Kale Juice Improves Coronary Artery Disease Risk Factors in Hypercholesterolemic Men[1]

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Objective To evaluate the effect of 3-month kale (*Brassica oleracea acephala*) juice supplementation on coronary artery disease risk factors among hypercholesterolemic men. **Methods** Thirty-two men with hypercholesterolemia (> 200 mg/dL) were recruited after annual health examinations among the faculty and staff at university. The subjects consumed 150 mL of kale juice per day for a 12-week intervention period. Dietary and anthropometric assessments were performed and blood samples were collected to evaluate biochemical profiles before and after supplementation. **Results** Serum concentrations of HDL-cholesterol, and HDL- to LDL-cholesterol ratio were significantly increased by 27% (P<0.0001) and 52% (P<0.0001), respectively. The LDL-cholesterol concentration and the atherogenic index were significantly reduced by 10% (P=0.0007) and 24.2% (P<0.0001), respectively without affecting body mass index, waist and hip circumferences, or nutrient intakes after three months of supplementation. While there was no difference in the concentration of malondialdehyde, significant increase in glutathione peroxidase activity (P=0.0005) were accompanied by a significant increase in the serum selenium level (P=0.0132). It was also found that the responses of these risk factors to kale juice administration were dependent on smoking status. **Conclusion** Regular meals supplementation with kale juice can favorably influence serum lipid profiles and antioxidant systems, and hence contribute to reduce the risks of coronary artery disease in male subjects with hyperlipidemia.

Key words: Kale juice; Hyperlipidemia; Coronary artery disease; Lipid profile; Antioxidant system

INTRODUCTION

There have been increasing interests in the prevention of chronic diseases, such as cardiovascular disease and cancer, by means of changing dietary factors, and accordingly the interest in nutritional supplementation has remarkably grown[1-3]. Researches focused on the effects of single nutrients, commonly antioxidant vitamins or minerals, have revealed their modes of action with respect to disease prevention[4-6]. However, it remains controversial as to whether the use of single nutrients helps to prevent chronic diseases, since adverse effects of single nutrients on the incidence of lung cancer in smokers have been reported[7-8].

Evidence is emerging on the roles of fruits and vegetables which are rich in antioxidant vitamins and minerals, with respect to reducing the risk of chronic diseases[9-11]. According to the US dietary guidelines[12], daily intakes of at least 5 servings of fruits and vegetables have been recommended rather than a single nutrient supplementation approach; however, in spite of importance of fruits and vegetables intake, only a relatively small proportion of the US population was reported to have intakes in the recommended range[13-14].

Green leafy vegetable juice can be conveniently used to meet the recommendations of daily fruits and vegetables. Kale (*Brassica oleracea acephala*) juice is one of the popular green leafy vegetable juices consumed in Korea and is known as a rich source of vitamins, flavonoids, and minerals[15]. The hypocholesterolemic effect of kale extract on the cholesterol metabolism through HMG-CoA inhibition and bile acid synthesis has been studied in *in vitro* system[16]. However, in spite of the popularity of kale juice in Korea, little is known about the potential effects of kale juice on human health.

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The present study was undertaken to examine whether kale juice beneficially alters cardiovascular disease risk factors, such as lipid profile, antioxidant system, and anthropometry, in male subjects with hypercholesterolemia.

METHODS AND MATERIALS

Subjects

Subjects were recruited after an annual health examination of faculty and staff at Yonsei University. Thirty-seven male subjects who met the following inclusion criteria were recruited: 1) subjects with hypercholesterolemia (>200 mg/dL) and normal triglyceridemia (<150 mg/dL); 2) subjects with no history of cardiovascular, hepatic, gastrointestinal, or renal disease; 3) subjects with no history of alcoholism; and 4) subjects who had not taken lipid-lowering medication or vitamins or mineral supplementation within 6 months prior to study commencement.

Experimental Protocol

Thirty-four eligible subjects willing to participate in this intervention study attended the health center and underwent a clinical examination, dietary and anthropometric assessments. Participants were advised to continue with their usual diets and normal life styles during a 2-week run-in phase. At the second visit, a set of blood samples were obtained from all subjects in addition to dietary and anthropometric assessments.

