

## Nattokinase decreases plasma levels of fibrinogen, factor VII, and factor VIII in human subjects<sup>☆</sup>

Chien-Hsun Hsia<sup>a</sup>, Ming-Ching Shen<sup>b</sup>, Jen-Shiou Lin<sup>c</sup>, Yao-Ke Wen<sup>d</sup>, Kai-Lin Hwang<sup>e</sup>,  
Thau-Ming Cham<sup>f</sup>, Nae-Cherng Yang<sup>g,\*</sup>

<sup>a</sup>Division of Cardiovascular Medicine, Changhua Christian Hospital, Changhua 500, Taiwan

<sup>b</sup>Division of Hematology-Oncology, Changhua Christian Hospital, Changhua 500, Taiwan

<sup>c</sup>Department of Laboratory Medicine, Changhua Christian Hospital, Changhua 500, Taiwan

<sup>d</sup>Division of Nephrology, Changhua Christian Hospital, Changhua 500, Taiwan

<sup>e</sup>Department of Public Health, Chung Shan Medical University, Taichung 402, Taiwan

<sup>f</sup>Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>g</sup>Department of Nutrition and Health Science, Chungchou Institute of Technology, Changhua 510, Taiwan

Received 17 December 2008; revised 21 January 2009; accepted 28 January 2009

### Abstract

Nattokinase, a serine proteinase from *Bacillus subtilis*, is considered to be one of the most active functional ingredients found in natto. In this study, we hypothesized that nattokinase could reduce certain factors of blood clotting and lipids that are associated with an increase risk for cardiovascular disease (CVD). Thus, an open-label, self-controlled clinical trial was conducted on subjects of the following groups: healthy volunteers (Healthy Group), patients with cardiovascular risk factors (Cardiovascular Group), and patients undergoing dialysis (Dialysis Group). All subjects ingested 2 capsules of nattokinase (2000 fibrinolysis units per capsule) daily orally for 2 months. The laboratory measurements were performed on the screening visit and, subsequently, regularly after the initiation of the study. The intent-to-treat analysis was performed on all 45 enrolled subjects. By use of mixed model analysis, a significant time effect, but not group effect, was observed in the change from baseline of fibrinogen ( $P = .003$ ), factor VII ( $P < .001$ ), and factor VIII ( $P < .001$ ), suggesting that the plasma levels of the 3 coagulation factors continuously declined during intake; also, the extents of decrease were similar between groups. After 2 months of administration, fibrinogen, factor VII, and factor VIII decreased 9%, 14%, and 17%, respectively, for the Healthy Group; 7%, 13%, and 19%, respectively, for the Cardiovascular Group; and 10%, 7%, and 19%, respectively, for the Dialysis Group, whereas blood lipids were unaffected by nattokinase. No significant changes of uric acid or notable adverse events were observed in any of the subjects. In summary, this study showed that oral administration of nattokinase could be considered as a CVD nutraceutical by decreasing plasma levels of fibrinogen, factor VII, and factor VIII.

© 2009 Elsevier Inc. All rights reserved.

### Keywords:

Nattokinase; Cardiovascular disease; Human; Fibrinogen; Factor VII; Factor VIII; Cholesterol

### Abbreviations:

CVD, cardiovascular disease; FU, fibrinolysis unit; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LSD, least significant difference; TC, total cholesterol; TG, triacylglycerol.

<sup>☆</sup> The Research Fund of Changhua Christian Hospital supported this study (Project No I050308).

\* Corresponding author. Tel.: +886 4 8395316; fax: +886 4 8395316.

E-mail address: [naeboy@dragon.ccut.edu.tw](mailto:naeboy@dragon.ccut.edu.tw) (N.-C. Yang).

## 1. Introduction

Nattokinase, a serine proteinase from *Bacillus subtilis*, has been reported to have potent fibrinolytic activity [1]. The enzyme is composed of 275 amino acids with a molecular weight of 27.7 kDa in its mature form [2] and is considered to be one of the most active functional ingredients found in natto, a traditional Japanese food prepared from fermented soybeans. Several studies in vitro have shown that nattokinase has potent fibrinolytic activity [2–4]. In rat studies, nattokinase was found to have an effect approximately 4 times stronger than plasmin in dissolving a thrombus [1,5]. A small trial on 12 adult Japanese subjects reported that oral administration of nattokinase produced a gradual enhancement of fibrinolytic activity in the plasma, as indicated by assays of euglobulin fibrinolytic activity and the production of tissue plasminogen activator [6]. The potent fibrinolytic activity of nattokinase suggests that it may be a nutraceutical for improving circulation and reducing the risk of cardiovascular disease (CVD). However, no evidence is available in the literature on whether a supplement of nattokinase is beneficial for humans with risk factors of CVD.

