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Effect of replacing sucrose by isomaltulose in green tea beverage on postprandial glucose level and antioxidant capacity in healthy subjects

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ABSTRACT

Green tea provides many health benefits such as decreasing postprandial glucose levels, lowering blood cholesterol, and preventing cardiovascular disease. However, the addition of sweeteners such as sucrose in the beverage may reduce antioxidant capacity and diminish health benefits of green tea. Therefore, the aim of this study was to examine the effects of replacing sucrose by isomaltulose, the alternative sweetener, in green tea beverage on postprandial glucose and antioxidant capacity in healthy subjects. Eighteen healthy subjects (male and female aged 18-35 years) were randomized into five groups to drink 400 mL of each beverage containing; 1) 50 g sucrose (SU), 2) 50 g isomaltulose (ISO), 3) green tea (GT), 4) green tea with 50 g sucrose (GTS), or 5) green tea with 50 g isomaltulose (GTI) with crossover-design. Total polyphenols content in GT, GTI, GTS (400 mL) were 481±7.76 mg gallic acid equivalent with no significant difference in ferric ion reducing antioxidant power (FRAP). After 12-h fasting, postprandial plasma glucose and FRAP of subjects were determined at each time point from 0 to 120 min after beverage consumption. We found that the incremental area under the curves of plasma glucose (iAUC-G) of ISO, GT, GTS and GTI were significantly lower than that of SU. Interestingly, iAUC-G of GTS was not different from ISO, whereas iAUC-G of GTI was significantly lower than that of ISO. Furthermore, GTS showed a significantly decrease in the iAUC of plasma FRAP level (iAUC-F), when compared with GT. Nevertheless, iAUC-F of GTI was significantly higher than GTS, but not significantly lower than GT. In conclusion, this study demonstrated that the substituting sucrose with isomaltulose in green tea beverage can help regulate postprandial plasma glucose level and improve antioxidant capacity.

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INTRODUCTION

Green tea (*Camellia sinensis*) has become to be one of the most widely consumed beverage in the world (Wang and Ho 2009). The major bioactive polyphenolic compound in green tea is catechins, especially epigallocatechin gallate (EGCG) (Khan and Mukhtar 2007; Taylor *et al.,* 2005). Previous studies found that tea polyphenols can decrease postprandial plasma glucose level in healthy humans (Bryans *et al.,* 2007; Tsuneki *et al.,* 2004). Beside its antihyperglycemic activity, green tea also has several beneficial health effects such as anti-carcinogenic, anti-inflammatory, anti-oxidative activity, hypocholesterolemic and preventing cardiovascular disease (Chacko *et al.,* 2010).

Nowadays, consumption of sugar-sweetened beverages has been dramatically increased especially adding refined sugars in the green tea beverage (Ng *et al.,* 2012; Won and Richard 2012). Several studies have shown that consumption of sugary beverages contributes to a rise of triglyceride concentration, body weight gain and the accumulation of visceral fat, involving in the progression of metabolic syndromes and obesity (Grundy *et al.,* 2004; Hu and Malik 2010; Stanhope *et al.,* 2009).

Isomaltulose (also known as Palatinose®), one type of alternative sweeteners, is a naturally occurring disaccharide that found in honey, sugarcane and molasses (Siddiqui and Furgala 1967). The sweetening strength of isomaltulose has 0.5 times that of sucrose (Okuno *et al.,* 2010). Taste and caloric value of isomaltulose (4 kcal/g) are similar to those of sucrose (Hamada 2002; Okuno *et al.,* 2010) but digestion rate of isomaltulose in the small intestine is slower than sucrose (Dahlqvist *et al.,* 1963; Kawai *et al.,* 1985). Furthermore, the consumption of isomaltulose (high doses up to 50 g in human) was found to be safe with no gastrointestinal discomfort side effects (Lina *et al*., 2002).

Replacing sucrose with isomaltulose (low-glycemic index of sweetener) is a possible alternative approach to control postprandial glucose and also retains health benefits of green tea. Therefore, this study was conducted to investigate the effects of isomaltulose in green tea beverage on postprandial plasma glucose concentration and antioxidant capacity in healthy subjects.