After washing, fresh kale was soaked in malic acid solution (pH 2.4) for 15 minutes and cut into 3-5 pieces for grinding. Kale juice was extracted, no water was added. Cooled crude kale juice was filtered twice using 100-mesh sieves and 60-mesh sieves consecutively to remove insoluble fibers. 150 mL of fresh kale juice (equivalent to 167 g of fresh kale, the product of the Pulmuwon Greenjuice Co., Ltd. Seoul, Korea) was individually packed and delivered every morning in a temperature-controlled container to subjects for three months. During the study period, all participants were encouraged to maintain their usual dietary and lifestyle habits, except for the kale juice supplementation.

Two out of the 34 initial participants dropped after the two-week trial due to non-compliance in two subjects and intermittent diarrhea in one. At the end of the three-month trial, the remaining 32 subjects underwent a second set of blood sampling, dietary and anthropometric assessments to compare if there were any changes during three months. The study protocol was approved by the Human Ethics Committee of Yonsei University and all subjects gave written informed consent. To check participants' compliance during the study period, all subjects were interviewed by telephone every two weeks to determine whether they were following the program.

Nutrient Composition of Kale Juice

General nutrient composition of kale juice is presented in Table 1. It was reported that kale juice contains several kinds of antioxidant nutrients, such as β-carotene, vitamin E, vitamin C, Se, Cu, Mn, Zn, and phenolics. Compared with other vegetable juices (such as *Angelica keiskei*, carrot, celery, cucumber), the compositions of vitamin E, vitamin C, and total phenolics are high, and kale juice is the second best source of antioxidant minerals, such as Se, Zn, Cu, and Mn[15,17].

TABLE 1

Compositions of Kale Juice per 150 mL Portion

Nutrients	Composition
Moisture (g)	143.0
Protein (g)	1.15
Fat (g)	0.3
Total Fiber (g)*	0.75
Ash (g)	1.59
Ca (mg)	101.0
Fe (mg)	0.32
Na (mg)	74.5
β-Carotene (μg)	2004
Vitamin B_1 (mg)	0.3
Vitamin B_2 (mg)	0.15
Vitamin C (mg)	172.5
Folate (μg)	523.6

Note. Source: R & D Center, Pulmuone Co. Ltd. *: Analyzed by Korea Health Industry Development Institute.

Anthropometry, Blood Collection, Blood Lipid Profile, and Blood Pressure Measurements

Anthropometric parameters (weight, height, and waist & hip circumferences) were measured. Body mass index (BMI) was calculated in kg/m^2. Venous blood samples were collected after a 12-hour fast in plain tubes, and the tubes were centrifuged to separate the serum, which was stored in aliquots at -70℃ until analyzed. Fasting total serum cholesterol and serum triglyceride levels were measured enzymatically, and the HDL-cholesterol fraction was measured after precipitating LDL and VLDL. LDL-cholesterol was estimated indirectly by using

the Friedwald formula. Blood pressure was read from the left arm with the subject seated. An average of three measurements was taken per subject.

Serum Malondialdehyde and Glutathione Peroxidase Activity

Serum malondialdehyde (MDA) was assayed as a parameter of the degree of lipid peroxidation, using the fluorometric method reported by Buckingham[18]. Glutathione peroxidase (GSH-Px) activity was measured using a modification of the coupled enzyme procedure reported by Paglia and Valentine[19]. Changes in absorbance of the system at 340 nm were recorded for 3 min and activity was defined as nmol of NADPH oxidized per min per mg of protein.

Serum Trace Elements

Serum Se, Cu, Zn, and Mn concentrations were measured using an atomic absorption spectrophotometer (Perkin Elmer Model 4110ZL) with a Zeeman background correction using the platform technique[20]. The monochromator slit was adjusted to 2.0 nm and the wavelength set to 196.0 nm. Samples were diluted, sampled with a matrix modifier, and injected directly into a graphite furnace. Concentrations were calculated using a calibration curve, which was based on aqueous standards.

