Original Article

Purple grape juice supplementation in smokers and antioxidant status according to different types of *GST* polymorphisms

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DNA damages and antioxidant status was assessed after 8 weeks of purple grape juice supplementation in male smokers depending on the glutathione *S*-transferase polymorphisms. Ninety-five smokers consumed 480 ml of purple grape juice for 8 weeks. The blood samples were collected before and after supplementation to measure lymphocyte DNA damages, plasma antioxidants, conjugated diene, and the erythrocyte antioxidant enzymes. The diastolic pressure, lymphocyte DNA damage, and plasma conjugated diene were significantly decreased but the plasma γ-tocopherol was increased in *GSTM1*-null genotype, while increased blood glutathione and decreased lymphocyte DNA damage were observed in *GSTM1*-present genotype. In case of *GSTT1* on the other hand, the decrease in diastolic pressure and lymphocyte DNA damage was observed in both null types and present types, but the erythrocyte catalase activity was decreased in *GSTT1*-null type and the plasma vitamin C level was increased in *GSTT1*-present type, suggesting that, the antioxidant effect of grape juice was greater in *GSTT1*-present type compared to *GSTT1*-null type. The intakes of 8-week purple grape juice affected diastolic blood pressures, DNA damage reductions and antioxidant status in smokers, mainly greater in *GSTM1*-null type and *GSTT1*-present type.

***Key Words*: purple grape juice, antioxidant status, blood pressure, DNA damage, *GST* polymorphism**

Smoking causes abnormal increases not only in free radicals but also in internal oxidation and the production of reactive oxygen species (ROS), which further increases oxidative stress and weakens the antioxidant and immune systems. Also, lipid peroxidation and oxidative DNA damage are increased[1] and the levels of antioxidant such as plasma vitamin C and vitamin E are decreased[2,3] due to the imbalance between pro-oxidant and antioxidant.

Recently, it has been reported that the degrees of DNA damages by carcinogens or oxidative stress, or the metabolism of carcinogens in the body, or the recovery from DNA damages by antioxidant administration, are different among individuals.[4] The reason for individual variation is that the individual genetic susceptibility for oxidative stress or antioxidant foods is different because of the presence of genetic polymorphisms of the enzymes that are responsible for inducing and suppressing oxidative stresses to individuals.[5] Thus, it is necessary to consider the genetic polymorphisms of the participants to find out individual differences in the antioxidant nutrients status for humans.[6]

Glutathione *S*-transferase (GST) is a phase II group enzyme that forms a protective system against carcinogens and plays a role in eliminating ROS and carcinogens from the external environments, and, thus, protecting the human body from oxidative stresses by binding glutathione to electrophilic compounds. GST has several genetic polymorphisms, such as α (*GSTA*), π (*GSTP*), μ (*GSTM*), and θ (*GSTT*), among which the *GSTM1* and *GSTT1* have been known to be related to smoking, DNA damage, and antioxidant. In participants with *GSTM1*-null genotype, it has been reported that the susceptibility to inflammation and the risk for smoking-related cancers are increased[7] and the DNA adduct level is high.[8] The risk for colon cancer and colorectal cancer are also high in *GSTT1*-null genotype.[9] And, it has been reported that the antioxidant nutrients status is low and the degree of DNA damages is high in the null genotype of both *GSTM1* and *GSTT1*,[10] and the risk for coronary artery diseases is high.[11] On the other hand, the aggravation of antioxidant status including increased DNA damages has been identified when *GSTM1* null genotype and *GSTT1* genotype coexisted.[12] A few observational studies on blood pressure changes and antioxidant status depending on GST genetic polymorphisms in smokers and adults have been reported in the country and abroad,[10,12] but interventional studies on the changes of blood pressure and antioxidant status after antioxidant supplementations are seldom being found. Recently, it has been reported that the effect of reducing DNA damages by antioxidant in meals is varied depending on the detoxification activity of *GSTM1* isoenzyme.[13] Also, it has been reported that the levels of urinary 8-hydroxydeoxyguanosine (8-OHdG) decreased in smokers after drinking more than 4 cups of green tea per day, and this effect is better observed in *GSTM1* and *GSTT1*-positive type,[14] while the effect of reducing DNA adduct was observed in *GSTM1* null genotype when fermented papaya was consumed for 3 months in the elderly.[8] However, changes in antioxidant nutrient status and blood pressure depending on *GSTM1* and *GSTT1* polymorphisms have not yet been reported in nutrition intervention studies in which antioxidant foods or nutrients are administered to smokers. Meanwhile, it has been recently reported that the intake of grape juice among antioxidant foods delays LDL oxidation, reduces DNA damages, and lowers the risk for cardiovascular diseases.[2] Thus, the study was performed to investigate the changes in blood pressure, DNA damage, and antioxidant nutrient status depending on GST genetic polymorphisms designing the experimental period to be 8 weeks according to the results of previous studies.[15,16]

Materials and Methods

Participants and dietary intake assessment. This research has been carried out for 8 weeks for the 95 male smokers, ages between 19–59, who were living in the area of Daejeon, in the

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central province of Republic of Korea. After being informed of its purpose, nature, and potential risks, they gave written consents to participate in the study. Approval for the study was obtained from the Institutional Review Board of the Hannam University, Korea (approval code: 2011-01k). Smokers of at least 8 cigarettes per day for at least 3 years were selected as participants. 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, waist, 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, Seoul, Korea).

