Nicotinamide mononucleotide (NMN) as an anti-aging health product – Promises and safety concerns

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HIGHLIGHTS

- Provides an overview of promises and safety concerns of NMN as an anti-aging product.
- Shows that NMN's beneficial effects supported by *in vivo* studies.
- Reveals that there is a lack of NMN's clinical safety and efficacy studies
- Suggests that proper clinical investigations are urgently needed on the effectiveness and safety of NMN supplementation.

GRAPHICAL ABSTRACT

ABSTRACT

Background: Elderly population has been progressively rising in the world, thus the demand for anti-aging heath products to assure longevity as well as to ameliorate age-related complications is also on the rise. Among various anti-aging health products, nicotinamide mononucleotide (NMN) has been gaining attentions of the consumers and the scientific community.

Aim of review: This article intends to provide an overview on the current knowledge on promises and safety concerns of NMN as an anti-aging health product.

Key scientific concepts of review: Nicotinamide adenine dinucleotide (NAD$^+$) levels in the body deplete with aging and it is associated with downregulation of energy production in mitochondria, oxidative stress, DNA damage, cognitive impairment and inflammatory conditions. However, NMN, as the precursor of NAD$^+$, can slow down this process by elevating NAD$^+$ levels in the body. A number of *in vivo* studies have indicated affirmative results of therapeutic effects for various age-induced complications with NMN supplementation. One preclinical and one clinical study have been conducted to investigate the safety concerns of NMN administration while a few more human clinical trials are being conducted. As there is a

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large influx of NMN based anti-aging products on the market, proper clinical investigations are urgently needed to find out the effectiveness and safety of NMN supplementation.

Introduction

The successful control of communicable diseases in the 20th century led to a sharp rise in the mean life expectancy of many countries. In 2019, number of persons, aged 65 or over was 702.9 million in the world and it is projected to be 1548.9 million by 2050 [1]. Percentage of global population aged 65 years or over in 2019 and future projections according to the medium-variant projection is illustrated in Fig. 1. Along with increasing elderly population, the prevalence of age-related diseases such as atherosclerosis, hypertension, osteoarthritis, neurodegenerative diseases including Alzheimer's and Parkinson's diseases, diabetes mellitus and cancers has gone up leading to heavy global socioeconomic and medical burden [2].

Therefore, age management medical practices have been mushrooming in the world for recommending nutritional supplements, various drugs, exercise programs, hormone therapies and other treatments to mitigate the effect of aging. Consequently, the consumer demand and the global market value for anti-aging health products are on the rise [3]. Excessive demand of consumers and high profit margin for manufacturers are the major driving force behind the release of anti-aging health products without adequate safety testing [4]. Thus, careful comprehensive and stepwise scientific preclinical and clinical investigations are crucial to be conducted.

Among various anti-aging health products, nicotinamide mononucleotide (NMN) has been gaining an increasing attention as a promising anti-aging product. The mitochondrial decay, which is responsible for aging, can be reversed by the increased levels of nicotinamide adenine dinucleotide (NAD^+) in the body. NMN is a precursor of NAD^+ that acts as an intermediate in NAD^+ biosynthesis, while dietary supplements of NMN are found to increase the NAD^+ levels in the body [5]. NMN is a bioactive nucleotide formed by the reaction between a nucleoside comprising nicotinamide and ribose and a phosphate group [6]. It naturally presents in a variety of plant and animal food sources. Furthermore, several studies have been carried out to investigate the potential of biotechnological production and purifying NMN from bacterial and yeasts [7].

Other than anti-aging potential of NMN, a wide range of pharmacological activities have been identified in a number of *in vivo* studies. The link between NMN and the incidence of Alzheimer's disease, obesity and associated complications, cerebral and cardiac ischemia, and age- and diet-induced type 2 diabetes has been studied extensively [8]. Though, previous attention of scientific community has been paid on NMN only as an intermediate in NAD^+ biosynthesis, recently, a number of pharmacological activities triggered by increasing NAD^+ levels in the body, especially anti-aging activity have been taken the centre of attention. As a result, a number of studies including cell culture, animal models and human clinical trials have been conducted to investigate the promises and the safety concerns of using NMN as an anti-aging health product and the potential of using NMN as a supplement to avoid age-related disease conditions. Hence, this review intends to present the most recent advances and current knowledge on promises and safety concerns of the use of NMN as an anti-aging health product, its other pharmacological and therapeutic uses and mechanism of action underlying the anti-aging properties with an interest to stimulate further research and offer an insight to the possibility of translating successful preclinical and clinical anti-aging outcomes of NMN into an effective treatment of aging and age-related diseases.

What is nicotinamide mononucleotide (NMN)?

