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Effects of Water-soluble and Water-insoluble Fractions of Kimchi on the Alteration of Plasma Lipids and Fibrinolytic Activity in Middle-aged Healthy Subjects

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Abstract

Freeze-dried kimchi fractions, water-soluble (WSK) versus water-insoluble (WISK), on their effects on plasma lipids and for their fibrinolytic activities were studied. Nineteen middle-aged healthy subjects were assigned to WSK, WISK, or placebo during 6 weeks of kimchi supplementation. Subjects in the WSK and WISK groups were supplemented with 3 grams of kimchi pills (equivalent to 60 grams of fresh kimchi), while the placebo group was on their normal diets. LDL/HDL and atherogenic index decreased in the subjects who received kimchi pills, but not in those in the placebo group. The levels of plasma triglyceride were significantly decreased in the WSK group compared with those in the WISK group ($p<0.05$). However, there was no difference in the levels of total cholesterol and HDL between the two groups. Fibrinolytic activity of WISK was significantly higher than that of WSK.

Key words: water-soluble and water-insoluble fractions of kimchi, cholesterol, triglyceride, atherogenic index, fibrinolytic activity

INTRODUCTION

Kimchi, a fermented Korean vegetable food, has been recognized as one of the top five healthiest foods in the world (1). Various vegetables can be used as major ingredients of kimchi, and condiments such as garlic, red paper powder, ginger, green onion and fermented fish sauce are generally added to the process of kimchi making (1). Kimchi can be considered a healthy food because it is a low-calorie (18 kcal/100 g) and low-fat (0.5 g/100 g) (2) plant-based food. In addition, kimchi has been spotlighted for its health desirable functionalities: anti-oxidative activity (3-7), anti-aging (8,9), anti-mutagenic (10-13), anti-tumor (13), anti-microbial activity (14), immune stimulating activity (15-17), anti-obesity (17-19), plasma lipid lowering and anti-atherogenic (19-27). While the mechanisms responsible for each of the above effects are not fully determined yet, vitamins, minerals, phytochemicals, dietary fiber, and organic acids are among the bioactive components believed to contribute to the desirable functionalities of kimchi (28).

According to the literature, Chinese cabbage kimchi suppressed plasma triglyceride (TG) concentrations in animal models when a normal diet was provided(20). By contrast, rabbits fed high cholesterol diet with kimchi supplementation showed a reduction in plasma total cholesterol and LDL cholesterol concentrations (22,29). The intima thickness of the aorta of rabbits fed a high cholesterol diet was reduced by kimchi supplementation (26). In a study using human subjects, lipid lowering effects of Chinese cabbage kimchi was also observed. An extra thirty grams of kimchi consumption in addition to a regular meal appeared to suppress the plasma TG and to lower the atherogenic index in middle-aged healthy subjects (23). Our previous works have shown that Chinese cabbage kimchi exhibited lipid lowering effects and anti-atherogenic effects in both animal models as well as in human subjects (19-27). In this study, we examined the effects of two kimchi fractions, water-soluble (WSK) versus water-insoluble fraction (WISK), on plasma lipid profiles and on fibrinolytic activity. As a control, a placebo group was included in which glutinous rice powder replaced the freeze-dried kimchi.

MATERIALS AND METHODS

Kimchi making

Cabbage (*Brassica pekiinesis,* average weight 3 kg) was cut into 3 cm×3 cm and soaked in 10% brine for 2 hours at 10°C and then rinsed three times with tap water

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and then drained for 2 hours. Mustard leaf (*Brassica juncea*) was prepared by the same process as the cabbage except for a briefer brining of 20 min. The recipe for kimchi preparation consisted of the following; brined Chinese cabbage 59.4%, brined mustard leaf 25.4%, red pepper powder 3.4%, garlic 3.1%, ginger 0.2%, pickled shrimp juice 1.9%, pickled anchovy juice 1.9%, glutinous rice paste 1.1%, and raw shrimp stock 3.6% (30). Mustard leaf was added to the Chinese cabbage kimchi in order to increase the anti-oxidative properties of kimchi (31). Kimchi was left at room temperature overnight and then stored at 5°C until it was well ripened. Fermented kimchi, pH 4.11, salinity 2.75% and acidity 1.18, was freeze dried.