MATERIALS AND METHODS

Subjects and Study design

Eighteen healthy subjects (aged 18-35 years) were recruited from local community through poster advertisement. Subjects were screened or they were eligible for this study if they had the following criteria: male or female with body mass index (BMI) ranged 18.5-22.9 kg/m^2, % body fat < 20% in male and < 30% in female, waist circumference ≤ 90 cm in male and ≤ 80 cm in female, blood pressure < 140/90 mmHg, fasting plasma glucose level ≤ 100 mg/dL, total cholesterol < 200 mg/dL, LDL-cholesterol < 150 mg/dL, triglyceride < 150 mg/dL, blood creatinine level ranged 0.7-1.4 mg/dL, and alanine aminotransferase (ALT) < 40 IU/L, no history of chronic disease, allergy, and gastrointestinal pathologies such as short bowel syndrome, non-smokers, non-heavy drinkers and not using medications or food supplements that could affect the results of study. This study protocol was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (No.135/56). Before the study was conducted, all subjects provided their written informed consents to participate this study after the study protocol was fully explained.

The randomized, five-visit crossover study was performed. Five types of beverage [SU: 50 g sucrose in 400 mL of water; ISO: 50 g isomaltulose in 400 mL of water; GT: 400 mL of green tea beverage; GTS: 50 g sucrose in 400 mL of green tea beverage; or GTI: 50 g isomaltulose in 400 mL of green tea beverage] were randomly administered orally to the subjects during five experimental visits with 2-wk washout period. During the experimental period, all subjects were instructed not to consume high-antioxidant diet, phenolic-rich foods (e.g. tea, coffee, berries, chocolate, etc.), alcoholic beverage, and not to practice intense physical activity within one week before each visit. Every visit, the subjects were asked to provide their food records and physical activity questionnaire. At each experimental visit, the subjects arrived at the clinical site after 12-hr fasting, and a serving portion (400 mL) of one type of beverages was provided for each subject to completely consume within 5 min from the starting time. Blood sample were collected by intravenous catheter inserted into a forearm vein for evaluation of postprandial plasma glucose and antioxidant capacity at each time points for total 120-min period from the starting point (0, 15, 30, 45, 60, 90 and 120 min). The incremental area under the curves (iAUCs) of plasma glucose and plasma FRAP were determined for the evaluation of the postprandial plasma glucose and antioxidant capacity, respectively.

Preparation of green tea beverages

Green tea leaf from one commercial brand was purchased from the local market. For a serving portion of green tea beverages, 4 grams (two bags) of the pure green tea leaf was infused in 400 mL of boiling water (95 ˚C) for 5 min. GTS was prepared by adding 50 g of sucrose into 400 mL of green tea beverage, whereas GTI was prepared by adding 50 g of isomaltulose (Rajburi Sugar Co., Ltd., Thailand) into 400 mL of green tea beverage.

Determination of total phenolic compounds in green tea beverages

The amount of total polyphenol in green tea beverage determined by Folin-Ciocalteu assay according to Singleton and Rossi (1965) method with some slight modification. Briefly, 10 µL of 10-fold diluted green tea beverages was mixed with 75 µL of 10-fold diluted Folin-Ciocalteu's reagent. After 5 min, 75 µl of 7.5% sodium carbonate solution was added to the mixture. The mixture was incubated for 90 min at room temperature and then measured the absorbance at the wavelength 725 nm. Total polyphenols contents were calculated by standard curve of gallic acid.

FRAP assay (ferric ion reducing antioxidant power or ferric reducing ability of plasma)

FRAP assay was performed according to the method described by Benzie and Strain (1996) with some slight modification. Briefly, the FRAP reagent was prepared freshly by mixing 0.3 M sodium acetate buffer solution (pH 3.6) with a solution containing 10 mM 2,4,6- tripyridyl-1-5-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ solution at the ratio of 10:1:1 (V/V/V), respectively. FRAP assay for

the beverages and the plasma were performed by mixing 10 µL of 10-fold diluted of each beverage or 10 µL of plasma with 90 µL of FRAP reagent, respectively. The FRAP mixture were incubated for 30 min at 37 °C in dark, and measured the absorbance at the wavelength 595 nm by spectrophotometer. The antioxidant capacity of samples was calculated by standard curve of $FeSO_4.7H_2O$. Each sample was determined for five replicates (n=5).

