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The effect of *glutathione S-transferase M1* and *T1* polymorphisms on blood pressure, blood glucose, and lipid profiles following the supplementation of kale (*Brassica oleracea acephala*) juice in South Korean subclinical hypertensive patients

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BACKGROUND/OBJECTIVES: Glutathione S-transferase (GST) forms a multigene family of phase II detoxification enzymes which are involved in the detoxification of reactive oxygen species. This study examines whether daily supplementation of kale juice can modulate blood pressure (BP), levels of lipid profiles, and blood glucose, and whether this modulation could be affected by the *GSTM1* and *GSTT1* polymorphisms.
SUBJECTS/METHODS: 84 subclinical hypertensive patients showing systolic BP over 130 mmHg or diastolic BP over 85 mmHg received 300 ml/day of kale juice for 6 weeks, and blood samples were collected on 0-week and 6-week in order to evaluate plasma lipid profiles (total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol) and blood glucose.
RESULTS: Systolic and diastolic blood pressure was significantly decreased in all patients regardless of their *GSTM1* or *GSTT1* polymorphisms after kale juice supplementation. Blood glucose level was decreased only in the *GSTM1*-present genotype, and plasma lipid profiles showed no difference in both the *GSTM1*-null and *GSTM1*-present genotypes. In the case of *GSTT1*, on the other hand, plasma HDL-C was increased and LDL-C was decreased only in the *GSTT1*-present type, while blood glucose was decreased only in the *GSTT1*-null genotype.
CONCLUSIONS: These findings suggest that the supplementation of kale juice affected blood pressure, lipid profiles, and blood glucose in subclinical hypertensive patients depending on their *GST* genetic polymorphisms, and the improvement of lipid profiles was mainly greater in the *GSTT1*-present genotype and the decrease of blood glucose was greater in the *GSTM1*-present or *GSTT1*-null genotypes.

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INTRODUCTION

Glutathione S-transferases (GSTs), a family of phase II enzymes found in all eukaryotic species, play a critical role in detoxifying both naturally occurring and xenobiotic compounds, including carcinogens, environmental toxins, and reactive oxygen species, by catalyzing the transfer and conjugation of glutathione [1]. Eight classes of mammalian cytosolic GSTs are currently recognized, designated as alpha (A), mu (M), kappa (K), omega (O), pi (P), sigma (S), theta (T), and zeta (Z) [2]. Among them, both *GSTM1* and *GSTT1* are known to be polymorphic in humans and both of them have null alleles resulting from gene deletion [3]. The null genotypes (homozygous for the non-functional allele) of *GSTM1* and *GSTT1* have a decreased capability of detoxifying some carcinogens. Also, *GST* genetic polymorphisms imply variations in enzyme activities that can result in oxidative stress susceptibility through alterations in GSH metabolism [4]. Because of the role of GSTs in detoxifying xenobiotics and the products of oxidative stress, the effect of *GST* deletion has been investigated for numerous conditions. Meta-analyses have indicated that the deletion of either *GSTM1* or *GSTT1* is associated with a significant increased risk of coronary heart disease [5], and several forms of cancer [6-8]. Several epidemiological studies indicated that cancer and cardiovascular diseases share common risk factors [9].

Cruciferous vegetables are widely consumed in people's diets. These vegetables include kale, as well as broccoli, cauliflower, radish, Brussels sprouts, watercress, and cabbage and are

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consumed either fresh (salads), cooked, or in vegetable juices. Kale, classified into the *Brassica oleracea* species (*Brassica oleracea acephala),* is one of the most popular cruciferous vegetable consumed in South Korea. Besides nutritional components, these vegetables are also rich in health beneficial secondary metabolites, which include sulfur containing glucosinolates, flavonoids, anthocyanins, coumarins, carotenoids, antioxidant enzymes, terpenes, and other minor compounds [10]. Glucosinolates upon hydrolysis form biologically active compounds such as indoles and isothiocyanates (ITC) [11]. ITC are potentially anticarcinogenic phytochemicals formed from the metabolism of glucosinolates and are found in cruciferous vegetables as well as a number of other foods [12]. ITC are both substrates for and inducers of glutathione S-transferase (GST) phase II metabolizing enzymes involved in carcinogen detoxification as well as effectors of phase I pathways [13]. A few human intervention trials have evaluated the ability of the *GST* genotype to modulate the response to cruciferous vegetable intake on biomarkers [13]. It has been suggested that the capacity of a moderate intake of watercress [14] or cruciferous vegetable [15] to induce detoxification is dependent in part on the *GSTM1* genotype. In the same manner, *GSTM1* genotypes have a significant effect on the metabolism of sulforaphane derived from glucosinolate broccoli, and this difference in metabolism may explain the greater protection that *GSTM1*-positive persons gain from consuming broccoli [16].

