NSK-SD® Nattokinase:
Improving circulation & promoting cardiovascular health

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What is Nattokinase?

Nattokinase is an enzyme with fibrinolytic activity, which is isolated from natto, a food made from fermented soybeans. Natto has been consumed as a food for more than 1,000 years and is currently available in almost every supermarket in Japan. Natto is considered a healthy addition to the diet that confers benefits to the cardiovascular system. In 1907, it was discovered that natto contained protease enzyme activity. In 1980, Dr. H. Sumi further characterized the enzyme as having the ability to dissolve thrombi (blood clots) and named it "nattokinase". In addition to fibrinolytic activity, the cardiovascular benefits of nattokinase include reducing elevated blood pressure, improving circulation and normalizing levels of CRP (a clinical marker for inflammation).

This paper focuses on a proprietary product produced by Japan Bio Science Laboratory Co, Ltd of Japan (JBSL), known as NSK-SD®. The effectiveness and safety of NSK-SD has been demonstrated in numerous human clinical studies.

Cardiovascular Health Benefits of Nattokinase

- **Reduces elevated blood pressure**
- **Fibrinolytic activity: dissolves blood clots without inhibiting wound healing**
- **Inhibits platelet & red blood cell aggregation**
- **Decreases blood viscosity**
- **Reduces clinical measure of inflammation (CRP)**

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Cardiovascular Health Recommendations

In 2010, the American Heart Association published goals and guidelines for improving cardiovascular health and preventing cardiovascular disease and stroke. The ideal behaviors and clinical parameters were defined as: non-smoking, body mass index <25 kg/m^2, physical activity at defined levels, a healthy diet, total cholesterol <200 mg/dl, blood pressure <120/80 mmHg and fasting blood glucose <100 mg/dl (Lloyd-Jones et al. 2010). Previously to this report, the focus on prevention had been to reduce the incidence of cardiovascular events (heart attack or stroke) in those already presenting with risk factors. Now the goal is for everyone to develop healthy habits that preempt the development of cardiovascular risk factors (elevated blood pressure, cholesterol, and fasting glucose).

Cardiovascular disease is associated with dysfunctional or injured blood vessels, which are prone to develop clots (thrombi). Thrombosis is a multi-step process wherein platelets adhere to the damaged vessel wall, secrete stimulatory mediators and start a cascade of biochemical events resulting in a clot. These clots can obscure blood flow, thereby decreasing oxygen supply to the tissues. The result can be heart attack, stroke or peripheral occlusive disease.

General dietary guidelines for cardiovascular health include the intake of fruits, vegetables, fish and whole grains, along with a reduction in sodium, sugar sweetened beverages and saturated fat (Lloyd-Jones et al 2010). Certain dietary interventions have been associated with reducing certain aspects of thrombosis. These foods include protein from soybeans, unsaturated fatty acids in olive oil and fatty fish, garlic, onions, ginger and tomatoes (Phang et al. 2011).

Nattokinase (NSK-SD) as an Aid to Cardiovascular Health

Natto (the food) and purified nattokinase have been shown to degrade fibrin clots in vitro. Nattokinase degrades fibrin directly in clot lysis assays with activity comparable to plasmin, which is the body's own anti-clotting mechanism. Nattokinase also degrades fibrin indirectly via plasminogen activator inhibitor 1 (PAI-1), which is the primary inhibitor of tissue-type plasminogen activator (t-PA). Importantly, nattokinase does not inhibit the formation of fibrin from fibrinogen, thus it does not inhibit the formation of blood clots. This is important as it means that nattokinase does not inhibit blood clotting in response to injury.

The cardiovascular benefits of nattokinase include reducing elevated blood pressure, improving circulation and normalizing levels of CRP, a clinical marker for inflammation (Table 1). Nattokinase (NSK-SD) has been shown in pharmacokinetic studies to be bio-available when taken orally with the peak plasma concentration occurring approximately 13 hours after ingestion (Ero et al. 2011). Several clinical studies have demonstrated the ability of nattokinase (NSK-SD) to reduce elevated blood pressure. The most conclusive study, a randomized, placebo-controlled study of 8 weeks conducted with hypertensive subjects, demonstrated that NSK-SD could reduce both systolic and diastolic blood pressure compared to placebo (Kim et al. 2008).

Human clinical studies have also demonstrated fibrinolytic activity. A single dose decreased ex-vivo euglobin (clot) lysis time (ELT) for up to 8 hours after oral intake and increased lysis area on a fibrin plate (euglobin fibrinolytic activity, EFA) for up to 12 hours (Sumi et al. 1990). Nattokinase has also clinically demonstrated the ability to inhibit platelet aggregation, reduce rouleaux formation in blood cells and decrease blood viscosity (Iuchi et al. 2006;Takaoka 2005).

Recent research suggests a role for of inflammation as a key pathogenic mechanism in cardiovascular disease. The American Heart Association recommends C-reactive protein (CRP) as an independent clinical marker for inflammation and cardiovascular disease risk (Pearson et al. 2003). Nattokinase has clinically demonstrated the ability to normalize levels of CRP (Jeske et al. 2011).

Development of NSK-SD

As previously stated, nattokinase is extracted from natto, a Japanese food that has been consumed for more than 1,000 years. Natto is made by fermenting cooked soybeans with a particular bacterium: *Bacillus subtilis natto*. The soybeans are fermented at 40 degrees C (104 degrees F) for 14 to 18 hours until the dark drown beans are covered with a sticky, viscous, string-like material. Because of this texture, natto has been called a vegetable cheese. Natto has a slightly musty flavor and characteristic odor.

It was discovered that natto contained protease enzyme activity in 1907, by Dr. S. Swamura. In 1925, Dr K. Oshima reported that the protease degraded fibrin and gelatin. In 1956, Dr. S. Miyake defined the amino acid composition of the enzyme. In 1980, Dr. H. Sumi confirmed the proteolytic activity when he tested 173 different foods for their effects on dissolving thrombi (blood clots) associated with heart attack and stroke. He named the enzyme “nattokinase” (Sumi et al. 1987).

The usual serving of natto food is 50 g, which has fibrinolytic activity that has been measured as 2,000 CU. (This measurement is approximate as there are several strains of *Bacillus subtilis natto* that produce varying potencies of enzyme activity.) CU is a measurement of activity compared to the action of plasmin, the endogenous fibrinolytic enzyme. The CU measurement has been replaced with FU activity units with a ratio of 1.33 to 1. Thus, 50 g of natto food has approximately 1500 units of FU activity.

Nattokinase is a serine protease with 275 amino acid residues and a molecular weight of 27,728 Daltons. Nattokinase is a member of the subtilisin family of enzymes and DNA sequencing shows 99.5 and 99.3% homology to subtilisin E and amylosacchariticus, respectively (Nakamura et al. 1992).