Dietary information provided by the participants was recorded using 24-h recall and a food frequency questionnaire. Total nutrients intake was estimated using the CAN Pro 3.0 (Nutrition Information Center, Korean Nutrition Society, Seoul, Korea), and evaluated using the Korean Dietary Reference Intakes (2010).[17]

Grape juice supplementation. Three bottles (total 480 ml) of commercialized 100% purple grape juice (Welch's Company, Concord, MA) were supplemented every day to the participants. Consuming 3 bottles of grape juice did increased the simple sugar intake, but, considering the cultural characteristics, 60–65% of carbohydrate is an acceptable amount. The % of daily carbohydrate intake in Korean are normally over 60% of the total kcal. The participants were instructed to consume grape juices daily with recordings on the daily log. Depletion period was set up for 2 weeks restricting the consumption of grape or grape products, fruits and vegetables with high antioxidant nutrients before the supplementation of grape juices. This period will keep the antioxidant vitamin status within similar levels at baeline. Also, they were reminded and advised over the phone individually to refrain from the supplementation of meals or foods during the experiment periods which may affect the antioxidant index.

Blood analysis. Blood was drawn from the participants at the beginning (0 week) and 8 weeks after the supplementation of grape juices. Blood samples drawn from the survey participants after a minimum 12 h overnight fasting was put in the 10 ml heparinated sterile tube (Becton Dickinson Co. Franklin, NJ) and brought to the laboratory. Some of the blood was put in separately for Comet analysis to measure the DNA damages. The remaining blood was centrifuged at 1,000 rpm for 15 min to collect the PRP (platelet-rich plasma) on the top, and then it was centrifuged again at 3,000 rpm for 30 min to collect the PDP (platelet-deficient plasma) for the separation of blood plasma. The blood plasma was divided for each analysis item and kept at −80°C in the freezer until use. The erythrocyte pellet was separated and stored at −70°C until analyzed, at which point it was thawed and re-suspended in ice-cold water and mixed thoroughly by vortex. An ice cold extraction reagent of ethanol and chloroform was added to the erythrocyte suspension and the suspension was mixed by vortex for 30 s. Samples were centrifuged at 3,000 rpm for 2 min at 4°C and the supernatants were divided into various concentrations.

Analysis of the *GSTM1* and *GSTT1* genetic polymorohisms. The *GSTM1* and *GSTT1* genotypes were determined essentially as previously described.[7,12] Briefly, the GSTM4-specific primer pair (sense: 5'-CGCCATCTTGTGCTACATTG-GCCG-3' and antisense: 5'-ATCTTCTCCTCTTCTGTCTC-3'), which was never deleted, was used as internal control. The primers for amplifying the *GSTM1* gene were (sense) 5'-CGCCATCTT-GTGCTA CATTGGCCGTC-3' and (antisense) 5'-TTCTGGATT-GTAGCAGATCA-3'. The primers for *GSTT1* gene were (sense) 5'-TTCCTTACTGGTCCTCACATCTC-3' and (antisense) 5'-TCACCGGAT CATGGCCAGCA-3'. The polymerase chain reaction (PCR) was performed in 50 μl reaction mix containing 0.1 μg DNA, 5 mM deoxyribonucleoside triphosphates, 30 pmol of each primer, 30 mM $MgCl_2$, and 0.5 U thermostable Taq DNA polymerase. After 15 min of pretreatment at 95°C the reaction was subjected to 30 cycles of amplification at 94°C for 1 min, annealing at 61°C for 1 min, 1 min of extension at 72°C and then store at 8°C. The products of the PCR amplification were separated by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. The internal standard fragment amplified from β-globin gene was 268 bp. A 215 bp fragment was amplified for the *GSTM1* gene, and the 480 bp fragment was obtained for *GSTT1* gene. The absence of amplified product was consistent with the null genotypes. All reagents and chemicals for the genetic polymorphims were purchased from Bioneer (Daejeon, Korea).

DNA damage determination by the alkaline comet assay. The alkaline Comet assay was conducted as described by Singh *et al.*,[18] with little modifications. Lymphocytes were separated by using 100 μl of Histopaque 1077, after mixing the fresh whole blood 70 μl in the 900 μl PBS. The isolated lymphocytes were subjected to oxidative stress by suspending in phosphate-buffered saline with 100 μmol/l H_2O_2 for 5 min. The lymphocytes were mixed with 75 μl of 0.7% low-melting agarose and added to the slides pre-coated with 0.5% agarose. The slides were then immersed in lysis solution and were then placed into an electrophoresis tank containing 300 mmol/l NaOH and 10 mmol/l Na_2-EDTA (pH 13.0) for 40 min. The slides were electrophoresed for 20 min, washed three times with a neutralizing buffer and then treated with ethanol for another 5 min before staining with ethidium bromide to view under fluorescent microscope (Leica, Wetzlar, Germany). The image of each nucleus received through the CCD camera (Nikon, Tokyo, Japan) was analyzed by the computer equipped comet image analyzing system (Kinetic Imaging, Liverpool, UK). DNA damage of lymphocyte and the damage restriction levels by the supplementation of grape juice were observed by the 3 analysis indexes of tail length (TL), which is the distance of the DNA fragment moved from the nucleus, DNA in tail (% DNA), and tail moment (TM), which is the multiplied value of TL and % DNA. The DNA damage degree was measured by total 100 lymphocytes 50 cells from each of two replicate slides.