Nicotinamide mononucleotide (NMN) exists as α and β anomeric forms while it is identified as nicotinamide ribotide, nicotinamide-1-ium-1-β-D-ribofuranoside 5′-phosphate, β-nicotinamide ribose monophosphate and 3-carbamoyl-1-[5-*O*-(hydroxyphosphinato)-β-D-ribofuranosyl] pyridinium, among others [9]. The β form is the active anomer and NMN is naturally structured as a result of a reaction, which catalysed by nicotinamide phosphoribosyltransferase enzyme, between a phosphate group and a nucleoside comprising nicotinamide (an amide form of vitamin B₃) and ribose [10]. NMN is a bioactive nucleotide with a pyridine base and its molecular weight is 334.22 g/mol. It is fairly acidic and water-soluble compound. The solubility has been reported to be 1.8 mg/mL [11] (Fig. 2).

NMN is mainly located in the nucleus, mitochondria and cytoplasm, whereas in the human body, it can be found in placenta tissue and body fluids such as blood and urine [9]. It is naturally found in a variety of fruits and vegetables including immature soybean pods, cabbage, cucumber, broccoli, tomato, mushroom and
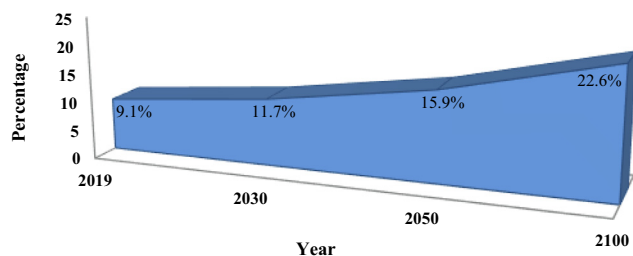

Fig. 1. Percentage of global population aged 65 years or over according to the medium-variant projection (UN, 2019).
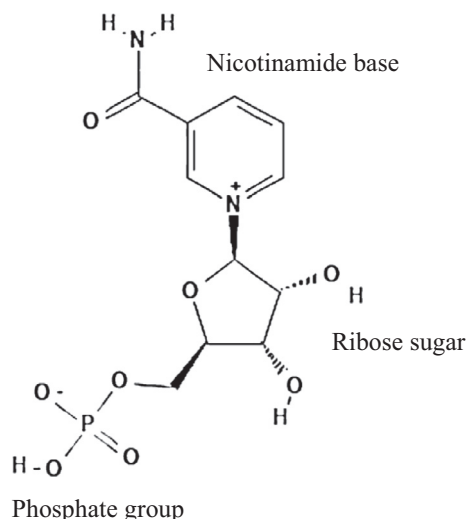

Fig. 2. Chemical structure of nicotinamide mononucleotide (NMN).

avocado as well as in raw beef and shrimp. The NMN content in these vegetables and fruits are 0.25–1.88 mg/100 g and 0.26–1.60 mg/100 g, respectively, whereas raw beef and shrimp contain comparatively lower level of NMN than those plant-based food (0.06–0.42 mg/100 g). Based on the evidence of the presence of NMN in red blood cells, it has been put forward that physiologically pertinent NMN contents, which are required for the biosynthesis of NAD$^+$ and many physiological functions, are absorbed from daily food sources [10].

NMN is an intermediate of NAD$^+$ biosynthesis. NAD$^+$ is a very important metabolic redox co-enzyme in eukaryotic organisms and is essential component for large number of enzymatic reactions. It plays a vital role in a variety of biological processes of the body including cell death, aging, gene expression, neuroinflammation and DNA repair, which indicating a significance role of NAD$^+$ in longevity and health of human life [12]. As revealed by many recent studies, deficiency of NAD$^+$ can be compensated by the NMN supplementation that affects a range of pharmacological activities in different disease conditions [13].

Several methods had been used to prepare and purify NMN: incubation of diphosphopyridine nucleotide in a non-phosphate buffer and fluoride with potato pyrophosphatase, synthesis of NMN from nicotinamide by human hemolysates and erythrocytes, and specific hydrolysis of pyrophosphate bond of NAD$^+$ using NAD$^+$ pyrophosphatase and metal catalysts [7]. However, these methods are not rather efficient and they produce low amounts of NMN, giving rise to high price of NMN. Currently, microbial biotechnologies are used to obtain NMN. Nevertheless, innovative methods and optimisations are essential in order to address the high cost and purity issues of NMN. Many studies have been performed using simple and efficient biotechnological production and purification methods using bacteria and yeast to make NMN production cost effective [14].

Though at first, NMN was only considered as a source of cellular energy and an intermediate in NAD$^+$ biosynthesis, currently, the attention of the scientific community has been paid on anti-aging activity and a variety of health benefits and pharmacological activities of NMN which are related to the restoring of NAD$^+$. Thus, NMN has therapeutic effects towards a range of diseases, including age-induced type 2 diabetes, obesity, cerebral and cardiac ischemia, heart failure and cardiomyopathies, Alzheimer's disease and other neurodegenerative disorders, corneal injury, macular degeneration and retinal degeneration, acute kidney injury and alcoholic liver disease [15].

