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RESEARCH ARTICLE

THE ACUTE EFFECT OF SUGAR- AND ARTIFICIAL- SWEETENER BEVERAGES ON PLASMA GLYCEMIA, PLASMA ANTIOXIDANT STATUS AND SELF-REPORT APPETITE IN HEALTHY MEN

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ABSTRACT

Excessive consumption of sugar-sweetener beverages is a cause of chronic disease such as obesity and diabetes because of the impairment of postprandial state and enhancement of energy intake. Therefore, plant-derived sweetener as substitution of sugar is an alternative sweetener but the evidence in human is limited. We aimed to determine the effects of carbohydrate sugar (high fructose syrup, organic brown rice syrup, honey, and sucrose) or sweetener (stevia leaf extract and aspartame) beverage on postprandial plasma glucose level, plasma antioxidant status, appetite, and *ad libitum* in healthy men. Thirteen healthy men were included in six randomized, crossover study. Each participant received breakfast meal together with either 400 mL of carbohydrate sugar (570 kcal/ serving) or sweetener (406 kcal/ serving) beverage. Visual analogue scale ratings for appetite (fullness, hunger, desire to eat, and satiety) and repeated blood sampling for plasma glycemia and antioxidant status were assessed for 240 min. Then, they were allowed to choose and consume foods at an *ad libitum* as a buffet-like meal at 240 min. We found that BRS-containing beverage was positively associated with postprandial plasma glucose, FRAP and ORAC ($P< 0.05$) compared to other beverages. In addition, it also significantly increased satiety and fullness ($P< 0.05$) compared to others but no significant difference was found for energy intake *ad libitum* meal. However, increased satiety and fullness in healthy subjects was associated with reduced energy intake an *ad libitum* meal. Therefore, decreased energy intake *ad libitum* may decrease risk of weight gain and other chronic disease.

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INTRODUCTION

The prevalence of obesity continues to increase and become public health problems in many countries around the world. In 2014, World Health Organization (WHO) reported that approximately 1.9 billion adults were overweight and over 600 million adults were obese. Excessive body weight can influence quality of life, education, and income potential, and also increase the risk of death (Katzmarzy and Ardern, 2000; Twells *et al*., 2014). Obesity is a complex condition that is caused by several factors, consisting of genetics, epigenetic, eating behaviors, physical inactivity, metabolism, psychosocial influences, and environmental factors (Hu, 2013). Moreover, the fundamental cause of weight gain is an imbalance between energy intake and energy output. Increasing of energy intake has been related to increase energy-dense food consumption that are high in claries, fats, and sugar (Hruby and Hu, 2014). Sugar-sweetened beverages (SSB) or carbohydrate sugar beverages including sucrose- and high fructose syrup-containing beverages have been received an attention on causing of weight gain, and increasing the risk of obesity because they contain high amount of calories and also added sugar (Hu and Malik, 2010).

Moreover, excessive intake of added sugar-sweetened foods and beverages can involve in increased postprandial glucose and insulin levels and likely induce in metabolic and hormone changes that stimulate hunger levels (O'Keefe and Bell, 2007). The consumption of SSB may affect to human appetite regulation, resulting in the selection of human foods after drinking the beverages (Sorensen *et al*., 2003). According to the previous data, sucrose-and high fructose-sweetener beverage and diets not only have the adverse effects on body weight but also are associated with other medical complications including cancer, cardiovascular diseases, and diabetes mellitus

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(Johnson *et al*., 2007; Anton *et al*., 2010). However, beverage-containing artificial sweetener is one of the alternative choice for reduction energy intake. Although artificial sweetener such as aspartame has no effect on energy, it may particularly stimulate appetite and further increase weight gain (Swithers and Davidson, 2008; Blundell and Hill, 1986). Organic brown rice syrup (BRS) is made from Jasmine rice No.105 (*Oryza sativa Linn*.). It is processed by fermenting cooked brown rice with enzymes that break down the rice starch polymers, after that, extracted liquid is evaporated and stored in a sterile container. However, It is a new product and not widespread in Thailand. Recently, it has not been studied in human. Thus, the presentstudy aimed to determine the effects of carbohydrate sugar (high fructose syrup, organic brown rice syrup, honey, and sucrose) or sweetener (stevia leaf extract and aspartame) beverage on postprandial plasma glucose level, plasma antioxidant status, self-reported appetite, and *ad libitum* in healthy men.

