Article

Nattokinase: Biological activities, pharmacological mechanisms, therapeutic applications, challenges and perspetives

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Abstract

Nattokinase (NK), a potent fibrinolytic serine protease derived from the traditional Japanese fermented soybean food natto, has garnered substantial scientific interest over the past four decades due to its remarkable cardioprotective and thrombolytic properties. First isolated and characterized in the 1980s, this enzyme (EC 3.4.21.62), also known as subtilisin NAT, is produced during the fermentation of soybeans by *Bacillus subtilis* var. *natto*. This comprehensive review synthesizes the current body of scientific literature on nattokinase, encompassing its historical discovery, molecular structure, physicochemical properties, extraction and purification methodologies, pharmacokinetic profile, and multifaceted pharmacological mechanisms. Particular emphasis is placed on the enzyme's established fibrinolytic and antithrombotic activities, its emerging antihypertensive, anti-inflammatory, antioxidant, lipid-modulating, and neuroprotective effects, as well as its potential applications in respiratory health and the management of COVID-19-associated coagulopathy. A critical appraisal of clinical trial evidence, including randomized controlled trials evaluating nattokinase alone and in combination with other nutraceuticals, is presented alongside an analysis of safety data from toxicological assessments. The review further examines the current landscape of quality control standardization, regulatory considerations, and the challenges posed by inconsistent manufacturing practices. Finally, the review identifies critical knowledge gaps, discuss the limitations of existing research, and propose future research directions to fully elucidate the therapeutic potential of this promising natural enzyme in the prevention and adjunctive management of cardiovascular, neurodegenerative, and other non-communicable diseases.

Keywords nattokinase; subtilisin NAT; fibrinolytic enzyme; cardiovascular disease; thrombolysis; hypertension; anti-inflammatory; antioxidant; *Bacillus subtilis* var. *natto*; clinical trial; fermentation.

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1 Introduction

Cardiovascular diseases (CVDs) remain the preeminent cause of premature mortality and disability on a global scale, imposing an immense burden on healthcare systems and economies worldwide (Selvarajan and

Bhatnagar, 2017; Li et al., 2023; Muric et al., 2024). The pathophysiological underpinnings of CVDs are multifactorial and complex, encompassing a constellation of interrelated risk factors including coagulation abnormalities, hypertension, dyslipidemia, endothelial dysfunction, chronic inflammation, and oxidative stress (Granito et al., 2024; Muric et al., 2024). While significant therapeutic advancements have been achieved through the development of synthetic anticoagulants, antiplatelet agents, statins, and antihypertensive medications, these pharmacological interventions are frequently associated with adverse effects, drug-drug interactions, and substantial financial costs, prompting a sustained and growing interest in identifying and validating safe, effective, and accessible natural alternatives or adjunctive therapies (Muric et al., 2024; Weng et al., 2017).

Among the diverse array of bioactive compounds derived from traditional fermented foods, nattokinase (NK) has emerged as a particularly compelling candidate for cardiovascular health promotion and disease prevention (Chen et al., 2018; Granito et al., 2024; Weng et al., 2017). Nattokinase is an alkaline serine protease (EC 3.4.21.62), alternatively designated as subtilisin NAT, that is produced abundantly by the bacterium *Bacillus subtilis* var. *natto* during the fermentation of boiled soybeans to produce natto, a traditional Japanese food that has been consumed for over a millennium (Dabbagh et al., 2014; Lampe and English, 2016; Sumi et al., 1987). The enzyme exhibits potent fibrinolytic activity, directly degrading fibrin, the primary proteinaceous constituent of blood clots, and simultaneously enhancing endogenous fibrinolytic pathways through multiple complementary mechanisms (Sumi et al., 1987; Weng et al., 2017). The recognition of nattokinase's thrombolytic potential has catalyzed an extensive and continuously expanding body of scientific inquiry, encompassing fundamental biochemical characterization, preclinical mechanistic investigations, animal model studies, and a growing portfolio of human clinical trials (Li et al., 2023; Liu et al., 2024; Muric et al., 2024).

Epidemiological observations have provided compelling circumstantial evidence supporting the cardiovascular benefits of natto consumption. Notably, studies have demonstrated that habitual natto intake is associated with a reduced risk of mortality from cardiovascular disease, an effect attributed primarily to the food's high content of nattokinase and its attendant fibrinolytic and antithrombotic properties (Granito et al., 2024; Muric et al., 2024). These population-level findings have been buttressed by a substantial corpus of experimental and clinical investigations that have systematically documented the positive effects of nattokinase on various facets of cardiovascular health, including the thinning of blood, dissolution of existing clots, reduction of blood pressure, and attenuation of atherosclerotic processes (Selvarajan and Bhatnagar, 2017; Muric et al., 2024). Beyond its well-established cardiovascular applications, recent research has unveiled a broader spectrum of biological activities attributable to nattokinase, encompassing potent anti-inflammatory and antioxidant effects, neuroprotective actions relevant to neurodegenerative disorders including Alzheimer's disease, and even potential antiviral properties against SARS-CoV-2 (Granito et al., 2024; Li et al., 2026; Tanikawa et al., 2022; Mahakalakar et al., 2025).

The escalating global prevalence of CVDs and other non-communicable diseases (NCDs) has created an urgent imperative for innovative, cost-effective, and well-tolerated preventive and therapeutic strategies (Granito et al., 2024; Li et al., 2023). Nattokinase, with its favorable safety profile, oral bioavailability, and pleiotropic mechanisms of action, is uniquely positioned to address this unmet need (Lampe and English, 2016; Weng et al., 2017). However, despite the substantial and growing body of evidence supporting its therapeutic potential, several critical challenges remain. These include the absence of standardized manufacturing and quality control protocols, significant heterogeneity in the design and outcomes of clinical trials, incomplete elucidation of its pharmacokinetic and pharmacodynamic properties, and a paucity of large-scale, long-term clinical outcome studies (Li et al., 2023; Feng et al., 2023; Yi et al., 2025). The present comprehensive review

aims to critically synthesize the existing scientific literature on nattokinase, providing a detailed and nuanced overview of its discovery, biochemical properties, pharmacological mechanisms, clinical evidence, and safety considerations, while also identifying key knowledge gaps and charting a course for future research endeavors.

2 Discovery and History

2.1 The Serendipitous Discovery by Dr. Hiroyuki Sumi

The discovery of nattokinase stands as a quintessential example of scientific serendipity intersecting with astute observation. The enzyme was first identified in 1980 by Dr. Hiroyuki Sumi, a Japanese researcher then affiliated with the Department of Physiology at the University of Chicago Medical School, who was engaged in a systematic search for natural agents capable of effectively dissolving thrombi associated with cardiac and cerebral infarction (Sumi et al., 1987). Driven by the hypothesis that traditional fermented foods might harbor potent fibrinolytic enzymes, Dr. Sumi and his colleagues undertook an ambitious and exhaustive screening program, testing more than 173 different natural foods for their ability to degrade artificial fibrin clots *in vitro* (Dabbagh et al., 2014; Sumi et al., 1987).

The pivotal experiment occurred on a day in 1980 when Dr. Sumi, inspired by the notion that natto, a food produced through fermentation, might contain fibrin-degrading enzymes, applied an extract derived from natto to an artificial thrombus (Sumi et al., 1987). Remarkably, within a period of approximately three hours, the thrombus had undergone substantial dissolution, losing approximately two centimeters in length (Sumi et al., 1987). This rate of thrombolysis was strikingly superior to that achieved with urokinase, a standard thrombolytic agent of the era, which required nearly two days to effect comparable dissolution of an identical thrombus under similar experimental conditions (Sumi et al., 1987). This dramatic and unexpected finding provided the initial, compelling evidence for the existence of a potent fibrinolytic principle within natto, an observation that would ultimately lead to the isolation, purification, and characterization of the enzyme that was subsequently designated "nattokinase" (Sumi et al., 1987).

The formal announcement of this discovery was made in 1987, when Sumi and colleagues published their seminal paper detailing the purification and characterization of the fibrinolytic enzyme from natto, thereby establishing the scientific foundation for all subsequent research on this remarkable biocatalyst (Sumi et al., 1987). The name "nattokinase" was coined to reflect both its origin in natto and its enzymatic nature, and it has since become the universally recognized designation for this enzyme, also known by its systematic name, subtilisin NAT (EC 3.4.21.62) (Lampe and English, 2016; Sumi et al., 1987).

2.2 Traditional Use of Natto and Cultural Significance

Long before its scientific characterization, natto held an esteemed place in traditional Japanese cuisine and folk medicine. Natto is a traditional fermented food produced by inoculating steamed soybeans with *Bacillus subtilis* var. *natto* (also referred to as *Bacillus natto*) and allowing the mixture to ferment under controlled conditions of temperature and humidity for a period of approximately 18 to 24 hours (Dabbagh et al., 2014; Ashiuchi et al., 2001; Fig. 1). The resultant product is characterized by its distinctive pungent aroma, stringy and viscous texture, and unique umami flavor profile, attributes that have secured its status as a staple of the Japanese diet for well over a thousand years (Dabbagh et al., 2014; Ashiuchi et al., 2001). Traditionally, natto is consumed as a breakfast food, served over steamed rice, incorporated into sushi preparations, or spread on toast; it is even available as an ice cream flavor in contemporary Japan (Dabbagh et al., 2014).