Mechanism underlying the anti-aging activity of NMN

Aging, as a natural process is identified by downregulation of energy production in mitochondria of various organs such as brain, adipose tissue, skin, liver, skeletal muscle and pancreas due to the depletion of NAD$^+$ [16]. NAD$^+$ levels in the body decrease as a consequence of increasing NAD$^+$ consuming enzymes when aging [17]. These NAD$^+$ consuming enzymes include NADase (CD38/CD157), poly (ADP-ribose) polymerase (PARP), NAD$^+$ dependent acetylase (sirtuins), BST and tankyrase (TNKS) [18]. Sirtuins consume NAD$^+$ in order to execute a variety of functions such as deacetylation, deglutarylase, lipoamidase, demalonylase and desuccinylase activities. Regulation of longevity, aging and age-associated physiological changes is one of the substantial aspects of sirtuin biology [19], while CD38 utilises NAD$^+$ to produce cyclic ADP-ribose and nicotinamide. Apart from that, PARP expends NAD$^+$ to form branched ADP-ribose polymers which assists in DNA repairing [20]. This depleted NAD$^+$ level by NAD$^+$ consuming enzymes can be compensated by administration of NMN to the body since NMN is an intermediate compound of the NAD$^+$ biosynthesis.

There are three different biosynthesis pathways to produce NAD$^+$ in mammalian cells including *de novo* synthesis from tryptophan, salvage and Preiss-Handler pathways. Among these three pathways, NMN is an intermediate by-product in salvage pathway, and it is involved in NAD$^+$ biosynthesis through salvage and Preiss-Handler pathways as illustrated in Fig. 3 [15]. The salvage pathway is the most efficient and the main route for the NAD$^+$ biosynthesis, in which nicotinamide and 5-phosphoribosyl-1-pyrophosphate are converted to NMN with the enzyme action of NAMPT followed by conjugation to ATP and conversion to NAD by NMNAT [21]. Furthermore, NAD$^+$ consuming enzymes are responsible for degradation of NAD$^+$ and consequent formation of nicotinamide as a by-product [13]. In the Preiss-Handler pathway, initially, nicotinic acid is converted to nicotinic acid mononucleotide (NAMN) by nicotinic acid phosphoribosyl-transferase enzyme (NAPRT) activity followed by biosynthesis of nicotinic acid adenine dinucleotide (NAAD$^+$) from NAMN using nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT 1/2/3). Subsequently, NAAD$^+$ is transformed to NAD$^+$ by NAD$^+$ synthetase (NADS) using ATP and ammonia [22].

Chronic inflammation and oxidative stress, which come along with aging, are the causes for reduction and inhibition of NAMPT-mediated NAD$^+$ biosynthesis [23]. The depletion of NAD$^+$ contents along with aging, which particularly of nuclear origin, is associated with interruption of mitochondrial regulation of PCG-$1\alpha/\beta$-independent pathway of oxidative-phosphorylation as well, causing pseudohypoxia. This incident can be overturned by raising the NAD$^+$ content [24].

Apart from reducing the functions of mitochondria, biological changes such as cognitive impairments, DNA damage and sirtulin gene inactivation, are brought about by aging which can be evaded by enhancing NAD$^+$ count in the body [24]. Apart from NMN supplementation, NAD$^+$ levels in the body can be increased as a response to conditions related to lower energy intake [25], calorie restriction, fasting, lack of glucose content in the body and exercise. Nevertheless, NAD$^+$ levels decrease as a consequence of intake of high-fat diets and aging [26].
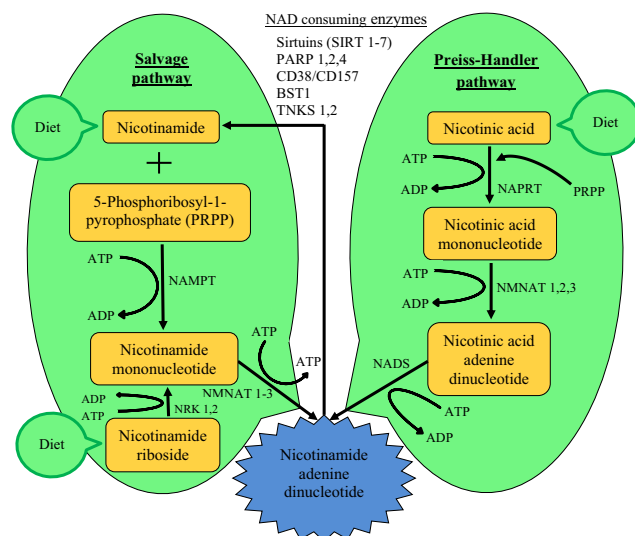
Fig. 3. NAD+ biosynthesis pathways in which NMN involves. Biosynthetic pathways of NAD+ in mammalian cells in which NMN involves are Preiss–Handler and salvage pathways, and the salvage pathway is the major source of NAD+. NAMPT-nicotinamide phosphoribosyltransferase; ATP-adenosine triphosphate; ADP-adenosine diphosphate; NMNAT-nicotinamide mononucleotide adenylyltransferase; NRK-nicotinamide riboside kinases; NAPRT-nicotinate phosphoribosyltransferase; NADS-nicotinamide adenine dinucleotide synthetases.