MATERIALS AND METHODS

***Study population*:** Participants were thirteen, non-smoking, healthy men (age 18-25 years old, body mass index ranging from 18.5-22.9 kg/m^2, fasting plasma glucose < 100 mg/dL, serum total cholesterol < 200 mg/dL, serum triglyceride < 150 mg/dL). The baseline characteristics of the participants are summarized in Table 1. Participants were excluded for any of the following reasons: (1) history of diabetes, cardiovascular disease, or other chronic illnesses, (2) taking any medication (including anti-diabetic drugs) or dietary supplements that could interfere with the study, and (3) dislike of or allergy to foods/sweeteners (organic brown rice syrup, honey, high fructose syrup, stevia, aspartame, or sucrose)used in test meals. All participants provided written informed consent before study participation. The study protocols and procedures were approved according to the Declaration of Helsinki by Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University

Table 1. Baseline characteristic of thirteen participants

Characteristic	Baseline	Reference[a]
Age , *years*	23.69 ± 0.26	
Weight, *kg*	65.95 ± 2.03	
Height, *cm*	174.62 ± 1.45	
BMI, *kg/m^2*	21.61 ± 0.54	
Plasma glucose, *mg/dL*	82.08 ± 1.79	70 - 100
BUN, *mg/dL*	11.15 ± 0.5	6 - 20
Creatinine, *mg/dL*	0.89 ± 0.03	0.5 – 1.2
Total cholesterol, *mg/dL*	163.15 ± 9.44	120 - 200
Serum triglyceride, *mg/dL*	63.77 ± 6.61	60 - 150
HDL cholesterol, *mg/dL*	45.69 ± 3.35	40 - 100
LDL cholesterol, *mg/dL*	108.46 ± 8.44	< 100
AST, *U/L*	19.15 ± 1.73	< 40
ALT, *U/L*	12 ± 1.17	< 40
ALK. Phosphatase, *U/L*	50.62 ± 3.43	26 - 117

Data are expressed as mean ± S.E.M, n = 13. [a]The normal range blood chemistry has been referred to The Health Sciences Service Unit in faculty of Allied Health Sciences, Chulalongkorn University.

***Test meals*:** The breakfast consisted of sandwiches with white bread (2 slides), tune steak in brine (75 g), margarine (12.5 g), and carbohydrate sugar (high fructose syrup, organic brown rice syrup, honey, and sucrose) or sweetener (stevia leaf extract and aspartame) beverage (400 mL). Amount of carbonate sugar beverages were equated for energy, whereas sweetener beverages were equated for sweetness intensity with sucrose (Table 2). After 4 hours of breakfast, participants were allowed to choose and consume foods *ad libitum* as a buffet-like meal. An *ad libitum* was provided by a portion size of white rice, cooked vegetable, fruits, cooked meat and egg, and milk. The participants were allowed 30 min for lunch and instructed to consume until comfortably full. Energy intake at *ad libitum* was analyzed using INMUCAL (version 3).

Table 2. Composition of the breakfast meal[1]

	BRS	HFS	Honey	Sucrose	SLE	ASP
Amount of sugar or artificial sweetener, *g*	40	40	40	40	0.8	0.2
Total energy, *kcal*	570	570	570	570	406	406
Protein, *g*	21.5	21.5	21.5	21.5	21.5	21.5
Fat, *g*	20	20	20	20	20	20
Carbohydrate, *g*	76	76	76	76	36	36

[1]BRS, organic brown rice syrup; HFS, high fructose syrup; SLE, stevia leaf extract; ASP, aspartame