Statistical analyses

The data are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA), followed by the LSD post-hoc test, was performed to assess the differences between FRAP values in each beverage. Data at each time point and iAUCs were analyzed using a repeated measurement ANOVA, followed by the LSD post-hoc for comparisons between groups. For all the statistical tests, significant difference was considered at *p-values* < 0.05.

RESULTS AND DISCUSSION

***In vitro* study**

Total phenolic content

Total phenolic content of green tea beverages in this present study contained 481±7.76 mg gallic acid equivalents (GAE) per one serving portion (400 mL).

Antioxidant capacity in green tea beverages

The FRAP values (antioxidant capacity) of unsweetened green tea beverage (GT), sucrose-sweetened green tea beverage (GTS) and isomaltulose-sweetened green tea beverage (GTI) were 13.15±1.40, 13.09±1.56 and 13.35±1.47 mmol Fe^{2+}/L, respectively with no significant differences among beverages.

Table 1 Baseline characteristics of the subjects in this study

	Mean ± SEM
Age (years)	23.50 ± 0.69
Weight (kg)	59.36 ± 1.84
BMI (kg/m2)	21.03 ± 0.38
% Body fat	Males: 13.58 ± 1.10
	Females: 22.56 ± 1.36
Waist circumference (cm)	Males: 80.00 ± 2.25
	Females: 69.94 ± 2.40
Systolic blood pressure (mmHg)	115.31 ± 1.97
Diastolic blood pressure (mmHg)	71.92 ± 2.34
Fasting glucose (mg/dL)	81.46 ± 2.29
Total cholesterol (mg/dL)	186.54 ± 3.17
LDL-cholesterol (mg/dL)	119.00 ± 5.16
Triglyceride (mg/dL)	76.54 ± 6.32
Creatinine (mg/dL)	0.98 ± 0.03
Alanine aminotransferase, ALT (IU/L)	10.76 ± 1.30

Results are expressed as the mean ± standard error of the mean (SEM), n = 15 (7 males and 8 females)

Human study

Eighteen healthy subjects were recruited to this study. Three subjects dropped out of the study after first week because of personal reasons. Fifteen subjects completed the study (7 males and 8 females). The baseline characteristics of all subjects are shown in Table 1. According to the data from food records and physical activity questionnaire, all subjects had no change on food and physical behavior during the experimental period. In addition, no gastrointestinal complaints from any provided beverages were reported.

Postprandial glycemic response

The incremental area under the curves (iAUCs) of postprandial plasma glucose level [iAUCs-G] after consumption of all beverages are shown in Table 2. According to this study, it demonstrated that the consumption of isomaltulose beverage caused the lower iAUCs of postprandial plasma glucose, compared with sucrose beverage (iAUCs-G of ISO vs. SU, *p-value < 0.001*). In several studies, isomaltulose was also found to decrease postprandial glycemic responses after intake (Arai *et al.,* 2007; Konig *et al.,* 2012). A randomized crossover study in healthy men found that the consumption of isomaltulose-based liquid diet could reduce the postprandial plasma glucose compared to the control liquid diet containing dextrin and sucrose (Arai *et al.,* 2007). The possible mechanism of isomaltulose for reduction of postprandial blood glucose concentration may be due to its slow digestion and absorption rate. The digestion rate of isomaltulose by the homogenate of human intestinal mucosa was shown to be only one-fourth that of sucrose (Dahlqvist *et al.,* 1963). In addition, the absorption rate of isomaltulose was also found to be slower than that of sucrose (Kawai *et al.,* 1985). Consequently, these may lead to a decrease on postprandial plasma glucose concentration.

Table 2 The incremental area under the curves of plasma glucose level (iAUCs-G) and iAUCs of FRAP (iAUCs-F) after the consumption of five different beverages containing: sucrose (SU); isomaltulose (ISO); green tea (GT); sucrose in green tea (GTS); or isomaltulose in green tea (GTI) for 2 hours.

