blood glucose measurement. The drop was dripped on the gluco-strip connected to the device. The number which appeared on the device was the blood glucose value.

2.5. RNA isolation

RNA isolation from adipose tissue was performed using RNeasy Lipid Tissue Mini Kit; RNA isolation from liver and brain was performed using RNeasy Plus Mini Kit; while RNA isolation from whole blood was performed using RNeasy Protect Animal Blood Kit. All the procedures were completed according to the manufacturer's protocol. The amount of samples used was $\pm$20 mg for liver and $\pm$100 mg for adipose and brain tissue. The blood samples' volume for RNA isolation was 200 µL.

2.6. DNA total extraction

Bacterial DNA was isolated from rats' feces samples using Power Fecal® DNA Isolation Kit (MO BIO, Carlsbad, USA) according to the manufacturer's protocol. After being isolated, the DNA concentration was measured with NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA).

2.7. cDNA synthesis

The RNA samples were converted to cDNA using Revert Aid First Strand cDNA Synthesis Kit. Samples of RNA with the concentration of 25 ng/µL were used as a template to create cDNA with the final volume 20 µL. The mixture containing 0.05 µg/µL control GAPDH, 0.5 µg/µL oligo-dT, 200 U/µL Revertaid Reverse Transcriptase, 20 U/µL Ribolock RNase inhibitor, 5X reaction buffer, 10 mM dNTP mix, and 10 µM of each primer. The PCR reaction was carried out as followed: 25˚C for 5 minutes, 42˚C for 1 hour, and 70˚C for 5 minutes.

2.8. Quantitative PCR

Gene expressions were analyzed by quantitative polymerase chain reaction (qPCR) method using Applied Biosystems StepOnePlus Real Time PCR (Thermo Fisher, Waltham, Massachusetts, United States). The DNA samples from cDNA synthesis were amplified in a final volume of 20 µL containing 2X SYBR Green mastermix and 0.8 µM of each primer. The primers used for qPCR were F:5-CCACGCTCTTCTGTCTACTG-3; R:5-GCTACGGGCTTGTCACTCGA-3 for *tnf-α* (Lv *et al*., 2012), F:5-AGCGTA GAAGGGGACTTGGT-3; R:5-ATGGTGGTCGACTTTCCATC-3 for *fabp4* (Eckertova *et al*., 2011), F:5-AGGCAGCTCAGTCCCATAAA-3; R:5-GTAG GTGCCTTCTCGCTGTC-3 for *alt2* (Techapiesancharoenkij *et al*., 2014), F:5-GCCGCATGTCAGACCTTCC-3; R:5-GACCTGTCCACCAAGTTCTCG-3 for *fto* (Wang *et al*., 2011), F:5-AGACCTCTATGCCAACACAGTGC-3; R:5-GAGCCACCAATCCACACAGAGT-3 for *β-actin* (Wang *et al*., 2011), F:5-CAAGGTCATCCATGACAACTTTG-3; R:5-GTCCACCACCCTGTTGCTGTAG-3 for *gapdh* (Wang *et al*., 2015). The data acquired would be analyzed using comparative C_T method ($-2^{\Delta\Delta C_T}$).The qPCR program was carried out as followed: pre-denaturation at 95˚C for 2 minutes, denaturation at 95˚C for 20 seconds, annealing at 64˚C for 45 seconds, elongation at 72˚C for 30 seconds, with the total number of cycles was 45 for *tnf-α*; pre-denaturation at 95°C for 3 minutes, 30 seconds at 95°C, 30 seconds at 65°C (*gapdh*) or 63°C (*fabp4* and *alt2*) and 30 seconds at 72°C with the total number of cycles was 45; pre-denaturation at 95°C for 2 minutes, denaturation at 95°C for 15 seconds, annealing with specific temperature of each primer for 30 seconds, and elongation at 72°C for 30 seconds with the total number of cycles was 40 for *fto,* β-actin (β), and *C. coccoides* groups. The specific annealing temperature for each primer were 64°C for *tnf-α*, 72°C for *fabp4*, 72°C for *alt2,* 55°C for *fto*, 59°C for *β-actin*, and 65°C for *gapdh*.