Plasma antioxidant vitamin level measurements. Plasma concentrations of vitamin C were measured spectrophotometrically by a chromogen using the 2,4-dinitrophenylhydrazine method.[12] Plasma was deproteinated with meta-phosphoric acids and the resulting protein-free plasma was treated with 2,4-dinitrophenylhydrazone-thiourea-copper sulfate reagent. The dehydroascorbic acids formed from the oxidation of ascorbic acids by copper was spectrophotometrically measured as the 2,4-dinitrophenylhydrazone derivative. Plasma levels of a-tocopherol, γ-tocopherol, α-carotene and β-carotene were analyzed with high-performance liquid chromatography with fluorometric detection using a modified version of a previously described method.[19]

Total radical-trapping antioxidant potential (TRAP) measurements. Plasma total antioxidant capacity was measured as the Trolox equivalent antioxidant capacity by the method of Rice-Evans and Miller.[20] The inhibition of absorbance was directly proportional to antioxidant capacity in the samples. The absorbance of samples was measured by UV/VIS spectrometry at 740 nm after incubating the samples at 30°C for 6 min. The concentrations of TRAP in plasma were calculated according to the calibration curve of Trolox.

Blood glutathione (GSH) analysis. Glutathione (GSH) was measured by modifying the DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] method (QuantiChrom Glutathione Assay kit; BioAssay Systems).[21] The whole blood was diluted 20 times with distilled water to prepare samples, and reagent A 120 μl and sample 120 μl were mixed and centrifuged at 14,000 rpm for 5 min. Reagent A contained EDTA to prevent blood coagulation and to eliminate color development and proteins. After centrifugation, the super-

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Table 1. General characteristics of the participants

Variables	Characteristics of the participants (n = 95)		
	0 week	8 weeks	p value
Age (years)	30.7 ± 1.0		
Height (cm)	174.5 ± 0.5		
Weight (kg)	73.1 ± 1.0	73.4 ± 1.0	ns
BMI (kg/m^2)	24.0 ± 0.3	24.1 ± 0.3	ns
WHR	0.869 ± 0.004	0.871 ± 0.004	ns
Body fat (%)	21.1 ± 0.7	21.1 ± 0.7	ns
Smoking habits			
Cigarettes/day	14.2 ± 0.6		
Smoking years	12.1 ± 0.9		
Pack years	9.0 ± 0.9		
Drinking habits			
Drinker n (%)	88 (92.6%)		
Alcohol (ml/day)	61.4 ± 6.6		
Exercise habits			
Regular exercisers, n (%)	61 (64.2%)		
Exercise time (min/day)	23.3 ± 2.4		

All values are means ± SE. WHR: waist hip ratio, Pack years = (Cigarettes smoked/day × years smoked)/20, p value by paired t test.

natant was collected and placed in a 96-well plate, and then reagent B was added and left at room temperature for 25 min. The absorbance was measured at 412 nm using enzyme-linked immunosorbent assay and enzyme-linked immunospecific assay (ELISA), and the calibration curve was made using GSH as a standard.

Determination of plasma lipid profiles. Plasma lipid, total cholesterol and triglyceride contents were analyzed using the Photometric Auto analyzer (*ERBA*CHEM-PRO) which had been kept in the –80°C freezer, with the enzyme solution 1 ml of the kit reagent produced by Somang Pharmaceutical Co., Ltd. (Suwon, Korea) and reactivated for 5 min in the constant temperature water bath. The HDL-Cholesterol was analyzed using the Photometric Autoanalyzer following the manual provided with the kit. LDL-Cholesterol was calculated using the Friedwald[22] equation.

Lipid peroxidation measurements. The amount of low-density lipoprotein (LDL) oxidation products was estimated based on the levels of conjugated dienes (CDs) in isolated LDL.[23] Plasma LDLs were isolated by precipitation buffer (0.064 mol/L of trisodium citrate, pH 5.05, with 5 N HCl, 5,000 IU/L of heparin). The pellet was suspended in 1 ml of 0.1 M Na-phosphate buffer, pH 7.4 and containing 0.9% of NaCl. Lipids were extracted from LDL samples by chloroform: methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm.