***Study design*:** The study was a randomized, six cross-over studies with a week wash out period. The test meals were served in a randomized order. The participants were introduced to maintain their usual diet and lifestyle during the study, avoided alcohol for 2 days of the study and intensive physical activity for 12 hours before the study. On test days, they reported to the Laboratory following an overnight fast (10-12 hours). An intravenous (IV) catheter was inserted for collection for venous blood. Blood samples were collected before the test meals (0) and 15, 30, 60, 90, 120, 180, and 240 min after staring to consume the meals. Furthermore, participants rated their score of fullness, hunger, desire to eat, and satiety at the same time points of blood collection and after an *ad libitum*. The rating scale was assessed using visual analogue scales (10-cm horizontal lines with Thai wording anchored at each end). Each end of the VAS line was expressed the most negative or most positive sensation of fullness, hunger, desire to eat or satiety. The participants drew a vertical line on the horizontal line corresponding to their feeling at -15, 30, 60, 90, 120, 180, and 240 min.

***Biochemical analyses*:** Plasma sample for glucose concentration (0-120 min) was collected in sodium fluoride-containing tubes and plasma antioxidant (0-240 min) in EDTA-containing tubes. Plasma samples were centrifuged for 15 min at 3000 rpm at 4^{O}C, and stored at – 80^{O}C until analyzed. Plasma glucose concentration was analyzed using glucose oxidase method (HUMAN GmbH, Germany). Plasma antioxidant status was determined using FRAP (Benzie andStrain, 1996) and ORAC (Wang *et al*., 2011) assay.

***Statistical analysis*:** The data are expressed as mean ± S.E.M. and were analyzed by using the SPSS for window software (version 18.0). Incremental plasma glucose concentrations plasma antioxidant status, and VAS levels were analyzed using repeated measures ANOVA, followed by LSD for multiple comparison. In addition, One-way ANOVA, followed by LSD for multiple comparisons was used for energy intake at *ad*

libitum. *P*-value less than 0.05 was considered statistically significant difference.

RESULTS

Plasma glucose concentration: The effect of different types of carbohydrate sugar and sweetener beverages on the incremental postprandial plasma glucose concentration is shown in Figure 1. After breakfast consumption, incremental plasma glucose concentrations were peaked at 30 minutes, followed by slightly declined afterward. Consumption of carbohydrate sugars increased higher postprandial plasma glucose than sweeteners. Comparing at individual time points, incremental plasma glucose concentrations after consumption of breakfast with aspartame-containing beverage were significantly decreased at 15 minutes compared to BRS (P = 0.031) and sucrose (P = 0.009). At 30 min, incremental plasma glucose concentrations after consumption BRS-containing beverage were significant increased compared to honey, SLE, and aspartame (P = 0.004, 0.005 and 0.006, respectively). Besides, consumption BRS-containing beverage significantly increased incremental plasma glucose at 60 min compared to HFS, SLE, aspartame, honey, and sucrose (P = 0.025, 0.014, 0.022, 0.017 and 0.016, respectively). At 120 min, only consumption sucrose-containing beverage significantly increased plasma glucose compared to honey (P = 0.048).

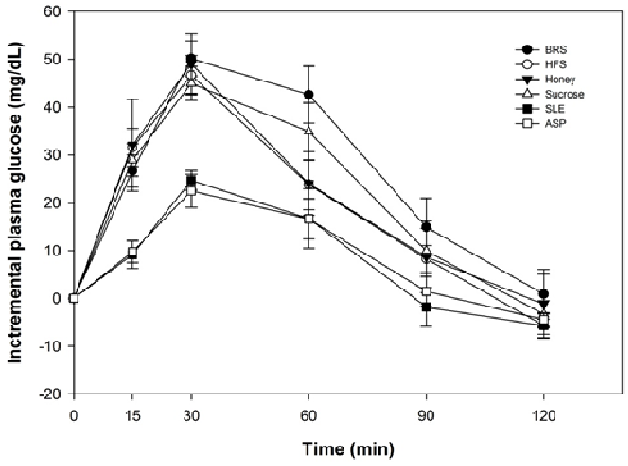

Figure 1. The changes in postprandial plasma glucose concentrations in healthy men after consumption breakfast together with organic brown rice syrup (BRS), high fructose syrup (HFS), honey, sucrose, stevia leaf extract (SLE), and aspartame (ASP). The baseline of plasma glucose concentrations in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 89.94 ± 7.97, 89.60 ± 2.07, 86.13 ± 1.57, 88.88 ± 1.64, 88.35 ± 1.07, and 86.95 ± 0.89 mg/dL, respectively. Data are expressed as mean ± SEM, n = 13.*P*< 0.05