2.9. Specific bacteria sequence amplification

From total bacteria DNA samples, specific bacteria sequence would be amplified with specific

primer according to its Tm in Polymerase Chain Reaction (PCR). The Reaction was prepared with 12.5 µL as total volume reaction and consisted of 6.25 µL GoTaq Green® Master Mix (Promega, Madison, USA), 2 µL template DNA feces, 0.5 µL for each forward and reverse primer (10 µM) *Ccoc* F:5-AAATGACGGTACCTGACTAA-3; R: 5-CTTTGAGTTTCATTCTTGCGAA -3 for *C. coccoides* (Kurakawa *et al*., 2015), and 3.25 µL nuclease free water. G-Storm GS482 Thermal Cycler (G-Storm, Somerton, Somerset, United Kingdom) instrument was used for this reaction with initial condition 94°C for 5 minutes, denaturation 94°C for 30 seconds, annealing condition 55°C for *Ccoc* according to Tm primer, extension 72°C for 1 minutes, and post elongation 72°C for 10 minutes. This reaction ran for 30 cycles. Afterwards, the amplification product was purified and verified. Verification was done by electrophoresis with 1% agarose gel in 1X buffer TAE, 100 V, 35 minutes. The agarose gel was stained with ethidium bromide (EtBr) in dark condition for 15 minutes followed by destaining with aquades for 5 minutes. Finally, the PCR product could be seen in the agarose gel with UV-transilluminator.

2.10. Plasmid construction

Plasmid construction method was done to make a standard curve for calculating the amount of specific bacteria in fecal samples. After getting specific bacteria sequence from PCR, the next step was ligation. Amplification product was purified with QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) before being ligated to pGEM-T Easy Vector Systems I (Promega, Madison, USA). On the ligation step, we used 20 µL ligation reaction consisted of 1 µL pGEM-T-Easy (Promega, Madison, USA), 1 µL T4 DNA ligase (Promega, Madison, USA), 2 µL buffer ligase 2X (Promega, Madison, USA), 5 µL purified amplification product, and nuclease free water. The ligation reaction was incubated at 4°C for 16 hours. After ligation process, the ligated plasmid was transformed into *Escherichia coli* DH5α. Transformation was divided into two main steps, making competent cells and transformation. Competent cells were made using magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) salts, while transformation process was done with heat shock method in 42°C for one minute. The transformed cells then were grown on Luria Agar media contained ampicillin (50 µg/mL) and X-Gal (40 µg/mL) for blue-white screening process. All media were incubated at 37°C overnight. Grown white colony bacteria indicated successful ligation process to vector. To clone the plasmid, the white colony was refreshed to Luria Agar media contained ampicillin in 37°C for 12 until 16 hours. The plasmid from refreshed bacteria was isolated with Wizard® Plus SV Minipreps DNA Purification System (Promega, Madison, USA).

2.11. Verification

To verify the isolated plasmid, plasmid was quantified with NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Then, plasmid was amplified with M13 forward-reverse primer. These amplification products were sequenced and checked with basic local alignment searching tools (BLAST) to make sure the plasmid was inserted into the right DNA template.

2.12. Standard curve construction

Standard curve was used to calculate the amount of specific bacteria in feces sample. The constructed plasmid was then diluted into five different concentrations from $5x10^1$ to $5x10^5$ to make the standard curve. Amount of specific bacteria DNA copy number was measured with the formula following according to Avogadro number and molecular mass for each nucleotide base as in.

$$number\ of\ copies = \frac{amount\ of\ DNA\ (ng) \times 6.022 \times 10^{23}}{DNA\ lenght\ (bp) \times 1 \times 10^{9} \times 650}$$

2.13. Data analysis

The amount of specific bacteria DNA copy number was converted into amount of specific bacteria by dividing the specific bacteria DNA copy number with operon number from 16S rRNA gene database. Livak or comparative Ct Mean method was used for gene expression analysis.