Hypertensive patients who may be genetically impaired in their ability to handle oxidative stress, by virtue of deletion of the *GSTM1* gene, are more susceptible to the impact of ROS exposure. Therefore, supplementation with antioxidants might compensate for this genetic susceptibility [2]. Recently, it has been reported that regular meals supplemented with kale juice could exhibit favorable influence on serum lipid profiles and antioxidant systems, and hence contribute to reduce the risks of coronary artery disease in male subjects with hyperlipidemia [17]. However, little is known about the potential effects of kale juice on human health, especially concerning the effect of *GST* polymorphisms on any health benefits that kale juice may provide. Therefore, the present study was undertaken to examine whether daily supplementation of kale juice modulates the blood pressure (BP), blood glucose, and levels of lipid profiles in subclinical hypertensive subjects, and whether this modulation could be affected by the *GSTM1* and *GSTT1* polymorphisms.

SUBJECTS AND METHODS

Participants and dietary intake assessment

This research was carried out for 6 weeks on male borderline isolated subclinical hypertensive patients [systolic BP (SBP) > 130 mmHg or diastolic BP (DBP) > 85 mmHg] who had never been treated for hypertension. Participants included members of the staff of Hannam University, government employees in Daejeon, and volunteers among the participants of a previous study [18]. The study was conducted according to a study protocol that passed the standards of the Institutional Review Board at Hannam University, South Korea (approval code: 2012-04k). Informed written consent including purpose, nature, and potential risks was obtained from all subjects. Information regarding individual characteristics, health status, and lifestyle factors including smoking, alcohol, and exercise were collected by questionnaires. Participants suffering from poor health or who were consuming prescribed medications were excluded from the study. The participants' weight, height, and waist and hip circumferences were measured using standard protocols. These measurements were then used for calculating body mass index (BMI) and waist-hip ratio (WHR). Body fat was measured by Bio-electrical Impedance Fatness Analyzer (Inbody 520, Biospace, South Korea). Blood pressure was the mean of three measurements in a seated position, using an automatic BP monitor (Watch BP Home, Microlife, Switzerland) on weeks 0, and 6. Dietary information provided by the participants was recorded using 24-hour recall and a food frequency questionnaire. Total nutrient intake was estimated using the CAN Pro 3.0 (Nutrition Information Committee, Korean Nutrition Society), and evaluated using the Korean Dietary Reference Intakes (2010) [19].

Kale juice supplementation

Two bottles (total 300 ml) of 100% pure kale juice (Pulmuone, South Korea) were freshly delivered and supplemented every day to the participants for 6 weeks. The participants were instructed to consume the kale juice daily and record their intake on a daily log. A depletion period restricting the consumption of kale products and fruits and vegetables with high antioxidant nutrients was established 2 weeks prior to the supplementation of kale juice. This period was intended to ensure antioxidant vitamin status within similar levels at baseline. Also, they were reminded and advised over the phone individually to refrain from consuming foods which may affect the antioxidant index during the experimental period.

Blood analysis

Blood was drawn from the participants at the beginning (0 week) and after 6 weeks of the supplementation of kale juice. Blood samples drawn from the survey participants after a minimum 12 hours overnight fasting were put in a 10 ml heparinated sterile tube (Vacutainer, Becton Dickinson, U.S.A.), and brought to the laboratory. Some of the blood (500 uL) was put in microcentrifuge tubes separately for the analysis of *GSTM1* and *GSTT1* polymorphisms. The remaining blood was centrifuged at 1,000 rpm for 15 minutes to collect PRP (platelet-rich plasma), and then it was centrifuged again at 3,000 rpm for 30 minutes to collect PDP (platelet-deficient plasma) following the separation of blood plasma. The blood plasma was divided for each analysis item and kept at -80°C in a freezer until use. The blood glucose level was measured immediately after drawing the blood using a testing device (GLUCOTREND, Roche Diagnostics GmbH, Germany).