***Postprandial plasma FRAP levels*:** Consumption of BRS-containing beverages with breakfast meal markedly increased incremental postprandial plasma FRAP levels compared to other carbohydrate sugars and sweeteners. After BRS consumption, we observed a significant increase at 15 and 30 min compared to SLE and aspartame (P = 0.047 and 0.012, respectively; Figure 2).

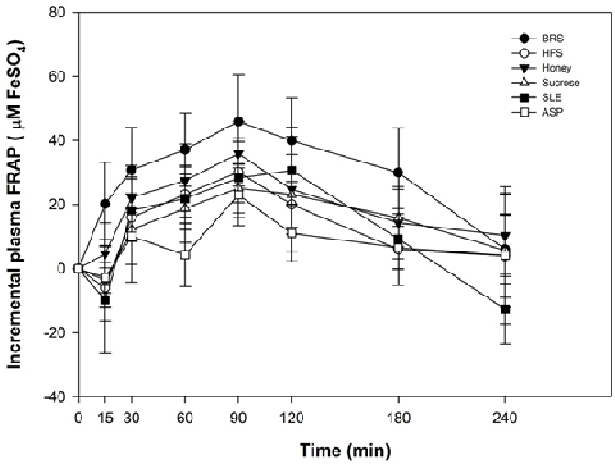

Figure 2. The changes in postprandial plasma FRAP concentrations in healthy men after consumption breakfast together with organic brown rice syrup (BRS), high fructose syrup (HFS), honey, sucrose, stevia leaf extract (SLE), and aspartame (ASP). The baseline of plasma FRAP concentrations in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 1027.85 ± 0.11, 1098.28 ± 0.08, 1116.04 ± 0.14, 1080.13 ± 0.09, 1013.76 ± 0.30, and 1054.73 ± 0.11μM $FeSO_4$, respectively. Data are expressed as mean ± SEM, n = 13. *P*< 0.05

Postprandial plasma ORAC levels: The changes in postprandial plasma ORAC levels are shown in Figure 3.

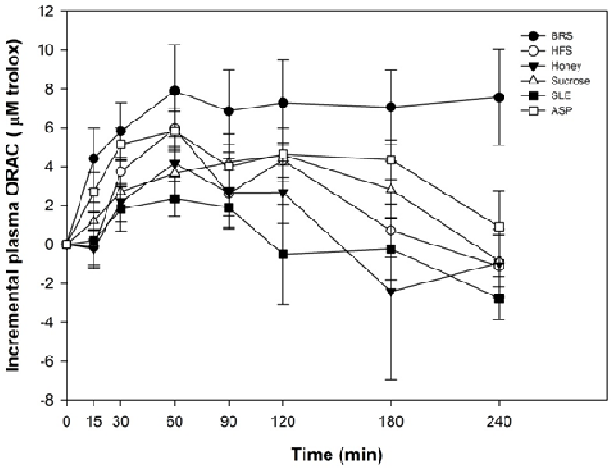

Figure 3. The changes inpostprandial plasma ORAC concentrations in healthy men after consumption breakfast together with organic brown rice syrup (BRS), high fructose syrup (HFS), honey, sucrose, stevia leaf extract (SLE), and aspartame (ASP). The baseline of plasma ORAC concentrations in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 59.15 ± 5.77, 64.61 ± 5.55, 57.42 ± 4.36, 59.77 ± 4.42, 64.48 ± 7.92, and 60.99 ± 4.36μMtrolox, respectively. Data are expressed as mean ± SEM, n = 13. *P*< 0.05

