Original Article

Effects of Nattokinase on Blood Pressure: A Randomized, Controlled Trial

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The objective of this study was to examine the effects of nattokinase supplementation on blood pressure in subjects with pre-hypertension or stage 1 hypertension. In a randomized, double-blind, placebo-controlled trial, 86 participants ranging from 20 to 80 years of age with an initial untreated systolic blood pressure (SBP) of 130 to 159 mmHg received nattokinase (2,000 FU/capsule) or a placebo capsule for 8 weeks. Seventy-three subjects completed the protocol. Compared with the control group, the net changes in SBP and diastolic blood pressure (DBP) were −5.55 mmHg (95% confidence interval [CI], −10.5 to −0.57 mmHg; $p<0.05$) and −2.84 mmHg (CI, −5.33 to −0.33 mmHg; $p<0.05$), respectively, after the 8-week intervention. The corresponding net change in renin activity was −1.17 ng/mL/h for the nattokinase group compared with the control group ($p<0.05$). In conclusion, nattokinase supplementation resulted in a reduction in SBP and DBP. These findings suggest that increased intake of nattokinase may play an important role in preventing and treating hypertension. (*Hypertens Res* 2008; 31: 1583–1588)

***Key Words*: nattokinase, blood pressure, renin activity, randomized, controlled trial**

Introduction

Hypertension has become a global public health challenge, affecting approximately 50 million individuals in the United States and one billion individuals worldwide (*1*, *2*). The prevalence of hypertension has increased dramatically in developing countries in recent decades (*3*). According to Choi *et al*. (*4*), the prevalence of hypertension was 31.6% in the Korean population.

High blood pressure is known to be one of the most important risk factors for cardiovascular disease (CVD), and it was shown that the reduction of highly or moderately elevated blood pressure levels resulted in decreased rates of stroke and myocardial infarction in 1993 and 2002 (*5*, *6*). Furthermore, Iseki *et al*. reported that hypertension is a significant predictor of stroke, acute myocardial infarction and end-stage renal disease (*7*).

Since the incidence of CVD in Asia is very low, traditional foods from Asia have been the subject of increased attention

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recently (*8*, *9*). For more than 1,000 years, people throughout Asia have consumed soybeans in a variety of traditional soy food products (*10*). The most popular soy foods in Western countries are tofu, soy milk (tonyu), soy burger, soy sauce, and miso. Natto, a traditional fermented vegetable cheese-like food, is another soy product. Natto extracts are known to include nattokinase (NK, formerly designated BSP, or subtilisin NAT, which is a serine proteinase from *Bacillus subtilis natto*), a potent fibrinolytic enzyme having an approximately 4-fold stronger activity than plasmin in clot lysis assays (*11*, *12*) In addition, it has a direct effect on thrombus cleavage, the mechanism by which this enzyme potentiates fibrinolysis of its reactive site (*13*).

Herein, we describe a randomized, double-blind, placebo-controlled trial conducted to test the effects of nattokinase supplementation on systolic blood pressure (SBP) and diastolic blood pressure (DBP) in subjects with pre-hypertension or stage 1 hypertension.

Methods

Subjects

For this study, subjects were recruited from volunteers who responded to advertisements for a nutrition study conducted by the Clinical Nutrition Research Team at Yonsei University in 2006. Trial participants were men and women from 20 to 80 years of age who had an average SBP of 130 to 159 mmHg based on an average of three readings. The criteria for exclusion were self-reported use of antihypertensive medications in the previous 6 months and a history of cardiovascular disease, diabetes mellitus, cancer, chronic obstructive pulmonary disease, psychiatric disease, or any other serious life-threatening illness that required regular medical treatment. We also excluded women who were pregnant or who intended to become pregnant during the study. Written informed consent was obtained from all subjects, and the protocol was approved by the Ethical Committee of the Yonsei University.

We invited 105 persons who met blood pressure and other criteria at prescreening and who were willing to participate in the trial to the study clinics for screening visits. Of these, 86 persons met all eligibility criteria and were randomly assigned.

Intervention

We randomly allocated 44 study participants to nattokinase supplementation and 42 study participants to the control group by using a computer-generated scheme. We stratified the randomization using a block size of four. The randomization assignment schedule was concealed in an ordered set of sealed envelopes, which were opened only after the study coordinator had confirmed a participant's eligibility. Apart from the study coordinator, all research personnel, including the blood pressure technicians and the study participants, were unaware of treatment assignment.