Analysis of GSTM1 and GSTT1 genetic polymorphisms

The *GSTM1* and *GSTT1* genotypes were determined as previously described without any modification [20,21]. Briefly, the *β-globin* primer pair (sense: 5'-CAACTTCATCCACGTTCACC-3' and antisense: 5'-GAAGAGCCAAGGACAGGTAC-3'), which had not been deleted, was used as an internal control. The primers for amplifying the *GSTM1* gene were (sense) 5'-CGCCATCTTGTGCTACATTGGCCGTC-3'

and (antisense) 5'-TTCTGGATTGTAGCAGATCA-3'. The primers for the *GSTT1* gene were (sense) 5'-TTCCTTACTGGTCCTCACATCTC-3' and (antisense) 5'-TCACCGGATCATGGCCAGCA-3'. The polymerase chain reaction (PCR) was performed in a 50 μL reaction mix containing 0.1 μg DNA, 5 mM deoxyribonucleoside triphosphates, 30 pmol of each primer, 30 mM MgCl2, and 0.5 U thermos table Taq DNA polymerase. After 2 minutes of pretreatment at 95˚C the reaction was subjected to 30 cycles of amplification at 94˚C for 1 minute, annealing at 64˚C for 1 minute, and 1 minute of elongation at 72˚C. A final extension step of 7 min at 72˚C terminated the process. The products of the PCR amplification were separated by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide (0.1 ug/mL). The internal standard fragment amplified from *β-globin* gene was 268 bp. A 215 bp fragment was amplified for the *GSTM1* gene, and a 480 bp fragment was obtained for the *GSTT1* gene. The absence of amplified product was consistent with the null genotypes. All reagents and chemicals for the genetic polymorphism were purchased from Bioneer (South Korea).

Determination of plasma lipid profiles

Plasma lipid, total cholesterol, triglyceride, and HDL-Cholesterol contents were analyzed using a semi-auto biochemistry analyzer (Shining Sun A6, Beijing Shining Sun Technology, China) with 1 ml of enzyme solution from a kit reagent produced by STANBIO Laboratory (U.S.A.) and reactivated for 5 minutes in a water bath under 37˚C. LDL-Cholesterol was calculated using the Friedewald equation [22].

LDL-Cholesterol
= Total cholesterol - HDL-Cholesterol - (Triglyceride/5) (mg/dl)

Statistical analysis

All the data were entered into a Microsoft Excel database and the statistical tasks were performed with the SPSS-PC+ statistics package (version 20.0). Mean and standard error of the mean (S.E) were obtained for each item, and the mean difference among the 4 types of polymorphism of the 2 groups (*GSTM1* null and present types; *GSTT1* null and present types) was verified by independent t-test, and all statistical significances evaluated at the level of $\alpha = 0.05$. The significance of the mean comparison before and 6 weeks after supplementations of kale juice was tested by paired t-test. Also, a chi-square test was performed on the frequency of smoking habits, existence of alcohol intake, and exercise habits.

RESULTS

General characteristics of the participants

General characteristics of the participants are shown in Table 1. All of the participants in this study were aged 20-57 years, and the average age was 38 years. After 6 weeks of kale juice supplementation, the percentage body fat of the participants was decreased significantly, regardless of *GSTM1* or *GSTT1* genotype. The WHR of the participants was decreased only in *GSTM1*-null genotype after 6 weeks of supplementation. Participants' smoking habits indicated a daily average of 13.9 ± 1.4 cigarettes with 11.1 ± 2.1 pack years. The drinking habits of the participants showed that the percentage of drinkers was 81.0% among all participants and the total alcohol consumption was 56.8 ± 5.1 mL/day. The portion of participants who regularly exercised was 79.8% and the average exercise time was 30.3 ± 2.9 min/day.

Nutrient intake of the participants

Nutrient intake before kale juice supplementation (0 week) and after 6 weeks of supplementation was surveyed using the 24-hour recall method to find out changes in dietary intake