Compared to SLE-containing beverages, consumption of BRS-containing beverages with breakfast meal significantly increased at 15, 30, 60, 90,120, 180, and 240 (P< 0.05). Compared to Honey-containing beverage, incremental plasma ORAC levels were significant increased after consumption of BRS-containing beverages with breakfast meal at 15, 30, 180,

and 240 (P< 0.05). Compared to sucrose-containing beverage, incremental plasma ORAC levels were significant increased after consumption of BRS-containing beverages with breakfast meal at 30, 60, and 240 (P< 0.05). Compared to HFS-containing beverage, incremental plasma ORAC levels were significant increased after consumption of BRS-containing beverages with breakfast meal at 15, and 240 (P< 0.05). Compared to aspartame-containing beverage, incremental plasma ORAC levels were significant increased after consumption of BRS-containing beverages with breakfast meal only at 240 (P< 0.05).

consumption BRS-containing beverage was significant lower compared to honey (at 120 and 180 min; P = 0.022 and 0.038, respectively) and SLE (at 180 min; P = 0.046). Moreover, incremental satiety rating was significant higher after consumption BRS-containing beverage compared to SLE at 180 min (P = 0.036) and after consumption Honey-containing beverage compared to aspartame at 180 min (P = 0.034).

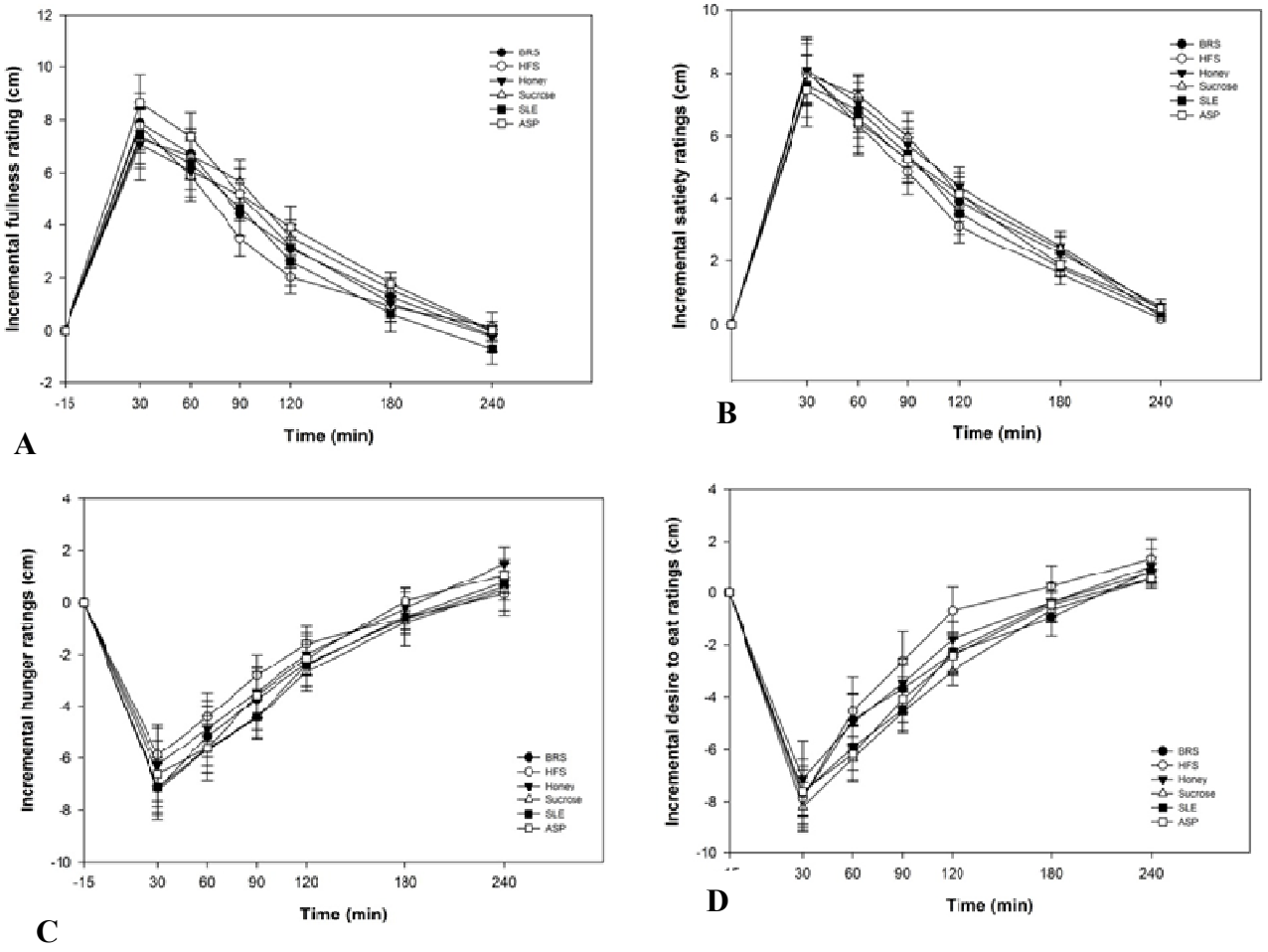

Figure 4. The changes in self-reported appetite in healthy men after consumption breakfast together with organic brown rice syrup (BRS), high fructose syrup (HFS), honey, sucrose, stevia leaf extract (SLE), and aspartame (ASP). *A*; Fullness ratings, the baseline of fullness ratings in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 0.52 ± 0.24, 0.60 ± 0.17, 1.34 ± 0.66, 0.44 ± 0.26, 