Characterization of NSK-SD

NSK-SD is a white (milk-white) colored powder with little to no smell. It has a nattokinase activity of more than 20,000 FU/g. The recommended intake level is 2,000 FU/day. All vitamin K_2 (which may increase blood coagulation) has been removed. NSK-SD is produced from non-genetically modified soybeans fermented with a proprietary strain of *Bacillus subtilis natto*. NSK-SD is stable in the pH range of 5.5 to 10 at 25 degrees C for 24 hours. NSK-SD in a soft gel capsule (NSK-II) retains 75 to 80% of activity when exposed to a pH of 2.0, mimicking gastric fluid, for 30 minutes. NSK-SD is stable at 50 degrees C for 1 hour. The optimal fibrinolytic activity occurs around 65 degrees C and pH 10.5. NSK-SD is stable under pressures up to 2000 kg/cm^2 and can therefore be pressed into tablet form.

NSK-SD is sold in soft gel capsules, known as NSK-II. The capsules contain 100 mg of NSK-SD with a minimum activity level of 2,000 FU. NSK-SD has replaced a previous product called NSK-FD (freeze-dried), which was a less purified product with an activity of 13,000 FU/g.

Benefits of NSK-SD

Reduction of Hypertension (Blood Pressure)

Blood pressure control is influenced by the renin-angiotensin hormonal complex. Angiotensinogen, a protein produced by the liver, is transformed in the blood to angiotensin I by the enzyme renin. Angiotensin I, in turn, is converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II increases blood pressure by constricting blood vessels. Two enzymes that exert control in this system, therefore, are renin and ACE. Inhibition of ACE is a common mechanism for hypertensive medications. However, renin, an enzyme that is released by the kidneys, is proposed to be the rate-limiting factor in the renin-angiotensin system.

Traditional knowledge is that natto in the diet tends to lower blood pressure. It has been suggested that the mechanism for this effect may be the inhibition of ACE (Maruyama and Sumi 1998). However, a recent clinical study found no difference in blood levels of ACE following treatment with nattokinase but did report a decrease in renin activity (Kim et al 2008). Thus, the mechanism whereby nattokinase decreases blood pressure may be through inhibition of renin activity.

Reduction of Hypertension: Animal Study

Nattokinase was demonstrated to decrease blood pressure in Wistar Rats. The animals (400-450 g; male) were administered intraperitoneally 0.5 ml of a lyophilized extract (80% ethanol; equivalent to 25 mg natto – roughly 0.8 FU total or 2 FU/kg body weight) and blood pressure was measured using the tail artery. The average systolic blood pressure of 6 rats decreased significantly 2 and 3 hours after administration of the natto extract by 12.6% and 13.2%,

respectively (both p<0.05). The systolic blood pressure decreased from 166 ±14 mmHg at baseline to 144 ± 27 mmHg after 3 hours (Maruyama & Sumi 1998).

Reduction of Hypertension: Clinical Studies

In an open label clinical study, 30g of lyophilized extract (80% ethanol; equivalent to 200 g natto, roughly 6,400 FU) was administered orally for 4 consecutive days to human volunteers with high blood pressure. In 4 of 5 volunteers the systolic as well as diastolic blood pressure decreased (measured in the supine position). The systolic average values decreased by 10.9% from 173.8 ± 20.5 to 154.8 ±12.6 mmHg. The diastolic blood pressure decreased by 9.9% from 101.0 ± 11.4 to 91.2 ±6.6 mmHg (Maruyama & Sumi 1998).

A randomized, placebo-controlled, crossover study was conducted with 20 men and women (ages 18-75 years old) with a variety of disease states (essential hypertension, hyper-coagulable states, auto-immune diseases and diabetes). Half of the study population received 4,000 FU (2,000 FU twice daily of NSK-SD) and the other half received placebo. After 4 weeks the groups crossed over and received the alternate intervention. There was a significant decrease in systolic blood pressure compared to baseline for the NSK-SD group (p=0.039) and no significant change in diastolic blood pressure compared to baseline. The placebo treatment did not cause any change in systolic or diastolic pressure (Krishnan Medical Association SC. 2003).

Another, more definitive, randomized, double-blind, placebo-controlled study was conducted with 73 hypertensive participants (20-80 years-old) with an initial systolic blood pressure between 130-159 mmHg. The participants received NSK-SD (2,000 FU per day) or placebo for 8 weeks. After 8 weeks of treatment there were significant decreases in systolic and diastolic blood pressure compared to placebo (both p<0.05). Both treatment and placebo groups had some reduction in blood pressure, with the net decreases for the treatment group being 5.5 mmHg in systolic blood pressure and 2.8 mmHg in diastolic blood pressure. There was also a net decrease in plasma renin activity (1.17 ng/ml/hr) in the treatment group compared to the control group (p<0.05). There was no significant difference in ACE levels between the two groups (Kim et al 2008).

Fibrinolytic Activity

Nattokinase has been shown to degrade fibrin clots both directly and indirectly. Clot lysis assays indicate nattokinase degrades fibrin directly with activity comparable to plasmin. Kinetic assays suggest nattokinase is 6 times more active than plasmin in degrading cross-linked fibrin. Nattokinase degrades fibrin indirectly by affecting plasminogen activator activity. Nattokinase does not directly stimulate plasminogen activator activity. Instead there are suggestions that it degrades plasminogen activator inhibitor 1 (PAI-1), which is the primary inhibitor of tissue-type plasminogen activator (t-PA). Importantly, nattokinase does not inhibit the formation of fibrin from fibrinogen, thus it does not inhibit the formation of blood clots in response to injury.

Human clinical studies have demonstrated that nattokinase has the ability to decrease euglobin (clot) lysis time (ELT) up to 8 hours after oral intake of a dose of natto estimated at 6,000 FU. In addition, euglobulin fibrinolytic activity (EFA: lysis area on a fibrin plate) was increased significantly up to 12 hours following administration. Nattokinase has also demonstrated the ability to dissolve experimentally-induced thrombi in animal experiments using dogs and rats. In addition, nattokinase has been shown to prevent thickening of vascular intima in a rat model. Details of the results summarized above are given below.

Fibrinolytic Activity: In Vitro

Initially, the fibrinolytic activity of natto was demonstrated when the vegetable cheese was applied directly to fibrin. The fibrinolytic activity was approximately 40 CU (plasma units)/g wet weight and the isolated protease was named nattokinase (Sumi et al 1987). Fibrinolytic activity of 40 CU is equal to 30 FU (Fibrin Degradation Units). Further experiments using a clot lysis assay (cross-linked fibrin) revealed that purified nattokinase had 4 to 5 times the fibrinolytic activity of plasmin. Nattokinase cleaved fibrinogen and fibrin, producing similar degradation products to those produced by plasmin.

Test preparations of pure nattokinase and capsule contents (bulk powder plus excipients) were tested in a series of in vitro experiments in human plasma. Test concentrations (0.2 to 1.6 FU/ml) were calculated as twice the plasma concentration of the highest recommended dose (4,000 FU) assuming 100% bioavailability in a 5-liter average blood volume. In this system, the functional ability of fibrinogen to form fibrin in response to thrombin was not altered by concentrations of 0.2 to 0.8 FU/ml nattokinase. Only at the highest concentrations of 0.8 and 1.6 FU/ml did nattokinase reduce the quantity of fibrinogen. This finding suggests that nattokinase will not affect the body's ability to respond to tissue wounding, when taken at the usual intake levels (Ero and Lewis 2008).