Beyond its culinary applications, natto has been empirically valued in traditional Japanese medicine for its purported health-promoting properties. Historical accounts indicate that natto was traditionally employed for the management of heart conditions, the alleviation of physical fatigue, and as an antiberiberi agent, reflecting an early, intuitive recognition of its beneficial effects on cardiovascular and overall health (Dabbagh et al.,

2014). The longevity and consistently high consumption of natto in Japan, coupled with the comparatively low incidence of cardiovascular disease in populations with high natto intake, have provided fertile ground for epidemiological investigations exploring the potential cardioprotective effects of this traditional food (Granito et al., 2024; Weng et al., 2017).

Fig. 1 *Bacillus subtilis* var. *natto* (Longxigong, 2024).

2.3 Evolutionary Trajectory of Nattokinase Research (1980–Present)

The scientific trajectory of nattokinase research has evolved through several distinct, overlapping phases since its initial discovery in 1980. The foundational period, spanning approximately from 1980 to 1990, was characterized by efforts focused on the isolation, purification, and preliminary biochemical characterization of the enzyme, culminating in the landmark publication by Sumi et al. in 1987 (Sumi et al., 1987). During this phase, the basic enzymatic properties of nattokinase, including its molecular weight, substrate specificity, and *in vitro* fibrinolytic activity, were established, laying the essential groundwork for subsequent investigations (Sumi et al., 1987; Sumi et al., 1990).

The second phase, extending through the 1990s and into the early 2000s, witnessed a significant expansion of research into the *in vivo* efficacy of nattokinase, employing a variety of animal models to explore its antithrombotic, antihypertensive, and anti-atherosclerotic effects (Fujita et al., 1993; Suzuki et al., 2003a; Suzuki et al., 2003b). Concurrently, investigations into the molecular mechanisms underpinning nattokinase's fibrinolytic activity deepened, revealing that the enzyme not only directly degrades fibrin but also potentiates endogenous thrombolysis by enhancing the production of tissue plasminogen activator (t-PA) and catalyzing the conversion of prourokinase to its active form (Sumi et al., 1990; Yatagai et al., 2007). Furthermore, this era saw the initiation of early-stage human clinical trials, which provided preliminary evidence for the safety and efficacy of orally administered nattokinase in reducing blood pressure and improving fibrinolytic parameters in human subjects (Kim et al., 2008).

The most recent phase, commencing around 2010 and continuing to the present day, has been marked by a dramatic diversification and deepening of nattokinase research. This period has been characterized by the conduct of larger, more rigorously designed randomized controlled trials (RCTs) evaluating the effects of nattokinase, both as monotherapy and in combination with other nutraceuticals, on a broad spectrum of cardiovascular risk factors and clinical outcomes (Hodis et al., 2021; Li et al., 2023; Liu et al., 2024). Concomitantly, high-resolution structural biology techniques, including X-ray crystallography, have yielded

atomic-level insights into the three-dimensional architecture of the enzyme, facilitating a more nuanced understanding of its catalytic mechanism and substrate interactions (Yanagisawa et al., 2010; Yanagisawa et al., 2013). Perhaps most significantly, this contemporary phase has witnessed the discovery of novel, unanticipated biological activities of nattokinase extending far beyond its canonical fibrinolytic function. These newly identified properties include potent anti-inflammatory and antioxidant effects mediated through activation of the Nrf2/HO-1 signaling axis (Granito et al., 2024; Li et al., 2026), neuroprotective actions relevant to the prevention and treatment of Alzheimer's disease and other neurodegenerative disorders (Tanikawa et al., 2024; Mahakalakar et al., 2025), and antiviral activity against the SARS-CoV-2 spike protein, suggesting potential utility in the context of COVID-19 (Tanikawa et al., 2022). These recent discoveries have fundamentally broadened the conceptual framework for nattokinase's therapeutic applications, positioning it as a pleiotropic agent with potential relevance to a diverse array of human diseases beyond the cardiovascular system (Granito et al., 2024; Weng et al., 2017).

3 Biochemical and Molecular Properties

3.1 Source and Microbial Production

Nattokinase is a naturally occurring extracellular enzyme produced predominantly by the bacterium *Bacillus subtilis* var. *natto*, a Gram-positive, spore-forming, rod-shaped microorganism that serves as the principal fermentative agent in the production of natto (Dabbagh et al., 2014; Lampe and English, 2016; Wang et al., 2009). The bacterium is typically isolated from natto starter cultures or directly from the fermented soybean product itself, and it is cultivated on a variety of nutrient media under aerobic or semi-aerobic conditions to achieve optimal enzyme yields (Dabbagh et al., 2014; Wang et al., 2009). While *Bacillus subtilis* var. *natto* is the most extensively characterized and industrially utilized source of nattokinase, it is noteworthy that other Bacillus species, as well as microorganisms present in analogous fermented soybean foods from other Asian culinary traditions, have also been reported to produce fibrinolytic enzymes with properties similar to or identical with nattokinase (Dabbagh et al., 2014; Ashiuchi et al., 2001; Peng et al., 2005). Examples of such traditional fermented foods include *chungkook-jang* in Korea, *douchi* in China, *thua nao* in Thailand, and *tempeh* in Indonesia, each of which harbors distinct microbial consortia that contribute to the production of bioactive peptides and enzymes with potential health benefits (Dabbagh et al., 2014; Ashiuchi et al., 2001).

The production of nattokinase by *Bacillus subtilis* var. *natto* is influenced by a complex interplay of nutritional and environmental factors. Optimal enzyme yields are typically achieved through fermentation on basal media enriched with carbon sources such as glucose or starch and nitrogen sources including peptone, yeast extract, or soybean meal hydrolysates (Dabbagh et al., 2014; Jagathy et al., 2017). The incubation temperature, pH, aeration rate, and duration of fermentation are critical parameters that must be carefully optimized to maximize enzyme production while minimizing the accumulation of undesirable metabolites (Dabbagh et al., 2014; Jagathy et al., 2017). Recent advances in fermentation technology, including the development of integrated bioprocessing strategies that couple biofilm-based fermentation with the use of low-cost agricultural byproducts such as soybean meal hydrolysates, have demonstrated significant potential for enhancing nattokinase yields and improving the economic viability of large-scale production (Yi et al., 2025). Notably, a study by Zhang et al. (2025) reported the development of an integrated bioprocessing strategy that achieved a nattokinase yield of 2949.3 U/mL using soybean meal hydrolysates prepared with trypsin under biofilm-based fermentation conditions, representing a substantial improvement over conventional submerged fermentation methods (Yi et al., 2025).

3.2 Molecular Structure and Amino Acid Sequence

At the molecular level, nattokinase is a single polypeptide chain composed of precisely 275 amino acid

residues, with a calculated molecular weight of approximately 27.7 kDa (Yanagisawa et al., 2010; Yanagisawa et al., 2013; Fig. 2). The enzyme is a member of the subtilisin family of serine proteases, a large and diverse group of endopeptidases characterized by a conserved catalytic triad consisting of aspartic acid, histidine, and serine residues that orchestrate the hydrolytic cleavage of peptide bonds (Yanagisawa et al., 2010; Yanagisawa et al., 2013). The amino acid sequence of nattokinase exhibits a high degree of homology with other bacterial subtilisins, including subtilisin E from *Bacillus subtilis* and subtilisin BPN' from *Bacillus amyloliquefaciens*, reflecting the evolutionary conservation of this catalytically efficient enzyme family (Yanagisawa et al., 2010).

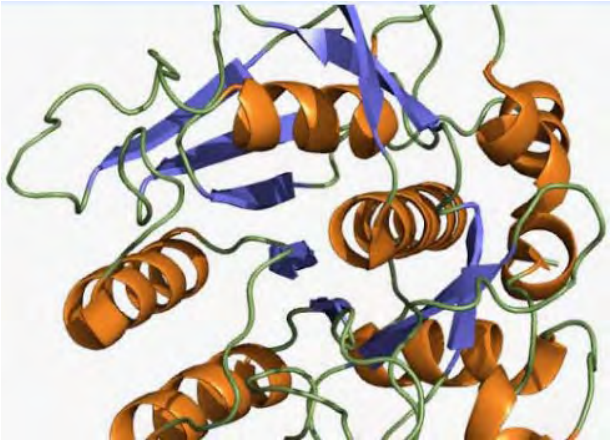

Fig. 2 Nattokinase molecule (Wikipedia, 2026).

The three-dimensional crystal structure of nattokinase has been determined at high resolution using X-ray crystallography, providing invaluable atomic-level insights into the structural determinants of its catalytic activity and substrate specificity (Yanagisawa et al., 2010; Yanagisawa et al., 2013). The overall fold of the enzyme is characteristic of the subtilisin family, featuring a central parallel β-sheet core flanked by α-helices and stabilized by multiple intramolecular interactions. The active site is located within a pronounced surface cleft and contains the canonical catalytic triad comprising residues Asp32, His64, and Ser221 (subtilisin BPN' numbering), which function in concert to effect the nucleophilic attack on the scissile peptide bond of the substrate (Yanagisawa et al., 2010). In addition to the catalytic triad, the substrate-binding pocket of nattokinase is lined by a series of hydrophobic and polar residues that confer specificity for particular amino acid sequences, thereby determining the enzyme's preference for cleavage at specific sites within target proteins such as fibrin (Yanagisawa et al., 2010; Yanagisawa et al., 2013). Notably, despite its close structural resemblance to other subtilisins, nattokinase displays distinct substrate specificity that distinguishes it from related enzymes and contributes to its unique fibrinolytic profile (Yanagisawa et al., 2013).