Study participants who were assigned to the intervention group received one capsule containing a nattokinase (2,000 FU/capsule) supplement per day for 8 weeks. The placebo was made to look identical to the test capsule. Japan Bio Science Laboratory Co., Ltd. (Ibaraki, Osaka, Japan) provided the nattokinase supplement.

During the intervention, we instructed study participants to continue their current food intake patterns and lifestyles so that the total energy intake and energy expenditure would be constant during the course of the trial.

Anthropometrical and Blood Pressure Measurements

Body weight and height were measured in the morning; the subjects were unclothed and barefoot. Blood pressure was read from the left arm while subjects were seated. An average of three measurements was recorded for each subject.

Blood Collection

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-h fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1–3 h) for separating plasma and serum. The separated plasma and sera were immediately stored at −70°C until analysis.

Measurement of Plasma Renin Activity and Serum Angiotensin Converting Enzyme Level

The plasma renin activity was measured by radioimmuoassay using the renin-RIA bead (Special Reference Laboratories, Tokyo, Japan), and activity was expressed as ng/mL per hour.

Measurement of serum angiotensin converting enzyme (ACE) level used was based on spectrofluorimetric determination of histidyl-L-leucine (HL) using *Z*-phenyl-histidyl-L-leucine (Bachem Bioscience Inc. Torrence, USA) as an ACE substrate (*14*). Briefly, 50 μL of plasma were incubated for 20 min at 37°C, after which 100 μL of cold trichloroacetic acid (10%) was added to stop the reaction. The samples were then centrifuged at 4°C and the supernatant was neutralized adding NaOH, followed by the addition of *o*-phthaldialdehyde solution. Samples were again incubated at 37°C for 10 min and the reaction was stopped by adding 2 mol/L HCl. Fluorescence was measured within 60 min in an Aminco-Bowman spectrofluorimeter (SLM Instruments, Urbana, USA).

Assessment of Food Intake and Physical Activity Level

Usual food intake was assessed with a 24-h recall method and a semi-quantitative food frequency questionnaire. Nutrient

Table 1. Baseline Characteristics of Study Participants According to Randomization

	Control group (n=42)	Nattokinase group (n=44)	p-value
Age (years)	46.5±1.65	47.6±1.78	0.662
Women (%)	53.8	43.2	0.283
Height (cm)	163.7±1.52	165.1±1.54	0.527
Body weight (kg)	68.0±2.56	68.8±1.81	0.792
Body mass index (kg/m^2)	25.0±0.54	25.1±0.43	0.875
Current cigarette smoking (%)	9.5	18.2	0.247
Current alcohol consumption (%)	64.3	59.1	0.620
Hyperlipidemia (%)	61.9	68.2	0.542

Mean±SEM. Tested by t-test or χ^2-test.

Table 2. Daily Dietary Nutrient Intake and Body Weight at Baseline and during Intervention, According to Randomization

	Control group		Nattokinase group	
	0 week	8 week	0 week	8 week
TCI (kcal/d)	2,298±62.1	2,300±60.9	2,378±51.2	2,372±51.8
TEE (kcal/d)	2,132±50.3	2,132±49.8	2,187±48.1	2,185±48.1
Carbohydrates (% of TCI)	62.8±0.26	62.9±0.22	62.7±0.29	62.7±0.24
Protein (% of TCI)	17.4±0.21	17.1±0.22	17.3±0.26	17.3±0.26
Fat (% of TCI)	20.4±0.38	20.1±0.29	20.2±0.24	20.2±0.31
Salt (g)	17.1±0.84	15.9±0.98	16.3±0.68	17.0±0.85
Na (mg)	6,829±335	6,353±392	6,507±271	6,801±341
Cholesterol (mg)	329.0±24.7	346.5±29.4	375.8±27.3	330.9±29.5
Body weight (kg)	68.0±2.56	67.9±2.50	68.8±1.81	68.7±1.82

Mean±SEM. TCI, total calorie intake; TEE, total energy expenditure.

intake data were calculated as mean values from the database referenced above. Total calorie expenditure (kcal/d) was calculated from activity patterns including basal metabolic rate, physical activity for 24 h (*15*), and specific dynamic action of food. The basal metabolic rate for each subject was calculated with the Harris-Benedict equation (*16*).

Statistical Analysis

We used SPSS version 12.0 for Windows (Statistical Package for the Social Science, SPSS Inc., Chicago, USA) for all statistical analyses.