Fibrinogen is a strong, consistent, and independent risk factor related to CVD [7–9] and even as a possible marker for CVD [10,11]. Several studies have also indicated that factor VII and factor VIII were highly related to the increase in risk of CVD [9,11–14]. Since the coagulation factors named are highly associated with CVD (hereafter referred to as “CVD-associated coagulation factors”), they were used here as indicators for assessment of the effects of nattokinase on CVD. In addition, it is well established that increases in total cholesterol (TC), triacylglycerol (TG), and low-density lipoprotein cholesterol (LDL-C) concentrations as well as a decrease in high-density lipoprotein cholesterol (HDL-C) concentration are important risk factors for CVD [15–17]. A study has shown that natto extracts could lower the levels of TG and TC in cholesterol-fed rats [18]. In this study, we hypothesized that nattokinase could reduce certain factors of blood clotting and lipids that are associated with an increase risk for CVD. To test this hypothesis, we investigated the effects of nattokinase on plasma levels of blood coagulation factors and blood lipids in human subjects. We hypothesized that, by administration of nattokinase, the CVD high risk group should be affected more on the indicators examined than healthy volunteers because this group might have more abnormal levels of CVD-associated coagulation factors and blood lipids. Therefore, we recruited 3 groups of subjects, including healthy volunteers, patients with CVD and/or more than 2 of CVD risk factors, and patients undergoing dialysis. Patients undergoing dialysis were included because it is well-known that CVD is the major cause of complication and mortality in these patients [19,20]. Furthermore, although natto has been consumed as a natural food supplement for years without safety concerns, an increase in uric acid concentration after soybean consumption had been reported

[21]. Hence, data on uric acid concentration and adverse events collected via a self-administered questionnaire were used to monitor the safety of nattokinase administration.

## 2. Methods and materials

### 2.1. Subjects

This study used an open-label, self-controlled design. Study protocols and materials were reviewed and approved by the institutional review board of Changhua Christian Hospital, Taiwan, before initiating the study. Forty-five adult men and nonpregnant women, 20 to 70 years of age, who met the enrollment criteria and gave written informed consent, were assigned to one of the 3 groups: the Healthy Group, the Cardiovascular Group, and the Dialysis Group, according to their conditions, aggregating 15 in each group. Study participants underwent screening evaluations up to 30 days before administration of the investigational nattokinase product. Eligible for inclusion into the Cardiovascular Group were patients with any one type of CVD such as coronary artery disease, peripheral arterial occlusive disease, history of stroke, transient ischemic attack, pulmonary embolism, deep vein thrombosis, and/or who had at least 2 major risk factors of CVD as listed by the Taiwan National Health Insurance guidelines. These risk factors were hypertension, smoking, diabetes mellitus, atrial fibrillation, lipid disorder, overweight, and physical inactivity. Patients undergoing regular dialysis at the same institute for at least 3 months were eligible for inclusion into the Dialysis Group. The healthy volunteers (Healthy Group) did not have any of the risk factors listed above or abnormal renal function as defined by serum creatinine above 1.4 mg/dL for males or above 1.3 mg/dL for females. Subjects with known allergies to the component products of the study, those currently using warfarin (an anticoagulant medication for thrombosis or embolism), those with active disease status, or those suffering from acute diseases were excluded from this study.

### 2.2. Investigational product

The study product of enteric-coated nattokinase capsule contained nattokinase 400 mg (2000 FU per capsule) and gelatin 100 mg. The suggested usage was 2 capsules once a day taken at about 30 minutes after dinner.

### 2.3. Efficacy assessment

After a 2- to 4-week screening period, subjects were provided an 8-week supply of the study product to evaluate the effects of oral intake of the nattokinase capsules. Since most of the assessments made in the study are known to fluctuate easily intra- and interindividually, subjects were requested to return for evaluations at the same time of day starting from the screening visit. All laboratory samples were collected after an overnight fast from all subjects. At every study visit, patients were asked to return all of the unused

trial products and empty bottles to account for consumption of the investigational product. Treatment compliance was measured by percentage of the trial products consumed. Laboratory tests including fibrinogen, factor VII, factor VIII, TG, TC, LDL-C, HDL-C, vital signs, and body weight were evaluated at the screening visit (baseline; before initiation of the investigational product intake) and regularly after the initiation of oral intake. Patients' self-evaluation of their tolerance and physical improvement was assessed by a structured patient questionnaire administered on days 3, 7, 28, and 56. Each patient was carefully monitored for the development of any adverse events.