Unlike urokinase, nattokinase does not stimulate fibrinolysis by directly stimulating plasminogen activator activity (Fujita et al. 1993). Instead, nattokinase is reported to degrade an important inhibitor of plasminogen activator activity. Plasminogen activator inhibitor 1 (PAI-1) is the primary inhibitor of tissue-type plasminogen activator (t-PA). Nattokinase cleaved active recombinant PAI-1 into low molecular weight fragments at concentrations of 0.02-1.0 nM (half maximal activity at 0.1 nM). In reducing the activity of the inhibitor, nattokinase enhanced t-PA induced lysis of the fibrin clot in a dose-related manner (0.06-1 nM) (Urano et al. 2001). In contrast with the above study, another group conducting an in vitro test in human plasma reported that nattokinase (0.8 and 1.6 FU/ml) slightly increased the presence of PAI-1 (Ero & Lewis 2008).

Fibrinolytic Activity: Animal Studies

The fibrinolytic activity of nattokinase was tested in dogs using an experimental thrombosis model in which bovine fibrinogen and thrombin were infused into the animals. Three dogs were treated with nattokinase and six dogs were given placebo, serving as controls. Four capsules of nattokinase (250 mg /capsule; 2.13 CU/mg; calculated to be a total of approximately 1,600 FU) or placebo were given orally. Angiograms were obtained before induction of the thrombus and from 2.5 to 24 hours afterwards. In the control group, there was no sign of lysis 18 hours after induction of thrombosis. By contrast, the dogs treated with nattokinase had complete restoration of blood circulation within 5 hours (Sumi et al 1990).

The fibrinolytic activity of nattokinase was also tested in a rat model, in which a thrombus was formed in the common carotid artery by damaging the endothelial cells of the vessel wall with acetic acid. In this model, urokinase or t-PA (given intravenously (iv), constant rate, 20 minutes) restored blood flow (45%) over 60 minutes. There was no restoration of blood flow with saline. Nattokinase was tested in this model in doses of 0.02, 0.04 and 0.12 mcmol/kg (iv) and its activity was compared to plasmin and elastase. Nattokinase caused a dose-dependent recovery of blood flow (18, 42 and 62%) after 60 minutes. When the activity of nattokinase and plasmin were compared on a molar basis, nattokinase was 4-fold more efficient than plasmin in restoring blood flow. Elastase did not restore blood flow. Degradation of cross-linked fibrin was determined through the presence of D-dimer gamma-gamma chain remnants in the plasma. D-dimer remnants were detected in the blood after treatment with nattokinase as well as with urokinase and t-PA. The feasibility of using nattokinase therapeutically for fibrinolysis depends upon its ability to digest fibrin without destroying fibrinogen. Values for residual plasma fibrinogen following administration of a dose of 0.12 mcmol/kg of plasmin, elastase or nattokinase were 33, 42 and 29%, respectively. When the dose of nattokinase was reduced by one-third to the approximate activity level of plasmin, the residual fibrinogen level was 53%. This is a greater amount of residual fibrinogen than the 33% remaining after treatment with plasmin at a comparable activity level. These results imply that nattokinase may be safer than plasmin at an appropriate dose level (Fujita et al. 1995a).

Thickening of vascular intima is thought to be part of the progression of arteriosclerotic plaques that can lead to heart attack and stroke. The ability of nattokinase to inhibit the progression of intimal thickening was tested in a rat model. In this model, endothelial damage to the femoral artery was induced by intravenous injection of rose-bengal followed by irradiation with transluminal green light. Twenty-one days after endothelial injury, significant intimal thickening was observed. Administration of nattokinase (50 or 100 CU/animal, calculated as 38 and 75 FU/animal) was started 3 weeks before endothelial injury and then continued for another 3 weeks following the injury. Nattokinase reduced the development of intimal thickening from an area of 1.28 ± 1.14 mm^2 in the control group to 0.79 ± 0.60 mm^2 and 0.71 ± 0.27 mm^2 in the low- and high-dose groups, respectively. The difference between intimal thickening in the control group and the high-dose group was significant ($p<0.05$). When the intima/media ratios were compared for the three groups, both treatment groups were different from the control group

($p<0.05$). There was no difference between the control and treatment animals in the time taken to develop occlusion following injury. However, differences were observed in the morphology of the mural thrombi. In the control group, the center of the vessel reopened with mural thrombi attached to the vessel walls. In the nattokinase groups, thrombi near the vessel walls showed lysis and most thrombi were detached from the vessel wall surface. The control group had thrombi attachment lengths measuring 858 ± 430 mm at 8 hours after injury. Nattokinase reduced the attachment length in a dose-dependent manner. The attachment length for the high-dose group was 173 ± 105 mm, significant shorter than the control group ($p<0.05$). Bleeding times for the three groups were not different (Suzuki et al. 2003).

Fibrinolytic Activity: Clinical Studies

Preliminary evidence that nattokinase would have an effect in humans was reported by Dr Sumi and co-workers in 1990. Twelve healthy volunteers (men and women, 21-55 years old) were given a single dose of 200 g natto (estimated to be 6,000 FU) or a control of boiled soybeans in a cross-over single-dose study with a 2-week interval. Blood was collected from 2 to 24 hours after ingestion. Euglobin (clot) lysis time (ELT) decreased significantly 2, 4 and 8 hours after intake of natto compared to the soybean control. Euglobulin fibrinolytic activity (EFA) was determined by measuring the lysis area on a fibrin plate. EFA increased significantly 2, 4, 8 and 12 hours after intake of natto compared to the soybean control. In another experiment the volunteers were given 2 enteric-coated capsules containing nattokinase (650 mg/capsule; 2.13 CU/mg) 3 times a day following meals (calculated to be a total of 3,000 FU per day) for 8 days. Blood was collected each day. EFA increased gradually but not significantly over that time. The degradation products from fibrin and fibrinogen (FDP) in the serum were also measured. The FDP levels in the serum spiked on the first day and then decreased slowly over the 8 days. The levels were significantly different from baseline on days 1 through 4 (Sumi et al 1990).

In another study, a single oral dose of 30 g lyophilized natto (ca. 200 g original wet weight; estimated to have 6,000 FU) was given to 5 volunteers (51-86 years old) and blood samples were taken from 2 to 24 hours after intake. Fibrinolysis was observed for 4 to 8 hours after intake. EFA increased significantly after 4 hours and FDP measurements increased significantly 6 and 8 hours after administration. EFA increased from 1.9 ± 2.7 mm^2 at baseline to 4.5 ± 3.3 mm^2 after 2 hours, 13.3 ± 7.2 mm^2 after 4 hours and 8.7 ± 7.4 after 8 hours. The FDP levels at baseline, 6 hours and 8 hours were 0.75 ± 0.52, 5.50 ± 2.74 and 2.75 ± 1.37 mcg/ml, respectively. The FDP was further decreased following additional intakes on the 2nd and 4th day (Sumi et al. 1996;Sumi and Maruyama 1998).