3.3 Physicochemical Characteristics

Nattokinase exhibits a set of well-defined physicochemical properties that are critical to understanding its stability, activity, and behavior under various environmental and physiological conditions. The enzyme functions optimally as an alkaline serine protease, exhibiting maximal catalytic activity at pH values ranging from approximately 8.0 to 10.0 (Dabbagh et al., 2014; Wang et al., 2009). The optimal temperature for nattokinase activity is typically reported to be in the range of 40°C to 50°C, although the enzyme retains substantial activity at physiological temperature (37°C) and displays a degree of thermostability that is relevant to its application in food processing and nutraceutical formulation (Dabbagh et al., 2014; Wang et al., 2009;

Lonxigong, 2024). The enzyme is soluble in water and aqueous buffer systems, a property that facilitates its extraction, purification, and incorporation into various dosage forms (Dabbagh et al., 2014).

The stability of nattokinase is influenced by a variety of environmental factors, including pH, temperature, and the presence of denaturing agents or proteolytic enzymes. The enzyme is relatively stable over a pH range of approximately 6.0 to 10.0, with significant loss of activity occurring under more acidic or alkaline conditions (Dabbagh et al., 2014). Thermal stability studies have demonstrated that nattokinase retains a substantial fraction of its activity following exposure to temperatures up to 50°C for extended periods, although irreversible denaturation and loss of activity occur at higher temperatures (Dabbagh et al., 2014). The presence of calcium ions has been shown to enhance the thermostability of nattokinase, an observation that is consistent with the known role of divalent cations in stabilizing the tertiary structure of subtilisin-family proteases (Dabbagh et al., 2014). These stability characteristics have important implications for the formulation, storage, and gastrointestinal survival of orally administered nattokinase supplements.

3.4 Extraction and Purification Methodologies

The extraction and purification of nattokinase from fermentation broths or directly from natto represent critical steps in the production of high-purity enzyme preparations suitable for research, nutraceutical, and potential pharmaceutical applications. A variety of chromatographic and non-chromatographic techniques have been developed and optimized for this purpose, with the choice of methodology dictated by considerations of yield, purity, scalability, and cost-effectiveness (Dabbagh et al., 2014; Wang et al., 2009; Yi et al., 2025).

The initial step in the purification process typically involves the removal of bacterial cells and particulate matter from the fermentation broth by centrifugation or filtration, yielding a clarified supernatant that contains the extracellular nattokinase along with a complex mixture of other proteins, peptides, polysaccharides, and small molecules (Wang et al., 2009). Subsequent purification steps are designed to selectively concentrate and isolate the nattokinase protein from this complex milieu. Ammonium sulfate precipitation is frequently employed as a preliminary fractionation technique, taking advantage of the differential solubility of proteins at varying salt concentrations to achieve a crude enrichment of nattokinase (Jagathy et al., 2017; Wang et al., 2009). This step is typically followed by one or more chromatographic separations, which exploit differences in the physicochemical properties of proteins to achieve higher degrees of purity.

Gel filtration chromatography, also known as size-exclusion chromatography, is commonly utilized to separate proteins on the basis of their molecular size and shape, effectively removing contaminating proteins of significantly different molecular weight (Wang et al., 2009; Yanagisawa et al., 2010). Ion-exchange chromatography, which separates proteins according to their net surface charge, represents another powerful purification tool that has been widely applied to nattokinase (Wang et al., 2009). A study by Wang et al. (2009) reported the purification of nattokinase from the supernatant of *Bacillus subtilis* natto B-12 culture broth to 56.1-fold homogeneity using a combination of ammonium sulfate precipitation, Sephacryl S-100 gel filtration, and S-Sepharose ion-exchange chromatography, achieving a yield of 100 mg of purified enzyme per liter of culture (Wang et al., 2009). Affinity chromatography, employing ligands that specifically bind to nattokinase, offers the potential for even higher degrees of purification, although its application is often limited by the cost and complexity of preparing suitable affinity matrices (Dabbagh et al., 2014).

Recent innovations in downstream processing have focused on the development of integrated, multi-product extraction strategies that enable the simultaneous recovery of nattokinase along with other valuable metabolites produced by *Bacillus subtilis* natto during fermentation (Yi et al., 2025). In a noteworthy study, Yi et al. (2025) described a sequential extraction system that capitalized on the differential precipitation characteristics of ammonium sulfate for various proteins and protein complexes to achieve the concurrent isolation of nattokinase, menaquinone-7 (vitamin K2), and γ-polyglutamic acid from a single fermentation

matrix. This integrated approach achieved an overall extraction efficiency of 70% for the three target metabolites, demonstrating the feasibility and economic advantages of multi-product recovery strategies for the sustainable production of nattokinase and related bioactive compounds (Yi et al., 2025).

3.5 Pharmacokinetics and Absorption

A comprehensive understanding of the pharmacokinetic behavior of orally administered nattokinase is essential for optimizing dosing regimens, predicting therapeutic outcomes, and ensuring the safe and effective use of this enzyme in clinical and nutraceutical applications. However, the pharmacokinetics of nattokinase have been relatively understudied compared to other aspects of its pharmacology, and significant gaps in knowledge remain regarding its absorption, distribution, metabolism, and excretion (ADME) in humans.

The oral bioavailability of nattokinase, like that of many protein-based therapeutics, is constrained by the formidable barriers presented by the gastrointestinal tract, including the denaturing effects of gastric acid, proteolytic degradation by digestive enzymes, and limited permeability across the intestinal epithelium (Ero et al., 2013; Zhou et al., 2025). Despite these challenges, a substantial body of evidence indicates that biologically relevant quantities of nattokinase and/or its active metabolites are absorbed into the systemic circulation following oral ingestion. A pilot study by Ero et al. (2013) investigated the serum pharmacokinetics of nattokinase in healthy human volunteers following the oral administration of a single daily dose of 2,000 fibrinolytic units (FU) of the enzyme. Using an immunological detection method, the study demonstrated that nattokinase and/or its immunoreactive metabolites were detectable in serum between 2 and 24 hours post-ingestion, confirming that the enzyme is absorbed from the gastrointestinal tract and reaches the systemic circulation in a form that retains immunological recognition (Ero et al., 2013).

The mechanisms underlying the intestinal absorption of nattokinase have been explored using a variety of *in vitro* and *ex vivo* model systems. Liu et al. (2023) investigated the transport and internalization mechanisms of nattokinase across the intestinal epithelium using Caco-2 cell monolayers and an animal everted intestinal sac model. The results of this investigation indicated that nattokinase is a moderately absorbed biomolecule, with its transport through enterocytes being an energy- and time-dependent process (Feng et al., 2023). A more recent and detailed study by Zhou et al. (2025) employed a rat everted intestinal sac model to quantify the small intestine absorption rate of nattokinase, reporting a value of 37.0% under the experimental conditions employed. Importantly, this study further characterized the nature of the absorbed species, revealing that the peptides crossing the intestinal barrier were predominantly hydrophobic, uncharged, and shorter than 10 amino acids in length (Zhou et al., 2025). This finding suggests that nattokinase undergoes significant proteolytic processing during transit across the intestinal epithelium, and that the biologically active species reaching the systemic circulation may consist of smaller peptide fragments rather than the intact, full-length protein.

The stability of nattokinase during gastrointestinal transit is a critical determinant of its ultimate bioavailability. *In vitro* digestion simulations conducted by Zhou et al. (2025) demonstrated that nattokinase retained approximately 25.8% of its initial enzymatic activity following exposure to simulated gastric and intestinal fluids, indicating that a substantial fraction of the ingested enzyme survives the harsh conditions of the upper gastrointestinal tract. These findings underscore the importance of formulation strategies, such as enteric coating or encapsulation, to protect nattokinase from gastric inactivation and enhance its delivery to the intestinal absorptive surface (Zhou et al., 2025). Further research is urgently needed to fully elucidate the pharmacokinetic profile of nattokinase, including the identification and characterization of its active metabolites, the determination of its volume of distribution and elimination half-life, and the evaluation of potential drug-drug interactions that may influence its absorption or clearance.

4 Pharmacological Actions and Mechanistic Insights

In the view of network biology or pharmacology, nattokinase is a regulatory and controlling factor in the human biological network (Zhang, 2016b, 2018, 2026, 2027a-d). Fig. 3 provides a schematic overview of nattokinase metabolic regulation pathways.

4.1 Fibrinolytic and Thrombolytic Activities

The fibrinolytic and thrombolytic activities of nattokinase constitute its most extensively characterized and clinically relevant pharmacological properties. The enzyme's capacity to degrade fibrin, the insoluble proteinaceous matrix that forms the structural backbone of blood clots, underpins its utility in the prevention and management of thrombotic disorders, including deep vein thrombosis, pulmonary embolism, ischemic stroke, and myocardial infarction (Granito et al., 2024; Muric et al., 2024; Weng et al., 2017). The mechanisms by which nattokinase promotes fibrinolysis and thrombolysis are multifaceted and operate at multiple levels within the hemostatic system.