2.14. Statistical analysis

Statistical analysis was performed using SPSS version 21.0 (IBM, Armonk, New York, United States). Shapiro-Wilk test was performed for normality test. Comparisons between groups were analyzed using One-Way ANOVA and posthoc Tukey test. For the comparison of blood glucose measurement within group, statistical analysis was performed using repeated measure ANOVA. Significance was assigned at P-value of <0.05.

3. Results

3.1. Sucrose increased the rats' body and liver weight

The body weight of each rat was measured every week during the seven weeks of treatment as shown in Figure 1A and 1B; while liver weight was measured after rats were sacrificed and the livers were excised at the end of the experiment as shown in Figure 1C. From the data, it was clear that body and liver weight of sucrose group were significantly higher ($p < 0.05$) compared to control and aspartame groups. From Figure 1A, the significant difference in body weight was started to appear from week 3, and continued to show the similar trend up to day 40. Meanwhile, the body weight during the treatment and on day 40; as well as liver weight in aspartame group on day 40 did not differ with the control group.

3.2. Aspartame maintained the blood glucose level

Blood glucose level for each rat was measured before the treatment was administered, in which all the rats had comparable basal blood glucose values (data not shown). Based on Figure 2, at the end of treatment (day 40), the group which received sucrose had a significantly greater blood glucose value ($p < 0.05$) compared to aspartame and control groups.

3.3. Aspartame did not affect the fto gene expression

Figure 3 demonstrated that, at the end of the experiment (day 40), sucrose treatment decreased the expression of *fto* gene; yet, the level of *fto* gene in aspartame group was comparable to the control group.

3.4. Aspartame showed lower expression of several metabolic syndrome-related markers

In this study, expression of several genes that was considered as metabolic syndrome-related biomarkers was measured. The target genes were *fabp4* gene from adipose tissue as shown in Figure 4A, *alt2* from liver tissue in Figure 4B, and *tnf-α* gene from blood samples in Figure 4C. Our data demonstrated that sucrose treatment gave the highest increase in expression level of those genes; while aspartame treatment showed much lower values. No significant difference was found between groups. However, these data showed a similar trend with the body weight and liver weight data.

3.5. Population of C. coccoides group was greatly decreased by sucrose administration

The difference between *C. coccoides* population between day 0 up to day 40 was observed. Our data showed that, after 40 weeks of administration, sucrose contributed a significant reduction in *C. coccoides* population ($p < 0.05$) as shown in Figure 5. Meanwhile, the *C. coccoides* population in aspartame group was not significantly affected compared to the control group.

4. Discussion

Our results clearly demonstrated that rats which were given sucrose treatment had a significantly higher body and liver weight, compared to the aspartame and control groups. Sucrose could induce body weight gain because it induced a higher amount of calorie intake to reach the same sweetness level as aspartame did. Each gram of both ingredients gave 4 kcal of energy. Yet, aspartame had 200 times higher sweetness level; contributing only 0.02 kcal for the same sweetness level with sucrose in food products (Mair *et al*., 2005; Magnuson *et al*., 2007). This might explain the major difference in body weight between rats in sucrose and aspartame group; in which the major difference started to appear after 3 weeks of