Extraction of water-soluble (WSK) and water-insoluble fractions (WISK) and kimchi pill preparation

Freeze-dried kimchi was extracted with 20 volumes of distilled water (w/v) with constant stirring for 6 hours and filtered. The extracts and residue were freeze fried. The yields for WSK and WISK were approximately 50% for each. Two types of kimchi pills, containing either 500 mg of freeze dried WSK or WISK, were prepared. Placebo contained the same amount of glutinous rice powder.

Subjects

The purpose and a detailed description of the study protocol were provided to recruit study participants. Middle-aged healthy adults who expressed an interest in participating in the study were then asked to answer a questionnaire about their medical history and life style. Those who reported any records of certain diseases or histories of medicinal intake that may affect the results of the study were excluded from the study. Informed consent forms were obtained prior to the initiation of the present study. Nineteen subjects (10 males and 9 females) who met our experimental criteria were divided into 3 groups depending on their sex, age and body mass index (BMI); 6 subjects for WSK (3 males and 3 females), 7 subjects for WISK (4 males and 3 females) and 6 for the placebo group (3 males and 3 females). Subjects of each group were asked to take six pills (500 mg/pill), preferably divided evenly for three meals, a day for 6 weeks. The amount of kimchi pills taken daily was 3 g in total, which was equivalent to 60 g of fresh kimchi. We instructed the subjects to continue their normal lifestyle, including dietary intake and physical activity, during the experimental periods.

Dietary intake

Three days of dietary records (32) were obtained both at baseline and at the end of the 6-week intervention. Subjects were instructed to record the items and amounts of foods consumed during two weekdays and one weekend day by registered dieticians. The subjects were trained with measuring cups and spoons, a ruler, and two-dimensional portion-size booklets as a visual aid for estimating amounts of foods eaten by the dieticians. The dietary data collected by food records were analyzed with CAN Pro program developed by the Korean Nutrition Society.

Body composition and blood pressure

Body composition was assessed by using the In Body 2.0 (Danil SMC, Korea). Body mass index (BMI), visceral fat content, and muscle mass were calculated. Blood pressure was measured by an automated blood pressure examiner (T.K.K, Japan).

Lipid profiles

Registered nurses obtained blood samples from the subjects after 12 hours of fasting. Plasma was separated immediately by centrifugation at $800\times g$ for 15 min and stored at -70°C until use. Blood glucose level, triglyceride (TG), total cholesterol (TC), HDL cholesterol (HDL), and other hematological parameters were analyzed (Olympus AU 5200 Analyzer, Japan) at Pusan National University Hospital. LDL cholesterol (LDL) concentration was calculated by the following formula: [$LDL=TC-(HDL+TG/5)$] (33). Atherogenic index (AI) was also calculated by [$(TC-HDL)/HDL$].

Fibrinolytic activity

Fibrinolytic activity of plasma obtained from the subjects was determined using a fibrin tube which is a modified version of the fibrin plate method (34). In brief, fibrin was prepared by mixing fibrinogen and thrombin in a tube. The plasma, 50 μL, was applied on top of the fibrin tube and then incubated for 18 hours at 37°C. The amounts of water released during the fibrinolysis were measured. Fibrinolytic activity (expressed as plasmin units) of plasma sample was calculated based on the standard curve obtained using a known amount of plasmin by the identical procedure used for plasma samples.