A double-blind, placebo-controlled study with 30 adults (men and women; average age 59) explored the administration of nattokinase to patients taking warfarin for maintenance purposes. The theory behind the combination of the two agents was that the addition of nattokinase might help stabilize the fibrinolytic effect of warfarin. The treatment group was given 2 capsules (1700 FU) nattokinase (NSK II) per day after breakfast for 26 weeks. As a result,

there were significantly decreased rate-of-changes in prothrombin and prothrombin-INR compared to placebo ($p<0.05$). Treatment was especially effective for those over 60 years of age. Activated partial thromboplastin time and prothrombin time were closer to reference values compared to the placebo group after 4 months ($p<0.05$). In addition, lower rates of change were observed for activated partial thromboplastin time, prothrombin time, prothrombin-INR time (Ninomiya 2006).

Reduction of Platelet Aggregation, Rouleaux formation and Blood Viscosity

In vitro experiments and human studies suggest that nattokinase may improve blood flow, decrease blood viscosity, reduce the stickiness of red blood cells and inhibit platelet aggregation.

RBC Aggregation; Blood Viscosity: In vitro

The effects of nattokinase on red blood cell aggregation and blood viscosity were measured in an in vitro experiment. Blood samples were incubated with nattokinase in concentrations of 15.6, 31.3, 62.5 and 125 activity units/ml, resulted in 21.9%, 25.9%, 49.7% and 62.0% inhibition of red blood cell aggregation, respectively, compared to the control. Nattokinase reduced blood viscosity at lower shear rates but there were no changes in viscosity at higher shear rates (Pais et al. 2006).

Platelet and RBC Aggregation: Case-studies

The effect of NSK-SD on platelet aggregation was determined in 4 subjects given a dose of 4,000 FU. Blood was drawn and platelet aggregation was measured ex-vivo before and after administration of nattokinase. Aggregation in platelet-rich plasma was induced with either collagen (1 µg/ml) or ADP (2 µM). ADP-induced aggregation was inhibited by approximately 50% by the blood from three men, 31, 34 and 59 years old, taken 6 hours after administration of nattokinase. The same blood samples had little effect on collagen-induced aggregation. Another individual's blood, from a 39-year-old male, demonstrated 50% inhibition of collagen-induced aggregation (12 hour blood sample), along with a smaller effect on ADP-induced aggregation (Takaoka 2005).

NSK-SD in a dose of 2,000 FU/day for 7 days was given to two subjects with red blood cells that were determined to be in active rouleaux formation (red cell stacking) by microscopic examination. The red blood cells were examined before treatment, after 1 week of treatment and then 3 weeks later. One subject was a 35-year-old male smoker, and the other a 42-year-old female who was a non-smoker. Treatment with NSK-SD returned the red blood cells to normal in both cases after 1 week of treatment. Three weeks after discontinuing treatment there were

signs of the red blood cells returning to their original rouleaux state. However, they had not returned to their baseline condition (Takaoka 2005).

Reduction of Blood Viscosity: Clinical Study

The effect of nattokinase on blood flow was studied in a placebo-controlled crossover clinical study with 15 healthy subjects aged 30-49 years old (7 men and 8 women). The participants were given 3 capsules NSK II (2,000 FU) in a single dose or placebo. There was a 2-week wash-out period before switching treatments. Blood flow was measured using the PeriScan PIM II method. In the nattokinase group there was a significant increase in blood flow in the right and left middle fingers 80, 120 and 180 minutes after intake ($p<0.01$). Compared to placebo there was a significant effect 180 minutes after intake (0.42 ±0.08V compared to 0.10 ± 0.11V; $p=0.034$). The nattokinase group also had an increase in blood flow in the back of the right and left hands at 40, 80, 120 and 180 minutes compared to baseline ($p<0.01$). When the participants were subdivided according to their BMI, those with a BMI over 23 treated with nattokinase had a statistical increase in blood flow compared to those given placebo ($p=0.046$)(Iuchi et al 2006).

Inflammation

A clinical study suggests that nattokinase has anti-inflammatory activity in subjects with levels of C-reactive protein (CRP) indicative of risk for cardiovascular disease. CRP is an acute-phase protein that is recognized as the most characterized biomarker for inflammation. In this acute study, 18 subjects, with three or more documented cardiovascular risk factors, took one dose of 100 mg NSK-SD. Blood was drawn before and up to 24 hours post-dose. CRP levels have been divided into three categories of low, moderate and high levels of risk for cardiovascular disease as follows: <1 mg/L, 1-3 mg/L and 3-10 mg/L. The subjects varied in their baseline CRP levels, including individuals in all three categories. Mean CRP levels decreased progressively for 12 hours post-dose; from 11.6 ± 14.6 mg/L at baseline to 8.0 ± 9.7 mg/L at 12 hours. After 24 hours the mean CRP levels rebounded to 10.7 ± 14.1 mg/L. When the subjects were divided into subgroups according to baseline CRP levels, it was evident that the effect from nattokinase was greatest in those with the most elevated baseline levels. For example, a single subject with an initial CRP level of 9.9 mg/L had a reduction of 72% at 24 hours post-dose. Three subjects with CRP levels of 3 to 10 mg/L at baseline had an average reduction of 2.8 mg/L at 24 hours post-dose. The study did not include a sufficient number of subjects to demonstrate a statistically significant effect (Jeske et al 2011).

Lipid Levels

A randomized, double-blind, placebo-controlled study included subjects with primary hypercholesterolemia (total cholesterol >200 mg/dl, triglycerides <350 mg/dl and blood pressure <180/110 mmHg) without any other metabolic disorders. Thirty adult men and women

(mean age 53.2 ± 9.87) received nattokinase (4000 FU) or placebo twice daily (total 8000 FU/day) for 8 weeks. All subjects were instructed to follow a low-cholesterol diet. There were no significant changes in lipid levels from baseline over time in the placebo group. The nattokinase group had greater reductions in total cholesterol than the placebo group, with 6 of the 15 subjects (40%) with cholesterol levels now <200 mg/dl after 8 weeks of treatment (compared with 2 of 14 in the placebo group. There were also greater reductions in high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) in the nattokinase group compared to placebo. However none of these differences reached statistical significance (Wu et al. 2009).

Bioavailability

The bioavailability of nattokinase was demonstrated in a rat study that measured transport of nattokinase across the intestinal tract. A dose of 80 mg purified nattokinase/kg was administered intraduodenally to the animals and blood was drawn at intervals. Nattokinase was detected in the plasma 3 and 5 hrs after administration. In addition, a half-hour after administration of nattokinase, fibrinogen degradation products were measured in the plasma. Coagulation time, determined as plasma re-calcification time, was prolonged compared to baseline at the 3 and 5 hour time points following administration of nattokinase (Fujita et al. 1995b).

A follow-up pharmacokinetic study was conducted with 11 healthy adults (male and female, ages 21-65 years old) who took a single dose of NSK-SD (100 mg, 2,000 FU). Blood was drawn before and up to 48 hours after ingestion of the nattokinase. The presence of nattokinase in serum was detected via an ELISA assay using a rabbit polyclonal anti-nattokinase capture antibody. The peak plasma concentration occurred at 13.3 ± 2.5 hrs post-dose. Nattokinase was significantly increased in serum from 2 to 24 hours post-dose compared to baseline ($p<0.05$) (Ero et al 2011).

