REVIEW ARTICLE

Therapeutic Potential of NAD^+ Precursors and Aging-related Diseases

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD^+) plays fundamental roles in human life and health as an essential cofactor and substrate for a great number of vital biological processes. A growing body of evidence has demonstrated that the development of many age-related degenerative diseases, including dementia, heart failure, and diabetes, are linked with an age-dependent depletion in cellular NAD^+ levels. Preclinical studies have shown that the restoration of NAD^+ levels through NAD^+ precursors, nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) supplementation, reduces the risk of heart failure, ameliorates the manifestations of Alzheimer's and Parkinson's, and improves insulin-sensitivity in type 2 diabetes. In recent years, multiple clinical studies have indicated that NR and NMN supplements are well tolerated and effectively stimulate NAD^+ metabolism in healthy subjects. Further efficacy studies of NR and NMN supplements in patients with degenerative diseases are under clinical trial. This article reviews the recent preclinical and clinical progress of NR and NMN supplements as potential therapies to treat aging-related diseases with a focus on cardiovascular disease, neurodegenerative disorders, and diabetes studies. (*Int J Biomed Sci* 2022; 18 (2): 35-40)

Keywords: aging; cardiovascular disease; diabetes; NAD^+ precursor; neurodegenerative disease; nicotinamide adenine dinucleotide; nicotinamide mononucleotide; nicotinamide riboside

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD^+), a crucial cofactor present in all living cells, plays fundamental roles in many important biological processes including ATP production, DNA repair, mitochondrial function, inflammatory response in immune cells, etc. (1-3). Recently, growing evidence has indicated an age-dependent decline of NAD^+ across multiple species including worms, rodents, and human tissues. Although the precise mechanism of the NAD^+ age-dependent decline remains unknown, the possible explanations include increased NAD+ consumption and/or decreased NAD+ synthesis (4-6). In addition, the decrease of NAD^+ levels have been demonstrated to be associated with many aging-related diseases such as skin disease, heart disease, neurodegenerative disorders, dia-

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betes, etc. (3). In line with these findings, NAD^+ replenishment through administration of NAD^+ precursors have been reported to display beneficial effects counteracting aging and aging-related diseases (7-9).

Four NAD^+ precursors have been extensively studied, including nicotinamide (NAM), nicotinic acid (NA), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). In contrast to NR and NMN, NAM and NA exhibit potential risks when used as supplementation to raise NAD^+ levels. For instance, NAM inhibits poly (ADP-ribose) polymerases (PARPs), an important enzyme in DNA repair for genomic integrity and programmed cell death (10), and long-term consumption of NA supplements increases the risk of type 2 diabetes (11). However, NR and NMN are much safer supplements and have been intensively studied in aging and aging-related diseases (Figure 1). NR is present in natural products such as cow milk and exhibits great bioavailability (12). NR can be directly converted into NMN intracellularly by nicotinamide riboside kinases 1 and 2 (NRK1 and NRK2) and is subsequently catalyzed to NAD+ by nicotinamide-nucleotide adenylyltransferase (NMNAT) (13) (Figure 1). In contrast to NR, which can readily cross cell membranes, NMN has to first be extracellularly converted to NR by a cell surface receptor named CD73 (14). Only then can the NAD^+ precursor molecule enters the cell via equilibrative nucleoside transporters (ENTs), which initiate a cascade of reactions to ultimately elevate intracellular NAD^+ levels (15).

In preclinical studies, both NMN and NR are orally bioavailable and effectively increase endogenous NAD^+ levels with beneficial or therapeutic effects (16). More recently, a number of clinical trials have been conducted to evaluate effects of NR and NMN on age-related diseases. This article reviews the recent advances in the therapeutical potentials of NR and NMN supplements for aging-related diseases with a focus on cardiovascular disease, neurodegenerative disorders, and diabetes in both preclinical and clinical studies.

PRECLINICAL STUDIES OF NR AND NMN IN AGING-RELATED DISEASES

The efficacies of NAD^+ precursors NR and NMN have been studied in multiple preclinical animal models for aging-related diseases, such as cardiovascular diseases, neurodegenerative disorders, diabetes, etc.