Statistical analysis

Values calculated in terms of percentage change in BMI, blood glucose level, plasma lipid concentration, AI, and fibrinolytic activity between the baseline and the end point of 6-week intervention were used for statistical analysis. One-way ANOVA followed by Duncan's multiple range test as post hoc analysis were used to compare the effects of WSK, WISK and placebo. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Characteristics of subjects

The average age, height and weight for the 19 subjects (10 males and 9 females) were 39 years old, 167 cm, and 66.8 kg, respectively. The average BMI of the subjects was 23.8 and 9 out of 19 subjects had BMI of 25 or higher. The degree of obesity was 111.4%. Only one or two subjects in each group smoked and the number of cigarettes smoked was less than 20. Eight males in total occasionally drank alcohol and the average alcohol consumption was 35 g/day. For physical activity, only 2 subjects reported that they exercised regularly (1 or 2 times/week). General characteristics of the subjects are described in Table 1.

During the 6 weeks of kimchi supplementation, changes in BMI among experimental groups were not significantly different (Table 1), although there were a slight reduction of BMI in WSK and WISK. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the subjects were in the range of 122~133 mmHg and 70~82 mmHg, respectively. SBP and DBP decreased in kimchi supplementation groups but not in the placebo group. The effect of kimchi on decreasing blood pressure was greater for DBP than SBP. DBP for the placebo was elevated by 8.1%, while DBP for WSK and WISK decreased by 4.5% and 0.9%, respectively. SBP for the placebo group was increased by 3.3%, whereas SBP for WSK and WISK decreased by 2.1% and 0.9%, respectively. However, these changes in SBP and DBP did not obtain the statistical significance, which may be attributable to the individual variations among subjects or the need for a larger study group. Overall, WSK was found to have a greater tendency to decrease blood pressure.

Table 1. General characteristics of subjects before and after 6 weeks of kimchi supplementation

		Group[1]		
		Placebo	WSK	WISK
Age (years old)		35.5±8.6[2]	38.5±5.7	41.6±5.8
Height (cm)		167.2±6.5	169.2±6.7	165.0±6.8
Weight (kg)		63.9±14.9	66.7±9.4	69.4±9.3
BMI	Pre	22.6±3.6	23.2±1.9	25.4±2.3
	Post	22.8±3.7	23.0±2.1	25.0±2.2
SBP[3]	Pre	122.8±9.8	133.7±21.6	123.1±11.3
	Post	127.2±21.5	129.8±15.8	121.4±6.3
DBP[4]	Pre	70.0±12.7	81.7±9.9	75.0±9.0
	Post	75.5±17.1	77.7±12.0	74.6±6.2

[1]Placebo: Parched glutinous rice powder was used, WSK: Water-soluble fraction of kimchi, WISK: Water-insoluble fraction of kimchi.
[2]Values are mean±SD.
[3]SBP: Systolic blood pressure. [4]DBP: Diastolic blood pressure.

Hematological parameters and blood glucose levels

The hematological parameters measured at baseline and at the end point of 6-week intervention indicated that the subjects' albumin, hemoglobin, hematocrit and platelet counts were in the normal range and these parameters were not changed significantly by kimchi supplementation (Table 2).

Blood glucose concentration of WISK decreased by 6.1% but there was little change in WSK and placebo groups (Table 3). Nonetheless, the effect of WISK on reducing blood glucose levels was not sufficiently different from the effects of placebo and WSK, which may be attributable to the individual variation and a small sample size. However, we observed the possible action of WISK on decreasing the blood glucose concentration. It might be that water-insoluble fiber in kimchi could inhibit the absorption of glucose in the intestinal tract.

Nutrient intake

The amounts of energy (kcal) and protein consumed by the subjects appeared to be less than the RDA (Table 4) and energy intake level met only two thirds of the RDA (35). Caloric intake of WSK and WISK appeared to be similar before and after the intervention, whereas energy consumption increased by 6.2% in the placebo group. There was no significant difference in lipid consumption in each group before and after the intervention. Cholesterol intake in placebo and WSK increased approximately 20% but the amount of dietary cholesterol in WISK remained similar. The magnitude of decrease in total cholesterol concentration before and after kimchi supplementation was greater in placebo and WSK than WISK. The amounts of kimchi consumed from diet in placebo, WSK and WISK were 68.3 g, 43.0 g, and 47.2 g, respectively. The placebo group consumed more kimchi than the other groups from their normal diet but the amount of kimchi intake remained same in each group during the experimental period. Thus, the effects of kimchi supplementation on altering serum lipid concentration would not be affected by kimchi intake since we compared the individual changes before and after kimchi supplementation.