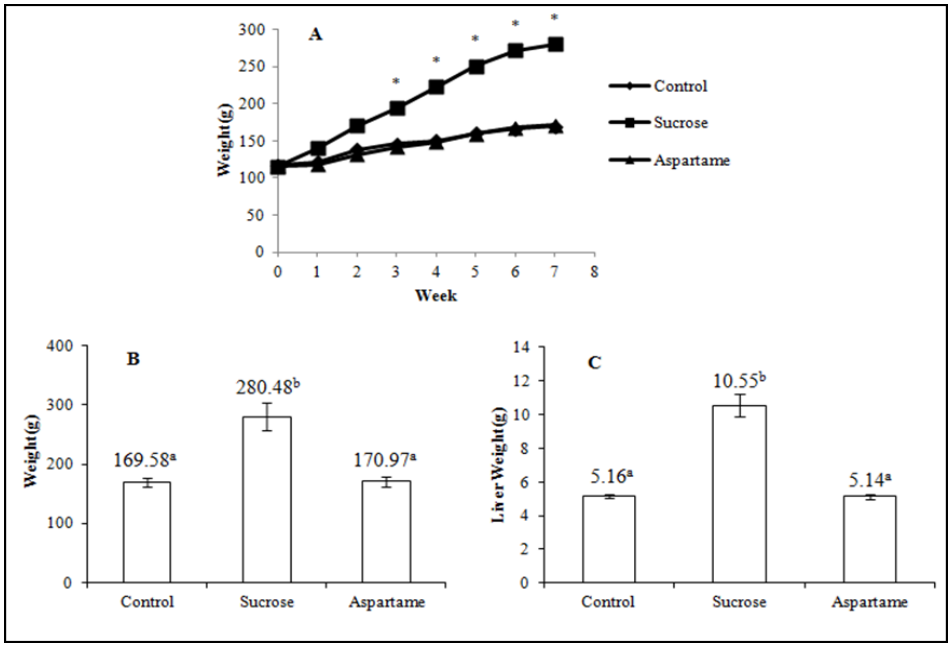

Figure 1. Body weight measurements of control, sucrose, and aspartame group during 7 weeks of treatment (A) and at the end of the treatment (day 40) (B). Liver weight measurement of three groups at the end of the treatment (day 40) (C). Data are expressed as mean ± SE (n=5). Values with different superscript or (*) superscript were significantly different ($p < 0.05$)

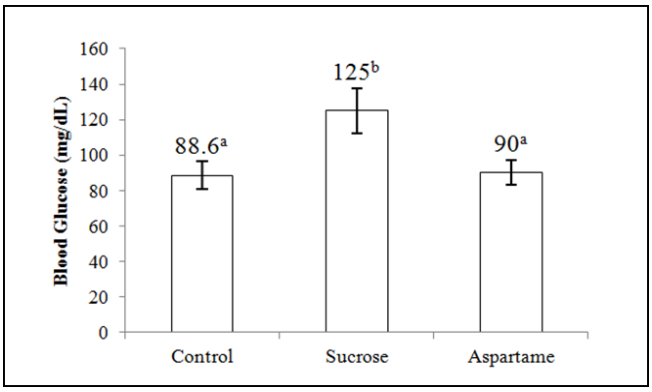

Figure 2. Blood glucose measurement at the end of the treatment (day 40). Data are expressed as mean ± SE (n=5). Values with different superscript were significantly different between groups ($p<0.05$)

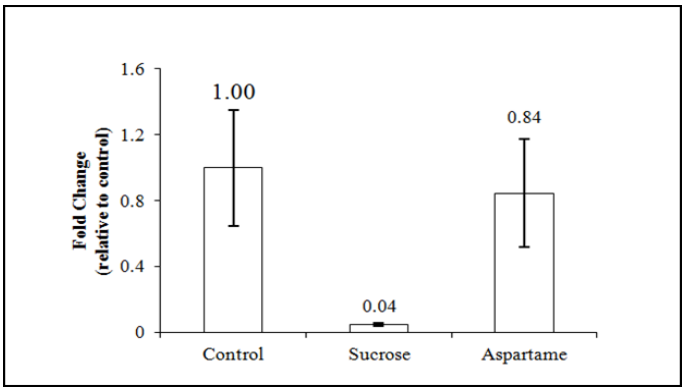

Figure 3. Expression of *fto* gene from the cerebellum. Data are expressed as mean ± SE (n=5). No values were significantly different ($p<0.05$)