NMN and NR in Cardiovascular diseases

Cardiovascular diseases are the leading killers globally, resulting in 17.9 million deaths each year as estimated

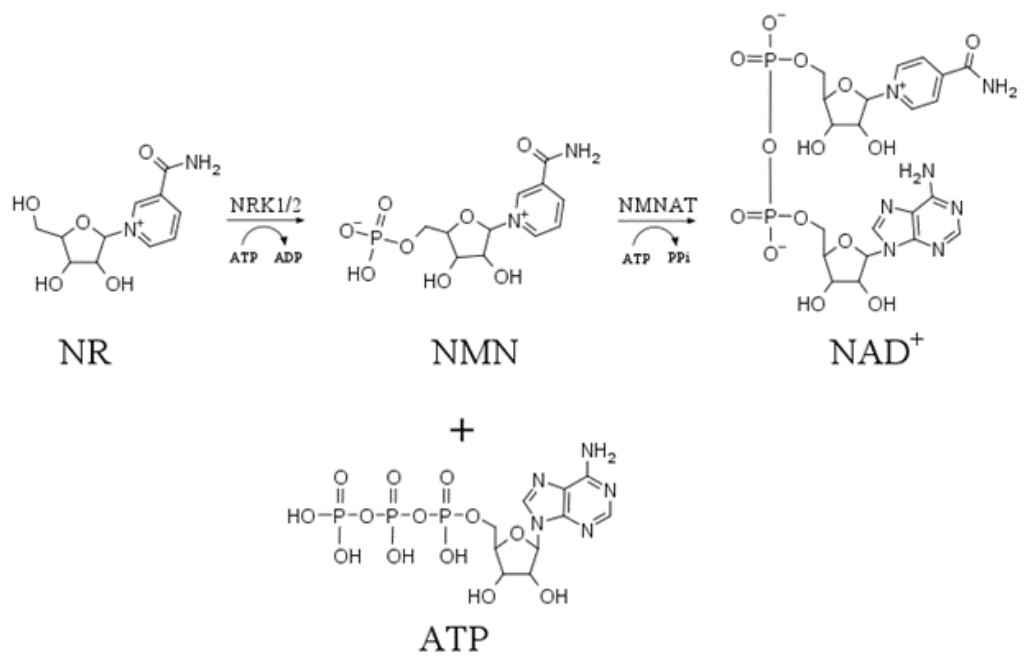

Figure 1. NAD+ Biosynthesis from its precursors. NR is phosphorylated to MNM by Nicotinamide Riboside Kinase ½ (NRK1/2), and subsequently, NMN reacts with ATP to produce NAD+ by Nicotinamide mononucleotide adenylyltransferase (NMNAT).

by the World Health Organization in 2022 (17). Raising NAD^+ by NMN and NR supplementation has been studied in many preclinical models. Yamamoto *et al* reported that administration of NMN supplementation at 500 mg/kg to mice, either once 30 minutes before the onset of ischemia or every 6 hours during the reperfusion for 24 hours, significantly decreased the heart infarct sizes by 44% and 29%, respectively, compared to the saline control (18). This result suggests that the exogenous administration of NMN greatly protects the heart from ischemia and reperfusion (I/R). Consistent with Yamamoto's report, Nie and colleagues in 2021 first assembled NR with antioxidant reagent resveratrol in nanocrystal microspheres (NR/RESms) and then orally delivered NR/RESms into mice (19). Their study indicated that NR/RESms significantly elevated NAD^+ levels in serum and attenuated myocardial infarction in a cardiac I/R injury mouse model (19). A similar result was observed in another study by Hosseini and colleagues when they administered NMN at 100 mg/kg/day with antioxidant reagent-Melatonin intraperitoneally in rats (20). The results showed that the combined therapy had significant cardioprotective effects in aged rats by reducing mitochondrial reactive oxygen species and oxidative status, as well as improvement of mitochondrial membrane potential (20).

Heart failure has been observed to be associated with mitochondrial dysfunction, and it has been well recognized that NAD^+ plays a critical role in mitochondrial function (21). Zhang and colleagues intraperitoneally injected NMN at 500 mg/kg/day for 5 days in mice with cardiac failure (21). They showed that NMN protects against pressure overload-induced heart failure through the preservation of mitochondrial ultrastructure, reduction of reactive oxygen species, and prevention of heart cell death (21). In parallel, Diguet *et al* studied NR administration through food in mice with dilated cardiomyopathy, showing that NR supplementation attenuates the development of mouse heart failure by stabilizing myocardial NAD^+ levels (22). Interestingly, a recent new study argued that high dosages of NR supplement may worsen the mitochondrial damage in the heart, warning that caution should be taken when administration of high doses of NR, particularly for the mitochondrial dysfunction-causing cardiomyopathy (23).

Neurodegenerative disorders

There were approximately 57.4 million people with dementia worldwide in 2019, and the number is predicted to increase to 152.8 million by 2050, causing large economic burden and healthcare challenges (24). Numerous preclinical studies have been done on the effect of raising NAD^+ through NR and NMN supplementation on such neurodegenerative disorders.