We compared baseline characteristics between the intervention and control groups by using the Student's t-test for continuous variables and χ^2 tests for categorical variables. We compared the difference in changes from baseline to follow-up in dietary nutrient intake, renin, ACE and body weight between the intervention and control groups by using the Student's t-test.

The primary outcome of interest was the net difference in change of SBP and DBP (final follow-up minus baseline) between the study groups (change in nattokinase supplement group minus change in control group) during the 8 weeks of intervention. In the main analysis, we included only study participants who completed the intervention. Results are expressed as the mean±SEM. A two tailed value of $p<0.05$ was considered statistically significant.

Results

Baseline Characteristics and Dietary Intake Change of the Study Participants

A total of 88.6% of the participants assigned to the nattokinase supplement group (39 of 44) and 81.0% of those assigned to the control group (34 of 42) completed the study and provided blood measurements at their 8-week visit. General characteristics at baseline were similar in the two groups (Table 1).

Table 2 presents dietary nutrient intake at baseline and during the intervention according to randomization. During the intervention period, total calorie intake (TCI), total energy expenditure (TEE) and dietary intake of macronutrients did not significantly differ between the nattokinase supplement and control groups ($p>0.05$). Changes in body weight from baseline to 8 weeks also did not significantly differ between the two groups ($p>0.05$).

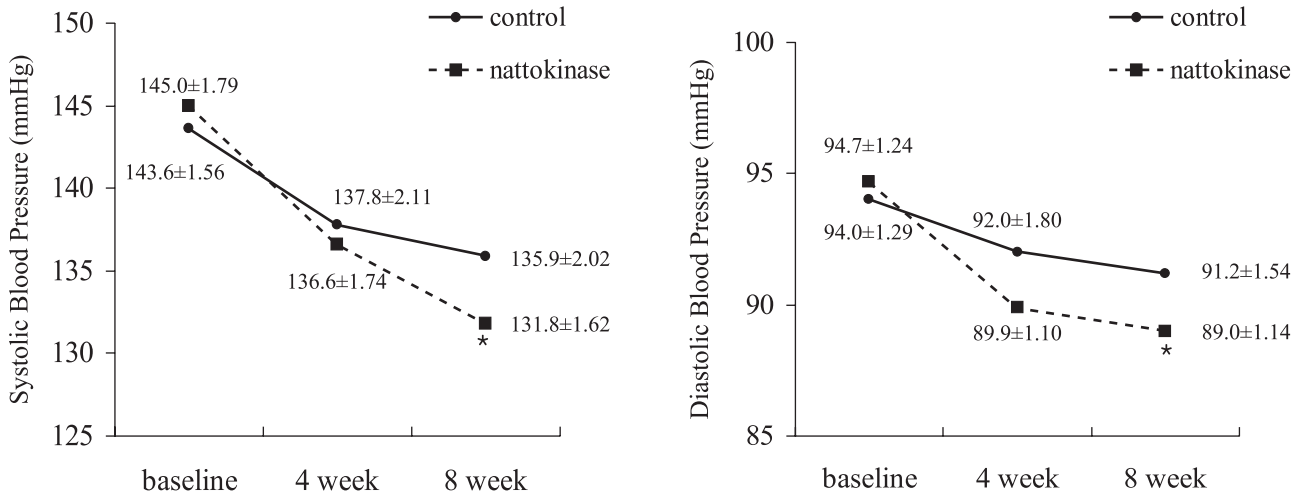

Fig. 1. *The effect of nattokinase supplmentation on systolic and diastolic blood pressure. Mean±SEM. *p<0.05 compared with the changed values (0–8 weeks) of control group.*

Table 3. Plasma Renin Activity and Angiotensin Converting Enzyme (ACE) Concentration at Baseline and during Intervention, According to Randomization

	Control group		Nattokinase group	
	0 week	8 week	0 week	8 week
Renin activity (ng/mL/h)	2.00±0.22	2.90±0.59	1.95±0.24	1.68±0.23†
ACE (U/L)	40.4±2.93	39.0±2.62	45.9±3.40	43.5±3.12

Mean±SEM. †$p<0.05$ compared with the changed values (0–8 weeks) of placebo group.