1.09 ± 0.65, and 0.24 ± 0.19 cm, respectively. *B*; Satiety ratings, the baseline of satiety ratings in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were zero. *C*; Hunger ratings, the baseline of hunger ratings in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 8.05 ± 0.73, 8.75 ± 0.40, 7.75 ± 0.88, 8.80 ± 0.40, 8.55 ± 0.57, and 8.54 ± 0.49 cm, respectively.*D*; Desire to eat ratings, the baseline of desire to eat ratings in the groups received BRS, HFS, honey, sucrose, SLE, and ASP were 8.16 ± 0.70, 8.48 ± 0.78, 7.86 ± 0.86, 9.19 ± 0.32, 8.75 ± 0.38, and 9.06 ± 0.37 cm, respectively. Data are expressed as mean ± SEM, n = 13.*P*< 0.05.

Self-reported appetite: As presented in Figure 4, incremental hunger rating was a significant decrease after consumption BRS-containing beverage with breakfast meal compared to honey-containing beverage at 30, 60, and 90 min (P = 0.049, 0.011, and 0.040, respectively). Incremental fullness rating was significant higher after consumption BRS-containing beverage compared to honey at 30 and 60 min (P = 0.016 and 0.031, respectively). Incremental desire to eat rating after

***Energy intake at ad libitum*:** The amount of energy intake at ad libitum after consumption of BRS (1598.52 ± 236.26 kcal), HFS (1981.66 ± 182.27 kcal), honey (1813.07 ±248.24), sucrose (1916.38 ± 189.11 kcal), SLE (1959.29 ± 213.35 kcal), and aspartame (1527.04 ± 303.37 kcal) were not significant difference. (Figure 5)

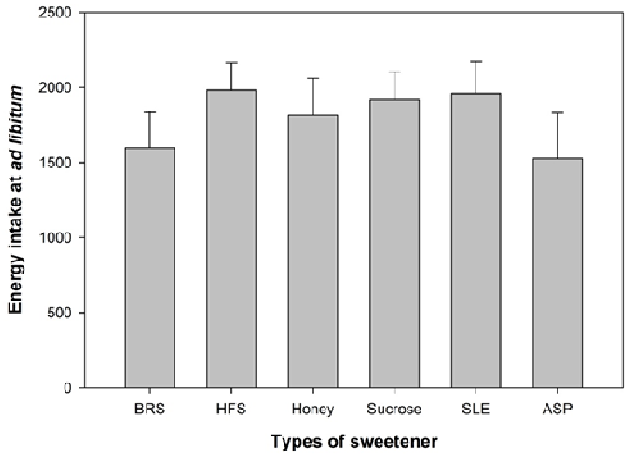

Figure 5. Ad libitum and total energy intakein healthy men after consumption breakfast together with organic brown rice syrup (BRS), high fructose syrup (HFS), honey, sucrose, stevia leaf extract (SLE), and aspartame (ASP). Data are expressed as mean ± SEM, n = 13.*P*< 0.05