#### 2.4. Laboratory methods

Plasma levels of fibrinogen were measured with a Star Diagnostica Stago by Fibri prest (Asnieres, France). Levels of factors VII and VIII antigens were measured using commercial enzyme linked immunosorbent assay kits (Diagnostica Stago, Asnieres, France). Plasma TC was determined by cholesterol esterase and cholesterol oxidase methods with a commercial kit (Wako Pure Chemical Co Ltd, Osaka, Japan). The concentrations of TG in the plasma were determined by commercial diagnostic kits (Triacylglycerol E-Test Wako, Wako Pure Chemical Industries). Determiner HDL-C kits (Kyowa Medex, Tokyo, Japan) were used to evaluate HDL-C levels. Plasma LDL-C was calculated using the Friedewald equation:  $\text{LDL-C (mmol/L)} = \text{TC} - \text{HDL-C} - \text{TG}/2.2$  [22]. A commercial kit (Shino-Test, Tokyo, Japan) was used for uric acid tests. Creatinine levels were determined using Creatinine-test-Wako (Wako Pure Chemical Industries). All laboratory tests were performed by the Laboratory Department of Changhua Christian Hospital, a medical center laboratory with a College of American Pathologists certification.

#### 2.5. Statistical analyses

All 45 subjects enrolled were included into the intent-to-treat [23] analysis set. Laboratory tests were tabulated or plotted against time. Means and standard deviation are presented for continuous data, while frequency and percentage are used for categorical variables. Changes from baseline of laboratory assessments are also presented to examine the effect of nattokinase administration by using the Wilcoxon signed-rank test [24] to compare the values on both days 28 and 56 after the initiation of the nattokinase administration and baseline. Measurements among the 3 groups at baseline were compared by using the Kruskal-Wallis test [25], followed by least significant difference post hoc comparisons of means [26]. The Fisher exact test [27] was used for the comparison of categorical variables. The mixed model [28] was used for repeated measured data analysis to evaluate both group and time effects. The study statistician conducted statistical analyses with the software SAS v8.02 (SAS Institute Inc, Cary, NC).  $P \leq .05$  was considered statistically significant.

### 3. Results

#### 3.1. Demographic characteristics and medical history

The demographic characteristics and medical history of all 45 subjects who received the investigational product are summarized in Table 1. There were 22 men (48.9%) and 23 females (51.1%) enrolled, with an average age of 56.0 years without significant differences between groups. The Cardiovascular Group enrolled 4 patients with diagnoses of coronary artery disease, 1 subject with both coronary artery disease and pulmonary embolism, and 10 subjects with more than 2 risk factors of CVD. All subjects of the Dialysis

Table 1  
Demographic characteristics and medical history of the subjects

Variables	Healthy Group (n = 15)		Cardiovascular Group (n = 15)		Dialysis Group (n = 15)		P
	n	%	n	%	n	%	
Gender							
Male	7	46.7	8	53.3	7	46.7	NS
Female	8	53.3	7	46.7	8	53.3	
Age							
Means $\pm$ SD (range)	50.5 $\pm$ 13.2 (27.8–71.3)		56.5 $\pm$ 15.3 (24.0–77.7)		61.0 $\pm$ 8.3 (44.6–75.3)		NS
CVD or >2 Risk Factors of CVD	0	0.0	15	100	12	80.0	<.001
Chronic renal diseases	0	0.0	0	0.0	15	100.0	<.001
Years since diagnosis							
Mean $\pm$ SD (range)					5.5 $\pm$ 3.4 (0.3–11.2)		
Years since initial dialysis							
Mean $\pm$ SD (range)					3.8 $\pm$ 4.0 (0.3–11.0)		
Creatinine >1.4 mg/dL for men or 1.3 mg/dL for women	0	0.0	2	13.3	15	100.0	<.001

All subjects took 2 capsules of nattokinase (2000 FU per capsule) daily orally for 2 months.

NS indicates not significant.

Comparisons of the 3 groups were analyzed by using Fisher exact test or Kruskal-Wallis test, when appropriate. Differences were considered statistically significant at  $P < .05$ .

Group had diagnosis of chronic renal diseases, including chronic renal failure, diabetes with renal manifestation, end-stage renal disease, and hypertensive renal disease, and all underwent regular hemodialysis at a Kt/V of 1.2–1.4. Three subjects (20%) in the Dialysis Group also had confirmed CVD diagnosis: 2 with coronary artery disease and 1 with coronary artery disease and pulmonary embolism. The average period since the first renal disease diagnosis was  $5.5 \pm 3.4$  years (range, 0.3–11.3 years), and the mean dialysis duration since the initial dialysis was  $3.8 \pm 4.0$  years (range, 0.3–11.0 years). Between the Cardiovascular Group and Dialysis Group, 15 subjects took concomitant medications for hypertension, and 5 subjects had medications for hyperlipidemia before the study.