Statistical Analysis

Statistical analysis was performed using SAS software version 6.12 for Windows[21]. For descriptive purposes, the mean values of untransformed and unadjusted variables are presented. The results are expressed as $\bar{x} \pm s$. Effects of kale juice supplementation on end points were tested using the paired student's t test in subjects before and after the experiment. To determine the effect of smoking on serum measurements, net differences between non-smokers and smokers were evaluated. A value of $P<0.05$ was considered statistically significant.

RESULTS

General Characteristics

No significant changes were observed in characteristics, such as BMI, waist and hip circumference, or blood pressure after the 12-week supplementation (Table 2). No changes in smoking, drinking, and exercise habits of subjects were observed during the study period.

TABLE 2

General Characteristics in Hypercholesterolemic Men

	0 Week (n=37)	12 Weeks (n=37)
Age	44.9±1.5	—
BMI (kg/m^2)	24.7±0.34	24.5±0.34
Waist Circumference (cm)	91.1±0.85	90.2±0.94
Hip Circumference (cm)	103.5±1.12	100.2±1.59
Smoking Habits (number, %)		
Non-smoker	22 (60%)	—
Smoker (5 or More Cigarettes/d)	15 (40%)	—
Drinking Habits (number, %)		
Non-drinker	24 (65%)	—
Drinker (2 or More drinks/d)	13 (35%)	
Exercise Habits (number, %)		
Non-exercise	21 (57%)	—
Regular Exercise	16 (43%)	—
(More Than 30 min/d and More Than 3 Times /week)		
Systolic Blood Pressure (mmHg)	132.2±2.60	131.9±2.86
Diastolic Blood Pressure (mmHg)	81.5±1.47	82.1±1.25

Note. Values are $\bar{x} \pm s$. None of the values are significantly different from the values at week 0.

Energy and Nutrient Intake

Subjects maintained their usual energy and nutrients intakes over the 12-week study period (Table 3).

TABLE 3

Daily Nutrient Intakes Estimated From Usual Diet at Baseline and 12 weeks of Kale Juice Supplementation in Hypercholesterolemic Men

	Nutrient Intake	
	0 Week	12 Weeks
Calorie (kcal)	1648.7±48.0	1677.9±33.3
Protein (g)	65.4±1.97	64.6±1.6
Carbohydrate (g)	263.4±7.28	268.3±7.31
Fat (g)	32.4±1.17	32.4±0.97
Fe (mg)	15.5±0.54	16.1±0.5
P (mg)	968.9±30.3	959.6±24.9
Ca (mg)	609.6±21.4	612.7±20.2
Vit A (RE)	691.8±22.4	695.6±23.3
Vit B_1 (mg)	0.99±0.03	1.0±0.03
Vit B_2 (mg)	1.42±0.04	1.43±0.04
Niacin (mg)	9.45±0.29	9.49±0.31
Vit C (mg)	154.6±6.96	164.2±7.21

Note. Values are $\bar{x} \pm s$. None of the values are significantly different from the values at week 0.

Lipid Profiles

Compared with the values measured at week 0, serum concentrations of HDL-cholesterol increased by 27% (P<0.0001) and LDL-cholesterol decreased by 10% (P=0.0007) over the study period. The ratio of HDL- to LDL-cholesterol was significantly higher on week 12 (P<0.0001), and the atherogenic index (AI) was significantly reduced (P<0.0001). However, kale juice supplementation was not found to influence serum total cholesterol and triglyceride levels.

Antioxidant Trace Elements, Glutathione Peroxidase, and Lipid Peroxidation

During the kale juice supplementation, the serum concentration of Se significantly increased by 22.5% (P=0.0132), but the other trace elements did not change. With kale juice consumption, serum GSH-Px activity increased by 74.8% (P=0.0005), but no significant change was observed in the serum malondialdehyde level (Table 5).