Alterations in plasma lipids

Changes in TG concentration in WSK appeared to be significantly different compared with the other groups ($p<0.05$). Approximately 16% of decrease in TG was observed in WSK, while TG in placebo and WISK were slightly elevated (Fig. 1). This observation was in line with the results from the previous studies in which the effects of kimchi on lipid profiles were examined using animal models or human subjects (20-26). Diets con-

Table 2. Hematological parameters of subjects before and after 6 weeks of kimchi supplementation

Group[1)]	Albumin (g/dL)		WBC[2)] ($\times 10^9$/L)		RBC[3)] ($\times 10^{12}$/L)		HGB[4)] (g/dL)		HCT[5)] (ratio)		PLT[6)] ($\times 10^9$/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Placebo	4.2±0.1[7)]	4.3±0.2	5.6±1.5	5.4±0.6	4.7±0.4	4.6±0.5	14.2±1.5	14.1±1.6	41.6±4.5	42.5±4.6	287.3±42	240.8±53
WSK	4.2±0.2	4.3±0.2	5.8±1.0	6.0±1.14	4.6±0.5	4.5±0.5	14.1±1.5	13.8±1.4	41.4±4.3	41.4±3.9	247.3±65	249.2±44
WISK	4.1±0.1	4.1±0.1	6.1±1.1	6.1±0.9	4.6±0.5	4.5±0.5	14.1±1.6	13.6±1.8	41.1±4.1	41.4±4.5	257.4±55	254.4±55

1)Groups are the same as in Table 1.
2)WBC: White blood cell. 3)RBC: Red blood cell. 4)HGB: Hemoblobin. 5)HCT: Hematocrit. 6)PLT: Platelet.
7)Values are mean±SD.

Table 3. Changes in blood glucose levels of subjects after 6 weeks of kimchi supplementation

Group[1)]	Glucose level (mg/dL)		% changes
	Pre	Post	
Placebo	90.50±5.21[2)]	90.33±11.08	-0.13[NS3)]
WSK	89.67±3.83	91.00±6.99	1.47
WISK	97.57±9.03	91.29±5.41	-6.10

1)Groups are the same as in Table 1.
2)Values are mean±SD.
3)Not significant.

Table 4. Dietary intakes of subjects before and after 6 weeks of kimchi supplementation

	Group[1)]					
	Placebo		WSK		WISK	
	Pre	Post	Pre	Post	Pre	Post
Calorie (kcal)	1720.9[2)]	1861.1	1446.0	1507.4	1711.8	1729.5
Carbohydrate (g)	254.2	269.0	200.5	216.4	245.5	245.8
Protein (g)	63.2	80.1	55.2	54.2	67.2	72.0
Lipid (g)	47.5	46.8	39.4	38.9	44.9	43.3
Cholesterol (mg)	228.2	275.3	259.2	308.4	240.6	236.8

1)Groups are the same as in Table 1.
2)Data were analyzed with three days of food records.

taining 5% or 10% kimchi effectively altered plasma TG concentration but not TC concentration in animal models. In this human study, supplementing healthy subjects with kimchi pills, equivalent to 60 grams of fresh kimchi per day, for six weeks decreased plasma TG concentration only. TC concentrations tended to decrease in all three groups but there was no significant difference between groups (Fig. 2). This result is consistent with others' report that both plasma TC and TG decreased in rabbits fed high cholesterol diets along with kimchi supplementation (22). The ratio of LDL to HDL (LDL/HDL) decreased radically with kimchi supplementation in WSK and WISK, whereas an elevated LDL/HDL was observed in the placebo group ($p<0.05$) (Fig. 3). The atherogenic index (AI), calculated by the formula [(TC − HDL)/HDL], in WSK and WISK decreased considerably more than in the placebo group ($p<0.05$) (Fig. 4).

