Nattokinase a.k.a NSK-SD

This white paper is designed to give those interested in the benefits behind NSK-SD a quick look inside this revolutionary nutritional supplement and food. For a deeper dive, please refer to our long-form white paper that takes a more clinical look.

What is NSK-SD?

NSK-SD is a, patented, branded, uniquely produced and studied enzyme with fibrinolytic activity. NSK-SD 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. More recently, health-focused specialty stores in the U.S. have begun selling Natto. Natto is considered a "heart-healthy" addition to the diet and for those with cardiovascular health concerns.

NSK-SD History of discovery

In 1907, it was discovered that natto contained protease enzyme activity. In 1956 the amino acid sequence of nattokinase was first identified. In the 80's Japan Bio Science Laboratories began to conduct investigative clinical work, which included: demonstrating the enzyme had the ability to dissolve thrombi (blood clots) and named it "nattokinase". In addition to being fibrinolytic, the cardiovascular benefits of nattokinase include reducing elevated blood pressure, improving circulation, improving circulation and normalizing levels of systemic inflammation as measured by C-Reactive Protein (CRP).

Proof is in the research

Nothing speaks louder than an ingredient with sound clinical research to prove its safety, efficacy, dosing and indications. NSK-SD has over 55 clinical studies of which 23 are human clinical trials on a variety of areas. Getting published is even more impressive when reviewing nutritional ingredients of which NSK-SD has 18 published papers. We will take a closer look at this research while covering the proven benefits and safety of NSK-SD.

Biochemical and Physiological Benefits of NSK-SD

The following areas have clinical research showing the physiological and biochemical benefits of NSK-SD:

- 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)
- Improve blood flow

Reduces elevated blood pressure

Elevated blood pressure or hypertension is one of the leading risk factors of cardiovascular disease. Having high blood pressure is defined by having either a systolic reading of 140mm of mercury or higher and/or a diastolic pressure of 90mm of mercury or higher. The first number, systolic, is the measure of pressure pushing against the arteries the moment the heart beats. The second number, diastolic, is the measure of pressure pushing against the arteries when the heart is resting (between beats). Typically more attention is given to the top number (systolic) as a major risk factor for cardiovascular disease for people over 50-year-old. In most people, systolic blood pressure rises steadily with age due to increasing stiffness of large arteries, long-term build-up of plaque, and increased incidence of cardiac and vascular disease.

There is also a condition called pre-hypertension. This condition impacts millions of Americans and is described as: Systolic between 120-139 and a diastolic reading between 80-89.

The precise mechanism of action of NSK-SD on its ability to help with elevated blood pressures has not completely been discovered to date. Regardless of knowing its mechanism, there is enough science to show that the use of NSK-SD can and will support the lowering of both Systolic and Diastolic readings. Currently there have been several human clinical trials evaluating this benefit. The following is a brief summary of this clinical research for hypertension:

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Sumi et al. 1998	6,400 FU/day 4-days	N=5 hypertensive people	Hypertension	Systolic and diastolic blood pressure decreased
Kim et al. 2008	2,000 FU/day 8-weeks	N=73, men and women, hypertensive 20-80 years old	Hypertension	Reduction is Systolic, Diastolic blood pressure and Renin all compared to placebo
Krishnan Medical Association 2003	4,000 FU/day 4-weeks	N=20, men and women, various illnesses	Hypertension	Reduction in systolic blood pressure compared to baseline
Fujita et al. 2011	0.2 mg/g diet, 2/6 mg/g diet, 0.2mg/g diet, 0.6 mg/g diet 3-weeks	SHR Rats, 63 total	Hypertension	Nattokinase may decrease blood pressure through cleavage of fibrinogen in plasma.