TABLE 4

Effects of Kale Juice Supplementation on Serum Lipid Profiles and Atherogenic Index in Hypercholesterolemic Men

	0 Week	12 Weeks	P-value
Total Cholesterol (mg/dL)	239.8±7.37	226.5±7.09	0.075
HDL-cholesterol (mg/dL)	40.3±1.53	49.0±2.26*	<0.0001
LDL-cholesterol (mg/dL)	178.1±7.35	150.2±8.62*	<0.0001
HDL/LDL	0.23±0.01	0.34±0.03*	<0.0001
Atherogenic Index†	5.05±0.26	3.72±0.22*	<0.0001
TG (mg/dL)	107.0±7.76	129.8±10.21	0.080

Note. Values are $\bar{x} \pm s$. †Atherogenic index: (Total cholesterol-HDL-cholesterol)/HDL-cholesterol. *Values are significantly different at P<0.05.

TABLE 5

Effects of Kale Juice Supplementation on Serum Concentrations of Antioxidant Minerals, Glutathione Peroxidase Activity, and Lipid Peroxidation in Hypercholesterolemic Men

	0 Week	12 Weeks	P-value
Se (μg/dL)	13.8±0.73	16.8±1.02*	0.0132
Zn (μg/dL)	92.6±9.68	98.7±10.8	0.2443
Cu (μ/dL)	67.8±8.42	65.1±8.49	0.3025
Mn (μg/dL)	0.42±0.09	0.32±0.02	0.2081
Cu/Zn	0.76±0.27	0.67±0.21	0.1253
GSH-Px (nmol of NADPH/min/mg pt)	31.23±3.69	54.6±5.79*	0.0005
MDA (nmol/mL)	4.18±0.13	4.02±0.19	0.7820

Note. Values are $\bar{x} \pm s$. *Values are significantly different at P<0.05.

Changes in Serum Variables According to Smoking Status

Subjects were divided into two groups according to smoking status, and changes in serum variables between smokers and non-smokers were compared. The effects of the three-month supplementation of kale juice on serum LDL, GSH-Px, and Se in non-smokers and smokers are shown in Fig. 1. Compared with data at week 0, a 13.2% reduction in LDL-cholesterol and a 88.1% increase in GSH-Px activity were observed in non-smokers, while smokers showed a 61.5% increase in Se during the same period. Net differences in LDL (P=0.0487) and GSH-Px (P=0.042) were significantly lower in smokers, however, net difference in Se (P=0.0111) was significantly higher in smokers. No changes in serum variables involved with drinking and exercise status after kale juice supplementation were found (data not presented).

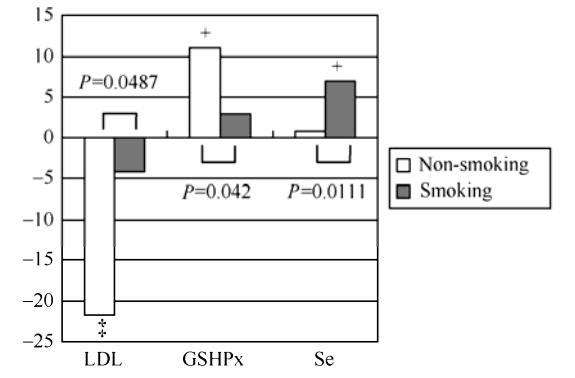

FIG. 1. Changes in LDL, GSH-Px activity, and Se level involved with smoking status after supplementation of kale juice. $^{+}P<0.01$, $^{‡}P<0.001$ compared with initial values in non-smoking and smoking groups.

DISCUSSION

Epidemiologic evidence is growing that foods rather than any single nutrient have beneficial effects on coronary artery disease[22-24]. Among several healthy foods or dietary patterns identified to date, vegetables, in particular, dark green leafy, cruciferous, and deep-yellow orange vegetables are now believed to protect against CAD. Accordingly, efforts to increase the consumptions of these foods may offer a practical way to reduce coronary artery disease (CAD)[10-11,25-26].

The present study shows that kale juice supplementation (150 mL/day for 12 weeks) resulted in substantial improvements in serum lipid profiles, especially with respect to HDL and LDL cholesterol levels, the ratio of HDL- to LDL-cholesterol, and in antioxidant status of hypercholesterolemic men. Biological activities of kale extract have been demonstrated in *in vitro* system, such as antioxidant activity, inhibitory effect of abnormal cell growth, and inhibition of HMG-CoA reductase activity[16,27]. In terms of the hypocholesterolemic effects of green vegetables, an animal study showed reduced cholesterol absorption and enhanced cholesterol catabolism to bile acid in rats fed *Angelica keiskei*, another dropwort family green vegetable[28].