Safety

Summary of the Evidence for the Safety of NSK-SD

Nattokinase is an enzyme present in a common Japanese food called natto. It has thus been consumed as a food without adverse effect for more than 1,000 years. NSK-SD, produced by JBSL, is a product that has been tested for safety in a number of studies elaborated below. NSK-SD was demonstrated to be non-mutagenic in the Ames assay with 5 strains of bacteria and in a chromosomal aberration study conducted in CHL/IU cells. NSK-FD and NSK-SD have been found to be non-toxic in a series of rodent studies with administration of a single dose and repeat dosing for 28 and 90 days with a dose of 20,000 FU/kg. The LD_{50} was determined to be more than 20,000 FU/kg body weight (more than 1,000 mg/kg).

The safety of NSK II has been tested in a randomized, double-blind human clinical study with 31 healthy men and women given a dose of 3 capsules per day (2,000 FU/day) for 4 weeks. The safety of nattokinase has also been tested in combination with heparin in acute stroke victims and in combination with warfarin in those taking it as a maintenance prophylactic. In both these studies nattokinase was safely administered along with the other fibrinolytic agents.

In conclusion, nattokinase appears to be safe to take at the recommended dose. However, it would be prudent for those who are taking other fibrinolytic agents, or have a bleeding disorder, to seek the advice of their physician before taking nattokinase

Traditional Use

Nattokinase is an enzyme present in a common Japanese food called natto. It has thus been consumed in a food without adverse effect for more than 1,000 years (Sumi et al 1987). According to the Japanese Ministry of Agriculture, Forestry and Fisheries, 125,000 tons of soybeans were used for natto production in 2009 and approximately 150,000 tons of natto (3 million servings) were consumed in Japan in 2010 (MAFF 2011).

Mutagenicity Studies

Nattokinase was demonstrated to be non-mutagenic in the Ames assay with 5 strains of bacteria and in a chromosomal aberration study conducted in CHL/IU cells. The mutagenic potential of nattokinase (20,000 FU/g) was tested in five strains of bacteria: *Salmonella typhimurium* TA98, TA1537, TA100, TA1535 and *Escherichia coli* WP2uvrA. Nattokinase was tested at 6 dose levels, the top level being 5,000 mcg/plate. Negative and positive controls were included. Positive controls included: AF-2 (2-(2-furyl)-3-(5-nitro-2-fyryl) acrylamide, 9-aminoacridine and sodium azide, as well as 2-aminoanthracene with metabolic activation (S9 liver homogenate enzymes). A dose ranging study, from 5,000 mcg/plate to 15.5 mcg/plate revealed neither mutagenicity nor growth inhibition. The main test using doses of 5,000µg to 156µg/plate did not reveal any colony counts exceeding 2 times the negative control at any dose level (Fuji Biomedix. 2003a).

Tests for cell growth inhibition and chromosomal aberration were conducted using CHL/IU cells originating from the lung of a female Chinese hamster. NSK- SD (20,000 FU/g) was found to inhibit cell growth abruptly at 0.156 mg/ml and higher concentrations. The chromosomal aberration test was performed at concentrations lower than those that caused inhibition of cell growth. NSK- SD was incubated with the cells for 6 hours (short term) with and without metabolic activation (S9) and for 25 hours (long term). The period of 25 hours was selected as it is 1.5 times the cell cycle for the CHL/IU cells. The chromosomal aberration test was conducted short term without metabolic activation at three doses (0.156, 0.110 and 0.078 mg/ml) and with metabolic activation at three slightly lower concentrations (0.110, 0.078 and 0.055 mg/ml). The

long term test was conducted using the latter concentrations. Both positive and negative controls were included. The results of the experiments were that chromosomal aberration was observed at less than 5% at all dose-levels and there were no dose-related trends. The researchers concluded that nattokinase did not produce chromosomal aberrations in CHL/IU cells at the concentrations tested (Fuji Biomedix. 2003b).

Animal Studies

Acute Single-Dose Study

NSK-FD freeze-dried powder (approximately 10,000 FU/g) was tested for toxicity in Sprague-Dawley rats given a single oral dose. A group of 10 rats (5 male and 5 female) were given 2,000 mg (20,000 FU) /kg bodyweight and another group, with the same number of animals, were given placebo. The animals were observed for 14 days and at the end of that time they were examined for gross pathology. The study methodology was based upon guidelines established by the Japanese Pharmaceutical Ministry of Health and Welfare (1997). No deaths occurred as a result of treatment. One day after dosing, diarrhea was observed in 2 males and soft stools in 3 males and all females. No abnormalities were observed in the remainder of the 14 days. Normal bodyweight gains proceeded during the observation period. No abnormalities were observed at necropsy (BILIS 1999).

Repeat-Dose 28-Day Study

A repeat-dose study of 28 days was conducted using Sprague-Dawley rats. A dose of 167 mg/kg/day nattokinase (20,000 FU/g nattokinase; 3,340 FU/kg bw) was administered orally to 6 males and 6 females. Another group, with the same number of animals, was given placebo. The amount of nattokinase was calculated as being equivalent to 100 times the usual intake of natto (50 g) taken by a 60 kg person. The animals were observed for clinical signs, body weight, food consumption, urinalysis and ophthalmological health. The study methodology was based upon guidelines established by the Japanese Ministry of Health and Welfare (1997). At the end of 28 days the animals were bled for hematological and blood chemistry analysis, as well as euthanized for necropsy and histopathological examination. No toxic effects were attributed to nattokinase (Fuji Biomedix. 2002).

Repeat-Dose 90-Day Study

A repeat dose of 90 days (13 weeks) was also conducted using Sprague-Dawley rats. This study included three oral doses of 100, 300 and 1000 mg/kg/day nattokinase (21,900 FU/g) and also included a control group. The 4 groups of animals consisted of 24 animals each: 12 males and 12 females. The study methodology was based upon guidelines established by the Japanese Ministry of Health and Welfare (1997). At the end of the study there were no deaths, no

nattokinase-related changes in clinical signs, body weight, food consumption, ophthalmological health, urinalysis (including water consumption), hematology, blood chemistry or pathology (Bozo Research Center 2004).

Clinical Studies

The safety of NSK II was tested in a randomized, double-blind human clinical study with 31 healthy men and women (20-64 years old; BMI between 18 and 28) (Ogasawara et al. 2006). Nine volunteers (5 men and 4 women) took a placebo and 22 volunteers (10 men and 12 women) took NSK II. The dose was three capsules (2,000 FU) per day for 4 weeks followed by a 2-week observation period. The volunteers visited the clinic at the beginning of the study, after 4 weeks of treatment and then 2 weeks after that. During visits to the clinic a health interview was conducted during which body weight, blood pressure and pulse rate were measured. In addition, blood was taken and urine was collected. Subjective symptoms were noted in a daily diary. No significant adverse effects were reported for either group. Mild adverse events reported for the placebo group were diarrhea (4 individuals) and back pain (1 individual). Mild adverse effects reported for the treatment group were diarrhea (3 individuals), common cold (2 individuals), constipation (1), pimples (1), stomach pain (1), menstrual cramps (1), constipation (1), and headache (1). Body weight increased by a small amount in both the placebo and treatment groups and it was not considered to clinically relevant. There were also minor changes in hematological profiles in both groups that were not deemed clinically significant. There was no effect on blood pressure or pulse and no significant changes in urine analysis. The researchers concluded that taking 3 capsules daily of NSK II for 4 weeks is safe.