Currently, several studies have shown that NMN, the NAMPT reaction product, is able to be utilised to trigger the SIRT1 activity [27]. It has been shown that when there is not adequate NAD$^+$ levels, SIRT1 becomes unable to block hypoxia-inducible factor 1 alpha (HIF-1) and elevated levels of which blocks the communication between mitochondria and nucleus at the cellular level and between adipose tissue and hypothalamus at the systemic level [28]. The resulting interruption in mitochondria and nuclear communication causes swift reduction in mitochondrial function that leads to the development of age-associated complications and diseases. Nevertheless, the particular communication and mitochondrial function can be restored by the administration of NMN as the NAD$^+$ precursor [24]. Causes for reducing NAD+ levels when aging and mechanism underlying anti-aging activity of NMN are illustrated in Fig. 4. The nutraceutical industry has already started to market NMN aggressively as a highly efficient and viable anti-aging health product to enhance the NAD$^+$ levels [15], thus to provide longevity to the general population. In addition, many studies are carried out to investigate the potential anti-aging activity of NMN and their applicability and usability.

Promises and efficacy as an anti-aging health product

Many studies have been carried out to investigate the promises and efficacy of NMN as an anti-aging health product for managing and regulating aging and age-associated complications and diseases using cell culture, animal models and human clinical

investigations. *In vivo* studies, which have been carried out to investigate anti-aging therapeutic effects of NMN administration, are summarised in Table 1, including animal models, given NMN dose, duration and observed effects. According to Yoshino et al. [23], during the aging process, NAMPT and NAD$^+$ levels significantly decrease in various organs and NMN administration could enhance NAD$^+$ levels (from 500 to 1550 pmol/mg-tissue), insulin secretion, insulin sensitivity and lipid profile in age-induced type 2 diabetic mice. Administration of NMN also can restore gene expression linked to circadian rhythm, inflammatory response and oxidative stress, and improve hepatic insulin sensitivity, partially by SIRT1 activation.

De Picciotto et al. [29] found that NMN supplementation was capable of restoring NAD$^+$ levels (by threefold), vascular SIRT1 activity, maximum carotid artery endothelium-dependent dilation, and nitric oxide-mediated carotid artery endothelium-dependent dilation in mice. Kawamura et al. [30] have reported that NMN retained in animals for longer period than nicotinamide. NMN resulted in a higher yield of NAD$^+$ (80 nmol/g of liver tissue) in salvage biosynthesis pathway activating higher response of SIRT1 than nicotinamide.

Mills et al. [10] found that devoid of any apparent deleterious effect or toxicity, NMN effectively suppressed aging-induced body weight gain and ameliorated eye dysfunction in mice. It maintained healthy plasma lipid profile, insulin sensitivity, physical activity, energy metabolism and other physiopathologies. Additionally, NMN supplementation averted alterations in age-

Fig. 4. Causes for reducing NAD+ levels when aging and mechanism underlying anti-aging activity of NMN. DNA damage, chronic inflammation, oxidative stress and increasing NAD+ consuming enzymes (sirtuins, CD38/CD157, PARP, TNKS and BST) accelerate NAD degradation. The reduced levels of NAD+ cause downregulation of energy production in mitochondria, leading to aging and various age-associated diseases. NMN supplementation can reinstate NAD+ levels in the body through biosynthesis pathways, reversing the aging process and preventing age-associated diseases.

Table 1
Anti-aging therapeutic effects of NMN administration *in vivo.*

Therapeutic effect/age-related complication	Model	NMN dose and duration	Effect	Reference
Age- and diet-induced diabetes	Age-induced and high-fat-induced diabetic mice	IP – 500 mg/kg body weight/day, age induced mice – 11 days, high-fat induced mice – 7–10 days	Enhanced insulin secretion, insulin sensitivity and lipid profile in age-induced type 2 diabetic mice. Improved hepatic insulin sensitivity and glucose tolerance in high-fat-induced diabetic mice	[23]
Age-associated vascular dysfunction and oxidative stress	Aged (26–28 months) C57Bl/6 mice	Drinking water – 300 mg/kg body weight/day, 8 weeks	Restored maximum carotid artery endothelium-dependent dilation and nitric oxide-mediated carotid artery endothelium-dependent dilation. Reduced vascular oxidative stress, normalised aortic stiffness and activated vascular SIRT1 activity	[29]
Anti-aging activity and longer retention in the body	Wistar rats (7 weeks)	IP – 45 μmol/kg body weight, Single	Retained in the body for longer period than nicotinamide Resulted a higher yield of NAD$^+$ activating higher response of SIRT1 than nicotinamide and better anti-aging activity and longevity than nicotinamide	[30]
Alzheimer's disease	APP$_{(swe)}$/PS1$_{(\Delta E9)}$ double transgenic (AD-Tg) mice	Subcutaneously – 100 mg/kg body weight/every other day, 28 days	Enhanced mitochondrial bioenergetic functions. Reduced expression of amyloid precursor protein (APP)	[47]
Age-associated physiological decline	Wild-type C57BL/6N mice	Drinking water – 100 and 300 mg/kg body weight/day, 12 months	Suppressed aging-induced body weight gain. Ameliorated eye functions, healthy plasma lipid profile, insulin sensitivity, physical activity, energy metabolism and other physiopathologies. Averted alterations in age-associated gene expression Enhanced mitonuclear protein imbalance and mitochondrial oxidative metabolism in skeletal muscles	[10]