### 3.2. Baseline assessments

As shown in Table 2, most of the baseline assessments were significantly different among the 3 groups; especially, those in the Dialysis Group tended to have the worse conditions (highest or lowest values) for several measures ( $P < .05$ ), including fibrinogen, factor VII, factor VIII, HDL-C, TG, systolic blood pressure, heart rate, and creatinine, compared with the other groups. With the Healthy Group as reference, the baseline levels of fibrinogen, factor VII, and factor VIII were higher in the subjects of the Cardiovascular Group by 1.12, 1.14, and 1.48 times, respectively, and also higher in the subjects of the Dialysis Group by 1.29, 1.26, and 2.23 times, respectively. In addition, TG in the Cardiovascular and Dialysis Groups was 2.02 and 2.61 times, respectively, higher than the level of Healthy Group. On the contrary, HDL-C in Cardiovascular and Dialysis Groups was 0.84 and 0.63 times, respectively, higher than the level of the Healthy Group. No significant differences in the blood levels of TC and LDL-C were observed between groups at baseline. Moreover, subjects in the Dialysis Group had creatinine levels that were, respectively, 11- and 8.5-fold higher compared with the Healthy and Cardiovascular groups.

### 3.3. Effects of nattokinase on the CVD-associated coagulation factors

Treatment compliance measured by accountability of investigational product was 97.5% for the Healthy Group, 96.6% for the Cardiovascular Group, and 99.1% for the Dialysis Group. Significant time effect but not group effect was observed in the changes from baseline of fibrinogen ( $P = .003$ ), factor VII ( $P < .001$ ), and factor VIII ( $P < .001$ ) by mixed model analysis. On day 56, fibrinogen decreased 30.4 (9.1%,  $P = .01$ ), 25.3 (6.7%,  $P = .49$ ), and 42.5 mg/dL (9.8%,  $P = .02$ ) from baseline for the Healthy, Cardiovascular, and Dialysis groups, respectively; factor VII decreased 17.4 IU (14.2%,  $P < .01$ ), 17.5 IU (12.5%,  $P < .01$ ), and 11.4 IU (7.4%,  $P = .15$ ) from baseline, respectively; and factor VIII decreased 17.7 IU (16.7%,  $P = .04$ ), 29.7 IU (19.0%,  $P < .01$ ), and 44.6 IU (18.9%,  $P < .01$ ) from baseline, respectively, for the 3 groups (Table 2 and Fig. 1).

Table 2  
Laboratory assessments and vital signs in treatment groups at baseline, days 28 and 56

Variable	Healthy Group (n = 15)			Cardiovascular Group (n = 15)			Dialysis Group (n = 15)		
	Baseline	Day 28	Day 56	Baseline	Day 28	Day 56	Baseline	Day 28	Day 56
SBP (mm Hg)	118.7 ± 12.0 <sup>†</sup>	116.9 ± 12.4	115.0 ± 11.9*	133.6 ± 17.5 <sup>†</sup>	132.6 ± 15.3	130.4 ± 16.0	148.5 ± 16.2 <sup>†</sup>	150.3 ± 19.8	151.3 ± 20.0
DBP (mm Hg)	73.2 ± 8.3 <sup>†</sup>	72.6 ± 6.9	69.4 ± 8.4	82.0 ± 10.6 <sup>†</sup>	81.6 ± 11.3	81.8 ± 9.8	78.3 ± 11.6	78.8 ± 8.8	76.8 ± 12.3
Heart rate (/min)	67.3 ± 4.9 <sup>†</sup>	66.3 ± 9.3	65.1 ± 6.7	74.6 ± 10.5	68.3 ± 8.3**	71.1 ± 10.8	75.7 ± 13.3 <sup>†</sup>	74.2 ± 11.7	72.4 ± 13.6
Body weight (kg)	60.0 ± 11.1 <sup>†</sup>	60.3 ± 11.2	59.9 ± 11.0	73.0 ± 15.3 <sup>†</sup>	72.9 ± 15.3	72.8 ± 14.8	66.3 ± 12.6	66.6 ± 12.2	65.7 ± 12.3
Creatinine (mg/dL)	0.98 ± 0.17 <sup>†</sup>	ND	ND	1.27 ± 0.76 <sup>†</sup>	ND	ND	10.8 ± 2.6 <sup>†*</sup>	ND	ND
Uric acid (mg/dL)	5.7 ± 1.6	ND	5.7 ± 1.4	6.6 ± 1.3	ND	6.6 ± 1.4	6.7 ± 1.8	ND	6.4 ± 1.6
TC (mg/dL)	202.3 ± 27.2	196.6 ± 23.1	201.0 ± 27.6	201.5 ± 35.2	191.3 ± 26.8	188.3 ± 28.0*	186.1 ± 37.6	181.9 ± 51.4	190.5 ± 40.4
LDL-C (mg/dL)	124.8 ± 32.3	120.5 ± 26.7	120.8 ± 29.2	122.5 ± 33.1	115.8 ± 28.1	112.3 ± 24.4	106.3 ± 27.6	101.0 ± 30.1	112.0 ± 26.4
HDL-C (mg/dL)	61.2 ± 17.8 <sup>†</sup>	60.2 ± 17.6	60.9 ± 19.3	51.5 ± 9.3 <sup>†</sup>	48.1 ± 6.8	47.9 ± 7.2	38.3 ± 12.3 <sup>†*</sup>	36.4 ± 13.0	36.6 ± 11.7
TG (mg/dL)	87.8 ± 29.5 <sup>†</sup>	91.3 ± 42.0	87.9 ± 31.3	177.3 ± 103.2	186.3 ± 128.9	171.7 ± 74.3	229.1 ± 181.5 <sup>†</sup>	222.2 ± 186.3	192.7 ± 141.8
Fibrinogen (mg/dL)	335.0 ± 68.3 <sup>†</sup>	338.9 ± 69.6	304.6 ± 59.2*	376.2 ± 80.5	364.9 ± 48.9	350.9 ± 63.5	433.5 ± 108.4 <sup>†</sup>	442.9 ± 116.0	391.0 ± 125.0*
Factor VII (IU)	122.5 ± 12.1 <sup>†*</sup>	113.8 ± 17.6	105.1 ± 19.5**	139.7 ± 22.9 <sup>†</sup>	128.5 ± 16.8*	122.3 ± 21.0**	154.8 ± 26.8 <sup>†</sup>	140.9 ± 36.9	143.4 ± 31.7
Factor VIII (IU)	106.1 ± 30.5 <sup>†</sup>	97.4 ± 35.6	88.3 ± 39.7*	156.7 ± 65.8 <sup>†</sup>	135.1 ± 63.8**	127.0 ± 43.8**	236.3 ± 105.2 <sup>†*</sup>	195.0 ± 127.0	191.7 ± 73.1**