Kang 2014	Nattokinase By Lotte: 2000 FU Nattokinase by KPX: 2000 FU/day 4-weeks	N=42, men and women with high blood pressure risk	Hypertension	Nattokinase by Lotte using NSK-SD had drastic reduction of blood pressure compared to nattokinase by KPX
Jensen 2016	2,000FU/day 8-weeks	N=79 adults with high BP	Hypertension	Nattokinase consumption in a North American population is associated with beneficial changes to BP in a hypertensive population
Ried (Wakunaga) 2012	Garlic with Nattokinase (70mg) and L-Theanine/day 12 weeks	N-79 adults with uncontrolled systolic hypertension	Hypertension	Garlic and NSK-SD blend is an effective and tolerable treatment in uncontrolled hypertension treatment.

Fibrinolytic activity

Before diving into the amazing studies of the effectiveness of NSK-SD with regards to its effects on Fibrin, it is good to clarify physiologically why having a positive fibrinolytic effect is important. Fibrinolysis (breaking down fibrin) is a process that prevents blood clots from growing and causing more serious health issues. Fibrin is a strong insoluble protein produced by our body in response to bleeding. Fibrin molecules combine together to form long fibrous chains that entangle the red blood cells and platelets at the site of a wound. When these blood clots form they can be beneficial like with external wound healing. In the case of cardiovascular disease it can be deadly because blood clots lead to heart attacks and strokes. Without proper control of blood clots and fibrin formation we open the body up to dangerous outcomes. We can now see why finding an ingredient that will help break down fibrin but won't inhibit wound healing is important.

NSK-SD has been shown to degrade fibrin clots both directly and indirectly. Clot lysis assays indicate NSK-SD degrades fibrin directly with activity comparable to plasmin. (In order to degrade fibrin blood clots the body naturally produces Plasmin.) Kinetic assays suggest nattokinase is 6 times more active than plasmin in degrading cross-linked fibrin. NSK-SD degrades fibrin indirectly by affecting plasminogen activator activity (the beginning step of plasmin production) but does not directly stimulate plasminogen activator activity. Importantly, NSK-SD does not inhibit the formation of fibrin from fibrinogen and therefore does not inhibit the

formation of blood clots in response to injury. Simply put, NSK-SD allows the body to heal without the potential for harm.

Taking a closer look at the research in summary format we can see that there are several clinical trials proving the benefits of NSK-SD on the degradation of fibrin.

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Sumi et al. 1990	6,000 FU single dose	N=12, healthy men and women 21-55 years old	Fibrinolytic	Euglobulin lysis time reduced 2,4,8 hours after consumption compared to control. Euglobulin Fibrinolytic Activity increased 2,4,8,12 hours after consumption compared to control
Sumi & Maruyama 1998	6,000 FU single dose	N=5, healthy men and women, 51-86 years old	Fibrinolytic	Euglobulin Fibrinolytic Activity increased 4 to 8 hours after consumption compared to baseline. Fibrin degradation products increased 6,8 hours compared to baseline.
Sumi et al. (PART B) 1990	1300 FU 3x daily 8-days	N=7 healthy volunteers, 21-55 years old	Fibrinolytic	Fibrin Degradation Products spiked on day 1, then decreased slowly over 8 days.
Hsia et al. 2009	4,000 FU/day 8-weeks	N=45, men and women 20-70 years of age	Fibrinolytic	Decrease in Plasma levels of Fibrinogen, factor VII and factor VIII in humans
Sumi et al. 1987	N/A	Natto from Samejima Co. Ltd. In Japan, Swine pancreas trypsin, (DFP), (Neguvon), (ε-ACA), (t-AMCHA), human plasmin and urokinase, DL-	Fibrolytic effects	Nattokinase was discovered to have strong fibrinolytic activity

		Arg-pNA, various enzymes from Kabi group, Inc. USA		
Fujita et al. 1995	Nattokinase in 0.02, 0.04 and 0.12 mcmol/kg (iv)	Male Wistar rats weighing 300g were used	Nattokinase will lyse thrombosis	Results imply that nattokinase may be safer than plasmin at an appropriate dose level
Fujita et al. 1995	N/A	Nattokinase	Fibrinolysis	Nattokinase is less sensitive on the cleavage of fibrinogen, but is more sensitive on the cleavage of cross-linked fibrin compared to plasmin
Urano et al. 2001	0.06-1 nM	Nattokinase	Plasminogen activator activity	Nattokinase degrades an important inhibitor of plasminogen activator activity.
Kurosawa 2015	2,000 FU single dose	12 young males	Coagulation/ Fibrinolytic effects	D-dimer concentrations and blood fibrin/fibrinogen degradation products were elevated significantly after administration. Factor VIII activity declined, blood antithrombin concentration was higher, and activated partial thromboplastin time prolonged. Therefore, a single dose of nattokinase enhances fibrinolysis and anti-coagulation via several different pathways simultaneously