Alzheimer's disease (AD) is the most common type of dementia, and amyloid β-peptide (Aβ) plaques and tau neurofibrillary tangles are the neuropathological hallmarks of AD (25). Wang *et al* reported that treatment with NMN (500 mg/kg) eliminated the accumulation of ROS, prevented the Aβ1–42 oligomer-induced neuronal death, and ameliorated learning and memory in rat AD model (31). In another study, the administration of NMN significantly decreased amyloid plaque burden, synaptic loss, and inflammatory responses, all of which are closely associated with AD (32-33). In addition, the effect of NR on AD has also been observed in preclinical studies. Hou *et al* treated AD mice with NR dissolved in drinking water (12 mM) for 6 months and they observed that NR supplement significantly normalized neuroinflammation, synaptic transmission, phosphorylated Tau, and DNA damage as well as improved learning and memory and motor function (34). Taken together, all the studies collectively indicate that NMN and NR supplementation hold great potential to treat AD.

Parkinson's Disease (PD), the most common neurodegenerative movement disorder, is characterized by the loss of dopaminergic neurons in the substantia nigra and by the accumulation of misfolded α-synuclein (35). In the PD model of *Drosophila,* NR treatment (500 μM for 30 days) significantly prevented dopaminergic neuronal loss (37). PC12 cells are a cell line derived from an induced, transplantable rat pheochromocytoma and are commonly used as a cellular model for PD. NMN treatment (0.1 or 1 mM for 24 hours) significantly improved survival rates in PC12 cells treated with the toxic compound rotenone, reducing rotenone-induced cell apoptosis (36).

Diabetes

According to the World Health Organization, diabetes was the direct cause of 1.5 million deaths in 2019 worldwide, and the number of people with the disease is only projected to increase to 643 million by 2030 and 783 million by 2045 (22). Aging has been identified as one of the most significant risk factors for the development of type 2 diabetes (T2D) (23). Patients are diagnosed as diabetic when they are hyperglycemic, with elevated fasting blood glucose of greater than 125 mg/dL (24). Yoshino *et al* reported that a single dose (500 mg/kg body weight/day) of NMN administrated intraperitoneally normalized the impaired glucose tolerance in aged, naturally occurring

diabetic male mice (26). As female mice with naturally developed T2D were more difficult to obtain, the study used aged female mice which had been given a high fat diet (HFD) for 7 weeks to induce diabetic conditions (26). These aged, diabetic female mice received 11 consecutive injections of NMN. Experimental results showed that these injections of NMN were able to completely normalize even severely impaired glucose tolerance, suggesting the potential for NMN to be effective in human T2D patients (26).

T2D is known to be caused in part by the inability of insulin-sensitive tissues to respond accurately to insulin, and long-term administration of NMN was studied in a 12-month long mouse model to determine NMN's ability to ameliorate age-associated decrease in insulin sensitivity (27). Blood glucose levels measured in T2D mice treated with various NMN concentrations (0 100, and 300 mg/kg/day) showed that NMN-administration significantly improved insulin sensitivity in T2D mice (27). These results indicate that NMN taken over a long duration of time could serve as a remedy for T2D patients.

Furthermore, Trammell *et al* studied the effects of NR supplementation on the fasting and non-fasting glucose levels in male mice with T2D (28). They found that NR supplementation dramatically decreased fasting glucose levels from 345 mg/dl to 194 mg/dl in the T2D mice and improved glucose tolerance, indicating that NR has notable effects on the metabolism of T2D mice (28).

In summary, preclinical studies have demonstrated that both NMN and NR supplements greatly help improve the conditions resulting from various aging-related diseases such as cardiovascular conditions, neurodegenerative disorders and diabetes.

CLINICAL STUDIES OF NMN AND NR AGING-RELATED DISEASES

Preclinical results strongly support the clinical potential of NR and NMN in age-related diseases. In recent years, the safety and bioavailability of NMN and NR have been examined in multiple clinical studies. Irie *et al* conducted a non-blinded clinical trial to investigate the safety of NMN administration in 10 healthy men who orally received a single administration of NMN at 100, 250 and 500 mg (29). Clinical parameters were then assessed at five hours after NMN administration, and the results demonstrated that single NMN doses are safe and successfully metabolized in healthy participants without causing any significant side effects (29). In addition, clinical studies of NR's safety and bioavailability have been conducted as well. Martens *et al* performed a randomized, double-blind, placebo-controlled clinical trial of NR supplementation (500 mg, twice/day) for six weeks (30). Their study demonstrated that NR is well tolerated and effectively stimulates NAD^+ metabolism in healthy subjects (30). Recently, Conze and colleagues conducted an 8-week randomized, double-blind, placebo-controlled clinical trial to study dose-dependency of NR oral availability and safety in overweight, but otherwise healthy human subjects (31). They found that NR administration at 100, 300, and 1000 mg dose-dependently increased blood NAD^+ and other NAD^+ metabolites without significant differences in adverse events between the NR and placebo-treated groups or between groups at different NR doses (31). The good NR safety and bioavailability at three single doses (100, 300, and 1000 mg /day) are reinforced by another clinical study conducted with 12 healthy human subjects (16).