Determination of antioxidant enzymes. The analysis of catalase, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the red blood cell was carried out as previously described.[2] GSH-Px catalyzes the oxidizing reaction of glutathione by the peroxide (tert-butyhydroperoxide). In the downstream reaction, oxidized glutathione is restored to the glutathione again with the existence of glutathione reductase and NADPH. Glutathione peroxidase was analyzed by measuring reduced NADPH concentrations, after the restoration, at 340 nm for 90 s with the UV/VIS spectrophotometer. For the SOD, ethanol and chloroform were added up after the suspension of red blood cell was hemolyzed with distilled water, which was centrifuged at 3,000 rpm for 2 min. Pyrogallol was added up, after incubating the solution on the top that had been divided into several concentrations, it was measured at 320 nm for 180 s by the UV/VIS spectrophotometer.

Statistical analysis. All the data were entered into Microsoft's Excel database, and the statistical tasks were performed with SPSS-PC+ statistics package (ver. 19.0). Mean and standard error of the mean (SE) was obtained for each item, then the mean difference among the 3 groups was verified by Scheffe after one-way ANOVA, and all the statistical significance was evaluated at the level of $\alpha = 0.05$. The significance of the mean comparison before and 8 weeks after supplementations of the grape juice was tested by paired t-test. Also, chi-square test was performed against the frequency of smoking habits, existence of alcohol intakes, etc.

Results

General characteristics of the participants. General characteristics of the participants are shown in Table 1. All of the participants in this study were smokers aged 19–59 years, and the average age was 30.7 ± 1.0 years. When compared before and after the 8-week grape juice supplementations, the BMI, WHR, and body fat of the participants were not significantly different. Participants' smoking habits indicated daily average of 14.2 ± 0.6 cigarettes with pack years (which was calculated on the basis of 1 pack a year) of 9.0 ± 0.9 years. The drinking habits of the participants showed that the percentage of drinkers was 92.6% in all participants and the total alcohol consumption was 61.4 ± 6.6 ml/day. The percentage of participants who regularly exercise was 64.2% and the average exercise time was 23.3 ± 2.4 min/day (Table 1).

Nutrient intake of the participants. The nutrients intake before grape juice supplementation (0 week), at 4 weeks of supplementation, and at 8 weeks of supplementation was surveyed using the 24-h recall method to find out changes in dietary intakes during 8 week of grape juice participants supplementations, as shown in Table 2. The nutrients 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 grape juice supplementation (Table 2). Nutrient contents of 480 ml of grape juices are; 300 kcal, 2 g of protein, 70 g of carbohydrates, 4.5 g of fiber, 25 mg of Vitamin C, 0.5 mg of Vitamin E, 20 μg of folate.

***GSTM1* and *GSTT1* polymorphism frequency analysis.** Among a total of 95 participants, *GSTM1*-null genotype was found in 56 participants (58.9%) and *GSTM1*-present genotype in 39 participants (41.1%) (Table 3), and *GSTT1*-null genotype in 61 participants (64.2%) and *GSTT1*-present genotype in 34 participants (35.8%). The number of participants who had both *GSTM1* and *GSTT1* present genotype was 15 (15.8%), who had either one of the genotype was 43 (55.3%), and who had none of

Table 2. Daily intakes of nutrients of the participants before and after grape juice supplementations

Nutrients	0 week	4 weeks	8 weeks	*p* value
Energy (kcal)	1,588.1 ± 68.7	1,620.4 ± 51.1	1,597.3 ± 62.3	ns
Protein (g)	77.0 ± 8.2	65.4 ± 2.6	67.2 ± 3.5	ns
Fat (g)	46.9 ± 2.8	52.7 ± 3.0	54.0 ± 3.6	ns
Carbohydrate (g)	218.0 ± 8.8	221.0 ± 7.4	207.7 ± 8.1	ns
Calcium (mg)	435.8 ± 28.7	406.1 ± 22.9	404.6 ± 23.2	ns
Vitamin C (mg)	85.6 ± 9.2	63.6 ± 4.0	66.9 ± 6.5	ns
Vitamin E (mg)	13.5 ± 0.8	13.9 ± 0.8	13.1 ± 0.8	ns
Vitamin A (μg RE)	705.9 ± 61.9	671.2 ± 44.2	721.4 ± 82.1	ns
Retinol (mg)	66.3 ± 7.8	96.8 ± 11.9	144.7 ± 54.0	ns

All values are means ± SE. Values with different letters within a row are significantly different at $p<0.05$ after Scheffe's test followed by ANOVA. *p* value by one-way ANOVA. The total amount of carbohydrate intake described is only from diet. Additional 3 bottles of grape juice added 70 g of carbohydrate which made ~300 g of carbohydrate/day which is still less than the maximum allowed intake of carbohydrate. (The 2010 dietary reference intake of carbohydrate is 55–70% of total calorie intake in adult).

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

GST genotypes	Frequency *n* (%)
GSTT1	
Null	61 (64.2%)
Present	34 (35.8%)
GSTM1	
Null	56 (58.9%)
Present	39 (41.1%)
Both null	37 (38.9%)
Null/present	43 (45.3%)
Both present	15 (15.8%)

the genotype was 37 (38.9%).