Therapeutic effect/age-related complication	Model	NMN dose and duration	Effect	Reference
Alzheimer's disease	Intracerebroventricular infusion of Aβ$_{1-42}$ oligomer in Wistar rats	IP – 500 mg/kg body weight/day, 10 days	Restored learning and cognition. Enhanced energy metabolism and neuron survival. Eliminated ROS accumulation	[48]
Age-associated susceptibility to acute kidney injury	Aged (20 months) wild-type 129S2/Sv and C57BL/6 mice	IP – 500 mg/kg body weight/day, 4 days	Boosted NAD$^+$ and SIRT1 levels in aged kidneys. Protected aged kidneys from both ischemia–reperfusion- and cisplatin-induced acute kidney injuries	[31]
Age-associated decline in DNA repair capacity	Aged (20–26 months) C57BL/6J mice	IP – 500 mg/kg body weight/day, 7 days	Reduced DND damage. Protected against changes in haemoglobin and white blood cell count including lymphocytes	[32]
Alzheimer's disease	APP$_{(swe)}$/PS1$_{(\Delta E9)}$ double transgenic (AD-Tg) mice	Subcutaneously – 100 mg/kg body weight/every other day, 28 days	Improved of behavioural measures of cognitive impairments. Reduced inflammatory responses, synaptic loss, amyloid plaque burden and β-amyloid production by inhibition of JNK activation	[49]
Age-related vascular aging	Aged (18 months) C57BL/6J mice	Drinking water – 400 mg/kg body weight/day, 2 months	Increased endurance. Improved blood flow in elderly mice by increasing capillary density	[34]
Age-related cognitive and behavioural dysfunction	Aged (20 months) C57BL/6 mice	Per oral – 300 mg/kg body weight/day, 3 weeks	Mitigated age-related decline in the sensory processing of hypersensitivity, some aversive stimuli and other related behaviours	[35]
Aging-induced cognitive impairment	Aged (24 months) Wistar rats	IP – 100 mg/kg body weight/every other day, 28 days	Stimulated neuroprotective effects. Reduced aging-induced cognitive decline. Alleviated age-associated memory and learning impairments	[36]

Therapeutic effect/age-related complication	Model	NMN dose and duration	Effect	Reference
Promoting micro-RNA profile in the aorta, epigenetic rejuvenation and anti-atherogenic effects	Aged (24 months) C57BL/6 mice	IP – 500 mg/kg body weight/day, 14 days	Overturned age-associated transformations in micro-RNA profile in the aged mouse aorta	[37]
Alzheimer's disease	C57BL/6 mice and transgenic mice with mitochondria-targeted yellow fluorescence protein (mito-eYFP) expression in neurons	IP – 62.5 mg/kg, Single	Enhanced mitochondrial bioenergetics. Overturned physiological decline. Restrained postischemic NAD$^+$ depletion and cellular death. Inhibited mitochondrial excessive fragmentation. Reduced mitochondrial protein acetylation and ROS in the hippocampus	[50]
Skeletal aging	Aged (12 months) C57BL/6J mice	Drinking water – 300 mg/kg body weight/day, 3 months	Protected from skeletal aging. Reduced osteogenesis. Increased adipogenesis by regulating mesenchymal stromal cells	[38]

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Table 1 (*continued*)