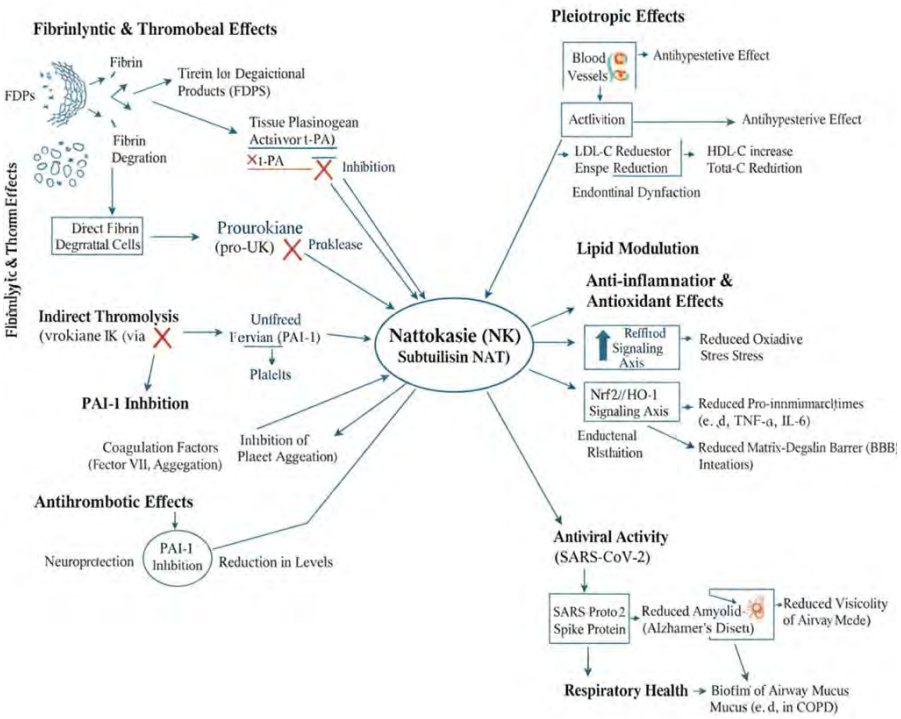

Fig. 3 Metabolic regulation pathways of nattokinase.

The most direct mechanism involves the enzymatic cleavage of fibrin itself. Nattokinase, as a serine protease, hydrolyzes peptide bonds within the fibrin polymer, thereby disrupting the structural integrity of the clot and facilitating its dissolution (Sumi et al., 1987). *In vitro* studies have consistently demonstrated the potent fibrin-degrading activity of nattokinase, with thrombolysis assays showing that the enzyme achieves fibrin clot lysis rates of 30-40% at concentrations of 5,000 μg/mL or higher (Zhou et al., 2025). Notably, this thrombolytic efficacy is observed irrespective of clot age, with nattokinase exhibiting comparable activity against both fresh and aged (stale) fibrin clots, a property that distinguishes it from some conventional

thrombolytic agents whose activity diminishes as clots mature and undergo structural remodeling (Zhou et al., 2025). This characteristic may have important clinical implications, as it suggests that nattokinase could be effective in the treatment of both acute and chronic thrombotic events.

Beyond its direct fibrinolytic action, nattokinase enhances endogenous thrombolytic pathways through several complementary mechanisms. The enzyme has been shown to stimulate vascular endothelial cells to produce and secrete tissue-type plasminogen activator (t-PA), a key endogenous fibrinolytic enzyme that catalyzes the conversion of the inactive zymogen plasminogen to the active protease plasmin, which in turn degrades fibrin (Sumi et al., 1990; Yatagai et al., 2007). By augmenting t-PA release, nattokinase indirectly amplifies the body's intrinsic capacity for fibrinolysis. Additionally, nattokinase has been reported to catalyze the activation of prourokinase (pro-UK) to its enzymatically active form, urokinase, which similarly promotes the conversion of plasminogen to plasmin (Sumi et al., 1990; Yanagisawa et al., 2010). This dual activation of endogenous plasminogen activators represents a powerful synergistic mechanism for enhancing thrombolysis.

A third important mechanism involves the degradation and inactivation of plasminogen activator inhibitor-1 (PAI-1), the primary physiological inhibitor of t-PA and urokinase (Urano et al., 2001). By reducing PAI-1 activity, nattokinase effectively removes a critical brake on the fibrinolytic system, allowing t-PA and urokinase to function with enhanced efficiency and prolonging the duration of their thrombolytic action (Urano et al., 2001). The combined effect of these multiple, convergent mechanisms—direct fibrin degradation, stimulation of t-PA production, activation of prourokinase, and inhibition of PAI-1—renders nattokinase a remarkably effective and versatile fibrinolytic agent.

In addition to its fibrinolytic properties, nattokinase exhibits antithrombotic activity through the inhibition of platelet aggregation and the reduction of coagulation factor levels. Studies have demonstrated that nattokinase can suppress platelet aggregation, a critical step in the initiation and propagation of thrombus formation (Jang et al., 2013; Kurosawa et al., 2015). Furthermore, nattokinase supplementation has been associated with reductions in plasma levels of coagulation factors, including factor VII and factor VIII, which are essential components of the intrinsic and extrinsic coagulation cascades, respectively (Hsia et al., 2009). By targeting both the cellular (platelet) and humoral (coagulation factor) arms of the hemostatic system, nattokinase provides comprehensive protection against pathological thrombus formation.

4.2 Antihypertensive Effects

Hypertension is a major modifiable risk factor for cardiovascular disease, and the identification of safe and effective blood pressure-lowering agents is a public health priority. A substantial body of evidence, derived from both preclinical animal studies and human clinical trials, indicates that nattokinase possesses significant antihypertensive properties (Li et al., 2023; Liu et al., 2024; Muric et al., 2024). The blood pressure-lowering effects of nattokinase have been consistently observed across multiple studies, with the magnitude of the reduction typically being in the range of 3-8 mmHg for systolic blood pressure and 2-5 mmHg for diastolic blood pressure (Kim et al., 2008; Li et al., 2023).

The most robust evidence for the antihypertensive efficacy of nattokinase comes from a systematic review and meta-analysis of randomized controlled trials published by Li et al. (2023). This comprehensive analysis, which included six eligible RCTs encompassing a total of 546 participants, found that nattokinase supplementation resulted in a statistically significant reduction in both systolic blood pressure (mean difference [MD] = -3.45 mmHg, 95% confidence interval [CI]: -4.37 to -2.18 mmHg, $p < 0.00001$) and diastolic blood pressure (MD = -2.32 mmHg, 95% CI: -2.72 to -1.92 mmHg, $p < 0.00001$) compared to placebo (Li et al., 2023). These findings were consistent across the included studies, which were judged to be of high methodological quality, providing strong support for the use of nattokinase as an effective adjunctive therapy for hypertension (Li et al., 2023).

The mechanisms underlying the antihypertensive effects of nattokinase are thought to involve the inhibition of angiotensin-converting enzyme (ACE), a key component of the renin-angiotensin-aldosterone system (RAAS) that plays a central role in the regulation of blood pressure (Fujita et al., 1993; Okamoto et al., 1995). ACE catalyzes the conversion of angiotensin I to the potent vasoconstrictor angiotensin II, and its inhibition leads to vasodilation, reduced peripheral vascular resistance, and a consequent lowering of blood pressure. Studies have demonstrated that nattokinase and natto-derived peptides can inhibit ACE activity *in vitro* and *in vivo*, providing a plausible mechanistic basis for the observed antihypertensive effects (Fujita et al., 1993; Okamoto et al., 1995). It is noteworthy that the antihypertensive action of nattokinase may be attributable not only to the intact enzyme but also to bioactive peptides generated during the fermentation process or during gastrointestinal digestion, which may exhibit potent ACE inhibitory activity (Fujita et al., 1993; Okamoto et al., 1995).

The clinical significance of the blood pressure reductions achieved with nattokinase should not be underestimated. Epidemiological studies have established that even modest reductions in blood pressure, on the order of 2-5 mmHg, are associated with clinically meaningful decreases in the risk of stroke, coronary heart disease, and all-cause mortality (Li et al., 2023). Therefore, the consistent and statistically significant antihypertensive effects of nattokinase observed in clinical trials suggest that this natural enzyme could play a valuable role in the comprehensive management of hypertension, particularly as an adjunct to lifestyle modifications and conventional pharmacotherapy.

4.3 Anti-Inflammatory and Antioxidant Properties

While the fibrinolytic and antihypertensive activities of nattokinase have historically garnered the greatest attention, a growing body of recent research has illuminated the enzyme's potent anti-inflammatory and antioxidant properties, which are now recognized as integral components of its broader cardioprotective and health-promoting effects (Granito et al., 2024; Li et al., 2026; Mahakalakar et al., 2025). Chronic inflammation and oxidative stress are fundamental pathogenic processes that underlie the development and progression of atherosclerosis, hypertension, endothelial dysfunction, and a host of other cardiovascular and non-communicable diseases (Granito et al., 2024; Zhang, 2027a-d). The ability of nattokinase to mitigate these processes represents a significant and previously underappreciated dimension of its therapeutic potential.

A comprehensive review by Granito et al. (2024) synthesized the available evidence regarding the anti-inflammatory and antioxidant effects of nattokinase, concluding that the enzyme can be employed as a novel adjuvant therapeutic strategy to mitigate inflammation and oxidative stress in non-communicable diseases, including cardiovascular disease. The authors emphasized that while nattokinase was initially recognized primarily for its fibrinolytic and antithrombotic activities, the more recent identification of its anti-inflammatory and antioxidant properties significantly expands its potential clinical applications and underscores its pleiotropic nature (Granito et al., 2024).