Change in Blood Pressure before and after Nattokinase Supplement

Figure 1 shows the changes in SBP and DBP from baseline to the 4-week and 8-week follow-up visits according to intervention assignment. At the 4-week visit, the mean change (±SEM) in SBP was −8.4±1.5 mmHg in the nattokinase group and −5.8±2.2 mmHg in the control group. The corresponding net change in SBP was −2.67 mmHg (95% confidence interval [CI], −7.86 to 2.52 mmHg) for the nattokinase group compared with the control group. At the 8-week visit, the mean change (±SEM) in SBP was −13.26±1.75 mmHg in the nattokinase group and −7.7±1.77 mmHg in the control group. The corresponding net change in SBP was −5.55 mmHg (95% CI, −10.5 to −0.57 mmHg) for the nattokinase group compared with the control group. These changes at the 8-week visit were statistically significant ($p=0.029$).

The average DBP was reduced by 4.77±0.94 mmHg at the 4-week follow-up visit and 5.67±0.91 mmHg at the 8-week follow-up visit in the nattokinase group and by 2.0±1.13 mmHg at the 4-week follow-up visit and 2.82±0.85 mmHg at the 8-week follow-up visit in the control group. The net change in DBP for the nattokinase group compared with the control group from baseline to 4 weeks and 8 weeks was −2.77 mmHg (CI, −5.68 to 0.14) and −2.84 mmHg (CI, −5.33 to −0.33), respectively. The net change at 8 weeks was statistically significant ($p=0.027$).

Plasma Renin Activity and ACE

Table 3 presents plasma renin activity and ACE concentration at baseline and during the intervention according to randomization. During the intervention period, mean renin activity decreased in the nattokinase group and increased in the control group but did not show a statistically significant difference. However, the corresponding net change in renin activity was −1.17 ng/mL/h for the nattokinase group compared with the control group. These changes were statistically significant ($p=0.026$).

Mean ACE concentration decreased in both the nattokinase group and the control group but was not statistically significant.

Discussion

Our results contribute important information toward nutritional intervention in lowering blood pressure. In the present study, we demonstrated that nattokinase supplementation reduced blood pressure in subjects with an average SBP of 130 to 159 mmHg.

Many studies have reported that natto suppresses intimal thickening and that a natto diet enhances the fibrinolytic system and thrombolytic effects (*17–20*). However, there has been almost no evidence that clinically proves the efficacy of natto in humans. Maruyama and Sumi (*21*) reported a change in SBP after administration of 0.5 mL of 80% ethanol extract into the peritoneal cavity of Wistar rats (400–500 g, male), where the average SBP of six rats was 166±14 mmHg before administration. After administration of the extract, SBP decreased significantly to 145±24 mmHg in 2 h ($p<0.05$) and to 144±27 mmHg in 3 h ($p<0.05$). They also reported a blood pressure change after oral administration of lyophilized product of 80% ethanol extract to human volunteers who had high blood pressure. Thirty grams of lyophilized extract (equivalent to 200 g of natto) was administered per orally for 4 consecutive days. In four of five volunteers, the SBP as well as the DBP decreased. The average values decreased from 173.8±20.5 mmHg to 154.8±12.6 mmHg in SBP and 101.0±11.4 mmHg to 91.2±6.6 mmHg in DBP.

Our study, with the largest sample size and several measurements of blood pressure, indicated that nattokinase supplementation reduced both SBP and DBP.

The underlying mechanisms by which nattokinase may influence blood pressure are not entirely clear.

The renin-angiotensin system is considered to be a blood pressure regulation system that is apt to be affected by food components (*22*). The system starts with the conversion of angiotensinogen to a pre-hypertensive hormone angiotensin I (DRVYIHPFHL) by the action of renin, which is secreted by the kidney. The angiotensin I is further converted to angiotensin II (DRVYIHPF), the active form of the hormone, by the action of ACE (EC 3.4.15.1). Angiotensin II raises blood pressure by acting directly on blood vessels, sympathetic nerves and adrenal glands. Okamoto *et al*. (*23*) reported that antihypertensive substances that inhibit ACE are also found in natto. Kuba *et al*. (*24*) also isolated ACE inhibitory peptides from Tofuyo, fermented soybean food.

In our study, we observed that a corresponding net change in renin activity for the nattokinase group compared with the control group was statistically significant. However, ACE concentration did not demonstrate a statistically significant difference.

In this study, a randomized, controlled trial in Korean adults documented a statistically significant reduction in blood pressure related to nattokinase supplementation. Results from our study provide new evidence supporting nattokinase supplementation to prevent and treat hypertension. For further confirmation of the blood pressure lowering effect of nattokinase or natto, we plan to increase the number of subjects in order to elucidate the mechanism of action.

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