Table 1. General characteristics of the subjects according to *GST* genotypes

Variables	*GSTM1* genotype				*GSTT1* genotype			
	null (n = 49)		present (n = 35)		null (n = 45)		present (n = 39)	
	0 week	6 weeks	0 week	6 weeks	0 week	6 weeks	0 week	6 weeks
Age (years)	38.5 ± 1.5[1)]		37.9 ± 1.8		38.1 ± 1.5		38.4 ± 1.8	
Height (cm)	171.0 ± 0.8		173.1 ± 1.2		171.8 ± 0.9		171.9 ± 1.1	
Body weight (kg)	73.2 ± 1.5	73.0 ± 1.5	78.0 ± 1.7	77.6 ± 1.7	76.1 ± 1.6	75.8 ± 1.6	74.2 ± 1.7	73.9 ± 1.7
BMI (kg/m^2)	25.0 ± 0.4	24.9 ± 0.4	26.0 ± 0.5	25.9 ± 0.5	25.7 ± 0.5	25.6 ± 0.5	25.1 ± 0.5	25.0 ± 0.5
Waist-hip ratio (WHR)	0.881 ± 0.006	0.877 ± 0.006*	0.898 ± 0.006	0.894 ± 0.006	0.892 ± 0.006	0.889 ± 0.006	0.883 ± 0.006	0.879 ± 0.006
Body fat (%)	23.4 ± 0.9	22.7 ± 0.9**	24.7 ± 1.3	23.9 ± 1.3**	25.1 ± 1.1	24.4 ± 1.1**	22.7 ± 0.9	21.9 ± 0.9**
Smoking habits								
Smokers (n (%))	20 (40.8%)		15 (42.9%)		17 (37.8%)		18 (46.2%)	
Cigarettes/day	11.5 ± 1.3		17.3 ± 2.7		10.8 ± 1.1		16.9 ± 2.4	
Smoking years	13.9 ± 2.0		14.3 ± 2.1		11.8 ± 1.5		16.2 ± 2.3	
Pack-years[2)]	7.7 ± 1.5		15.3 ± 4.3		7.0 ± 1.2		14.7 ± 3.8	
Drinking habits								
No. of drinker (n (%))	41 (83.7%)		27 (77.1%)		33 (73.3%)		35 (89.7%)	
Drinks (ml/day)	57.8 ± 6.7		55.4 ± 8.2		52.1 ± 8.2		61.3 ± 6.4	
Exercise habits								
Regular exercisers (n (%))	39 (79.6%)		28 (80.0%)		37 (82.2%)		30 (76.9%)	
Exercise time (min/day)	29.7 ± 3.6		31.0 ± 4.7		28.8 ± 3.5		32.0 ± 4.8	

[1)] All values are means ± SE
[2)] Pack-years: (Cigarettes smoked/day × years smoked)/20
* $P < 0.05$, ** $P < 0.01$

Table 2. Dietary intake of nutrients and cholesterol before and after six weeks of kale juice supplementation

Variables	0 week (n = 84)	6 weeks (n = 84)	*P*-value[2]
Energy (kcal)	1681.4 ± 77.9[1]	1669.7 ± 62.7	NS[3]
Protein (g)	73.4 ± 8.0	65.9 ± 3.0	NS
Fat (g)	49.1 ± 3.4	44.1 ± 2.5	NS
Carbohydrate (g)	230.4 ± 9.1	241.1 ± 9.4	NS
Fiber (g)	17.2 ± 0.9	18.8 ± 0.9	NS
Calcium (mg)	483.1 ± 48.6	449.1 ± 25.4	NS
Iron (mg)	14.6 ± 0.9	13.7 ± 0.8	NS
Sodium (mg)	3911.0 ± 221.0	3985.3 ± 209.4	NS
Potassium (mg)	2512.6 ± 154.9	2545.4 ± 116.6	NS
Vitamin A (μg RE)	854.9 ± 66.1	919.6 ± 68.2	NS
Retinol (μg)	134.4 ± 16.8	137.2 ± 27.8	NS
β-carotene (μg)	4327.9 ± 377.2	4702.2 ± 387.6	NS
Vitamin B1 (mg)	1.3 ± 0.1	1.2 ± 0.1	NS
Vitamin B2 (mg)	1.1 ± 0.1	1.1 ± 0.1	NS
Vitamin B6 (mg)	1.3 ± 0.1	1.5 ± 0.1	NS
Niacin (mg)	14.1 ± 0.8	15.5 ± 0.8	NS
Folate (μg)	438.9 ± 26.1	447.2 ± 21.9	NS
Vitamin C (mg)	75.7 ± 5.6	82.2 ± 5.3	NS
Vitamin E (mg α-TE)	14.4 ± 1.2	14.1 ± 0.9	NS
Cholesterol (mg)	333.3 ± 39.2	263.5 ± 21.9	NS

[1] All values are means ± SE
[2] Statistical significance between baseline (0 wk) and after six weeks of supplementation within each group by paired t-test, $P < 0.05$.
[3] NS: Not significant.

during the 6 weeks of kale juice supplementation. The result is shown in Table 2. The nutrient intake showed that the participants maintained their usual intake in energy, protein, fat, carbohydrate, calcium, vitamin C, vitamin E, vitamin A, and retinol before and after kale juice supplementation (Table 2). The nutrient contents of 300 ml of kale juice are: 30 kcal of energy, 4 g of carbohydrate, 4 g of sugars, 4 g of protein, 1253.4 ug RE of vitamin A, 262 mg of vitamin C, 1 mg of iron, 334.6 mg of calcium, and 230 mg of total polyphenols.