Following safety and bioavailability studies in healthy human subjects, many clinical studies on the efficacies of NMN and NR in various aging-related diseases have been or are being conducted as well. To date, two clinical trials of NMN in hypertension are ongoing, and one is currently being conduct ed in the Integrative Physiology of Aging Laboratory in Colorado, United States (NCT03821623) and the other is performed at the First Affiliated Hospital of Sun Yat-sen University in China (NCT04903210, Table 1). No outcomes of these two studies have been released. About ten clinical trials of NR have also been initiated to treat various cardiovascular diseases including atherosclerosis, heart failure, and hypertension (Table 1), but no study results have been reported yet.

The use of NMN and NR in treating neurodegenerative disorders has still yet to be studied extensively, as only a couple of clinical trials have been done on NR and none yet have been reported on NMN. Studies investigating the effects of supplementation are beginning to emerge, however (43-44, Table 1).

The efficacy of NMN and NR in treating diabetes has not been studied to a great extent. So far, only a 10-week, randomized, placebo-controlled, double-blind clinical trial was conducted in postmenopausal women with prediabetes, who were overweight or obese, and the results showed that NMN supplementation (250 mg/day) increased their muscle insulin sensitivity, insulin signaling, and remodeling (45). These positive effects have opened the door to the possibility of NMN as a viable treatment for diabetes (45).

Table 1. Clinical trials of NAD^+ precursors, NMN and NR FOP (by May 2022)

Precursor	Disease	Dosage	Clinical Phase	Status and Results	NCT
NMN	Hypertension	800 mg total per day (400 mg capsules)	Phase 4	Recruiting	NCT04903210
NR	Hypertension, Aging	500 mg of NIAGEN, BID (1,000 mg per day)	Phase 2	Recruiting	NCT03821623
	Atherosclerotic, Cardiovascular Disease, Dyslipidemia, Cardio-metabolic Diseases	2 capsules of 250 mg twice daily, PO	Phase 2	Recruiting	NCT04271735
	Vascular Diseases,	500 mg PO BID	Phase 2	Recruiting	NCT04040959
	Heart Failure, Congestive Heart Failure,	Day 1: 250 mg (1 capsule) BID Day 2: 500 mg (2 capsules) BID Day 3-14: 1000 mg (4 capsules) BID	Early Phase 1	Completed, results not yet published	NCT03727646
	Heart Failure, Systolic Heart Failure,	Day 1: 250 mg (1 capsule) BID Day 2: 500 mg (2 capsules) BID Day 3-14: 1000 mg (4 capsules) BID	Early Phase 1	Recruiting	NCT04528004
	Hypertension	1,000 mg/day	Phase 1	Recruiting	NCT04112043
	Systolic Heart Failure,	250 mg capsules, PO Initial dose of 1 capsule BID, weekly up-titration by 1 capsule/dose to final dose of 4 capsules (1000 mg) BID at end of Week 4, final dose continued to Week 12	Phase 1 Phase 2	Completed, results not yet published	NCT03423342
	Diabetes, Coronary Artery Disease, Atherosclerosis	1000 mg/day for 7-10days	n/a	Completed, results not yet published	NCT02812238

PO, Oral Administration; BID, twice a day; AD, Alzheimer Disease; PD, Parkinson Disease.

CONCLUSION

A decline in NAD^+ levels is associated with numerous aging-related diseases including cardiovascular diseases, neurodegenerative disorders, and diabetes. Cumulative preclinical studies strongly evidence the beneficial effects of NAD^+ precursors, NMN and NR supplements, in the amelioration of the conditions of aging-related diseases. Nevertheless, the mechanistic details of how extracellular NAD precursors interact with cells to exert their effects remains to be discovered. Recently, a number of clinical trials have been conducted, with results showing that NMN and NR are safe and bioavailable to humans. Currently, several clinical trials are underway to investigate whether NMN and NR supplementation can effectively treat aging-related disease such as cardiovascular diseases, neurodegenerative disorders, and diabetes. The next few years will witness whether the promising results observed in preclinical studies can be successfully translated to human clinical trials.

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CONFLICT OF INTERESTS

The authors declare that no conflicting interests exist.

ABBREVIATIONS

NR	Nicotinamide Riboside
NMN	Nicotinamide Mononucleotide
NAD^+	Nicotinamide Adenine Dinucleotide
T2D	Type 2 Diabetes
PD	Parkinson's Disease
AD	Alzheimer's Disease

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