Therapeutic effect/age-related complication	Model	NMN dose and duration	Effect	Reference
Age-associated cerebromicrovascular dysfunction and cognitive decline	Aged (24 months) C57BL/6 mice	IP – 500 mg/kg body weight/day, 14 days	Restored NAD$^+$ levels mitochondrial energetic Reduce oxidative stress Reversed cerebrovascular endothelial dysfunction Improved neurovascular coupling responses and cognitive performance	[39]
Rescuing female fertility during reproductive aging	Aged (12–14 months) C57BL/6 female mice	Drinking water – 2 g/L, 4 weeks	Restored NAD$^+$ levels and fertility Rejuvenated oocyte quality Supported the embryo development reversing the adverse consequences of elevated maternal age	[43]
Therapeutic effect/age-related complication	Model	NMN dose and duration	Effect	Reference
Mitochondrial function and cardioprotection in myocardial ischemia/reperfusion injury	Aged Wistar rats (22–24 months old)	IP – 100 mg/kg body weight/every other day, 28 days	Enhanced hemodynamic parameters Decreased dehydrogenase release and infarct size Ameliorated mitochondrial membrane potential Declined mitochondrial ROS and oxidative stress Restored NAD$^+$/NADH ratio	[40]
Promoting neurovascular rejuvenation	Aged (24 months) C57BL/6 mice	IP – 500 mg/kg body weight/day, 14 days	Promoted SIRT1activatio in the neurovascular unit Reversed age-associated alterations in neurovascular transcriptome which predicted to be mediated by the involvement of genes in anti-apoptosis, anti-inflammatory and mitochondrial rejuvenation pathways	[37]
Oocyte quality reduction with advanced maternal age	Aged (64–68 weeks) ICR female mice	IP – 200 mg/kg body weight/day, 10 days	Restored NAD$^+$ levels Increased ovulation, fertilisation capability and meiotic competency Promoted cytoplasmic and nuclear maturation Suppressed accumulation of DNA damage and ROS Declined apoptosis	[42]
Werner syndrome	young (Day 2) and old (Day 10) *wrn-1(gk99)* and wild type N2 *Caenorhabditis elegans* and *Drosophila melanogaster* worms	1 mM from the L4 stage	Extended lifespan and health-span Improved proliferative potency and number of mitotic cells by the NAD$^+$ repletion	[52]
Ataxia telangiectasia	Ataxia-telangiectasia mutated B6;129S4-*Atm^{tm1Bal}*/J mice	Drinking water – 12 mM, 2 weeks	Normalized neuromuscular function Delayed memory loss Extended lifespan Stimulated neuronal DNA repair Improve mitochondrial quality via mitophagy	[54]

IP-Intraperitoneal.

associated gene expression in main metabolic organs, while enhancing mito-nuclear protein imbalance and mitochondrial oxidative metabolism in skeletal muscles. Guan et al. [31] elaborated that NMN supplementation restored reduced contents of renal protective molecule, SIRT1 and its cofactor, NAD$^+$. The heightened NAD$^+$ and SIRT1 levels in kidneys of aged mice protected aged kidneys from both ischemia–reperfusion- and cisplatin-induced acute kidney injuries.

As explained by Li et al. [32], nudix homology domains (NHDs) are binding domains of NAD$^+$ and through binding to them, NAD$^+$ is able to regulate protein–protein interactions. The modulation of these interactions may lead to protecting the human body from aging, radiation and cancer. PARP1 is a critical protein that involves in DNA repairing. The inhibition of PARP1 is prevented by binding of NAD$^+$ to the NDH domain of DBC1 (deleted in breast cancer 1) nuclear protein. Nevertheless, when NAD$^+$ concentration is reduced with the age, DBC1 is progressively bound to PARP1, leading to accumulate DNA damage. This process of DNA damage can be reversed by restoring NAD$^+$ levels in the body. Results of this *in vivo* study showed that, NMN treatment increased hepatic NAD$^+$ contents, disrupted DBC1-PARP1 complex, reduced DNA damage and defended against changes in haemoglobin and white blood cell count including lymphocytes.

Tsubota [33] showed that NMN, as a sirtuins activating agent had protective effects against age-related ocular diseases such as glaucoma, dry eye and macular degeneration. As elaborated by Das et al. [34], reduction of blood flow and capillary density with

aging is a main cause of morbidity and mortality, whereas NMN as a NAD$^+$ precursor can reverse these to a certain extent by triggering sirtuin deacylases (SIRT1-7). NMN supplementation could increase NAD levels in liver and gastrocnemius tissues by nearly 5 and 1 folds, respectively. Johnson et al. [35] observed that *in vivo* occurrence of age-associated cognitive and behavioural dysfunctions was induced by declined NAMPT-mediated NAD$^+$ biosynthesis causing 40% gradual decrease of NAD$^+$ levels in the hippocampus, predominately in CA1 region. It showed that, even by short term supplementation of NMN, NAD$^+$ levels could be restored, while mitigating the age-related changes in the sensory processing of hypersensitivity, several other aversive stimuli and other associated behaviours, enhancing the quality of later lives. A prospective downstream effector was identified, namely, calcium/calmodulin-dependent serine protein kinase, which got reduced in hippocampus along with age-related NAD$^+$ drop. The promoter activity of this effector was regulated in a SIRT1 reliant manner, while its expression could be enhanced by NMN supplementation.