An open label study evaluated the safety of nattokinase as an additional oral fibrinolytic agent for those who had suffered a stroke. The study included 12 adults (men and women; average age 53.3) who presented to the hospital in a conscious state with acute mild to moderate ischemic stroke of non-cardiac origin. All patients were administered heparin s.c. (7,600 IU/day) and an anti-platelet drug (low dose aspirin 150 mg-325 mg or Clopidogrel). They were also treated for 7 days with nattokinase (6,000 FU/day; 3 doses of 2,000 FU). The subjects were then monitored for 3 months (90 days). No deaths occurred during the course of the study. There were no reported incidents of hemorrhagic transformation of the infarct as confirmed by CT scan. The outcomes of the patients were evaluated using three internationally recognized scales: National Institute of Health Stroke Scale, Modified Rankin Scale and Barthel Index. According to these scales, 5 patients had an overall favorable response. Coagulation and fibrinolytic assays were performed on days 1, 2 and 7. Significant changes compared to day 1 were are follows: bleeding time increased on day 7, clotting time increased on days 2 and 7, prothrombin time decreased on day 7, activated partial thromboplastin time decreased on day 2, and D-dimmer levels decreased on days 2 and 7. There were three adverse events that may possibly have been attributed to nattokinase: 1) prolonged activated partial thromboplastin time, 2) moderate hematemesis and 3) an abnormal liver function test. All of these events were

temporary. The study authors declared that the study showed that nattokinase could be safely administered to stroke patients as an adjunct to standard medical treatments (Shah et al. 2004).

A further study explored the safety of the administration of nattokinase to patients taking warfarin for maintenance purposes. This was a double-blind, placebo-controlled study with 30 adults (men and women; average age 59). The treatment group was given 2 capsules (1700 FU) nattokinase (NSK II) per day after breakfast for 26 weeks. There were no adverse effects reported due to the combination of the two agents and the authors suggested that parallel administration of nattokinase and warfarin may be possible (Ninomiya 2006).

Case Report

A case report was published recently describing a 52-year old woman in Taiwan who experienced an acute cerebellar hemorrhage that may have been linked to consumption of 400 mg nattokinase for 7 consecutive days (Chang et al. 2008). The report was complicated by the fact that the patient was taking low-dose aspirin and anti-hypertensive agents. She also had high blood pressure and a family history of cerebral hemorrhage. No information was given on her prognosis or progress after discontinuing nattokinase. The brand and activity of the nattokinase was also not reported.

Bacillus subtilis natto

The safety of the bacteria used to make natto, Bacillus subtilis, was tested in mice (ICR-strain; 5-weeks old). A single oral inoculation of control or 7.55 x 10^8 CFU were given to two groups of 10 animals each (5 males and 5 females in each group). The mice were observed for 14 days after inoculation. As a result, no deaths occurred, there were no abnormalities in general health, body weight, no treatment-related abnormalities in the histopathology examination during autopsy and no bacteria in any of the tissues examined during autopsy. The researchers concluded that the bacteria used in the production of NSK-SD held no potential for infectivity, pathogenicity or toxicity (Japan Biological Science Inc. 2003).

Allergenicity

NSK-SD is derived from soybeans, which as a potential allergy-provoking ingredient must be declared as such in labels. However there is no soy left in the final NSK-SD product.

MSDS

A Material Safety data Sheet for NSK-SD is available and it describes the material as not having a Hazards Classification.

How NSK-SD differs from competitors' proteins

The characteristics of nattokinase are dependent on the strain of the bacteria and the characteristics of the soybeans used to produce it, as well as the industrial processing techniques. JBSL has discovered and enhanced a proprietary strain of *Bacillus subtilis natto* that produces maximal yield and potency of nattokinase when exposed to a select soybean crop using proprietary processing and growth techniques.

The distinctiveness of nattokinase products can be described with physical characteristics of the protein, activity of the protein and vitamin K_2 content. Several competitors' products were compared to NSK-SD and found to have different characteristics.

Proteins such as nattokinase can be characterized by mass, charge and purity using gel electrophoresis. Other nattokinase products were compared to NSK-SD using several gel electrophoresis techniques (SDS-PAGE, IEF and 2-DIGE). The SDS-PAGE run depicted differences in the molecular weight and the IEF revealed differences in electric charge (pI). The 2-DIGE compared the proteins in the same gel using fluorescent dyes. The results show that the molecular weights and pI's of the competitors' products were different from that of NSK-SD. These results suggest that the proteins are different. These physical differences could result in functional differences.

The functional (enzymatic activity) profile of the nattokinase in NSK-SD was also compared to that of other nattokinase products. Enzymatic degradation products created by incubating the nattokinases with oxidized insulin B-chain protein at 37^0C were characterized using HPLC. The results showed different degradation patterns of the oxidized insulin B-chain with different products. Another difference between NSK-SD and other products was the amount of fibrinolytic activity. In addition, NSK-SD has no vitamin K_2 content, whereas competitors' products showed measureable amounts of vitamin K_2.

Table 1: Nattokinase (NSK-SD) Clinical Data

Reference (Author, date)	Study Design	Subjects	Primary Endpoint	Preparation/ Dose	Main Results
Ero et al, 2011	Single dose	n=11, healthy men & women, 21-65 yrs	Pharmacokinetics	NSK-SD 2,000 FU	T-max 13.3 ± 2.5 hrs
Maruyama & Sumi, 1998	Open 4 days	n=5, high blood pressure	Hypertension	30 g extract 6400 FU	↓systolic & diastolic blood pressure
Krishnan Med Assoc, 2003	R,PC x-over 4 weeks	n=20, men & women, various illnesses	Hypertension	NSK-II 4000 FU	↓systolic blood pressure compared to baseline (p=0.039)
Kim et al, 2008	R,PC 8 weeks	n=73, men & women, hypertensive, 20-80 yrs	Hypertension	NSK-II 2000 FU	↓systolic & diastolic blood pressure, ↓plasma renin, all compared to placebo (p<0.05)
Sumi et al, 1990	PC x-over single dose	n=12, healthy men & women, 21-55 yrs	Fibrinolytic	200 g Natto 6000 FU est.	ELT ↓2,4,8 hrs after compared to control (p<0.005); EFA ↑ 2,4,8, 12 hrs after compared to control (p<0.005)
Sumi et al, 1990	CT 8 days	n=12, healthy men & women, 21-55 yrs	Fibrinolytic	Natto, 3000 FU est. (divided dose following meals)	EFA ↑ gradual over days, ns; FDP spiked on day 1 (p<0.001), then decreased slowly over 8 days
Sumi et al, 1996; Sumi & Maruyama 1998	Single dose	n=5, healthy, 51-86 yrs	Fibrinolytic	Natto, 6000 FU est.	EFA ↑ 4 hrs post dose (p<0.05); FDP ↑ 6 & 8 hrs post dose (p<0.05), this effect was less after 2nd & 3rd dose 2 and 5 days later