Changes in blood pressure. The changes in blood pressure of the participants after grape juice supplementation showed that the systolic pressure was not changed but the diastolic pressure was decreased in participants when divided according to the GST polymorphism (Table 4). The diastolic pressure was significantly decreased in *GSTM1* compared to *GSTM1*-null genotype. In case of *GSTT1*, on the other hand, the systolic pressure was significantly increased after grape juice supplementations in *GSTT1*-null type but the diastolic pressure was significantly decreased. In *GSTT1*-present type, the diastolic pressure was significantly decreased after grape juice supplementation.

Changes in human lymphocyte DNA damage. The lymphocyte DNA damage in all participants was significantly decreased in TD, TL, and TM after grape juice supplementations (Table 5). The results of possible changes in lymphocyte DNA

Table 4. Changes of systolic and diastolic blood pressure of the participants by *GSTM1* or *GSTT1* genotype after 8 weeks of grape juice supplementation

	All participant (*n* = 95)	*GSTM1* genotype		*GSTT1* genotype	
		Present (*n* = 39)	Null (*n* = 56)	Present (*n* = 34)	Null (*n* = 61)
SBP					
0 week	123.5 ± 1.3	122.6 ± 2.1	124.2 ± 1.6	125.7 ± 2.4	122.3 ± 1.5
8 weeks	125.9 ± 1.4	125.1 ± 2.3	126.5 ± 1.8	124.0 ± 2.2	127.0 ± 1.8*
DBP					
0 week	80.5 ± 1.0	79.4 ± 1.5	81.2 ± 1.4	81.1 ± 1.7	80.1 ± 1.3
8 weeks	76.9 ± 1.0*	77.6 ± 1.6	76.5 ± 1.4*	76.3 ± 1.6*	77.3 ± 1.4*

All values are means ± SE. SBP: systolic blood pressure; DBP: diastolic blood pressure. *Statistical significance at $p<0.05$ after paired *t* test.

Table 5. Changes of lymphocyte DNA damage of the participants by *GSTM1* or *GSTT1* genotype after 8 weeks of grape juice supplementation

	All participant (*n* = 95)	*GSTM1* genotype		*GSTT1* genotype	
		Present (*n* = 39)	Null (*n* = 56)	Present (*n* = 34)	Null (*n* = 61)
Tail DNA (%)					
0 week	15.5 ± 0.2	15.4 ± 0.2	15.5 ± 0.3	14.9 ± 0.3	15.8 ± 0.2
8 weeks	7.8 ± 0.1*	7.7 ± 0.3*	7.8 ± 0.2*	7.7 ± 0.2*	7.8 ± 0.2*
Tail length (μm)					
0 week	100.6 ± 1.4	100.9 ± 2.2	100.4 ± 1.8	101.6 ± 2.0	100.0 ± 1.9
8 weeks	92.1 ± 1.4*	95.0 ± 2.2	90.0 ± 1.7*	91.2 ± 2.0*	92.6 ± 1.8*
Tail moment					
0 week	16.2 ± 0.3	16.1 ± 0.4	16.2 ± 0.4	15.7 ± 0.4	16.5 ± 0.4
8 weeks	7.8 ± 0.2*	8.1 ± 0.4*	7.6 ± 0.2*	7.7 ± 0.2*	7.9 ± 0.3*

All values are means ± SE. *Statistical significance at $p<0.05$ after paired *t* test.

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Table 6. Changes of plasma levels of antioxidant vitamin, TRAP and glutathione of the participants by *GSTM1* or *GSTT1* genotype after 8 weeks of grape juice supplementation

	All participant (n = 95)	*GSTM1* genotype		*GSTT1* genotype	
		Present (n = 39)	Null (n = 56)	Present (n = 34)	Null (n = 61)
Ascorbic acid (mg/dl)					
0 week	1.15 ± 0.03	1.20 ± 0.05	1.11 ± 0.04	1.09 ± 0.07	1.18 ± 0.04
8 weeks	1.19 ± 0.04	1.21 ± 0.05	1.18 ± 0.05	1.29 ± 0.06*	1.14 ± 0.05
α-tocopherol (μg/dl)					
0 week	1,888 ± 251	1,801 ± 531	1,949 ± 217	1,677 ± 569	2,006 ± 233
8 weeks	1,536 ± 158	1,185 ± 227	1,781 ± 212	1,091 ± 200	1,784 ± 214
γ-tocopherol (μg/dl)					
0 week	244.1 ± 21.6	257.2 ± 36.4	235.0 ± 26.7	273.8 ± 38.2	227.6 ± 26.1
8 weeks	286.0 ± 22.9	264.8 ± 33.5	300.8 ± 31.2*	306.8 ± 40.2	274.4 ± 27.9
α-carotene (μg/dl)					
0 week	16.1 ± 1.5	15.5 ± 1.5	16.5 ± 2.4	13.9 ± 1.6	17.3 ± 2.2
8 weeks	17.2 ± 2.3	20.9 ± 5.1	14.6 ± 1.5	22.4 ± 6.10	14.3 ± 1.1
β-carotene (μg/dl)					
0 week	86.2 ± 5.7	90.4 ± 9.1	83.3 ± 7.2	83.9 ± 9.6	87.6 ± 7.1
8 weeks	82.7 ± 7.2	99.8 ± 14.7	70.8 ± 6.4	90.8 ± 16.7	78.2 ± 6.4
TRAP (mM)					
0 week	1.497 ± 0.002	1.497 ± 0.002	1.497 ± 0.002	1.498 ± 0.002	1.496 ± 0.002
8 weeks	1.496 ± 0.001	1.495 ± 0.002	1.497 ± 0.002	1.496 ± 0.003	1.496 ± 0.002
Glutathione (μM)					
0 week	633.2 ± 15.2	599.0 ± 16.8	657.0 ± 22.6	661.3 ± 29.6	617.6 ± 16.9
8 weeks	670.5 ± 17.0	678.4 ± 22.0*	665.0 ± 24.7	681.1 ± 22.9	664.6 ± 23.4