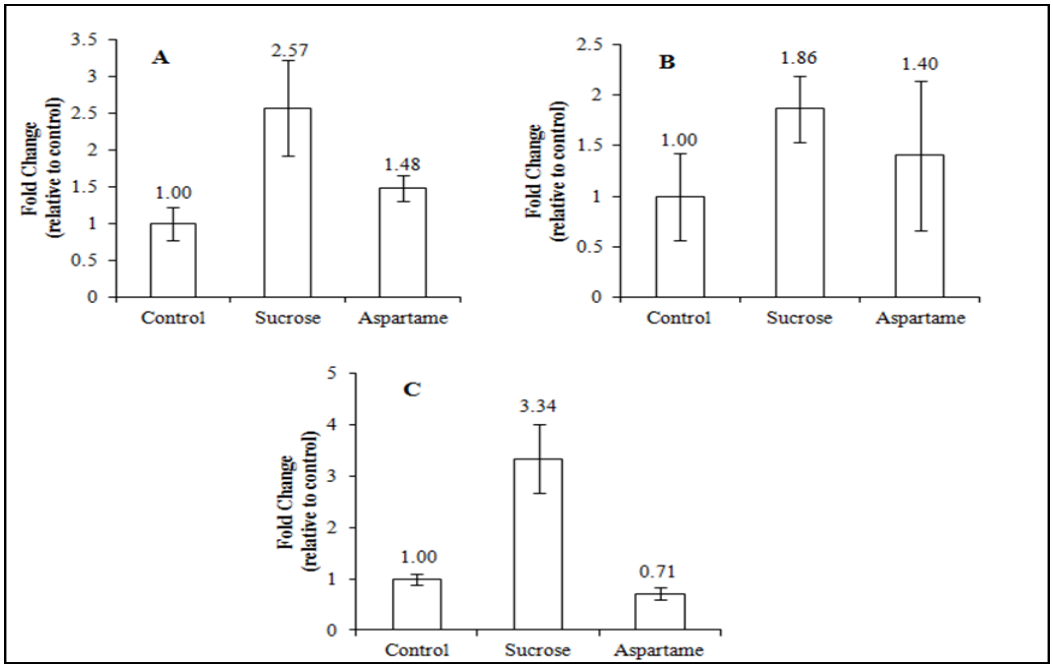

Figure 4. Gene expression measurement from adipose tissue, liver, and blood samples. Expression of *fabp4* gene from adipose tissue (A). Expression of *alt2* from liver tissue (B). Expression of *tnf- α* gene from blood (C). Data are expressed as means ± SE (n=5). No values were significantly different ($p<0.05$)

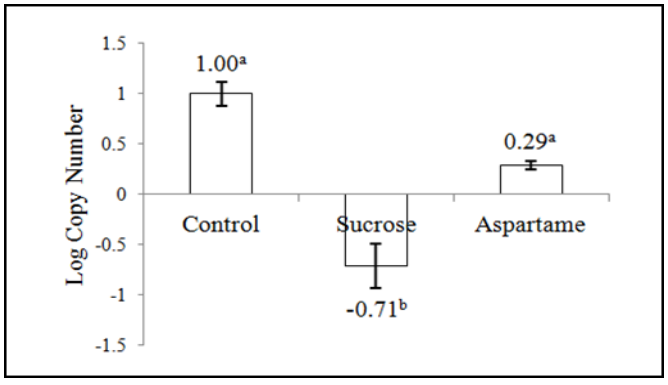

Figure 5. Difference of *C. coccoides* number in control, sugar and aspartame group between day 40 and day 0 (n=5 in each group). Values with different superscript were significantly different ($p<0.05$)

treatment, and continued to be significantly different until the end of experimental period (day 40).