Ninomiya, 2006	R,DB,PC 26 wks	n=30 men & women (avg. 59 yrs) on warfarin	Fibrinolytic	NSK II 1700 FU	Stabilized INR, ↓ rate of change ($p<0.05$)
Takaoka, 2005	Single dose	n=10, men & women, 22-59 yrs	Platelet aggregation	NSK-SD 4000 FU	Ex-vivo ADP induced platelet aggregation ↓50%; collagen induced aggregation no effect in 75% subjects
Takaoka, 2005	1 wk	n=2, man & woman, RBC in rouleaux formation	RBC in rouleaux formation (stacked)	NSK II 2000 FU	RBC to normal after 1 week, effect partially reversed 2 weeks after discontinuing treatment
Iuchi et al, 2006	PC x-over single dose	n=15, healthy men & women, 30-49 yrs	Blood viscosity	NSK II 2000 FU	↑blood flow in fingers & back of hands compared to baseline, 80, 120, 180 min afterwards
Jeske et al, 2011	single dose	n=18, men & women, cardiovascular disease	Inflammation (CRP)	NSK-SD 2000 FU	CRP levels ↓ for those with elevated levels (8-40 mg/L) at baseline

ELT = euglobin lysis time; EFA = euglobin fibrinolytic activity; FDP = fibrinogen degradation product; RBC = red blood cells

Appendix: Blood Clotting Biochemistry & Pharmacology

Clotting mechanisms

Blood coagulation or blood clotting is the transformation of blood into a solid gel called a clot or thrombus. The clot consists of a lattice of a protein polymer known as fibrin in combination with activated platelets. Clotting occurs in response to injury in the blood vessel.

Blood clots are produced as a result of a complex cascade of biochemical reactions. At each step of the cascade, an inactive plasma protein is converted to an enzyme or coenzyme, which in turn catalyzes the generation of the next enzyme in the sequence. At the end of the clotting cascade, the plasma protein prothrombin is converted to the enzyme thrombin. Thrombin causes several polypeptides to be split from fibrinogen. The fibrinogen remnants then bind to each other to form fibrin. Fibrin is strengthened by cross linking caused by an enzyme called factor XIIIa.

Two pathways can initiate the formation of fibrin: the intrinsic pathway in which everything needed is in the blood and the extrinsic pathway in which a cellular component (thromboplastin, also called tissue factor) is needed. The intrinsic pathway involves factor XII which becomes activated to factor XIIa following contact with damaged endothelium. Factor XIIa catalyzes the activation of XI to factor XIa, which in turn activates factor IX to factor IXa, and factor X to factor Xa, which is the enzyme that converts prothrombin to thrombin. The extrinsic pathway begins with a protein called tissue factor (which is not a plasma protein). Tissue factor is located on the outer plasma membrane of various tissue cells including fibroblasts and other cells below the endothelium. Tissue factor binds to plasma protein factor VII, which is activated to factor VIIa, which in turn catalyzes the activation of factor X to Xa, and in turn factor IX.

The liver plays a role in clotting by producing many of the plasma clotting factors. The liver also produces bile salts that are important for the intestinal absorption of vitamin K. The liver requires vitamin K for the production of fibrinolytic proteins and several clotting factors (factors II, VII, IX and X).

Anti-clotting mechanisms

Anti-clotting mechanisms include factors that limit clot formation and the fibrinolytic system that dissolves the clot once it is formed. Mechanisms that limit clot formation include plasma proteins such as tissue factor pathway inhibitor (TFPI), protein C, protein S and antithrombin III.

TFPI is secreted mainly by endothelial cells and acts during the ignition phase of clotting. It binds to complexes between tissue factor and factor VIIa, inhibiting the ability of these complexes to generate factor Xa.

Thrombin can bind to an endothelial cell receptor called thrombomodulin, eliminating its clot-producing effects. The bound thrombin then binds to a particular plasma protein, protein C. The binding of thrombin activates protein C, which in combination with another plasma protein inactivates factors VIIIa and Va. (Thrombin directly activates factors VIII and V and indirectly inactivates them via protein C). Antithrombin can inactivate thrombin after binding to heparin. There is an endogenous heparin present on the surface of endothelial cells.

The fibrinolytic (thrombolytic) system contains a plasma proenzyme, plasminogen, which can be transformed to its active form by plasminogen activators. The active form of plasminogen is the enzyme plasmin. Once formed, plasmin digests fibrin thereby dissolving the clot. An example of a plasminogen activator is tissue plasminogen activator (t-PA), which is secreted by endothelial cells. During clotting, both plasminogen and t-PA bind to fibrin and become incorporated throughout the clot. t-PA is a weak enzyme that requires the presence of fibrin to catalyze the generation of plasmin from plasminogen.

Anti-clotting drugs

A brief summation of current drugs (aspirin, warfarin, heparin and streptokinase) used to inhibit blot clotting is included here to place in context the activity of nattokinase. Aspirin inhibits the cyclooxygenase enzyme, which, in turn, prevents the generation of prostaglandins and thromboxanes. Thromboxane A_2, which is produced by platelets, causes platelet activation and aggregation. Low doses of aspirin cause a steady state inhibition of platelet cyclooxygenase activity, which reduces platelet aggregation in the blood. Vitamin K is required for the synthesis of clotting factors by the liver. Drugs that interfere with the action of vitamin K are a class of pharmaceutical known as oral anticoagulants. The most well known is warfarin (Coumadin®). Heparin, a naturally occurring endothelial cell co-factor for antithrombin III, can be administered as a drug which then binds to endothelial cells. Heparin facilitates the action of antithrombin III and reduces platelet function through inhibition of thrombin agonists. Plasminogen activators dissolve a clot after it is formed (known as thrombolytic therapy). Administration of t-PA or a proteolytic drug called streptokinase reduces the amount of tissue damage when injected into the blood within 3 hours of a heart attack or occlusive stroke.

References

BILIS 1999, *Final report: Single-oral dose toxicity study of biozyme NSK-FD powder in rats.*, Environmental Biological Life Science research Center, Inc.

Bozo Research Center, Inc. 2004, *Report: 13-week oral toxicity study of Nattokinase DS (NSK-SD) in rats*. B-5170.

Chang, Y.Y., Liu, J.S., Lai, S.L., Wu, H.S., & Lan, M.Y. 2008. Cerebellar hemorrhage provoked by combined use of nattokinase and aspirin in a patient with cerebral microbleeds. *Intern.Med*, 47, (5) 467-469 available from: PM:18310985

Ero, M., Ng, C., Chou, R., Mihailovski, T., Ballard, C., Esplana, A., Harvey, N., & Lewis, B. A pilot study on the serum pharmacokinetics of nattokinase in humans following a single oral daily dose. 2011.
Ref Type: Unpublished Work

Ero, M. & Lewis, B. 2008, *Coagulation profile of nattokinase.* Machaon Diagnostics, Inc..