Hosseini et al. [36] reported that *in vivo* intraperitoneal injection of NMN and NMN together with melatonin stimulated neuroprotective effects and alleviated age-associated memory and learning impairments. Furthermore, the administration of them separately or in combination enhanced mitochondrial function and decreased apoptosis cell count both in hippocampus and prefrontal cortex regions of aged rats. Kiss et al. [37] illustrated that age-associated NAD$^+$ decline was linked with mis-regulation of

vascular mico-RNA expression, NMN intraperitoneal treatments resulted in anti-aging transformations mouse aorta mico-RNA expression profile. It was predicted that epigenetic rejuvenation and anti-atherogenic effects were some of regulatory consequences of NMN. NMN treatment distinctively expressed mico-RNAs in aged vessels.

The role of NMN in fighting against age-associated disorders, such as skeletal aging associated with reduced osteogenesis and increased adipogenesis by regulating mesenchymal stromal cells (MSCs), was studied by Song et al. [38]. MSCs are non-hematopoietic stem cells that contain regeneration capacity. NMN supplementation led to self-renewal of MSCs along with decreased adipogenesis and strengthened osteogenesis through upregulating SIRT1 activity in mice. In addition, NMN has been identified as a promising and potential therapeutic agent for skeletal aging which is able to regulate bone-fat imbalance via SIRT1 and rejuvenation and expansion of aged MSCs.

Tarantini et al. [39] found that age-related increase of oxidative stress and cerebromicrovascular dysfunction, which exacerbated neurovascular coupling responses and age-related cognitive decline, were caused by reduced NAD$^+$ availability with aging. NMN supplementation could restore tissue NAD levels by fold change of 1 and overturn these processes which led to improved cognitive performance in aged mice. Hosseini et al. [40] examined the individual and the combined outcome of NMN preconditioning and melatonin postconditioning on mitochondrial function and cardioprotection in myocardial ischemia/reperfusion injury of aged Wistar rats, because ischemic heart diseases are the foremost reasons for mortality and disability in elderly. This treatment ameliorated mitochondrial membrane potential, declined mitochondrial ROS and oxidative stress and restored the balance between the oxidised and reduced forms of NAD. The consequences of the combined therapy of NMN and melatonin on these beneficial effects were greater than those of individual treatments.

According to Kiss et al. [41] *in vivo* NMN supplementation enhanced NAD$^+$ levels followed by promoting SIRT1 activation and improving neurovascular functions and cognitive performances. These protective effects caused by NMN treatments on neurovascular function were predicted to be mediated by the involvement of genes in anti-apoptosis, anti-inflammatory and mitochondrial rejuvenation pathways which are attributable to versatile sirtuin-regulated anti-aging alterations in the neurovascular gene expression.

Miao et al. [42] showed that NMN supplementation improved the oocyte quality through restoring NAD$^+$ levels (nearly 50%) in mice. It increased ovulation, fertilisation capability and meiotic competency, while promoting cytoplasmic and nuclear maturation in order to maintain euploidy. Furthermore, NMN reinstated mitochondrial functions of aged oocytes to mitigate accumulation of DNA damage and reactive oxygen species, leading to low levels of apoptosis. This finding is echoed by Bertoldo et al. [43] who further indicated that restoration of fertility in aged mice and other benefits of NMN treatment could be recapitulated by the transgenic overexpression of SIRT2. Apart from rejuvenating oocyte quality of aged animals, NMN supported the embryo development, reversing the adverse consequences of elevated maternal age on developmental milestones by increasing NAD levels in ovarian tissue from nearly 200 to 300 pmol/mg. Fu and Zhang [44] conducted an experiment for a patent application for using β-NMN in preparation of anti-aging health-care products or drugs using aged mice. It was found that the NMN administration could extend the life span of mice by ~29%. Aging is the most influential determinant and the greatest known risk factor for neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [45]. It has been illustrated that elevation of NAD$^+$, sirtuins, CD38, PARP and SARM1 (sterile alpha and TIR motif containing 1) protein levels

through NMN supplementation were capable of inducing neuronal mitophagy to ameliorate cognitive decline in *Caenorhabditis elegans* model of AD [46].

Long et al. [47] suggested that depletion of cellular and mitochondrial NAD$^+$ contents induced mitochondrial abnormalities, activation of NAD glycohydrolases and bioenergetic failure as well as cell death. NMN supplementation decreased mitochondrial fragmentation and mutant amyloid precursor protein levels (38%) in mice without observing any detrimental side effects, while it enhanced mitochondrial bioenergetic functions.

Wang et al. [48] indicated that intraperitoneal administration of NMN restored learning and cognition in AD model Wistar rats, while enhancing energy metabolism and neuron survival and preventing ROS accumulation and the central biomarker (amyloid beta) induced neuronal death. Yao et al. [49] has also reported improvements of behavioural measures of cognitive impairments and reduction of multiple AD-linked pathological characteristics such as inflammatory responses, synaptic loss, amyloid plaque burden and β-amyloid production by inhibition of JNK activation in mice after subcutaneous administration of NMN.