GSTM1 and GSTT1 polymorphism frequency analysis

Among 84 participants, the *GSTM1*-null genotype was found in 49 (58.3%) and the *GSTM1*- present genotype in 35 (41.7%) (Table 3), The *GSTT1*-null genotype was found in 45 participants (53.6%) and the *GSTT1*-present genotype in 39 (46.4%). The number of participants who had both the *GSTM1* and *GSTT1* present genotypes was 13 (15.5%), and those who had either one of the present genotypes was 48 (57.1%), and who had neither of the present genotypes was 23 (27.4%).

Changes in blood pressure

The changes in blood pressure of the participants after kale juice supplementation showed that the systolic and the diastolic pressures were decreased in the participants when divided according to *GST* polymorphism (Table 4). Systolic pressure was decreased by 5.0%, and diastolic pressure by 3.7% in the *GSTM1*-null genotype, and also the systolic and diastolic pressures were decreased by 5.2% and 3.1%, respectively, in the *GSTM1*-present genotype. In the case of *GSTT1*, on the other hand, the systolic

Table 3. Frequency of *GSTM1* and *GSTT1* genotypes in the participants

GST genotypes	Frequency (%)
GSTM1	
Null	49 (58.3%)
Present	35 (41.7%)
GSTT1	
Null	45 (53.6%)
Present	39 (46.4%)
GSTM1/GSTT1	
Both null	23 (27.4%)
Null/present	48 (57.1%)
Both present	13 (15.5%)

Table 4. Change of blood pressure of the subjects by *GSTM1* and *GSTT1* genotype after kale juice supplementation

Variables	*GSTM1* genotype		*GSTT1* genotype	
	null (n = 49)	present (n = 35)	null (n = 45)	present (n = 39)
SBP (mm Hg)				
0 week	136.7 ± 1.5[1]	140.4 ± 2.2	136.5 ± 1.5	140.4 ± 2.2
6 weeks	129.8 ± 1.7***	133.1 ± 1.9***	130.5 ± 1.7***	132.0 ± 2.0***
Difference	-6.9 (5.0%)	-7.3 (5.2%)	-6.0 (4.4%)	-8.4 (6.0%)
P-value[2]	0.000	0.000	0.000	0.000
DBP (mm Hg)				
0 week	91.8 ± 1.2	91.2 ± 1.4	91.1 ± 0.9	93.1 ± 1.7
6 weeks	88.4 ± 1.3**	88.4 ± 1.5**	87.8 ± 1.1**	89.2 ± 1.7**
Difference (%)	-3.4 (3.7%)	-2.8 (3.1%)	-3.3 (3.6%)	-3.9 (4.2%)
P-value	0.002	0.001	0.001	0.002

[1] All values are means ± SE
[2] Statistical significance between baseline (0 wk) and after six weeks of supplementation within each group by paired t-test, $P < 0.05$
** $P < 0.01$, *** $P < 0.001$

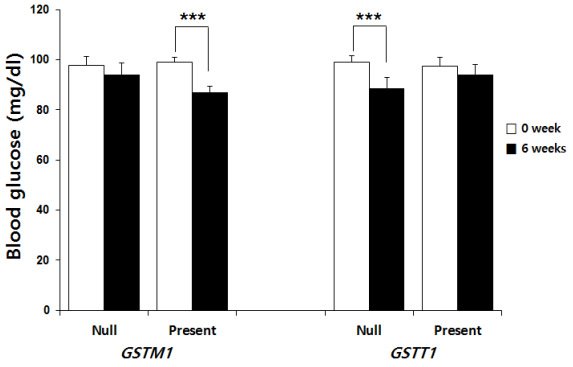

Fig. 1. Change of blood glucose of the subjects by *GSTM1* and *GSTT1* genotype after kale juice supplementation. All values are means ± SE, * Statistical significance between baseline (0 wk) and after six weeks of supplementation within each group by paired t-test, $P < 0.05$, *** $P < 0.001$

and diastolic pressures were significantly decreased by 4.4% and 3.6% in the *GSTT1*-null genotype, and 6.0% and 4.2% in the *GSTT1*-present genotype, respectively, after kale juice supplementation.