According to the literature, kimchi exhibited beneficial effects on the alteration of plasma lipids in healthy subjects as well as hyperlipidemic subjects. The hypolipidemic effects of WSK might be due to the soluble dietary fiber or unidentified compounds in kimchi. Mechanisms of dietary fibers, both soluble and insoluble, in disturbing nutrients absorption are well established. In addition, other substances in kimchi than the dietary fiber could be responsible for the observed alteration in plasma lipid profiles. According to the literature, β-sitosterol and cystein sulfoxide in Chinese cabbage (36), capsaicin and decapsaicin in red pepper (37), allicin in garlic (38), gingerol in ginger (39), and lactic acid bacteria (27) produced in kimchi are well

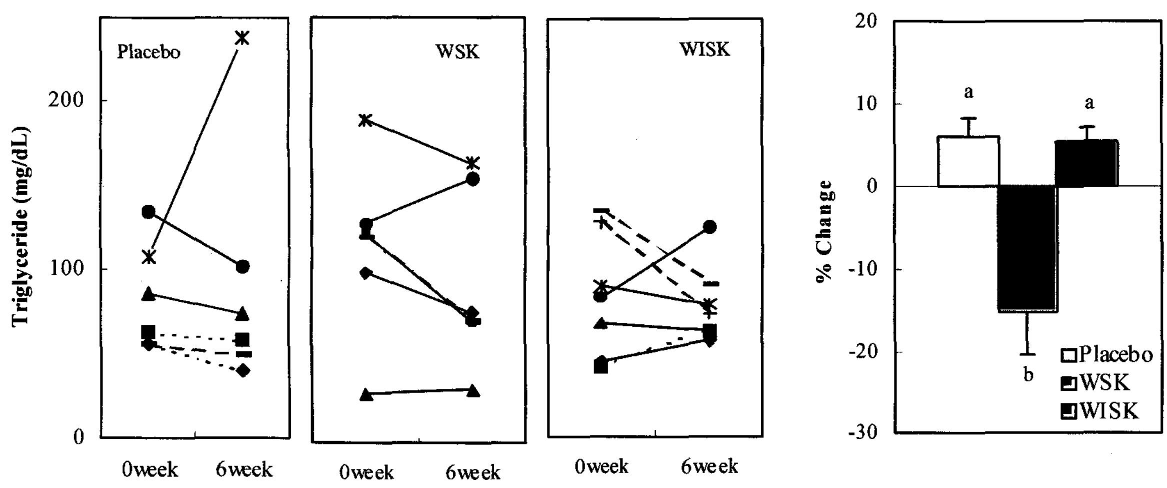

Fig. 1. Changes in plasma triglyceride concentrations of subjects after 6 weeks of kimchi supplementation. Groups are the same as in Table 1. Values with different letters are significantly different at $p<0.05$.