Classic modifications of dietary patterns aimed at preventing chronic diseases through improving lipid profiles have targeted at reduction of energy, dietary saturated fat, and cholesterol intakes[29]. Recently, some data have been made available on the benefits of fruits and vegetables to the plasma concentrations of lipids[30-32]. One of the most plausible mechanisms that have been proposed to explain the effect of fruits and vegetables consumption on CAD risk involves the intake of several antioxidant nutrients[11]. Changes in serum antioxidant biomarkers, such as increased levels of Se (P=0.0132) and the activity of GSH-Px (P=0.0005), after kale juice supplementation imply the improvement of the additional serum antioxidant defense system, rather than the only effect due to antioxidant vitamins, as suggested in other intervention studies using single or mixed vegetables[32-33]. Several antioxidant vitamins, minerals and other nutrients in kale juice could be independently or jointly responsible for the apparent reduction in CAD risk factors[34].

It was expected that the supplementation of antioxidant nutrient-rich kale juice might reduce the extent of lipid peroxidation through two possible mechanisms: by reducing the amount of serum lipid substrate available for peroxidation and by increasing the concentrations of antioxidants derived from kale juice. In this trial, no difference was observed in MDA level, a measure of *in vitro* lipid peroxidation. It is in accord with the findings of a previous randomized clinical trial that demonstrated the effect of fruit and vegetables only on *in vivo* measures of lipid peroxidation, such as breath ethane levels, but not on MDA levels[35]. However, the significant increase in the HDL- to LDL-cholesterol ratio found in the present study implies protection against the possible deleterious effect of oxidized-LDL, which has implications in atherosclerosis. In addition to the mechanism through the ability to reverse cholesterol transport, anti-atherogenic property of HDL has also been proposed to protect LDL from oxidation[36].

Hu *et al.*[26] suggested that the combined effects of diet and better lifestyle are more powerful than any single factor alone in terms of disease prevention. Among several modifiable CAD risk factors, such as smoking, obesity, hypertension, and alcohol drinking, smoking has been suggested as the most significant risk factor in Korean men[37].

We compared changes in serum variables and net differences in serum variables with respect to smoking status to evaluate if there was a difference in response to kale juice supplementation. No adverse effect of kale juice was found in smokers. However, whereas LDL-cholesterol levels and the activities of GSH-Px responded more to kale juice supplementation in the non-smokers, serum Se responded more in the smokers.

This difference in serum Se with respect to smoking status seems to be due to the lower basal level of Se in smokers (baseline Se level in smoking group: 10.27+3.04 μg/dL and in non-smoking group: 15.46+3.82 μg/dL). This depletion may be due primarily to the toxic effect of tobacco smoke[38], i.e. the high oxidant content of smoke which could lower the levels of antioxidant nutrients and increase oxidative stress. The serum Se level response in

smokers was very similar to that found in a previous study, which demonstrated that plasma antioxidant vitamins are replenished more effectively by the supplementation of antioxidant nutrients in smokers than in non-smokers[39-40].

On this basis it has been suggested that smokers, in particular, would benefit from increasing their dietary intake of antioxidant nutrients[6,12], and in terms of the sources of antioxidant nutrient, considering the harmful effect of a single antioxidant nutrient supplementation reported in smokers in several studies[7-8], the consumption of fruits and vegetables is highly recommended.

There are some limitations in this study that should be considered. Although significant changes were detected after supplementation, the numbers of subjects were comparably small and there was no placebo group to compare with; therefore, our results need to be confirmed by a larger-scaled, controlled study. Nonetheless, present study demonstrated that kale juice supplementation favorably influences serum lipid profiles and antioxidant systems. And these results provide an additional scientific rationale for recommendations to increase the consumption of fruits and vegetables as a means of reducing the risks of CAD.

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