Comparison of blood glucose and plasma lipid levels

The results of blood glucose, plasma total cholesterol (TC), LDL-C, HDL-C, and triglyceride (TG), before and after 6 weeks of kale juice supplementation are shown in Fig. 1 and 2. The

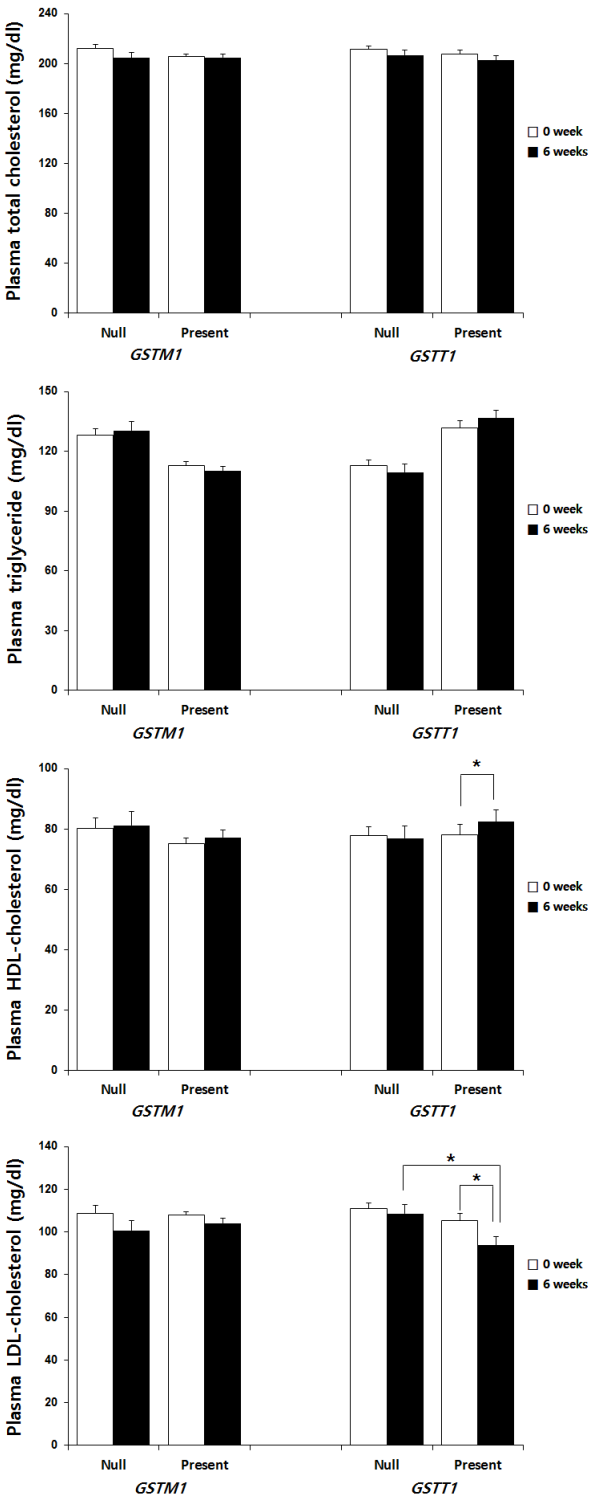

Fig. 2. Change of plasma lipid levels of the subjects by *GSTM1* and *GSTT1* genotype after kale juice supplementation. All values are means ± SE, * Statistical significance between baseline (0 wk) and after six weeks of supplementation within each group by paired t-test, $P < 0.05$. * $P < 0.05$

blood glucose levels of the participants were significantly decreased in the *GSTM1*-present and *GSTT1*-null genotypes (Fig. 1). The plasma TC and TG levels of the participants were not changed after kale juice supplementation regardless of *GST* polymorphisms. The plasma HDL-C level was significantly increased, and the plasma LDL-C level was significantly decreased after kale juice supplementation in the *GSTT1*-present genotype, but those levels in the *GSTM1*-null, *GSTM1*-present, and *GSTT1*-null genotypes were not significantly changed after kale juice supplementation (Fig. 2).

DISCUSSION

Lack of consistent GSTM1 and GSTT1 modulation of cruciferous vegetable intervention studies is probably due to multiple factors, including tissue-specific responses, differences in end points measured, and the type and amount of crucifers fed [13]. It is unknown whether these differences in glucosinolate profiles, and therefore ITC, lead to different biological effects in humans; however, several laboratories have shown differences in the potency and function of ITC *in vitro* [23-25]. Navarro *et al*. [13] reported that individuals with one or more null genotypes of *GSTM1* or *GSTT1* responded to a greater extent than individuals with both genotypes intact. These results also suggest that the intact *GSTT1* allele may be compensating for the lack of active GSTM1 enzyme activity by playing a larger role in ITC metabolism among *GSTM1*-null individuals; when both alleles are absent, this compensation is no longer possible [13].