At the cellular and molecular level, the anti-inflammatory and antioxidant effects of nattokinase are mediated, at least in part, through the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling axis (Li et al., 2026; Mahakalakar et al., 2025). Nrf2 is a transcription factor that serves as a master regulator of the cellular antioxidant response, controlling the expression of a battery of cytoprotective genes, including HO-1, that function to neutralize reactive oxygen species (ROS) and restore redox homeostasis. A recent study by Li et al. (2025), published in the Journal of Dental Sciences, provided detailed mechanistic insights into the Nrf2/HO-1-dependent protective effects of nattokinase in human gingival fibroblasts (HGF-1 cells) exposed to particulate matter (PM)-induced inflammation and oxidative stress. The study demonstrated that nattokinase pretreatment markedly suppressed the PM-induced expression of pro-inflammatory mediators, including cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), as well as

the matrix-degrading enzyme matrix metalloproteinase-1 (MMP-1) (Li et al., 2025). Mechanistically, nattokinase was shown to enhance Nrf2 activation and HO-1 expression, thereby attenuating the PM-induced activation of NADPH oxidase-derived ROS generation and the downstream PI3K/Akt and MAPK signaling cascades (Huang and Zhang, 2012; Li and Zhang, 2013; Zhang, 1016a, 2017; Li et al., 2026). Crucially, pharmacological inhibition of Nrf2 or HO-1 abolished the protective effects of nattokinase, establishing the essential role of this signaling pathway in mediating the enzyme's anti-inflammatory and antioxidant actions (Li et al., 2026).

These findings are further corroborated by studies conducted in other experimental systems. A research has extended the Nrf2/HO-1 activation paradigm to the context of neurodegenerative disease, demonstrating that nattokinase protects against blood-brain barrier dysfunction and neuroinflammation through mechanisms involving the Nrf2/HO-1 pathway. The convergence of evidence from diverse cellular and animal models strongly implicates Nrf2/HO-1 activation as a central and conserved mechanism by which nattokinase exerts its anti-inflammatory and antioxidant effects across multiple tissue types and disease contexts.

4.4 Lipid-Modulating Effects

The effects of nattokinase on lipid metabolism and plasma lipid profiles have been the subject of considerable investigation, yet the evidence remains somewhat equivocal and appears to be contingent upon the specific formulation, dosage, and combination with other nutraceuticals. While some studies have reported favorable effects of nattokinase on total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), other investigations have failed to demonstrate significant lipid-lowering benefits, and a systematic review and meta-analysis has cast doubt on the efficacy of nattokinase monotherapy for improving lipid parameters (Li et al., 2023).

The meta-analysis by Li et al. (2023) provided a comprehensive and rigorous assessment of the available randomized controlled trial evidence concerning the effects of nattokinase on cardiovascular risk factors, including lipid profiles. The analysis revealed that relatively low total dosages of nattokinase were associated with a negative effect on blood total cholesterol (MD = 5.27, 95% CI: 3.74 to 6.81, $p = 0.00001$), HDL-C (MD = -2.76, 95% CI: -3.88 to -1.64, $p < 0.00001$), and LDL-C (MD = 6.49, 95% CI: 0.83 to 12.15, $p = 0.02$) (Li et al., 2023). Nattokinase groups receiving relatively high total dosages also exhibited higher total cholesterol (MD = 3.18, 95% CI: 2.29 to 4.06, $p < 0.00001$) compared to control interventions, although no significant differences were observed in HDL-C or LDL-C levels at these higher doses (Li et al., 2023). Furthermore, no significant correlation was found between nattokinase supplementation and triglyceride levels ($p = 0.71$) (Li et al., 2023). Based on these findings, the authors concluded that relatively low-dose supplementation of nattokinase may have no significant lipid-lowering effect and may even be associated with unfavorable changes in lipid profiles (Li et al., 2023).

In contrast to the equivocal findings for nattokinase monotherapy, studies evaluating nattokinase in combination with other lipid-modulating nutraceuticals have yielded more promising results. A four-month randomized, double-blind, placebo-controlled clinical trial by Liu et al. (2023) examined the effects of nattokinase-monascus supplements (NMSs) on dyslipidemia. The study demonstrated that NMSs significantly ameliorated lipid levels, including reductions in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and non-high-density lipoprotein cholesterol (non-HDL-C), as well as a decrease in the LDL-C to HDL-C ratio (Feng et al., 2023). However, no significant effects were observed on triglyceride levels, HDL-C, or carotid intima-media thickness (CIMT) (Feng et al., 2023). The beneficial effects of the combination product are likely attributable to the inclusion of monascus, a source of naturally occurring statin-like compounds (monacolins) that are known to inhibit HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis.

Similarly, a 90-day randomized, double-blind, placebo-controlled trial by Liu et al. (2024) investigated the effects of nattokinase combined with red yeast rice (RYR) in patients with stable coronary artery disease (CAD). Red yeast rice is another natural source of monacolins and is recognized for its cholesterol-lowering properties. The study found that the combination of NK + RYR produced the maximum effect in reducing triglyceride (-0.39 mmol/L), total cholesterol (-0.66 mmol/L), and diastolic blood pressure (-7.39 mmHg), while simultaneously increasing HDL-C (0.195 mmol/L) compared to other groups (all p for multiple group comparisons < 0.01) (Liu et al., 2024). Both the NK + RYR and NK alone groups exhibited significantly improved lactate dehydrogenase levels compared to the other groups (-29.1 U/L and -26.4 U/L, respectively) (Liu et al., 2024). The NK + RYR group also demonstrated more potent reductions in thromboxane B2 and increases in antithrombin III compared to placebo (both $p < 0.01$), suggesting that the combination may favorably alter antithrombin and COX-1 pathways, potentially reducing thrombosis risk in CAD patients (Liu et al., 2024).

Mechanistic insights into the potential lipid-modulating effects of nattokinase have been provided by a recent study by Zhou et al. (2025), which employed a high-fat diet-induced atherosclerosis model in ApoE-/- mice. Proteomic analysis of mouse liver tissues identified that high-dose nattokinase treatment (900 FU/kg body weight) down-regulated the expression of peroxidasin (PXDN) and pancreatic lipase (PNLIP), proteins that are involved in promoting lipid oxidation and intestinal lipid absorption, respectively (Zhou et al., 2025). The down-regulation of these proteins may contribute to the amelioration of atherosclerosis and dyslipidemia, providing a plausible molecular basis for the observed effects. Collectively, the available evidence suggests that while nattokinase as a standalone therapy may have limited or inconsistent effects on lipid profiles, its combination with other lipid-modulating nutraceuticals, such as red yeast rice or monascus, produces more robust and clinically meaningful improvements in lipid parameters.

4.5 Neuroprotective Effects and Alzheimer's Disease

An exciting and rapidly evolving frontier in nattokinase research concerns its potential neuroprotective effects and its application in the prevention and treatment of neurodegenerative disorders, most notably Alzheimer's disease (AD) (Tanikawa et al., 2024; Mahakalakar et al., 2025). Alzheimer's disease is a progressive and devastating neurodegenerative condition characterized by the accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles, chronic neuroinflammation, oxidative stress, and synaptic dysfunction, culminating in profound cognitive decline and dementia. The identification of safe and effective agents capable of targeting these pathogenic processes is a critical unmet need. Emerging evidence from preclinical studies suggests that nattokinase may possess a constellation of properties that render it a promising candidate for neuroprotection in AD and related disorders.

A landmark study by Tanikawa et al. (2024) investigated the effects of nattokinase in a rat model of Alzheimer's disease induced by the combined administration of D-galactose and aluminum chloride. The results of this investigation were highly encouraging, demonstrating that oral administration of nattokinase to AD model rats resulted in increased levels of free-form β-amyloid in the cerebrospinal fluid (CSF), a finding that is consistent with the mobilization and clearance of Aβ deposits from the brain parenchyma (Tanikawa et al., 2024). Furthermore, nattokinase treatment was associated with improved aluminum and amyloid plaque accumulation in the brain, suggesting that the enzyme may facilitate the removal of these neurotoxic aggregates (Tanikawa et al., 2024). The authors concluded that nattokinase has potential therapeutic applications in the treatment of Alzheimer's disease (Tanikawa et al., 2024).

Complementary and convergent findings have been reported by Mahakalakar et al. (2024), who investigated the neuroprotective effects of nattokinase in a mouse model of Aβ1-42-induced neuropsychiatric complications, neuroinflammation, and disruption of brain-derived neurotrophic factor (BDNF) signaling. The

study demonstrated that nattokinase administration improved learning and memory impairment and ameliorated neuropsychiatric complications, effects that were attributed, at least in part, to the downregulation of neuroinflammatory pathways and the restoration of BDNF signaling (Mahakalakar et al., 2025). BDNF is a critical neurotrophin that supports the survival, differentiation, and synaptic plasticity of neurons, and its disruption is implicated in the pathophysiology of AD and other neurodegenerative conditions. The ability of nattokinase to preserve BDNF signaling represents a potentially important mechanism of neuroprotection.