Non-alcoholic fatty liver disease (NAFLD) is a liver disease characterized by excessive fat accumulation in liver (triglyceride >5% hepatocyte). Fatty liver or steatosis is the first stage of NAFLD. It could progress into more severe condition, such as non-alcoholic steatohepatitis (NASH) and fibrosis, cirrhosis, and sometimes, carcinoma (Serfaty and Lemoine 2008). Based on NAFLD characteristic, enlargement of liver could be one of the metabolic disorder symptoms. Our experiment showed that the average liver weight in sucrose group was higher than aspartame and control group. In sucrose-treated group, rats were given high concentration of sucrose (30% w/v). The excessive

calorie and sucrose intake caused insulin resistant state; in which the adipocyte lipolysis increased independently from nutritional status, leading to abundant free fatty acids in plasma pool. This caused increased fatty acid uptake and fat accumulation in liver (Bechmann *et al*., 2012); and finally resulted in liver enlargement, as observed in our experimental data. Meanwhile, the liver weight in groups treated with aspartame (0.15% w/v) did not show any notable difference with the control group.

High blood glucose level (hyperglycemia) has been long associated with the higher probability of type 2 diabetes mellitus development. The blood glucose value is widely used to classify subjects into several states: normal (<100 mg/dL), pre-diabetes (between 100 – 125 mg/dL), and diabetes (>125 mg/dL) (Genuth *et al*., 2003). This study showed a significantly higher blood glucose level in sucrose-treated rats (125 mg/dL), compared to the aspartame (90 mg/dL) and control (88.6 mg/dL) group on day 40. Moreover, all rats belonged to sucrose group could be classified as "pre-diabetes" at the end of the treatment, correlated to the blood glucose value states. Thus, these results confirmed previous studies about the beneficial effect of aspartame in maintaining blood glucose better than sucrose did.

Several studies remarked the possibility of aspartame in inducing craving behavior (Yang 2010). A study demonstrated that *fto* gene expression in rats' hypothalamus was up-regulated when they were fasting (Vujovic *et al*., 2013). Moreover, the *fto* expression was correlated positively with the level of ghrelin as hunger-inducing hormone (Karra *et al*., 2013). Therefore, in this experiment, we investigated the level of *fto* gene expression in *cerebellum* tissue as a marker to identify the craving or hunger level of the experimental rats.

Our results showed that sucrose reduced the level of *fto* gene expression; indicating sucrose did deliver the satiety effect to the experimental rats. The expression of *fto* gene in aspartame-treated rats was higher than sucrose group, but it was still a bit lower compared to the control group. This implying that, although aspartame did not give satiety effect as high as the sucrose did; yet, aspartame did not induce hunger in the rats either. So, previous studies which suggested that aspartame induced craving was not proven in this research.

As already mentioned above, sucrose consumption was likely to contribute extra calories compared to aspartame. These excessive calories would be stored as fat via a process called lipogenesis. Lipogenesis mechanism involved several regulatory proteins, like sterol regulatory element-binding protein 1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP), as the major transcriptional regulators of lipogenesis in the liver. SREBP isoforms (SREBP-1a, SREBP-1c and SREBP2) and ChREBP were engaged in both transcriptional and post-transcriptional mechanisms. The monomers of sucrose acted as substrates for fatty acid synthesis and influenced the transcriptional regulation of lipogenesis. Fructose induced SREBP-1 and ChREBP, while glucose only induced ChREBP (Moore *et al*., 2014). Studies reported that the expression of *fabp4* was high when lipogenesis took place; since FABP4 would act as lipid transporter which accommodates the fatty acid transfer (Serr *et al*., 2009). Therefore, in this experiment, we hypothesized that sucrose treatment would raise the expression of *fabp4* in adipose tissue.