Data are means ± SD. All subjects took 2 capsules of nattokinase (2000 FU per capsule) daily orally for 2 months. Significant changes from baseline were determined by the Wilcoxon signed-rank test.

Common symbols (daggers and double daggers) at baseline within a row indicate significant differences by LSD post hoc comparison of means for the comparison of 3 groups at  $P < .05$ .

SBP, systolic blood pressure; DBP, diastolic blood pressure; ND, not done.

\*  $P < .05$ .

\*\*  $P < .01$ .

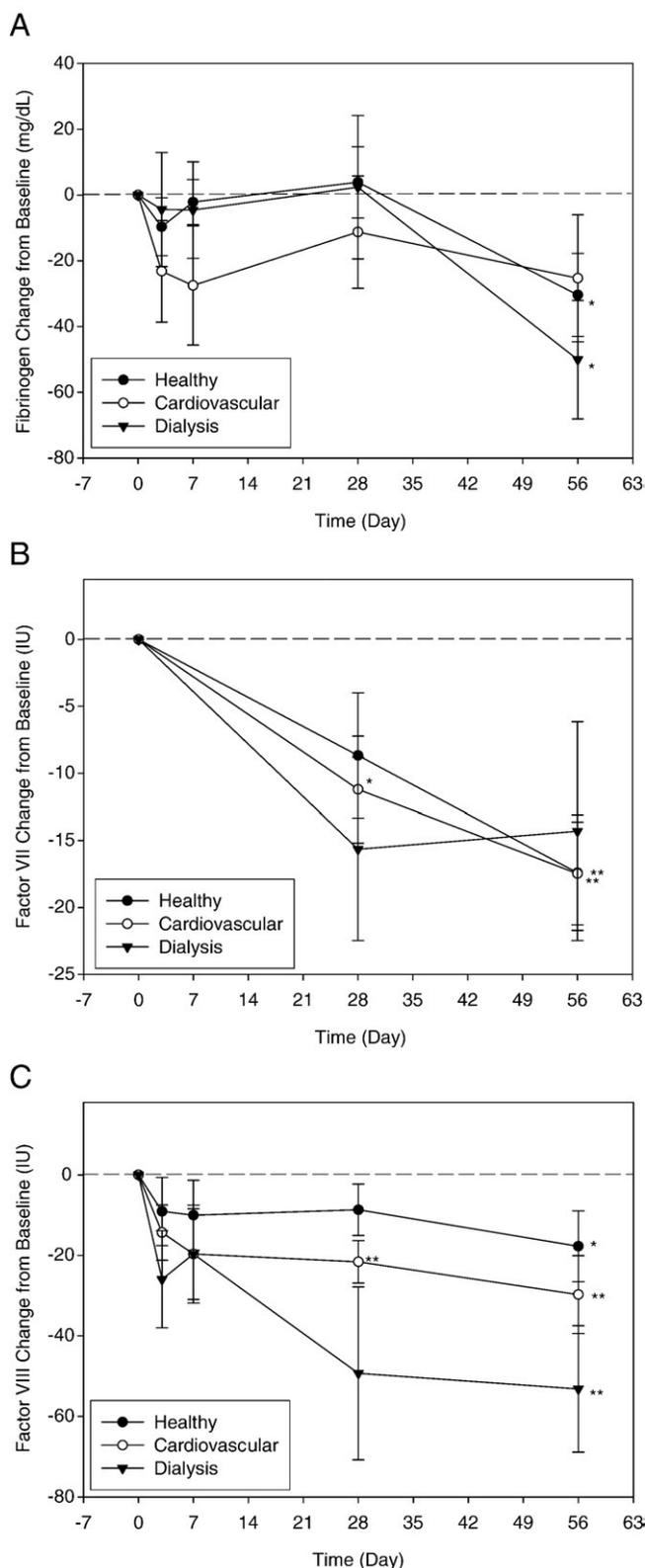


Fig. 1. Changes in coagulation factors from baseline for fibrinogen (A), factor VII (B), and factor VIII (C). Error bars represent S.E. Significant changes from baseline were determined by the Wilcoxon signed-rank test at  $P < .05$  (asterisk) and  $P < .01$  (double asterisk).