The neuroprotective effects of nattokinase are further supported by a comprehensive review by Mahakalakar et al. (2024), which synthesized the available preclinical evidence regarding the potential of nattokinase to prevent blood-brain barrier (BBB) dysfunction in neurodegenerative disorders. The review concluded that there is sufficient evidence from experimental animal studies to indicate that nattokinase supplementation can alleviate BBB dysfunction, reduce brain inflammation, and improve cognitive ability (Mahakalakar et al., 2024). The blood-brain barrier is a highly specialized and tightly regulated endothelial interface that protects the brain from circulating pathogens, toxins, and inflammatory mediators. Disruption of BBB integrity is a common feature of many neurodegenerative diseases and contributes to the propagation of neuroinflammation and neuronal injury. The observation that nattokinase can preserve BBB function and mitigate neuroinflammation positions it as a potentially valuable therapeutic agent for a spectrum of neurodegenerative conditions.

The mechanisms by which nattokinase exerts its neuroprotective effects are likely multifaceted and may include: (1) the degradation of amyloid-β aggregates, as suggested by the increased CSF Aβ levels observed in the study by Tanikawa et al. (2024); (2) the attenuation of neuroinflammation through the Nrf2/HO-1 pathway, as demonstrated by Hsu et al. (2023) and Mahakalakar et al. (2024); (3) the preservation of BBB integrity and the prevention of vascular dysfunction, which may involve the enzyme's fibrinolytic and antithrombotic properties; and (4) the enhancement of neurotrophic support through the restoration of BDNF signaling (Mahakalakar et al., 2024). Further research, including well-designed clinical trials in human subjects with AD or mild cognitive impairment, is urgently needed to validate these promising preclinical findings and to fully elucidate the therapeutic potential of nattokinase in the realm of neurodegenerative disease.

4.6 Respiratory Health and COVID-19 Applications

The emergence of the COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has catalyzed intense interest in identifying safe and effective therapeutic agents capable of mitigating viral infection, reducing disease severity, and preventing the development of long-term complications. A notable and unexpected development in this context has been the investigation of nattokinase as a potential antiviral agent with activity against SARS-CoV-2 (Tanikawa et al., 2022). The rationale for exploring nattokinase in the context of COVID-19 is grounded in two complementary lines of evidence: its direct degradative effect on the SARS-CoV-2 spike protein and its potential to address the hypercoagulable state and microvascular thrombosis that characterize severe COVID-19.

A pivotal study by Tanikawa et al. (2022), published in the journal Molecules, examined the effect of nattokinase on the spike (S) protein of SARS-CoV-2. The S protein is a critical structural component of the viral envelope that mediates viral attachment to host cell receptors (angiotensin-converting enzyme 2, ACE2) and facilitates viral entry into target cells. The study demonstrated that nattokinase is capable of degrading the S protein of SARS-CoV-2 *in vitro*, an effect that was observed under physiologically relevant conditions (Tanikawa et al., 2022). The authors concluded that their findings suggest that nattokinase exhibits potential for the inhibition of SARS-CoV-2 infection via S protein degradation (Tanikawa et al., 2022). By disrupting the structural integrity of the S protein, nattokinase may interfere with the virus's ability to bind to and enter host cells, thereby limiting the establishment and propagation of infection.

In addition to its direct antiviral potential, nattokinase may confer benefit in COVID-19 through its well-established fibrinolytic and antithrombotic activities. Severe COVID-19 is frequently complicated by a profound hypercoagulable state, characterized by elevated levels of D-dimer and fibrinogen, increased thrombin generation, and impaired fibrinolysis, which can lead to the formation of microvascular thrombi and the development of acute respiratory distress syndrome (ARDS) and multi-organ failure (Okeahialam, 2024). Nattokinase, with its potent fibrinolytic properties and its ability to enhance endogenous thrombolysis, has been proposed as a potential therapeutic agent to address the coagulopathy associated with COVID-19 (Okeahialam, 2024). By degrading fibrin clots, inhibiting platelet aggregation, and reducing coagulation factor levels, nattokinase could theoretically improve microvascular perfusion, alleviate tissue hypoxia, and mitigate the systemic inflammatory response that drives disease progression.

The hypothesis that nattokinase may be beneficial in COVID-19 has been articulated in several publications. Okeahialam (2024), writing in the Santosh University Journal of Health Sciences, proposed nattokinase as a "low-hanging fruit" for the treatment of COVID-19, emphasizing its capacity to counter plasminogen activator inhibitor-1 (PAI-1) and thereby liberate tissue plasminogen activator (t-PA) to lyse fibrin deposits that obstruct the vasculature (Okeahialam, 2024). The author argued that the fibrinolytic properties of nattokinase could be particularly valuable in addressing the microvascular thrombosis that contributes to the morbidity and mortality of severe COVID-19.

It is important to emphasize that the evidence supporting the use of nattokinase for COVID-19 is currently limited to *in vitro* studies and theoretical considerations; rigorous clinical trials evaluating its efficacy in human subjects with COVID-19 have not been reported. Furthermore, caution is warranted, as some sources have noted that there is no solid proof that nattokinase effectively removes spike proteins in real human scenarios (THIP Media, 2023). The translation of *in vitro* findings to clinical benefit requires careful validation through well-designed, randomized, placebo-controlled trials. Nevertheless, the existing data provide a compelling rationale for further investigation and underscore the potential of nattokinase to contribute to the armamentarium of strategies for combating COVID-19 and its associated complications.

4.7 Additional Biological Activities

Beyond the extensively studied cardiovascular, anti-inflammatory, antioxidant, neuroprotective, and antiviral properties detailed above, nattokinase has been reported to exhibit a range of additional biological activities that further underscore its pleiotropic nature and expand the potential scope of its therapeutic applications.

Improvement of Blood Rheology and Microcirculation. Nattokinase has been shown to improve blood rheological properties, including the reduction of blood viscosity and the enhancement of red blood cell deformability, which collectively promote more efficient microcirculatory blood flow (Pais et al., 2006). Improved microcirculation is essential for adequate tissue oxygenation and nutrient delivery and may contribute to the observed benefits of nattokinase in conditions such as peripheral artery disease and diabetic microangiopathy.

Ocular Health and Retinal Protection. Emerging evidence suggests that nattokinase may confer benefits in the realm of ocular health, particularly through the reduction of pathological retinal neovascularization (Weng et al., 2017). Retinal neovascularization is a hallmark of several vision-threatening conditions, including diabetic retinopathy and age-related macular degeneration. The mechanisms underlying this protective effect are not fully elucidated but may involve the anti-inflammatory, antioxidant, and anti-angiogenic properties of nattokinase.

Enhancement of Skin Temperature Recovery. An intriguing study by Nara et al. (2023) investigated the effects of a single oral dose of nattokinase on skin temperature recovery following cold water immersion. This double-blind, placebo-controlled crossover study conducted in nine healthy men found that nattokinase

accelerated the recovery of skin temperature after cold exposure, an effect that may reflect improved peripheral blood flow and microcirculatory function (Nara et al., 2023). While the clinical significance of this finding remains to be determined, it provides additional evidence for the vascular and thermoregulatory effects of nattokinase.

Gastrointestinal and Intestinal Health. Nattokinase has been suggested to have applications in regulating gastrointestinal and intestinal function, including potential antibacterial actions (Core Pure Bio & Tech, 2021). The enzyme may contribute to the maintenance of a healthy gut microbiota and the prevention of gastrointestinal infections, although rigorous studies in this area are currently lacking.

5 Clinical Trials and Therapeutic Efficacy

5.1 Clinical Applications and Formulations

Nattokinase is currently marketed and consumed primarily as a dietary supplement and functional food ingredient, rather than as a regulated pharmaceutical agent. It is available in a variety of oral dosage forms, including powders, pills, capsules, and softgels, and is frequently formulated alone or in combination with other bioactive ingredients, such as red yeast rice, coenzyme Q10, omega-3 fatty acids, and various vitamins and minerals (Dabbagh et al., 2014; Liu et al., 2024). The enzyme is promoted for a range of health applications, most notably for the support of cardiovascular health, the maintenance of healthy blood pressure and circulation, and the prevention of thrombotic events (Dabbagh et al., 2014; Muric et al., 2024). However, it is important to acknowledge that clinical trials to definitively support such uses are still evolving, and the level of evidence varies across different indications (Drugs.com, 2026).

The clinical dosing of nattokinase has not been standardized, and optimal dosing regimens remain to be firmly established through rigorous clinical investigation. Clinical studies have employed a wide range of nattokinase doses, typically expressed in fibrinolytic units (FU), with daily doses ranging from 2,000 FU to 10,000 FU or higher (Ero et al., 2013; Li et al., 2023). A commonly used dosage in clinical trials evaluating thrombolytic/fibrinolytic and blood pressure effects has been 100 mg/day of nattokinase, which is equivalent to 2,000 FU, administered for a duration of 8 weeks (Drugs.com, 2026). However, the optimal dose for specific indications and patient populations may vary and requires further investigation.

5.2 Overview of Clinical Research Studies

The clinical evidence base for nattokinase has expanded considerably over the past two decades, encompassing a growing number of randomized controlled trials (RCTs) that have evaluated its effects on a variety of cardiovascular risk factors and clinical outcomes. The most comprehensive and rigorous synthesis of this evidence is provided by the systematic review and meta-analysis of randomized controlled trials conducted by Li et al. (2023). This analysis, which included six eligible RCTs with a total of 546 participants, concluded that nattokinase can be used as an effective adjunctive therapy for hypertension, but that relatively low-dose supplementation may have no significant lipid-lowering effect (Li et al., 2023). The methodological quality of the included studies was assessed as high, lending credibility to the findings (Li et al., 2023).