Our result demonstrated a similar trend; in which *fabp4* gene expression was almost three-times higher compared to the control group. Meanwhile, the expression of the *fabp4* gene in aspartame-treated mice was about half the sucrose group. The higher level of *fabp4* gene expression might induce a higher level of FABP4 protein, which accommodated the fatty acid transport from and into the adipose tissues; and led to stimulation of higher adipocyte lipogenesis. Furthermore, a lower *fabp4* gene expression in aspartame group suggested that aspartame induced lipogenesis in a minor level compared to sucrose. The level of *fabp4* gene expression was also positively correlated with inflammation marker (Terra *et al*., 2014); proposing a higher inflammatory state was induced by sucrose compared to the two other treatments.

The expression of *alt2* gene in liver tissue was also observed. The sucrose group showed the highest expression level, which was almost two-times fold compared to control. Meanwhile, the expression of *alt2* gene in aspartame-treated mice was lower compared to the sucrose. High concentration of sucrose might lead to excessive energy intake and insulin resistance in rats. As described above, insulin resistant state induced the up-regulation of gluconeogenic enzymes, such as ALT (Saltiel and Kahn 2001). This could explain the up-regulation of *alt2* expression in sucrose group. Taken together with the blood glucose value, the data from *alt2* gene expression suggested the beneficial effect of aspartame in maintaining blood glucose level.

Excessive sucrose consumption had been stated to elevate the *tnf-α* gene expression in human blood; of which was associated with metabolic syndrome development (Kempf *et al*., 2007). In our experiment, the

highest *tnf-α* gene expression was observed in sucrose-treated mice; while aspartame seemed to lower *tnf-α* expression compared to sucrose, and even the control group. This result agreed previous study by Sharma *et al*. (2005) which showed aspartame had a possible anti-inflammatory effect based on the paw volume measurement (Sharma *et al*., 2005). In overall, our result confirmed previous data proclaiming that TNF-α concentration would elevate when sucrose consumption was high; yet, sucrose replacement with aspartame would lower the TNF-α concentration (Kempf *et al*., 2007; De Koning *et al*., 2012; Jung and Choi 2014; Choudhary and Devi 2015). However, further studies are needed to understand the detailed mechanism.

In this study, the effect of sucrose and aspartame on *C. coccoides* population was also monitored. The sucrose treatment significantly decreased the *C. coccoides* population at the end of the experimental period; while the variation in aspartame group was not notably different compared to the control group. These results, taken together with the body weight data, agreed on the previous research about *C. coccoides* group; which stated the excessive calorie consumption in obese people could suppress the growth of *C. coccoides* (Schwiertz *et al*., 2009). However, although the number was not significantly different; a slight reduction in *C. coccoides* population was observed in aspartame group. Interestingly, the average body weight values between aspartame and control group in day 40 was actually comparable. These data corresponded to the study from Noble *et al.* (2017) which stated that dietary pattern altered the gut microbiota composition, independently of obesity status.

To our knowledge, there was still limited information about the effects of sucrose and artificial sweetener consumption using metabolic syndrome-related gene expression and gut microbiota population, as the potential biomarkers. Along with the increment of worldwide diabetes prevalence, this might lead to the increase artificial sweetener consumption; these research topics deserve further investigations. Furthermore, although this study implied that the artificial sweetener seemed to offer more beneficial effects compared to sucrose; more studies are still needed to understand the detailed mechanisms.

5. Conclusion

In summary, this study confirmed that aspartame had more beneficial effects in maintaining body weight and blood glucose level, compared to sucrose consumption. Besides, aspartame group also demonstrated a less fat accumulation in liver; and did not show elevation in craving behavior as well. Several markers related with inflammation, lipogenesis and insulin resistance were also lower in aspartame group. Moreover, although it was proven that dietary pattern did alter the gut microbiota population; our data indicated that sucrose consumption might lead to a more severe dysbiosis. Taken together, this study concluded that aspartame might be considered as a safe and favorable as sugar substitute; especially in the era of raise of diabetes prevalence worldwide.

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