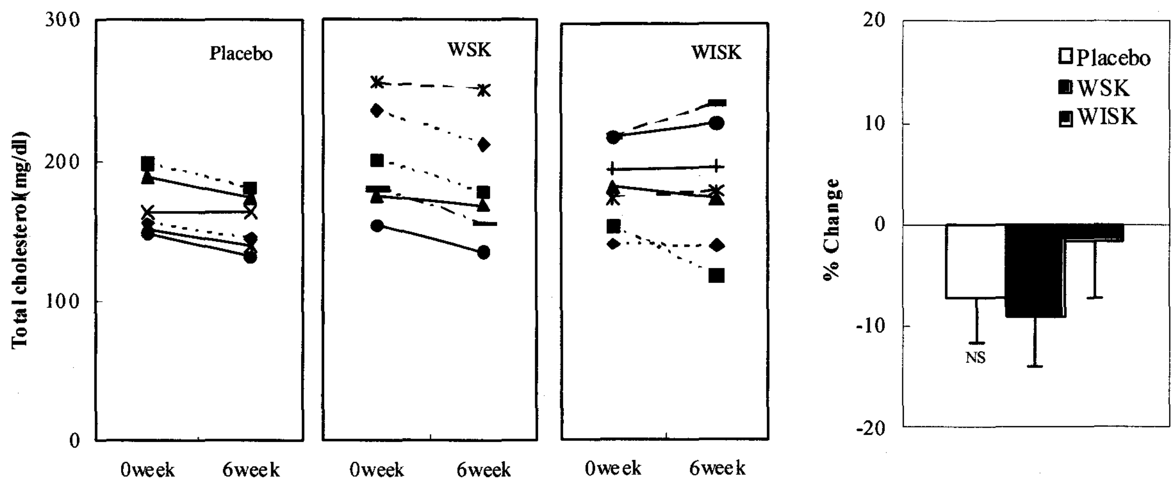

Fig. 2. Alterations in plasma total cholesterol concentrations of subjects after 6 weeks of kimchi supplementation. Groups are the same as in Table 1. NS: Not significant.

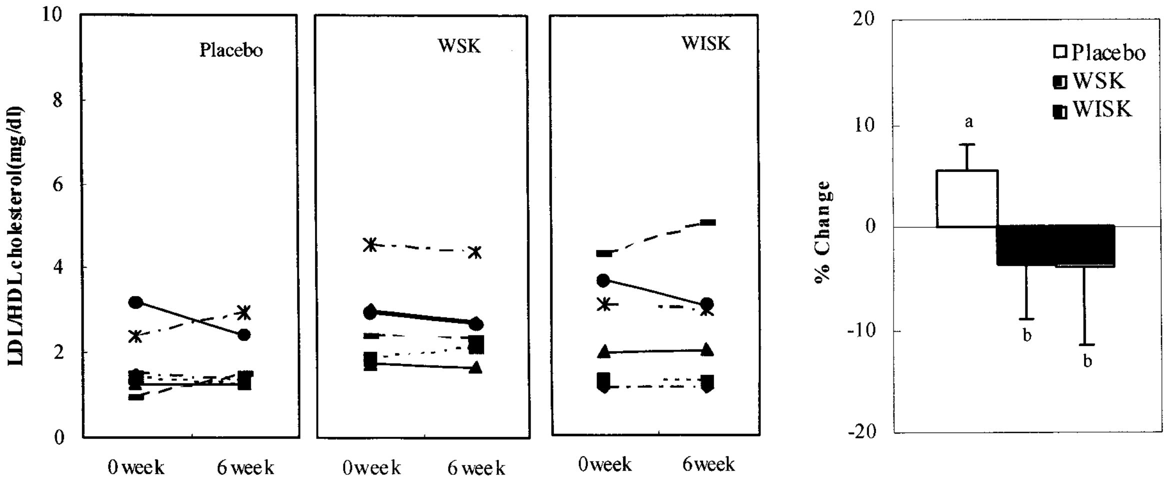

Fig. 3. Changes in LDL/HDL of subjects after 6 weeks of kimchi supplementation.
Groups are the same as in Table 1. Values with different letters are significantly different at $p<0.05$.