### 3.4. Effects of nattokinase on the blood lipids

Only subjects from the Cardiovascular Group showed a significant decrease in TC on day 56 (mean change from baseline,  $-13.1 \pm 22.2$  mg/dL,  $P = .037$ ) with a percent reduction of 6.5% (Table 2). No other significant changes in blood lipids were observed among all subjects or the subset, excluding those who were under hyperlipemia treatment (data not shown).

### 3.5. Safety and self-evaluations

The levels of uric acid, body weight, and heart rate remained stable during the entire period (Table 2). Blood pressure also remained stable over the entire period except for a very slight decline in the level of systolic blood pressure (mean change from baseline,  $-3.73 \pm 8.95$  mm Hg,  $P = .022$ ) that was observed on day 56 in the healthy volunteers (Table 2). No notable adverse events were reported. Self-stated improvement after consuming the investigational product included vitality (8 [17.8%]), blood pressure monitored at home (8 [17.8%]), bowel movement (6 [13.3%]), and shoulder-neck ache (5 [11.1%]).

## 4. Discussion

To investigate the beneficial effects of nattokinase on human subjects, we evaluated the change of the CVD-associated coagulation factors and blood lipids during oral administration of nattokinase for 2 months in healthy volunteers, patients with cardiovascular risk factors, and patients under regular dialysis. Among the 3 groups tested, oral intake of nattokinase capsules showed a similar effect on the plasma levels of these CVD-associated coagulation factors, that is, a continuously declining pattern in fibrinogen, factor VII, and factor VIII during the intake period. However, blood lipids were not affected significantly. These results, namely, the observed reduction of the coagulation factors of fibrinogen, factor VII, and factor VIII, suggest that nattokinase administration can be considered as a CVD nutraceutical. To our knowledge, this study is the first clinical trial reported in the literature to investigate the effects of nattokinase on the CVD-associated coagulation factors.

Consistent with the literature currently available, the baseline differences among the 3 groups revealed a close relationship between fibrinogen, factor VII, and factor VIII with CVD. As the results show, the plasma levels of fibrinogen and factors VII and VIII in the Cardiovascular Group were all higher than those in the Healthy Group. In addition, dialysis patients (who are often associated with complications related to CVD [19,20]) also had the highest plasma levels of the blood factors evaluated. In the Dialysis Group, there were 12 subjects with more than 2 risk factors of CVD and 3 subjects with confirmed CVD diagnoses among the 15 subjects, which exemplified the close relationship between dialysis and CVD. Furthermore, the efficacy of nattokinase on the coagulation factors showed no

difference between groups by mixed model analysis, suggesting that nattokinase has a similar effect on the healthy population, CVD high-risk patients, and dialysis patients. In the literature, the proposed beneficial effects of nattokinase on thrombosis or CVD are possibly attributable to 2 major mechanisms: (1) fibrin clots directly dissolving through proteolysis and/or via cleavage and (2) inactiveness of the plasminogen activator inhibitor-1 protein [1]. In this study, we found that nattokinase has the ability to decrease plasma levels of the 3 CVD-associated coagulation factors. It can be assumed that a significant decrease in these coagulation factors would reduce the clotting activity in circulation and may result in further beneficial effects to patients with CVD patients. Although not knowing the precise responsible mechanism, we propose here a third possible way for nattokinase to benefit CVD, that is, by decreasing the coagulation factors of fibrinogen, factor VII, and factor VIII.

Fibrinogen is involved in primary hemostasis and is the major determinant of whole blood and plasma viscosity; elevated plasma fibrinogen levels are known to be independently associated with CVD [7,8,11]. According to the present results, nattokinase supplementation can ameliorate blood viscosity and may reduce the incidence of CVD. In addition, both factors VII and VIII have been reported to have cross-sectional association with atherosclerosis and coronary heart disease and, prospectively, with coronary heart disease, stroke, ischemic heart disease, and diabetes mellitus [13,29,30]. Several studies also indicated that factor VII and factor VIII were highly related to increased CVD risk [9,12–14]. The potent effects of nattokinase on lowering blood level of factor VII and factor VIII may imply that it can be considered as a nutraceutical for several types of CVD. However, a limitation that should be noted here is that the data need to be interpreted with caution because of its open-label, self-controlled design, which may contain other confounding factors whose effects cannot be excluded. Thus, further investigations and trials with a randomized, double-blind, and placebo-controlled design are merited. In addition, the decline of fibrinogen levels has been considered as a sensitive measure of clinical coagulopathy. Decreased fibrinogen levels of about 20% in fibrinogen concentration can be amplified by hemorrhage and resuscitation, as what occurs in patients after a trauma injury or post surgery. Therefore, it should be a concern that a decrease in coagulation factors may have a favorable effect in the Dialysis and CVD groups; on the other hand, it may also have a deleterious effect that might lead to hemorrhage in a healthy population.