DISCUSSION

This is the first study to demonstrate the effect of organic brown rice syrup (BRS) on postprandial plasma glucose, antioxidant status, appetite and *ad libitum* energy intake in healthy men. Brown rice is a source of starch for human diets and it has more nutritional values than white rice. Owing to outer layer of the rice, it also contains carbohydrate, protein, vitamins, minerals, as well as fiber and phytochemical compounds (Tian *et al*., 2004). BRS is sweet syrup made from brown rice that can affect to plasma glucose levels after consumption as same as other carbohydrate sugars. Some study showed that the glycemic index (GI) to indicate the level of postprandial plasma glucose concentration after ingesting brown rice is 66 while GI after ingesting white rice is 72 (Jenkins *et al*., 1981). Based on this study, consumption of carbohydrate sugar-containing beverages with breakfast meals increased postprandial plasma glucose concentrations more than artificial sweetener-containing beverages. There was little difference between brown rice syrup, sucrose, high fructose syrup, and honey because fiber from rice had less effect on postprandial plasma glucose and on effect on glucagon regulation (Madar, 1983; Jenkins *et al*., 1977). Oxidative stress is an imbalanced condition between free radicals and antioxidant defense mechanism. It plays an important role in the development and progression of several human diseases such as diabetes mellitus, cancer, atherosclerosis, and neurodegenerative diseases (Chang and Chuang, 2010; Madamanch *et al*., 2005; Spector, 2000). Consumption of sugar or high GI foods and beverages induced postprandial hyperglycemia can be a cause of oxidative stress, resulting from inducing oxidative stress and reducing antioxidant defenses (Ceriello *et al*., 1998). A previous study found the positive correlation between acute hyperglycemia and cardiovascular risk (Ceriello, 2000). Our results showed that consumption BRS-containing beverage with breakfast meal, compared to other carbohydrate sugar- and sweetener-containing beverages, increased postprandial plasma antioxidant status indicated in plasma FRAP and ORAC. FRAP assay is assessed as antioxidant power that can indicate a putative index of antioxidant, or reducing, potential of biological fluids (Benzie and Strain, 1996). ORAC assay can be used to investigate the total antioxidant activity of biological fluids (Wang *et al*., 2011). In addition, Imam *et al*. (2012) found that brown rice and germinated brown rice improved glycemia and kidney hydroxyl radical scavenging activities, and prevented the deterioration of total antioxidant status in type 2 diabetic rats. They indicated that brown rice and germinated brown rice have high antioxidant potential to scavenge the free radical-induced damage. Concentration of postprandial plasma glucose level has been associated with fullness, hunger, desire to eat, and satiety. After a meal, the highest postprandial plasma glucose increment and its earliest decline seem to be the key for the onset of self-reported appetite (Niwano *et al*., 2009). In present study, consumption of BRS-containing beverage with breakfast markedly increased fullness and satiety ratings, and decreased hunger and desire to eat ratings compared to other carbohydrate sugar- and sweetener-containing beverages. In addition, the finding found that articifical-sweetener beverage have less effect on fullness and hunger rating compared with sugar-sweetened beverages. However, several short-term studies suggested that artificial, nonenergetic sweeteners can increase appetite through cephalic stimulation and further that aspartame may have a paradoxical stimulation effect on appetite (Blundell and Hill, 1986; Rogers and Blundell, 1989; Raben *et al*., 2002). Increased fullness and satiety did not result in a significant reduction in an *ad libitum* energy intake at 4 hours after consumption of BRS-containing beverage. Nevertheless, when the participants ate *ad libitum*, consumption of BRS resulted in slightly lower energy intakes than high fructose syrup, stevia leaf extract, sucrose and honey.

In conclusion, our results indicate that consumption of BRS-containing beverage with breakfast is related to increase antioxidant status and satiety but this effect is not related to plasma glycemic response. However, increased satiety and fullness in healthy subjects was associated with reduced energy intake an *ad libitum* meal. Therefore, decreased energy intake *ad libitum* may decrease risk of weight gain and other chronic disease.

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