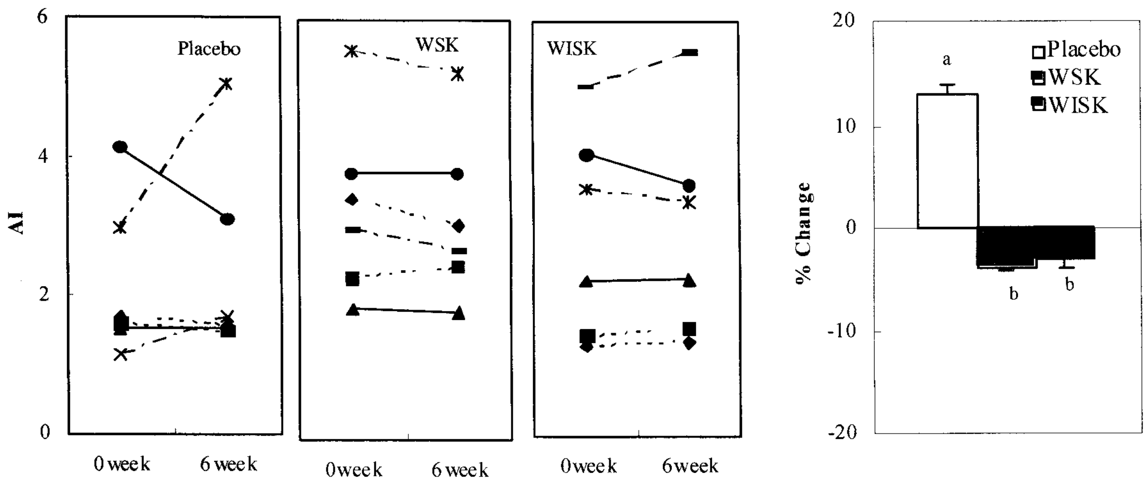

Fig. 4. Changes in atherogenic index (AI) of subjects after 6 weeks of kimchi supplementation.
Groups are the same as in Table 1. Values with different letters are significantly different at $p<0.05$.

known compounds that have TG or cholesterol lowering properties. Given that the subjects were allowed to continue their normal dietary intake, potential factors stemming from the subjects' diets need to be taken into account as well when factors influencing plasma lipid profiles are examined. It is possible that the increased caloric intake in placebo group (6.2%) during the intervention period elevated the TG concentration (6.0%). Interestingly, an increased TG concentration by 5.3% was observed in WISK, although caloric intake in this group was not significantly increased between the two dietary assessments. A decrease in TC concentration was observed in all three groups. This result was not anticipated because dietary cholesterol intake in placebo and WSK was increased about 20% during the intervention. There might be other factors than the dietary intakes that altered the plasma lipids concentrations. Findings from this study suggest that kimchi supplementation may have played an important role for the alteration of lipid profiles.

Fibrinolytic activity

The fibrinolytic activity of WISK after 6 weeks of kimchi supplementation was remarkably increased compared with the other groups and it was 8 folds greater than before the intervention ($p<0.05$) (Table 5). By contrast, an elevated fibrinolytic activity was not observed in the WSK group. These results are in good agreement with a previous study (27). An increased fibrinolytic activity in rats fed semi-synthetic diet, supplemented with 10% of kimchi for 6 weeks was observed but this effect was very small in diets with

Table 5. Changes in fibrinolytic activities of subjects after 6 weeks of kimchi supplementation

Group[1)]	Fibrinolytic activity (plasmin unit)		% changes
	Pre	Post	
Placebo	0.082±0.006[2)]	0.093±0.007	0.011[a3)]
WSK	0.031±0.017	0.073±0.070	0.042[a]
WISK	0.056±0.024	0.432±0.375	0.376[b]

[1)]Groups are the same as in Table 1.
[2)]Values are mean±SD.
[3)]Values with different letters are significantly different at $p<0.05$.

less than 5% kimchi (27). A subsequent study carried out by same authors using the water and methanol extracts of kimchi have shown that methanol extracts of kimchi exhibited fibrinolytic activity 6 times greater than that of water extracts. Among ingredients commonly used for making kimchi, methanol extracts of red pepper powder exhibited the highest fibrinolytic activity followed by methanol extract of radish, water soluble extracts of dropwort, and methanol extracts of green onion in decreasing order (27). WISK used in this experiment was prepared with the residue part of the kimchi after water extraction. Therefore, water-insoluble components in kimchi may have remained in WISK. Both our results and previous reports suggest that WISK may be more responsible for the fibrinolytic activity of kimchi than the water-soluble fraction.

Findings from this study advocate further work to discover the active components that are responsible for the effects of kimchi in regulating plasma lipid profiles and atherosclerosis-related parameters. Furthermore, the results can be used in nutritional education programs for the younger generation in Korea who does not consume kimchi as much as the older generation.

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