Blood lipids are also known as important risk factors for CVD [15–17]. In this study, only TC showed a slight decrease ( $P < .05$ ) in the Cardiovascular Group on day 56, while TG, LDL-C, and HDL-C were all unaffected. Although studies on animals have shown that natto extract possesses beneficial effects on TG and TC in cholesterol-fed rats [18], the present results did not demonstrate an obvious

effect of nattokinase on blood lipids in humans. The possible explanation might be that the intake period was insufficient to observe such effects. Another reason might be that the impurities in the tested nattokinase product were mostly removed from the purifying process. It is known that crude extracts of natto contain many other ingredients that possibly cause an additive effect on blood lipids. Indeed, natto extract has been reported to contain large amounts of isoflavones that are known to profoundly affect blood cholesterol [31,32]. Since our investigational product of nattokinase was purified by ultrafiltration and, consequently, might have contained fewer impurities, we thus assumed that the purity of the product might cause inconsistency, at least marginally, between our results and those of others.

Furthermore, nattokinase is extracted from a Japanese traditional food called natto, which is produced from the fermentation of soybeans by *Bacillus subtilis* [1]. However, soybean protein consumption may be harmful by increasing plasma levels of uric acid [21]. This study showed that a daily intake of 800 mg nattokinase for 2 months did not cause any harmful effects. These results supported the safety of nattokinase intake, at least for the 2-month ingestion period. On the other hand, most of the treatment options for thrombosis, such as use of anticoagulants, antiplatelet or clot-dissolving agents, might cause abnormal bleeding and, therefore, result in serious consequences [33–35]. In this study, the plasma levels of fibrinogen and factors VII and VIII showed a continuous decreasing pattern during the administration period. As for concerns on effects similar to those of medicines for thrombosis, there is a need for studies on safety with a longer period of evaluation, especially on the coagulation factors.

In summary, we found that nattokinase, in CVD risk subjects, reduced fibrinogen, factor VII, and factor VIII, but not on blood lipids after a daily oral intake of 4000 FU of nattokinase for 2 months. Nattokinase administration was safe and in healthy volunteers, patients with high-risk CVD factors, and dialysis patients. These results support our hypothesis that oral administration of nattokinase would have a beneficial action on risk factors associated with CVD (causing a decrease in fibrinogen and factors VII and VIII). Because of the study design, further investigations following a randomized, double-blind, and placebo-controlled design are needed for validation of the results.

## Acknowledgment

We thank Su-Feng Kuo and Min-Lin Cheng for their help with the laboratory tests, and Savelife Bioscience Co, Ltd, for providing the nattokinase product (clinical trial registration no. NCT00447434).

## References

- [1] Tai MW, Sweet BV. Nattokinase for prevention of thrombosis. *Am J Health Syst Pharm* 2006;63:1121–3.