All values are means ± SE. TRAP: Total radical-trapping antioxidant potential. *Statistical significance at $p<0.05$ after paired t test.

Table 7. Changes of erythrocyte antioxidant enzyme activities of the participants by *GSTM1* or *GSTT1* genotype after 8 weeks of grape juice supplementation

	All participant (n = 95)	*GSTM1* genotype		*GSTT1* genotype	
		Present (n = 39)	Null (n = 56)	Present (n = 34)	Null (n = 61)
Catalase (K/g Hb)					
0 week	43.6 ± 0.6	44.2 ± 0.8	43.1 ± 0.9	44.5 ± 1.1	43.0 ± 0.7
8 weeks	42.5 ± 0.6	42.5 ± 1.1	42.5 ± 0.8	44.3 ± 1.3	41.5 ± 0.7*
SOD (U/g Hb)					
0 week	1,814 ± 35.1	1,797 ± 46.1	1,827 ± 50.4	1,739 ± 43.8	1,856 ± 48.3
8 weeks	1,944 ± 72.4	1,948 ± 115.4	1,941 ± 93.7	1,884 ± 121.6	1,977 ± 90.5
GSH-Px (U/g Hb)					
0 week	12.8 ± 1.4	12.2 ± 2.2	13.3 ± 1.9	10.0 ± 1.6	14.4 ± 2.0
8 weeks	11.9 ± 1.2	11.4 ± 2.0	12.3 ± 1.4	11.8 ± 1.6	12.0 ± 1.6

All values are means ± SE. SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase. *Statistical significance at $p<0.05$ after paired t test.

damage after 8 weeks of grape juice supplementations depending on GST polymorphism showed that all of TD, TL, and TM were significantly decreased in *GSTM1*-null genotype, and TD and TM were significantly decreased in *GSTM1*-present genotype. In case of *GSTT1* genotype, all of TD, TL, and TM were significantly decreased in both null genotype and present genotype. From the above results, it was found that the intakes of 8-week grape juice supplementation significantly decreased the DNA damages in smokers.

Changes in antioxidant nutrient status and TRAP levels. The antioxidant nutrients status and TRAP levels were not significantly changed after grape juice supplementations in all smokers (Table 6). However, the plasma antioxidant nutrient status depending on GST polymorphism after grape juice supplementation showed that the plasma γ-tocopherol concentration was significantly increased in *GSTM1*-null genotype after grape juice supplementations as compared to previous supplementations and the glutathione concentration was increased in *GSTM1*-present genotype. In case of *GSTT1* genotype, the plasma vitamin C concentration was significantly increased after grape juice supplementations in *GSTT1*-present genotype.

Changes in erythrocyte antioxidant enzyme activities. The activities of erythrocyte antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were not significantly different after grape juice supplementations as compared to the baseline levels. And these results were not different depending on *GSTM1* polymorphism (Table 7). However, when the participants were categorized by *GSTT1* genotype, the erythrocyte catalase activity was significantly decreased after grape juice supplementations in *GSTT1*-null genotype.

Comparison of plasma lipid levels and LDL oxidation. The results of comparing plasma total cholesterol (TC), LDL-C, HDL-C, triglyceride (TG), and conjugated diene (CD) before and after 8 weeks of grape juice supplementation are shown in Table 8. The lipid levels of the participants were not changed after grape

Table 8. Changes of plasma lipid profiles and conjugated diene (CD) of the participants by *GSTM1* or *GSTT1* genotype after 8 weeks of grape juice supplementation