The aims of this study were to assess a *GSTM1* and/or *GSTT1* genotype-dependent effect of kale juice consumption on the blood pressure, glucose, and lipid profiles found in human plasma in subclinical hypertensive patients. In this study, there was a significant reduction of blood pressure after kale juice supplementation in both *GSTM1*-null and -present as well as *GSTT1*-null and -present individuals, however, the genetic polymorphisms of *GSTM1* and *GSTT1* are not associated with SBP and DBP reduction in subclinical hypertensive patients. From these results, it is suggested that this blood pressure reducing effect is more dependent on the composition of kale juice than on *GSTM1* or *GSTT1* polymorphisms. Furthermore, it is worth noticing that the reduction of blood pressure of *GSTT1*-present individuals was higher than those of *GSTT1*-null, as well as those of *GSTM1*-present or *GSTM1*-null individuals. Several studies have shown that blood pressure variation between 30% and 40% in a population is thought to have a genetic basis [26]. The results of our previous study of smokers indicated that the reduction of DBP was only observed in *GSTM1*-null, *GSTT1*-null, or *GSTT1*-present individuals after the 8-weeks of grape juice supplementation [27]. However, Saadat *et al*. [28] showed that alteration in SBP was only observed in subjects who possess the *GSTM1*-null, *GSTT1*-present combination genotype. Conversely, Delles *et al*. [29] did not find an association between *GSTM1* gene variants and hypertension. A number of genome-wide linkage analyses concerned with blood pressure have been reported, and most studies have reported linkage with SBP rather than DBP, but there was no obvious explanation for that [30]. The conflicting results for the *GST* genes and blood pressure could be due not only to publication bias and sample size but also to extreme gene-

environment interactions characterizing the hypertensive phenotypes [31]. Moreover, this discrepancy could be due to differences in the ethnic, genetic, and environmental background of the population studied [32].

A significant reduction of blood glucose was seen in the *GSTM1*-present and *GSTT1*-null genotypes after kale juice consumption. Several researchers have shown that consumption of vegetables in the *Brassica* family may improve insulin resistance and glycemic control in type 2 diabetes. Bahadoran *et al*. [33] observed that the consumption of 10g of broccoli sprout powder a day resulted in a significant decrease in serum insulin concentration and improved insulin resistance in type 2 diabetic patients. Supplementation of type 2 diabetics with high sulforaphane content broccoli sprouts resulted in increased plasma total antioxidant capacity and decreased oxidative stress index, serum insulin, and insulin resistance [34]. However, there has been no research which observes the modulation of blood glucose level after consumption of *Brassica* plants in hypertensive patients linked with *GST* polymorphisms. Several observational studies reported an association between *GSTM1* or *GSTT1* polymorphisms and the risk of diabetes mellitus. Amer *et al*. [32] demonstrated that the *GSTT1*- and *GSTM1*-null genotypes, alone or combined, are associated with increased risk of type-2 diabetes mellitus. The *GSTM1*-null genotype had an effect on glycemic control in type-2 diabetes patients, but they did not observe any significant effect of *GSTT1*-null on glycemic control. US-based epidemiologic study have correlated broccoli or crucifer consumption with the risk of cancer stratified by *GSTM1* genotype, which suggests that *GSTM1*-present persons gain a greater protection than do *GSTM1*-null persons [16]. So, it is hypothesized that, due to the potential differences in ITC metabolism between *GSTM1*-present and *GSTM1*-null individuals [35], blood glucose differs by *GSTM1* genotype. However, in this study's result, a decrease of blood glucose in the *GSTT1*-null genotype was also observed in addition to the decrease in the *GSTM1*-present genotype after kale juice supplementation. Reasons for these modulations are unclear. Further research is needed on the modulation of blood glucose by *GSTM1* and/or *GSTT1* polymorphisms and on the difference of the mechanism by which kale juice contributes to blood glucose.