Hamaoka 2013	2,000 FU single dose	N=3	Thrombosis	Coagulability increased when not taking nattokinase, compared to not much change when taken nattokinase
Urano 2007	4,000 CFU/day 8 weeks	N = 45, slightly overweight individuals (BMI: 23-28)	Fibrinolytic	Fibrinolytic effect in NK group compared to Placebo group was not significant. However, when limited to participants with BMI higher than average (25), there was a significant decrease in PAI-1.

Whether animal or human trials, the benefits of NSK-SD on fibrin and fibrinolytic effects are well researched and documented.

Inhibits platelet & red blood cell aggregation

The inhibition of platelet and red blood cell aggregation is another key mechanism in which NSK-SD benefits cardiovascular health. 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. Why is this so important? Being able to manage this area of cardiovascular health is linked to decreasing the risk of strokes and heart attacks. Platelet aggregation is the clumping together of platelets in the blood, which is part of the pathway to the formation of blood clots/thrombus. When red blood cells aggregate (clump together) in a special way they form something called a rouleaux. Red blood cell aggregation is the main determining factor of the viscosity of blood. The formation of rouleaux is on indicator that there may be an issue with disease. Both platelet and red blood cell aggregation are of great importance for those concerned with cardiovascular disease. NSK-SD has clinical research showing its benefits in both of these areas. The following are a few of the studies and the results realized:

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Takaoka 2005	4,000 FU single dose	N=10, men and women, 22-59 years old	Reduction in platelet aggregation	Ex-vivo ADP induced platelet aggregation down 50%; collagen induced aggregation no effect in 75% of the subjects

Takaoka 2004	4,000 FU single dose	N=9, subjects with spontaneous platelet aggregation caused by smoking or hyperlipemia	Platelet aggregation	There is a strong inhibitory effect on platelet aggregation after 6 hours from ingestion of NSK-SD. In addition, inhibition of spontaneous aggregation was observed in most of the subjects also in this study.
Takaoka 2004	4,000 FU single dose	N=7 healthy subjects (6 males 28 – 59 years, 1 female: 27 years)	Platelet aggregation	With all the subjects, more than 50% of inhibitory action/rate were identified.

Decreases blood viscosity

Blood viscosity is the thickness and stickiness of blood. It is a direct measure of the ability of blood to flow through the vessels. It is also a key-screening test that measures how much friction the blood causes against the vessels, how hard the heart has to work to pump blood, and how much oxygen is delivered to organs and tissues. Increased blood viscosity is the only biological parameter that has been linked with all of the other major cardiovascular risk factors, including high blood pressure, elevated LDL cholesterol, low HDL, type-II diabetes, metabolic syndrome, obesity, smoking, age, and male gender (Sloop 1996). Look at this way, if you have a squirt gun and use glue it is very difficult to shoot the glue out but if you used water the volume of fluid would move easily. This same issue happens in the veins, arteries and capillaries of the body. The efforts used have to be increased and can lead to other more serious health issues.

A unique study done with NSK-SD showed a marked increase in blood flow in the fingers and back of the hands compared to the original baseline with just one dose. (See below)

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Luchi et al. 2006	2,000 FU single dose	N=15, healthy men and women, 30-49 years old	Blood Viscosity	Increased blood flow in fingers and backs of hands compared to baseline, 80, 120, and 180 minutes after consumption

Reduces clinical measure of inflammation (C-Reactive Protein or CRP)

C-reactive protein (CRP) is a substance produced by the liver in response to inflammation. A high blood level of CRP indicates there is an increased state of inflammation in the body. This inflammation can be caused by a wide variety of conditions, from infection to cancer. High CRP levels can also indicate that there's inflammation in the arteries of the heart, which can mean a higher risk for heart attack. However, it's important to remember that the CRP test is an extremely nonspecific test, and CRP levels can be elevated in any inflammatory condition.