	All participant (n = 95)	*GSTM1* genotype		*GSTT1* genotype	
		Present (n = 39)	Null (n = 56)	Present (n = 34)	Null (n = 61)
TC (mg/dl)					
0 week	195.6 ± 4.1	189.5 ± 6.1	199.8 ± 5.5	195.2 ± 7.0	195.8 ± 5.2
8 weeks	192.2 ± 3.8	191.0 ± 5.7	193.0 ± 5.1	194.7 ± 6.3	190.8 ± 4.8
TG (mg/dl)					
0 week	145.5 ± 4.3	149.3 ± 6.6	142.9 ± 5.8	149.7 ± 8.4	143.1 ± 4.9
8 weeks	152.2 ± 5.7	156.5 ± 9.7	149.4 ± 6.9	155.4 ± 11.6	150.4 ± 6.0
HDL (mg/dl)					
0 week	75.6 ± 1.6	75.3 ± 1.9	75.8 ± 2.3	76.6 ± 3.0	75.0 ± 1.8
8 weeks	73.4 ± 1.4	73.8 ± 1.9	73.2 ± 1.9	73.6 ± 2.6	73.3 ± 1.6
LDL (mg/dl)					
0 week	93.0 ± 3.6	89.0 ± 5.6	95.5 ± 4.8	94.2 ± 6.4	92.3 ± 4.5
8 weeks	89.8 ± 3.5	89.5 ± 5.4	90.0 ± 4.7	93.8 ± 5.8	87.6 ± 4.4
CD (μM)					
0 week	38.8 ± 0.8	39.1 ± 1.4	38.6 ± 0.9	40.5 ± 1.3	37.8 ± 1.0
8 weeks	38.3 ± 0.8	40.6 ± 1.7	36.7 ± 0.8	40.3 ± 1.8	37.2 ± 0.9

All values are means ± SE. CD: conjugated diene.

juice supplementations. When the participants were grouped based on their *GSTM1* genotype, the plasma CD level was significantly decreased after grape juice supplementations in the *GSTM1*-null genotype, but other plasma TC, LDL-C, HDL-C, and TG levels were not significantly changed after grape juice supplementation. When the participants were divided by *GSTT1* genotype, plasma lipids and CD levels were not significantly changed after grape juice supplementations in both the *GSTT1*-null and present genotype.

Discussion

In an intervention study on the administration of antioxidant food or nutrient in participants with high oxidative stress, the changes in blood pressure and antioxidant nutrient status are different depending on GST genetic polymorphisms of the subject. It has been reported that the frequency of GST genetic polymorphisms varies in different races and in case of Koreans, the number of people with *GSTM1* and *GSTT1* null type is higher than those with present type, and the antioxidant status in *GSTM1* null type is lower compared to *GSTM1* present type.[10,12,24] Meanwhile, the intake of grape juices has been reported not only to decrease blood pressure but also to reduce DNA damages, and further contribute to the improvements of antioxidant nutrient status.[2,15,16,25,26] Thus, this study was conducted to find out whether the improvement in blood pressure and antioxidant nutrient status is greater in *GSTM1* and *GSTT1* null type after grape juice supplementation, which was assumed to demonstrate blood pressure-reducing effect and antioxidant effect in smokers. Comparing the effect of grape juice with placebo was already proven and published elsewhere.[15,16,25]

In the study, blood pressure changes in the participants showed that the diastolic pressure was decreased in all participants after 8 weeks of grape juice supplementation, and for GST polymorphisms, the diastolic pressure was decreased in *GSTM1*-null genotype, and *GSTT1*-null and *GSTT1*-present genotype (Table 4). This was similar to the results in the study by Park *et al.*[15] showing that the systolic pressure was significantly decreased after grape juice supplementations in men with hypertension and in the studies by Park *et al.*[25] and by Kim *et al.*[16] showing that the diastolic pressure was decreased after 8 weeks of grape juice supplementation in adult males with hypertension and 67 adult males and females, respectively. Similarly, several studies have reported decreased blood pressure after grape juice supplementations in adult smokers or patients with hypertension. But studies on the possibility of blood pressure reducing effects can vary as GST genetic polymorphisms are seldom found. It is thought that the reason for significantly higher blood pressure reducing effects in *GSTM1*-null genotype compared to *GSTM1*-present genotype in this study was partially from the lower antioxidant nutrients status of *GSTM1*-null type as compared to *GSTM1*-present type as previously suggested by Jo *et al.*[10] It can be also interpreted that the nutritional intervention effect of grape juice supplementations was more useful in *GSTM1*-null genotype. When looking at the result of diastolic blood pressure decrease with a systolic blood pressure increase after grape juice supplementations in *GSTT1*-null genotypes, this should be confirmed in the future through careful repetitive studies even if the increment range was in the normal level and may not indicate significance.

The degree of lymphocyte DNA damage was examined using Comet assay in this study and the results showed that the DNA damage was significantly decreased after grape juice supplementations for all smokers, and DNA damages depending on GST polymorphisms were all observed in *GSTM1*-null and *GSTM1*-present genotype, and *GSTT1*-null and *GSTT1*-present genotype. This result was consistent with those by Park *et al.*[2] showing that DNA damages were decreased after 8 weeks of grape juice supplementations for adult smokers, and by a preliminary study in which DNA adduct and 8-OHdG were significantly decreased in participants with *GSTM1*-null type after 3 months of fermented papaya supplementation in males and females aged 72–84 years.[8] On the contrary, Hakim *et al.*[14] reported that the urinary 8-OHdG excretion was decreased in participants with *GSTM1* and *GSTT1* present genotype after 4 months of green tea supplementations for 143 adult male smokers, and the beneficial effects of green tea was rather observed in *GSTM1*- and *GSTT1*-present genotype, which had conflicting results to ours.