Beverages	Parameters	
	iAUCs-G (mg×min/dL)	iAUCs-F (µM×min)
SU	3095.5±234.7^{a}	-920.1±311.9^{a}
ISO	1741.2±134.3^{b}	-623.1±291.1^{a}
GT	-80.1±139.8^{c}	4087.6±419.3^{b}
GTS	1752.3±231.8^{b,d}	2437.2±418.2^{c}
GTI	1385.8±127.9^{d,e}	3412.2±398.2^{b,d}

Data are expressed as mean ± SEM, n=15.

$^{a, b, c, d, e}$ Different superscript letters indicate significant difference of mean values between beverages in the same parameter *(p-value <0.05)*.

Moreover, the present study also demonstrated that the consumption of green tea together with sucrose led to reduction of postprandial plasma glucose, compared with sucrose (iAUCs-G of GTS vs. SU, *P-value =0.001*). Green tea contains high amount of phenolic compounds, especially flavonoids and catechins; mainly epigallocatechin-3-gallate (EGCG) (Khan and Mukhtar 2007; Taylor *et al.,* 2005). Several *in vitro* studies found that polyphenols can inhibit intestinal glucose transport SGLT-1 and GLUT-2 in the Caco-2 intestinal cell (Farrell *et al.,* 2013; Kwon *et al.,* 2007; Shimizu *et al.,* 2000), and inhibit alpha-glucosidase (Koh *et al.,* 2010). These inhibitory activities of polyphenols may lead to decreasing on the digestion and absorption

of monosaccharide, and consequently lowering postprandial plasma glucose concentration. When isomaltulose was combined in green tea beverage, it showed greater reduction of postprandial plasma glucose than when isomaltulose was consumed alone (iAUCs-G of GTI vs. ISO, *p-value = 0.027*). This result was confirmed by our *in vitro* study (data not shown) that found green tea could inhibit the isomaltase activity, which may result in delaying digestion of isomaltulose. In addition, the replacing of sucrose by isomaltulose in green tea beverage (GTI) showed the significantly higher reduction of postprandial plasma glucose concentration at some time points (15, 30 and 45 min) compared with sucrose-sweetened green tea (GTS) [data not shown].

Postprandial plasma antioxidant capacity

The incremental area under the curves (iAUCs) of postprandial plasma antioxidant status (FRAP level) [iAUCs-F] after consumption of all beverages are shown in Table 2. The present results demonstrated that the consumption of green tea increased plasma FRAP level which was consistent with previous studies (Benzie *et al.,* 1999; Leenen *et al.,* 2000; Pecorari *et al.,* 2010). This could be explained by high polyphenols, especially flavonoids (e.g. catechins) in green tea which is positively correlated with anti-oxidative activities (Frei and Higdon 2003).

Interestingly, the FRAP level was significantly decreased after the consumption of sucrose-added green tea, compared with green tea alone (iAUCs-F of GTS vs. GT, *p-value < 0.001*). However, there was no significant difference of FRAP level after the consumption of isomaltulose-added green tea vs. green tea alone (iAUCs-F of GTI vs. GT, *p-value =0.180*). On the other hand, the replacing of sucrose by isomaltulose in green tea (GTI) showed higher FRAP level than sucrose-added green tea (GTS) (*p-value = 0.032*). The underlying mechanism how refined sugar attenuates the antioxidant capacity of green tea that found in this study remains unclear. It is postulated that polyphenols and flavonoids in green tea may compete with glucose for the absorption at the same transporter (sodium-dependent glucose transporters, SGLT-1) (Walgren *et al.,* 2000). When sucrose was replaced with isomaltulose, it may result in the reduction of glucose absorption as a consequence the competitive absorption between glucose and polyphenol may also decline. This could be probably explained the improvement of depleting antioxidant status when sucrose was replaced with isomaltulose in green tea.

CONCLUSION

This study demonstrated that green tea could suppress postprandial plasma glucose. Moreover, when isomaltulose was used to replace for sucrose in green tea beverage, it showed a higher reduction of postprandial plasma glucose than that of green tea containing sucrose. In addition, the consumption of green tea increased total antioxidant status whereas sucrose decreased the antioxidant capacity of green tea. Interestingly, isomaltulose increased the antioxidant status when used for sucrose substituting in green tea beverage. Therefore, the replacing sucrose by isomaltulose improves postprandial plasma glucose and increases total antioxidant status which it may be alternative sweeteners for beverage manufacturers worldwide in response to perceived consumer demand.

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