If we have inflammation in the blood stream, we are more likely to develop plaque in our arteries. Simply put, inflammation triggers mild abrasion in the veins and arteries. This "wound" triggers a cascade of events that can lead to plaque buildup and more.

CRP is one of the best ways to predict cardiovascular risk. In fact several studies have now linked elevated CRP levels to cardiovascular disease. One published in *Circulation* found that among healthy adult men, those with a high level of CRP were three times more likely to have a heart attack than those with low levels of CRP. This was among men who had no previous history of heart disease. (Ridker 2007) Additionally, according to the Cleveland Clinic the Harvard Women's Health Study showed that high CRP levels were more predictive of coronary conditions and stroke in women than were high cholesterol levels. (https://my.clevelandclinic.org/health/diagnostics/16792-blood-tests-to-determine-risk-of-coronary-artery-disease/c-reactive-protein)

Clinical studies with NSK-SD have shown that it can help reduce the levels of CRP in the bloodstream by taking 100mg or 2000 FU per day. One study in particular (Jeske et al 2011) evaluated the effects of NSK-SD in 18-subjects with 3 or more cardiovascular risk factors. C-Reactive Protein Levels decreased for those with elevated CRP levels (8-40 mg/L) at baseline. Also, it was evident that the effect from NSK-SD was greatest in those with the most elevated baseline levels.

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Jeske et al. 2011	2,000 FU Single dose	N=18, men and women with cardiovascular disease	Reduction of C-Reactive Protein	C-Reactive Protein Levels decreased for those with elevated CRP levels (8-40 mg/L) at baseline

Lipid lowering

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Wu et al. 2009	8,000 FU/day 8 weeks	N=30, hypercholesterolemia patients	Reduction of Cholesterol	Slight reduction in HDL-C and LDL-C

Other pertinent health condition studies information with NSK-SD

As mentioned previously, there have been 55 clinical trials evaluating the safety, dosing and efficacy of NSK-SD. Granted the majority of the benefits are mentioned above, but it is important to share some of the other less talked about areas other than cardiovascular health. With regards to other health concerns, NSK-SD has been studied in solution for biofilm inhibition (effects dental carries) and nasal polyps and mucus viscosity. Note the study summaries below:

Inhibition of Biofilm (Dental Carries)

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Narisawa 2014	0.1mg/ml to 1.0mg/ml	N/A	Inhibition of Biofilm	Nattokinase was found to inhibit the sucrose-dependent biofilm formation of cariogenic streptococci. The presence of nattokinase resulted in the reduction of water-insoluble glucan

Nasal Polyp with Chronic Rhinosinusitis with Nasal Polyps (CRSwNP)

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Takabayashi	10-1000FU/ml	N=12 patients undergoing nasal surgery	Nasal Polyp with CRSwNP	NK effectively shrinks the nasal polyp tissue through fibrin degradation and the viscosity of the nasal discharge and sputum from patients with CRSwNP and asthma

Safety

One of the most important aspects of a great nutritional ingredient is proving its safety. There is no shortage of documentation proving the safety of NSK-SD. Not only have studies been done on oral safety with NSK-SD, but also for topical

applications and eye irritation. Below you will find a summary table of studies done with NSK-SD on the topic of safety.