Fuji Biomedix., L. 2002, *Final Report: Oral repeated dose 28-day toxicity study of Nattokinase DS in rats.* FBM 02-2704 .

Fuji Biomedix., L. 2003a, *Final Report: Reverse-Mutation Assay in bacteria.* FBM 02-8766.

Fuji Biomedix., L. 2003b, *Final Report: Chromosomal Aberration Test.* FBM 02-8767.

Fujita, M., Hong, K., Ito, Y., Fujii, R., Kariya, K., & Nishimuro, S. 1995a. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. *Biol Pharm Bull*, 18, (10) 1387-1391 available from: PM:8593442

Fujita, M., Hong, K., Ito, Y., Misawa, S., Takeuchi, N., Kariya, K., & Nishimuro, S. 1995b. Transport of nattokinase across the rat intestinal tract. *Biol Pharm Bull*, 18, (9) 1194-1196 available from: PM:8845803

Fujita, M., Ito, Y., & Nishi 1995c. Characterization of nattokinase-degraded products from human fibrinogen or cross-linked fibrin. *Fibrinolysis*, 9, 157-164

Fujita, M., Nomura, K., Hong, K., Ito, Y., Asada, A., & Nishimuro, S. 1993. 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*, 197, (3) 1340-1347 available from: PM:8280151

Iuchi, S., Dokai, H., & Shionoya, K. 2006. Improvement of blood flow increase by ingestion of NSK-II. *Food Style 21.*, 10(8), 78-90.

Japan Biological Science Inc. 2003, *Final report: pathogenicity study of nattokinasr DS (NSK-SD) producing bacteria in mice.* JBS-03-MOPG-549.

Jeske, W., Ng, C., Ballard, C., Harvey, N., Mihailovski, T., Lewis, B., & Ero, M. Evaluating the effects of nattokinasem on C-reactive protein levels in adults at risk for cardiovascular disease. 2011.
Ref Type: Unpublished Work

Kim, J.Y., Gum, S.N., Paik, J.K., Lim, H.H., Kim, K.C., Ogasawara, K., Inoue, K., Park, S., Jang, Y., & Lee, J.H. 2008. Effects of nattokinase on blood pressure: a randomized, controlled trial. *Hypertens.Res*, 31, (8) 1583-1588 available from: PM:18971533

Krishnan Medical Association SC. 2003, *Effect of NSK-SD on blood pressure by oral administration*.

Lloyd-Jones, D.M., Hong, Y., Labarthe, D., Mozaffarian, D., Appel, L.J., Van, H.L., Greenlund, K., Daniels, S., Nichol, G., Tomaselli, G.F., Arnett, D.K., Fonarow, G.C., Ho, P.M., Lauer, M.S., Masoudi, F.A., Robertson, R.M., Roger, V., Schwamm, L.H., Sorlie, P., Yancy, C.W., & Rosamond, W.D. 2010. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*, 121, (4) 586-613 available from: PM:20089546

MAFF. Ministry of Agriculture, Forestry and Fisheries Annual Report. 2011.
http://www.maff.go.jp/e/index.html.
Ref Type: Online Source

Maruyama, M. & Sumi, H. 1998, "Effect of natto diet on blood pressure.," Japan Technology Transfer Association (JTTAS), pp. 1-3.

Nakamura, T., Yamagata, Y., & Ichishima, E. 1992. Nucleotide sequence of the subtilisin NAT gene, aprN, of Bacillus subtilis (natto). *Biosci Biotechnol Biochem*, 56, (11) 1869-1871 available from: PM:1369081

Ninomiya, J. 2006, *Clinical Study report: Investigative research for effects of ingestion of natural Super Kinase II; a nattokinase soft gel capsule.* Japan Medical School.

Ogasawara, K., Iuchi, S., & Shionoya, K. 2006. Report of Research: an oral safety study of nattokinase containing food, Natural Super Kinase II: a randomized, placebo controlled, double-blind study. *Progress in Medicine*, 26, (5) 5

Pais, E., Alexy, T., Holsworth, R.E., Jr., & Meiselman, H.J. 2006. Effects of nattokinase, a pro-fibrinolytic enzyme, on red blood cell aggregation and whole blood viscosity. *Clin Hemorheol.Microcirc.*, 35, (1-2) 139-142 available from: PM:16899918

Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., III, Criqui, M., Fadl, Y.Y., Fortmann, S.P., Hong, Y., Myers, G.L., Rifai, N., Smith, S.C., Jr., Taubert, K., Tracy, R.P., & Vinicor, F. 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*, 107, (3) 499-511 available from: PM:12551878

Phang, M., Lazarus, S., Wood, L.G., & Garg, M. 2011. Diet and thrombosis risk: nutrients for prevention of thrombotic disease. *Semin.Thromb Hemost.*, 37, (3) 199-208 available from: PM:21455854

Shah, AB., Rawat, S., Mehta, S., Takaoka, S., Sato, K., & Ogasawara, K. 2004. An Open Clinical Pilot Study to Evaluate the Safety and Efficacy of Natto Kinaseas an Add-on Oral Fibrinolytic Agent Tolow Molecular Weight Heparin & Anti-plateletsin Acute Ischeamic Stroke. *Japanese Pharmacology & Therapeutics*, 32, (7) 437-445

Sumi, H., Banba, T., & Krishimoto, N. 1996. Strong pro-urokinase activators proved in Japanese soybean cheese Natto. *Nippon Shokuhin Kagaku Kaishi*, 43, (10) 1124-1127

Sumi, H., Hamada, H., Nakanishi, K., & Hiratani, H. 1990. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. *Acta Haematol.*, 84, (3) 139-143 available from: PM:2123064

Sumi, H., Hamada, H., Tsushima, H., Mihara, H., & Muraki, H. 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. *Experientia*, 43, (10) 1110-1111 available from: PM:3478223

Sumi, H. & Maruyama, M. 1998, "Enhancement of fibrinolytic system and thrombolytic effect by natto diet," Japan Technology Transfer Association (JTTAS), pp. 5-10.

Suzuki, Y., Kondo, K., Matsumoto, Y., Zhao, B.Q., Otsuguro, K., Maeda, T., Tsukamoto, Y., Urano, T., & Umemura, K. 2003. Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery. *Life Sci*, 73, (10) 1289-1298 available from: PM:12850244

Takaoka, S. 2005, *Platelet aggregation inhibitory effect of nattokinase (NSK); Studies by Dr Keiko Hoshi at St. Marianna University School of Medicine*.

Urano, T., Ihara, H., Umemura, K., Suzuki, Y., Oike, M., Akita, S., Tsukamoto, Y., Suzuki, I., & Takada, A. 2001. The profibrinolytic enzyme subtilisin NAT purified from Bacillus subtilis Cleaves and inactivates plasminogen activator inhibitor type 1. *J Biol Chem*, 276, (27) 24690-24696 available from: PM:11325965

Wu, DJ., Lin, CS., & Lee, MY. 2009. Lipid-lowering effect of nattokinase in patients with primary hypercholesterolemia. *Acta Cardiol Sin*, 25, 26-30