In this study, the antioxidant vitamin status in all participants was not changed after 8 weeks of grape juice supplementations, but when the participants were divided by GST genotype, the plasma γ-tocopherol concentration was increased in *GSTM1*-null genotype and the plasma vitamin C concentration was significantly increased in *GSTT1*-present genotype (Table 6). Previous studies on different plasma vitamin C levels depending on GST genotype have reported conflicting results. While Block *et al.*[27]

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and Dusinska *et al.*[28] reported that the vitamin C concentration was higher in *GSTM1*-null type compared to the present type, Horska *et al.*[29] observed that the plasma vitamin C level was low in *GSTM1*-, *GSTT1*-null genotype, and some study results shows that the blood vitamin C level was not related to the GST genotype.[10,30] In our study, the plasma γ-tocopherol concentration was increased in *GSTM1*-null genotype and the plasma vitamin C concentration was increased in *GSTT1*-present type, suggesting that the antioxidant intervention effects of grape juice was partially greater in the null genotype as compared to *GSTM1*-wild genotype and the present genotype as compared to *GSTT1*-null type, thus, should be studied more carefully in the future.

Glutathione (GSH) is the most abundant thiol antioxidant in the mammalian cells. GSH-Px and GST joins with GSH and GSH-reductase to prevent the productions of the harmful hydroxyl radicals and also the H_2O_2 and alkyl hydroperoxidase. Therefore, the reduction of GSH decreases the activity of GST in addition to GSH-Px,[31] and the GSH levels in patients with ankylosing spondylitis was lower when compared to normal individuals.[32] The GSH concentration was measured for all participants in this study and was not changed significantly after grape juice supplementations, but, the GST polymorphisms may increase the *GSTM1*-present genotype. It is thought that increased GSH in *GSTM1*-present genotype was caused by the binding of glutathione S-transferase with GSH, and thus, increased GST genes in *GSTM1*-present genotype.

Antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) form the primary defense system against oxidative damages, and are activated when the organisms with such enzymes are exposed to oxidative stresses.[33] In this study, the activity of antioxidant enzymes was changed in all participants after grape juice supplementations, but the erythrocyte catalase activity was significantly decreased in participants with *GSTT1*-null genotype (Table 7). Park *et al.*[2] reported that the activity of erythrocyte catalase and GSH-Px significantly increased in both male smokers and nonsmokers after 8 weeks of grape juice supplementations which conflicted with our results. The study on the relationship between the intakes of fruits and vegetables and GST genes in 190 male and female adults indicated that the erythrocyte GST activity was lower in *GSTM1*-null genotype due to low intakes of fruits and vegetables.[34] Therefore, investigators have reported different results on the activity of antioxidant enzymes, because antioxidant enzymes and antioxidant nutrients are under a homeostatic control in which the activity is decreased after being greatly used to overcome oxidative stress or increased to alleviate the oxidative stress.[35] In conclusion, it is hard to simply determine the nutritional intervention effects only by the increase or decrease of antioxidant enzymes. Also in this study, although the erythrocyte catalase activity was decreased after grape juice supplementations in *GSTT1*-null genotype, it is difficult to relate this result to the antioxidant system for interpretations.

The lipid profiles of the participants measured in this study were not significantly different, and the difference by *GSTM1* and *GSTT1* polymorphisms was not observed either. But in case of *GSTM1*-null genotype, CD level, which shows the degree of LDL peroxidation, was significantly decreased. While a high level of CD in participants with *GSTM1*-null genotype was reported,[36] some studies showed no differences in plasma CD levels depending on *GSTM1* and *GSTT1* genotype.[10] In this study, the plasma CD level in all participants was not different after grape juice supplementation but, on the other hand, it was decreased in participants with *GSTM1*-null genotype, suggesting that the antioxidant effects of grape juices will be more effective in *GSTM1*-null type. It is necessary to perform more extensive studies in the future to find out the differences in the antioxidant nutrition intervention effects depending on GST genetic polymorphisms.

The limitation of this study was due to an insufficient number of participants whom observed the relationship between changes of the antioxidant nutrient status and GST genetic polymorphisms after grape juice supplementations. Since there are not many antioxidant-related nutrition intervention studies that analyze its effects depending on *GSTM1* and *GSTT1* genotype, it is necessary to perform studies on the possibility of changes in the nutritional interventions for antioxidant nutrients or antioxidant foods depending on GST genetic polymorphisms more extensively in the future.

In conclusion, the 8-week grape juice supplementations in adult male smokers demonstrated effects in reducing diastolic blood pressure and in protecting DNAs damaged by smoking for all participants; these effects were varied in detail when the participants were further divided by GST genetic polymorphisms. That is, in case of *GSTM1*, the beneficial antioxidant effect of grape juice was greater in *GSTM1*-null type as compared to *GSTM1*-present type; in case of *GSTT1*, on the other hand, the antioxidant effect of grape juices was partially greater in *GSTT1*-wild type as compared to *GSTT1*-null type, on the contrary to *GSTM1*.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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