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Ninomiya & Yamada 2008	Low dose group: 1700 FU/day High dose group: 3400 FU/day 26-weeks	2 dose groups, n=60 men and women (average 59 years old) on Warfarin	Side effects when taken with Warfarin	No adverse effects when taken with Warfarin
BILIS 1999	20,000 FU/kg of body weight (2000 mgs/kg) single dose	N=20 rats (10 males and 10 females, healthy, six weeks old)	Side Effects	No adverse effects observed
Bozo Research Center Inc. 2006	24,700 FU/KG & 49,400 FU/kg of body weight (1000 OR 2000 mgs/kg) single dose	N=24 rats (12 healthy males and 12 healthy females, all six weeks old)	Side Effects	No adverse effects were observed
Bozo Research Center Inc. 2004	Three oral doses of 100, 300 and 1000 mg/kg/day nattokinase (21,900 FU/g) for 90-days	N = 96 rats [4 groups of animals; consisted of 24 animals each: (12 males and 12 females)] all healthy, 5 weeks of age	Toxicity	No adverse effects were observed
Fuji Biomedix Co., Ltd. 2002	A dose of 167 mg/kg/day nattokinase (20,000 FU/g nattokinase; 3,340 FU/kg bw) for 28 days	N=24 rats, healthy, 6 weeks of age	Toxicity	No adverse effects were observed

Ogasawara Kazuya et al. 2006	2200 FU/day for 28-days	N=31 healthy men and women (20-64 years old; BMI between 18 and 28)	Safety	No adverse effects were observed
Fuji Biomedix Co., Ltd. 2003	Nattokinase was tested at 6 dose levels, the top level being 5,000 mcg/plate. 5+ months	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.	Mutagenicity	Nattokinase was demonstrated to be non-mutagenic.
Fuji Biomedix Co., Ltd. 2003	NSK- SD (20,000 FU/g)	Used CHL/IU cells originating from the lung of a female Chinese hamster.	Safety	Did not produce chromosomal aberrations.
Gifu Research Laboratories , JBS Inc. 2003	7.55 x 108 CFU	N=20 mice (10 males and 10 females. ICR-strain; 5- weeks old))	Safety	No adverse effects were observed
Kowatari 2015	10,000FU/day and 2,000FU/day 4-weeks	5 males, 6 females	Safety	No concern over the safety of the intake of 10,000FU/day for 4 weeks
Lampe 2015	N/A	N/A	Toxicology	Animal and human studies suggest that the oral consumption of nattokinase is of low toxicological concern
Shelanski 2018	Cream containing 5% NSK-SD 3	N=10, healthy adults	Skin-Irritation - Topical Use	After three 24-hour exposure sessions, the effect of NSK-SD

	24hour applications			on scarified skin was substantially greater than control but still possessed a **low irritation potential**.
Shelanski 2018	Cream containing 5% NSK-SD 24-hours	N= 61 females, 48 males	Skin Irritation (Topical - Safety Study)	NSK-SD was found to be neither a clinically significant skin irritant nor a skin sensitizer and is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.
Yasso (MB Research) 2018	Cream containing 5% NSK-SD	N= 5 corneas (BCOP); 40 Eggs (CAMVA)	Eye-Irritation Test	The calculated In Vitro score of NSK-SD EL Cream is -1.74; therefore, the test article is considered a mild irritant according to Gautheron et al. No category can be assigned regarding the UN GHS Category, as per the OECD Test Guideline No. 437.

Techie Stuff

Characterization of NSK-SD

NSK-SD is a white, odorless, tasteless, water-soluble, free flowing powder. Its enzymatic activity is measured in Fibrin Degrading Units (FU) and is standardized to an activity level 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, particularly in patients taking warfarin) 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 65 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.

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.

SDS

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

Bioavailability

If the studies above haven't proven that NSK-SD is bioavailable based on its effectiveness, here are a few bioavailability studies:

Author Year	Preparation Dose and duration	Subjects	Primary Endpoint	Main Results
Ng et al. 2011	2,000 FU single dose	N=11, healthy men and women, 21-65 years old	Bioavailability	13.3±2.5 hour for peak blood concentration
Fujita et al. 1995	80 mg purified nattokinase/ kg single dose	20 Male Wistar rats (250g) and male New Zealand White rabbits (2.5kg)	Bioavailability	Nattokinase is absorbed from the rat intestinal tract and cleaves fibrinogen in plasma after intra-duodenal administration of the enzyme.

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 patented a 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.

For more in-depth information on all of these studies please consult with the larger technical white paper.

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