In addition to the trials included in the Li et al. (2023) meta-analysis, several more recent RCTs have further expanded the clinical evidence base. A four-month randomized, double-blind, placebo-controlled trial by Liu et al. (2023) evaluated the effects of nattokinase-monascus supplements (NMSs) in patients with dyslipidemia. The study enrolled 104 participants (56 in the NMS group and 58 in the placebo group), of whom 48 and 45, respectively, completed the intervention (Feng et al., 2023). The results demonstrated that NMSs significantly ameliorated lipid levels, including reductions in TC, LDL-C, and non-HDL-C, as well as a decrease in the LDL-C to HDL-C ratio (Feng et al., 2023). The study was well-designed and the results were clear and consistent, although the authors noted the need for further research with larger sample sizes and

longer follow-up periods (Feng et al., 2023).

A 90-day randomized, double-blind, placebo-controlled trial by Liu et al. (2024) investigated the effects of nattokinase combined with red yeast rice (RYR) in 178 patients with stable coronary artery disease (CAD). The study employed a four-group design (NK + RYR, NK alone, RYR alone, and placebo), which allowed for the evaluation of both individual and combined effects. The findings were striking: the NK + RYR combination produced the maximum effect in reducing triglyceride (-0.39 mmol/L), total cholesterol (-0.66 mmol/L), and diastolic blood pressure (-7.39 mmHg), while simultaneously increasing HDL-C (0.195 mmol/L) compared to all other groups (all p for multiple group comparisons < 0.01) (Liu et al., 2024). Furthermore, the NK + RYR group showed more potent reductions in thromboxane B2 and increases in antithrombin III compared to placebo (both $p < 0.01$), suggesting that the combination may favorably alter antithrombin and COX-1 pathways, potentially reducing thrombosis risk in CAD patients (Liu et al., 2024). No adverse effects due to the interventions were reported (Liu et al., 2024).

An ongoing clinical trial, registered on ClinicalTrials.gov (NCT05200234), is evaluating the effects of nattokinase on cerebral blood flow and cognitive function in patients with asymptomatic intracranial/carotid stenosis (ClinicalTrials.gov, 2023). This randomized controlled trial will provide valuable insights into the potential neuroprotective and cerebrovascular benefits of nattokinase in a high-risk population and will contribute to the evidence base supporting its use for brain health.

5.3 Quality Control and Standardization

The quality control and standardization of nattokinase products represent critical challenges that have significant implications for the safety, efficacy, and reproducibility of clinical research findings and consumer experiences. The absence of universally accepted and enforced standards for nattokinase manufacturing, purity, and potency has resulted in substantial variability in the composition and biological activity of commercially available supplements (Li et al., 2023; Yi et al., 2025).

The primary metric used to quantify the potency of nattokinase products is enzymatic activity, which is typically expressed in fibrinolytic units (FU) or international units (IU) (Demeter Herb, 2026; Nutriavenue, 2025). The fibrin plate method is widely regarded as the gold standard for determining nattokinase activity, as it provides a direct and visual measure of the enzyme's ability to degrade fibrin. However, alternative assay methods, including HPLC-based assays such as the S-2444 test, are also employed by some manufacturers to ensure consistent potency and reliable thrombolytic performance across production batches (Nutriavenue, 2025). The lack of harmonization across different assay methodologies can complicate the comparison of product potencies and the interpretation of clinical study results.

In addition to enzymatic activity, other quality indicators that are relevant to the characterization and standardization of nattokinase products include molecular weight and amino acid sequence confirmation, which can be achieved through methods such as SDS-PAGE electrophoresis, HPLC chromatography, or mass spectrometry (Demeter Herb, 2026). The purity of the enzyme preparation, the presence of potential contaminants (such as residual bacterial cells, endotoxins, or fermentation byproducts), and the stability of the enzyme under various storage conditions are also important quality parameters that require careful monitoring and control (Yi et al., 2025).

Regulatory frameworks for nattokinase vary considerably across different global markets. In the United States, nattokinase is regulated as a dietary supplement under the Dietary Supplement Health and Education Act (DSHEA) of 1994, which places the responsibility for ensuring product safety and accurate labeling on the manufacturer but does not require pre-market approval or demonstration of efficacy (Demeter Herb, 2026). In other jurisdictions, such as the European Union, nattokinase may be subject to more stringent regulations, particularly if it is intended for use as a novel food ingredient or if specific health claims are made (Demeter

Herb, 2026). The development and adoption of internationally recognized quality standards and reference materials would greatly facilitate the rigorous evaluation of nattokinase products and enhance the credibility of the clinical evidence base.

5.4 Safety Profile and Toxicological Assessment

The safety of nattokinase has been evaluated in a comprehensive series of toxicological studies and has been monitored in numerous human clinical trials. The preponderance of available evidence indicates that nattokinase is of low toxicological concern and is well-tolerated when administered orally at the doses typically employed in clinical practice (Lampe and English, 2016).

The most thorough and authoritative toxicological assessment of nattokinase was conducted by Lampe and English (2016) and published in the journal Food and Chemical Toxicology. This investigation included a battery of GLP-compliant studies in rodents and human volunteers. *In vitro* assays demonstrated that nattokinase was non-mutagenic in bacterial cells (Ames test) and non-clastogenic in mammalian cells (chromosomal aberration test), indicating a lack of genotoxic potential (Lampe and English, 2016). *In vivo* studies conducted in Sprague-Dawley rats revealed no adverse effects in 28-day and 90-day subchronic toxicity studies at doses up to 167 mg/kg-day and 1,000 mg/kg-day, respectively (Lampe and English, 2016). The 90-day oral subchronic no-observed-adverse-effect level (NOAEL) for nattokinase in male and female Sprague-Dawley rats was determined to be 1,000 mg/kg-day, the highest dose tested (Lampe and English, 2016). Furthermore, mice inoculated with 7.55×10^8 CFU of the enzyme-producing bacterial strain (*Bacillus subtilis* var. *natto*) showed no signs of toxicity or residual tissue concentrations of viable bacteria (Lampe and English, 2016). Finally, the consumption of 10 mg/kg-day nattokinase for 4 weeks was well-tolerated in healthy human volunteers, with no indication of adverse effects (Lampe and English, 2016). Based on these comprehensive data, the authors concluded that the oral consumption of nattokinase is of low toxicological concern (Lampe and English, 2016).

The favorable safety profile of nattokinase is further corroborated by the findings of numerous human clinical trials. A systematic review and meta-analysis of randomized controlled trials by Li et al. (2023) reported that no notable adverse events were observed in any of the included studies due to the intake of nattokinase. Similarly, the randomized controlled trials by Liu et al. (2023) and Liu et al. (2024) reported no adverse effects attributable to the nattokinase interventions.

Despite this strong safety record, certain precautions and contraindications must be recognized. The European Food Safety Authority (EFSA) has noted that nattokinase at commonly used doses is not associated with adverse effects (Examine.com, 2026). However, due to its fibrinolytic and antithrombotic properties, nattokinase has the potential to increase the risk of bleeding, particularly when administered in conjunction with anticoagulant and antiplatelet agents such as warfarin, aspirin, clopidogrel, and direct oral anticoagulants (Drugs.com, 2026; MSKCC, 2022). There is a theoretical risk that nattokinase could cause an existing clot to dislodge, resulting in a stroke or embolus at a distant location (MSKCC, 2022). Consequently, patients with a history of deep vein thrombosis, ischemic stroke, peptic ulcer disease, or coagulation disorders, as well as those in the perioperative period, are advised to avoid the use of nattokinase (Drugs.com, 2026; MSKCC, 2022).

An additional consideration relates to the potential presence of vitamin K2 (menaquinone-7) in natto-derived products. Natto is naturally rich in vitamin K2, which is also produced by *Bacillus subtilis* var. *natto* during fermentation. High concentrations of vitamin K2 can antagonize the anticoagulant effects of warfarin, leading to a reduction in the international normalized ratio (INR) and an increased risk of thrombotic events (Drugs.com, 2026). This interaction may also occur with nattokinase supplements if vitamin K2 is not adequately removed during the manufacturing process (Drugs.com, 2026). Therefore, patients receiving

warfarin therapy should exercise caution and consult with their healthcare provider before initiating nattokinase supplementation.

Rare cases of late-onset anaphylaxis associated with natto consumption have been reported and have been attributed to poly-gamma-glutamic acid (γ-PGA), a product of the fermentation process that may be present in nattokinase supplements (Drugs.com, 2026). While these reactions are exceedingly uncommon, they underscore the importance of vigilance for hypersensitivity reactions in susceptible individuals.

In summary, nattokinase exhibits a favorable safety profile when used appropriately, with a low incidence of adverse effects reported in both preclinical toxicological studies and human clinical trials. The primary safety concern relates to its potential to increase bleeding risk, particularly in the context of concomitant anticoagulant or antiplatelet therapy. Prudent clinical practice dictates that nattokinase supplementation should be undertaken under the guidance of a qualified healthcare professional, with careful consideration of individual patient risk factors and potential drug interactions.

6 Unresolved Issues, Limitations, and Challenges

Despite the substantial and growing body of evidence supporting the therapeutic potential of nattokinase, several critical issues remain unresolved, and significant challenges must be addressed to fully realize the clinical promise of this natural enzyme.