- [2] Urano T, Ihara H, Umemura K, Suzuki Y, Oike M, Akita S, et al. The profibrinolytic enzyme subtilisin NAT purified from *Bacillus subtilis* Cleaves and inactivates plasminogen activator inhibitor type 1. *J Biol Chem* 2001;276:24690-6.
- [3] Sumi H, Hamada H, Tsushima H, Mihara H, Muraki H. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. *Experientia* 1987;43:1110-1.
- [4] Fujita M, Nomura K, Hong K, Ito Y, Asada A, Nishimuro S. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan. *Biochem Biophys Res Commun* 1993;197:1340-7.
- [5] Fujita M, Hong K, Ito Y, Fujii R, Kariya K, Nishimuro S. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. *Biol Pharm Bull* 1995;18:1387-91.
- [6] Sumi H, Hamada H, Nakanishi K, Hiratani H. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. *Acta Haematol* 1990;84:139-43.
- [7] Koenig W. Fibrin(ogen) in cardiovascular disease: an update. *Thromb Haemost* 2003;89:601-9.
- [8] Lefevre M, Kris-Etherton PM, Zhao G, Tracy RP. Dietary fatty acids, hemostasis, and cardiovascular disease risk. *J Am Diet Assoc* 2004;104:410-9 [quiz 492].
- [9] Kannel WB. Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. *Lipids* 2005;40:1215-20.
- [10] Eidelman RS, Hennekens CH. Fibrinogen: a predictor of stroke and marker of atherosclerosis. *Eur Heart J* 2003;24:499-500.
- [11] Canseco-Avila LM, Jerjes-Sánchez C, Ortiz-López R, Rojas-Martínez A, Guzmán-Ramírez D. Fibrinogen. Cardiovascular risk factor or marker? *Arch Cardiol Mex* 2006;76:S158-72.
- [12] Cushman M, Yanez D, Psaty BM, Fried LP, Heiss G, Lee M, et al. Association of fibrinogen and coagulation factors VII and VIII with cardiovascular risk factors in the elderly: the Cardiovascular Health Study. *Cardiovascular Health Study Investigators. Am J Epidemiol* 1996;143:665-76.
- [13] Tracy RP, Arnold AM, Ettinger W, Fried L, Meilahn E, Savage P. The relationship of fibrinogen and factors VII and VIII to incident cardiovascular disease and death in the elderly: results from the cardiovascular health study. *Arterioscler Thromb Vasc Biol* 1999;19:1776-83.
- [14] Kamphuisen PW, Eikenboom JC, Bertina RM. Elevated factor VIII levels and the risk of thrombosis. *Arterioscler Thromb Vasc Biol* 2001;21:731-8.
- [15] Nicolosi RJ, Wilson TA, Lawton C, Handelman GJ. Dietary effects on cardiovascular disease risk factors: beyond saturated fatty acids and cholesterol. *J Am Coll Nutr* 2001;421S-7S [discussion 440S-442S].
- [16] Després JP, Arsenault BJ, Côté M, Cartier A, Lemieux I. Abdominal obesity: the cholesterol of the 21st century? *Can J Cardiol* 2008;24 (Suppl D):7D-12D.
- [17] Arsenault BJ, Pibarot P, Després JP. The quest for the optimal assessment of global cardiovascular risk: are traditional risk factors and metabolic syndrome partners in crime? *Cardiology* 2008;113:35-49.
- [18] Iwai K, Nakaya N, Kawasaki Y, Matsue H. Antioxidative functions of natto, a kind of fermented soybeans: effect on LDL oxidation and lipid metabolism in cholesterol-fed rats. *J Agric Food Chem* 2002;50:3597-601.
- [19] Chow FY, Polkinghorne KR, Chadban SJ, Atkins RC, Kerr PG. Cardiovascular risk in dialysis patients: a comparison of risk factors and cardioprotective therapy between 1996 and 2001. *Nephrology (Carlton)* 2003;8:177-83.
- [20] Bergesio F, Monzani G, Guasparini A, Ciuti R, Gallucci M, Cristofano C, et al. Cardiovascular risk factors in severe chronic renal failure: the role of dietary treatment. *Clin Nephrol* 2005;64:103-12.
- [21] Garrel DR, Verdy M, PetitClerc C, Martin C, Brule D, Hamet P. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. *Am J Clin Nutr* 1991;53:665-9.
- [22] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density cholesterol in plasma without the use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- [23] Hollis S, Campbell F. What is meant by intention to treat analysis? Survey of published randomized controlled trials. *BMJ* 1999;319:670-4.
- [24] Rice JA. A nonparametric method—the signed rank test. *Mathematical statistics and data analysis*. Belmont: Wadsworth & Brooks; 1988. p. 374-6.
- [25] Rice JA. A nonparametric method—the Kruskal-Wallis test. *Mathematical statistics and data analysis*. Belmont: Wadsworth & Brooks; 1988. p. 406-8.
- [26] Dawson B, Trapp RG, editors. *Multiple-comparison procedures*. Basic & Clinical Biostatistics. 3rd ed. Singapore: McGraw-Hill; 2001. p. 169-72.
- [27] Dawson B, Trapp RG, editors. *Multiple-comparison procedures*. Small expected frequencies & Fisher's exact test. 3rd ed. Singapore: McGraw-Hill; 2001. p. 169-72.
- [28] Louis TA. General methods for analysing repeated measures. *Stat Med* 1988;7:29-45.
- [29] Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986;2:533-7.
- [30] Duncan BB, Schmidt MI, Offenbacher S, Wu KK, Savage PJ, Heiss G. Factor VIII and other hemostasis variables are related to incident diabetes in adults. *Diabetes Care* 1999;22:767-72.
- [31] Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. *Am J Clin Nutr* 2007;85:1148-56.
- [32] Xiao CW, Mei J, Wood CM. Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression. *Front Biosci* 2008;13:2660-73.
- [33] Levine MN, Raskob G, Landefeld S, Kearon C. Hemorrhagic complications of anticoagulant treatment. *Chest* 1998;114:511S-23S.
- [34] Lee AY, Hirsh J. Diagnosis and treatment of venous thromboembolism. *Annu Rev Med* 2002;53:15-33.
- [35] Landefeld CS, Beyth RJ. Anticoagulant-related bleeding: clinical epidemiology, prediction, and prevention. *Am J Med* 1993;95:315-28.