In individuals from the general population, triglycerides, HDL-cholesterol, and the triglycerides/HDL ratio were significantly associated with a double-deleted genotype, suggesting that individuals without any copy of both the *GSTM1* and *GSTT1* genes are at increased risk for cardiovascular disease [36]. Amer *et al*. [32] attempted to evaluate the association of the *GSTM1* (present, null) and *GSTT1* (present, null) genotypes with different lipid profiles in diabetic subjects. Patients with the *GSTT1*-null genotype had higher levels of triglycerides and very low-density lipoprotein cholesterol compared to those with the *GSTT1*-present genotype. In the same manner, our previous observation study showed that TC and LDL-C levels were significantly higher in non-smokers with the *GSTT1*-null genotype than those with the *GSTT1*-present genotype [37]. Thus, it was hypothesized that the plasma lipid profiles of individuals with the *GSTT1*-null genotype who might be susceptible to coronary diseases responded to a greater extent after kale juice intervention. Contrary to this study's hypothesis, an increase of plasma HDL-C and a decrease of LDL-C with kale juice supplementation were seen among *GSTT1*-present individuals only, and not among individuals with the *GSTT1*-null genotype. Reasons for these modulations are unclear. However, these *GSTT1*-genotype specific responses of plasma lipid profiles to kale juice intake suggest that a differential response to ITC exposure in *GSTT1*-present and *GSTT1*-null individuals may influence plasma lipid levels. Whether this genotype difference is due to a difference in ITC metabolism [16] or another factor remains to be determined. To date, studies have not shown consistent pharmacokinetic differences in ITC handling by the *GSTT1* genotype. *Brassica* ITCs induce Phase II enzymes, and, in turn, Phase II enzymes conjugate ITCs leading to excretion. Seow *et al*. [38] observed a statistically significant difference in levels of urinary excretion of total ITC between *GSTT1*-positive versus *GSTT1*-null individuals with similar intakes of dietary ITC; the null genotype was associated with a lower excretion level. From these results, they suggested that *GSTT1* may be a key enzyme in the metabolism of ITCs in humans, and suggest the presence of *GSTT1* inducer(s) in cruciferous vegetables. At present, however, it is not known if cruciferous vegetables contain *GSTT1* inducers. Conversely, Fowke *et al*. [39] found that urinary ITC excretion was marginally higher with the *GSTT1*-null genotype and trends between ITC levels and habitual *Brassica* intake was more consistent within subjects with the *GSTT1*-null genotypes. Reasons for these inconsistencies are unclear but may include differences in urine collection protocols, dietary assessment methods, types or amounts of *Brassica* consumed, or genetic profiles between populations [39]. On the other hand, it has been reported that *GSTT1* and *GSTM1* genotypes are not likely to be involved in the rate of excretion of ITCs in watercress juice [40]. The demonstrated differences in protection among subjects with the two genotypes are not likely due to differences in overall ITC excretion rates. Other yet to be identified mechanisms(s) may underlie the diet and gene interactions between dietary ITCs and *GST* genotypes in human populations [40]. Meanwhile, Gasper *et al*. [16] suggested that the differences from contrasting patterns of crucifer consumption and the most prevalent isothiocyanate (i.e. sulforaphane or 2-propenyl isothiocyanates) in the diet within different locations may be critical in interpreting the effect of the *GSTM1* and *GSTT1* genotypes. Hence the enzymology of sulforaphane and 2-propenyl isothiocyanates with *GST* isoenzymes is different [41], so it is expected that a *GSTM1* and/or *GSTT1* deletion will have contrasting effects on the metabolism of sulforaphane and alkenyl isopthiocyanates. This may explain the apparent paradoxical diet-gene interactions observed in different locations and in different studies. Further research is needed to evaluate the protective metabolism of the main metabolites of kale against hyperlipidemia with the *GSTM1* or *GSTT1* genotypes.

While the strengths of this study include the recruitment of participants based on *GSTM1* and *GSTT1* genotype, and the 6-week duration of kale juice supplementation, the limitation of the study is that there was no placebo group to compare with, although this study's purpose was to observe the effect of kale juice according to different *GSTM1* and/or *GSTT1* genotypes. The authors have already proven that green vegetable juice

(*Angelica keiskei*) has an effect compared to the placebo [42]. Another potential limitation is modest sample sizes, which limited our power to further stratify the data by *GST* genotype and other possibly confounding factors, although significant changes were detected after supplementation. Thus, this study's results need to be confirmed by a larger-scaled, controlled study in the future.

In summary, our findings suggest that the supplementation of kale juice affected blood pressure, blood glucose, and lipid profiles in subclinical hypertensive patients depending on their *GST* genetic polymorphisms, and the decrease of blood glucose was greater in the *GSTM1*-present and *GSTT1*-null genotypes, and the improvement of lipid profiles were mainly greater in the *GSTT1*-present genotype. This finding suggests that kale juice intervention might be effective in plasma lipid profile control in the subgroup of hypertensive patients who are *GSTT1*-present, although the strength of this study's findings is limited by the sample size of the study. Much larger studies will be required to accurately measure the modest effects of genes, such as *GSTT1*, and identify the extent of gene-diet interactions. The relationship between genetic susceptibility biomarkers and the expression of a specific genotype needs to be assessed *in vitro*, in vivo, or in clinical trials of human volunteers [43]. Further investigation is necessary to establish the mechanism by which kale juice contributes to blood pressure, blood glucose, and lipid profile changes in relation to the *GSTM1* and *GSTT1* genotypes.

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