6.1 Heterogeneity of Clinical Trial Design and Outcomes

A major limitation of the current clinical evidence base is the considerable heterogeneity in trial design, including variations in patient populations, inclusion and exclusion criteria, nattokinase dosage and formulation, duration of intervention, and choice of outcome measures (Li et al., 2023; Muric et al., 2024). This heterogeneity complicates the synthesis and interpretation of findings across studies and limits the ability to draw definitive conclusions regarding the optimal use of nattokinase for specific clinical indications. The meta-analysis by Li et al. (2023) represents an important step toward synthesizing the available evidence, but the authors themselves acknowledged that more work is needed to determine whether the positive efficacy of nattokinase on cardiovascular risk factors is dose-dependent. Future clinical trials should strive for greater standardization in design and reporting to facilitate robust meta-analyses and to inform evidence-based clinical practice guidelines.

6.2 Lack of Standardized Quality Control and Product Consistency

As discussed in Section 5.3, the absence of universally accepted and enforced quality control standards for nattokinase products poses a significant challenge to both clinical research and consumer safety. The potency, purity, and composition of commercially available nattokinase supplements can vary widely, making it difficult to compare results across studies and to ensure that consumers receive a consistent and reliable product (Li et al., 2023; Yi et al., 2025). The development and adoption of internationally recognized reference standards and validated analytical methods for assessing nattokinase activity, purity, and identity are urgently needed to address this critical gap.

6.3 Incomplete Understanding of Pharmacokinetics and Metabolism

While important progress has been made in elucidating the pharmacokinetic behavior of nattokinase (Ero et al., 2013; Feng et al., 2023; Yi et al., 2025), significant gaps in knowledge persist. The precise nature of the biologically active species that reach the systemic circulation following oral ingestion remains to be fully characterized. The absorbed fractions appear to consist primarily of small hydrophobic peptides (Zhou et al., 2025), but the identity, pharmacokinetic properties, and biological activities of these peptides have not been comprehensively defined. Furthermore, the distribution, metabolism, and elimination of nattokinase and its metabolites have not been systematically investigated in humans. A more complete understanding of

nattokinase's ADME profile is essential for optimizing dosing regimens, predicting potential drug interactions, and ensuring the safe and effective use of this enzyme.

6.4 Need for Large-Scale, Long-Term Outcome Trials

The vast majority of clinical trials evaluating nattokinase have been of relatively short duration (typically 8-24 weeks) and have focused on surrogate markers of cardiovascular risk, such as blood pressure, lipid profiles, and fibrinolytic parameters, rather than on hard clinical outcomes, such as the incidence of myocardial infarction, stroke, or cardiovascular mortality (Li et al., 2023; Feng et al., 2023; Liu et al., 2024). While these surrogate markers are valuable and provide important mechanistic insights, they do not constitute definitive proof of clinical benefit. Large-scale, long-term randomized controlled trials with hard clinical endpoints are required to definitively establish the efficacy of nattokinase for the primary and secondary prevention of cardiovascular events and to justify its broader adoption in clinical practice.

6.5 Underdeveloped Research on Non-Cardiovascular Applications

The investigation of nattokinase's potential applications beyond the cardiovascular system, including its neuroprotective, antiviral, and anti-inflammatory effects, is still in its nascent stages. The promising findings from preclinical studies in models of Alzheimer's disease (Tanikawa et al., 2024; Mahakalakar et al., 2025) and the *in vitro* evidence of activity against SARS-CoV-2 (Tanikawa et al., 2022) provide a strong rationale for further investigation, but these findings have not yet been translated into human clinical trials. Rigorous clinical research in these emerging areas is needed to validate the preclinical observations and to determine whether nattokinase can offer tangible benefits for patients with neurodegenerative disorders, viral infections, and other non-communicable diseases.

6.6 Potential for Interactions and Contraindications

While nattokinase has a generally favorable safety profile, the potential for clinically significant drug interactions, particularly with anticoagulant and antiplatelet agents, requires further investigation and careful clinical monitoring (Drugs.com, 2026; MSKCC, 2022). Well-designed pharmacokinetic and pharmacodynamic interaction studies are needed to quantify the magnitude of the bleeding risk associated with the concomitant use of nattokinase and antithrombotic medications and to develop evidence-based guidance for the safe co-administration of these agents.

7 Future Research Directions and Perspectives

The field of nattokinase research is dynamic and rapidly evolving, with numerous exciting opportunities for future investigation. Based on the current state of knowledge and the identified limitations and challenges, several key research directions can be prioritized.

7.1 Conduct of Large-Scale, Pivotal Clinical Outcome Trials

The highest priority for future nattokinase research is the design and execution of large-scale, multicenter, randomized, double-blind, placebo-controlled trials evaluating the effects of nattokinase on hard clinical outcomes, including the incidence of major adverse cardiovascular events (MACE), in high-risk patient populations. Such trials would provide the definitive evidence needed to establish nattokinase as a first-line adjunctive therapy for the prevention of cardiovascular disease.

7.2 Comprehensive Pharmacokinetic and Pharmacodynamic Characterization

Detailed pharmacokinetic studies in humans are needed to fully characterize the absorption, distribution, metabolism, and elimination of nattokinase and its active metabolites. The application of advanced analytical techniques, such as mass spectrometry-based proteomics and peptidomics, should be employed to identify and quantify the specific peptide species that reach the systemic circulation and to correlate their plasma concentrations with pharmacodynamic effects.

7.3 Elucidation of Molecular Mechanisms in Diverse Disease Contexts

Continued investigation into the molecular mechanisms by which nattokinase exerts its pleiotropic effects is essential. Particular emphasis should be placed on delineating the role of the Nrf2/HO-1 signaling pathway in mediating the anti-inflammatory and antioxidant effects of nattokinase across different tissues and disease states, as well as on identifying additional molecular targets and signaling pathways that may be modulated by the enzyme.

7.4 Clinical Translation of Neuroprotective and Antiviral Findings.

The promising preclinical data supporting the neuroprotective and antiviral activities of nattokinase should be translated into well-designed human clinical trials. Pilot studies in patients with mild cognitive impairment or early Alzheimer's disease could evaluate the effects of nattokinase on biomarkers of amyloid pathology, neuroinflammation, and cognitive function. Similarly, clinical trials in patients with mild to moderate COVID-19 could assess the impact of nattokinase on viral load, disease severity, and the incidence of thrombotic complications.

7.5 Development of Standardized Quality Control Protocols and Reference Materials

Concerted efforts should be directed toward the development and validation of standardized, internationally recognized methods for the assessment of nattokinase activity, purity, and identity. The establishment of certified reference materials and the harmonization of regulatory standards across global markets would greatly enhance the reliability and comparability of nattokinase products and research findings.

7.6 Investigation of Synergistic Formulations and Personalized Approaches

The evidence suggests that nattokinase may be most effective when used in combination with other bioactive nutraceuticals, such as red yeast rice and monascus. Future research should systematically evaluate the optimal combinations and ratios of nattokinase with other cardioprotective agents and should explore the potential for personalized, biomarker-guided approaches to nattokinase therapy.

7.7 Long-Term Safety Surveillance and Pharmacovigilance

Although the available toxicological and clinical data support the safety of nattokinase, ongoing post-marketing surveillance and pharmacovigilance are warranted to detect any rare or long-term adverse effects that may emerge with widespread and prolonged use.

8 Conclusion

Nattokinase, a fibrinolytic serine protease derived from the traditional Japanese fermented food natto, has emerged as a remarkably versatile and promising natural enzyme with a broad spectrum of biological activities and therapeutic applications. Since its serendipitous discovery by Dr. Hiroyuki Sumi in 1980, a substantial and continuously expanding body of scientific research has established nattokinase as a potent fibrinolytic and antithrombotic agent, an effective adjunctive therapy for hypertension, and a pleiotropic modulator of inflammation, oxidative stress, lipid metabolism, and neuroprotection. The enzyme's favorable safety profile, oral bioavailability, and multiple convergent mechanisms of action position it as a valuable candidate for the prevention and management of cardiovascular disease and a range of other non-communicable diseases.

The evidence supporting the cardiovascular benefits of nattokinase is robust and is derived from a combination of compelling epidemiological observations, mechanistic *in vitro* and *in vivo* studies, and a growing portfolio of randomized controlled clinical trials. Systematic review and meta-analysis have confirmed the antihypertensive efficacy of nattokinase, while recent clinical trials have demonstrated that its combination with lipid-modulating nutraceuticals such as red yeast rice and monascus produces clinically meaningful improvements in lipid profiles and other cardiometabolic parameters. Beyond its cardiovascular applications, exciting new research has unveiled the enzyme's potential to protect against neurodegeneration,

degrade the SARS-CoV-2 spike protein, and mitigate inflammation and oxidative stress through activation of the Nrf2/HO-1 signaling axis.

Despite this remarkable progress, significant challenges remain. The heterogeneity of clinical trial designs, the lack of standardized quality control and product consistency, incomplete understanding of nattokinase's pharmacokinetic properties, and the absence of large-scale, long-term clinical outcome trials constitute critical barriers to the full realization of nattokinase's therapeutic potential. Addressing these limitations through rigorous, well-designed future research is an urgent priority.

In conclusion, nattokinase represents a compelling example of the untapped therapeutic potential harbored within traditional fermented foods. With continued scientific investigation and the translation of promising preclinical findings into rigorous clinical trials, nattokinase is poised to make a significant and lasting contribution to the prevention and management of cardiovascular disease and other chronic conditions that afflict humanity. The journey that began with a simple observation in a Chicago laboratory in 1980 has evolved into a rich and multifaceted scientific enterprise that holds the promise of improving human health on a global scale.

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