According to Klimova et al. [50], *in vivo* NMN administration enhanced mitochondrial bioenergetics, overturned physiological decline and restrained postischemic NAD$^+$ depletion and cellular death. Furthermore, increasing NAD$^+$ levels in mitochondria (from 3.12 to 5.51 nmol/mg) by NMN supplementation caused many metabolic changes such as SIRT3-mediated decline in mitochondrial protein acetylation, which defended mitochondria by detrimental effects of ROS and excessive and impetuous fragmentation. In addition, according to Lu et al. [51], NMN could cause considerable beneficial effects, attenuate apoptosis and enhance mitochondrial inhibitor induced declining of energy metabolism in PD-like neuropathological and behavioural changes as resulted in cellular model of PD, utilising rotenone-treated PC12 cells. They investigated increased ATP1 and NAD$^+$ levels (30%) in PC12 cells, necrotic and apoptotic cell death and cell survival with NMN supplementation.

Fang et al. [52] investigated that approximately two folds repletion of NAD$^+$ through NMN unusually delayed accelerated aging and extended lifespan of *Caenorhabditis elegans* and *Drosophila melanogaster* models of Werner syndrome. A short-term treatment of NAD$^+$ precursor, nicotinamide riboside (NR) improved cochlear health, restored outer hair cell loss and prevented hearing loss in $CSB^{m/m}$ mice [53]. It showed that, as a precursor of NAD$^+$, NMN may have the same potential to prevent the age-related and Cockayne syndrome-related hearing loss. Fang et al. [54] demonstrated that boosting NAD$^+$ through NMN supplementation evidently extended *in vivo* lifespan, normalized neuromuscular functions, stimulated neuronal DNA repair and ameliorated neuropathological defects.

Safety concerns and challenges

The ultimate goal of geroscience is to discover approaches to boost natural defence mechanisms and prolong healthy lifespan through better management of the risks posed to cells and tissues of humans. Due to the continuous enhancement of the living standards of people, the desire of healthy longevity has become progressively stronger. Conversely, there is a possibility to achieve the maximum life span specified by the nature through decelerating the rate of aging. Using tiny molecules to slow down the aging process and enhancing aging-associated outcomes is a flourishing research area at the present [55]. Furthermore, at the present, the number of anti-aging medicines and health products are commercially available and popular among elderly consumers [56]. Along with the current concerns on aging as a natural biological

process, many researches on longevity are being conducted to understand and manage the aging process through anti-aging interventions, while complying with gerontological and biogerontological aspects.

Fu and Zhang [44] have applied for a patent application for using β-NMN in the preparation of healthcare products or anti-aging drugs as a single dose of 1–500 mg/kg body weight/day of β-NMN. The healthcare products or anti-aging drugs was in the forms of injections, enteric-coated preparations, aqueous solutions, granules, capsules or tablets. A number of NMN anti-aging health products have been produced and launched by various pharmaceutical, nutraceutical, biotechnology and health food companies. The quantity of NMN in available commercial products vary from 50 to 150 mg/capsule, whereas some consumers take two 150 mg capsules per day [57]. Nowadays, there are NMN products, marketed as supplements for anti-aging and longevity in the form of capsules, which are very high in dose such as ≥500 mg. The safety of these doses cannot be assessed since required clinical and toxicological studies have not been completed yet to establish the recommended safe levels for long term administration. Nevertheless, their safety and efficacy are uncertain and unreliable since most of them have not been backed up by rigorous scientific preclinical and clinical testing. This issue has been arisen as manufacturers are hesitant to pay for research and clinical trials due to potential lower profit margin, and there is no authorising agency to regulate NMN products because it is often sold as functional food product rather than heavily regulated therapeutic drug. Therefore, more strict approval process has been demanded by consumer advocacy groups requesting regulatory agencies to set standard and restrictions for marketing anti-aging health products, considering safety, health and wellbeing of consumers [58]. NMN should not be considered as a panacea for the elderly, because boosting NAD levels when not required may yield some detrimental effects. Therefore, the dose and frequency of NMN supplementation should be carefully prescribed depending on the type of age-related deficiency and all other confronting health conditions of the people [59]. Other NAD precursors have been studied to discover the efficacy for diverse age-related deficiencies and they are used for particular deficiencies, only after they are proven for effectiveness and safe to use. Therefore, the same principle should be applied to NMN as well [60,61].

Grozio et al. [62] reported that NMN was speedily absorbed in the small intestine by a specific transporter, which was encoded by the *Slc12a8* gene as demonstrated in *in vitro* and *in vivo* studies. Even though a large number of preclinical studies have provided a highly promising possibility for developing NMN as an evidence-based anti-aging health product, its safety and effectiveness on human physiology remains unclear. The toxicological and clinical evidence to back up its utility is currently scarce. Despite this, NMN supplements are already available in the market and considerably used by consumers as anti-aging health products. Therefore, in addition to animal models, human trails to investigate the safety and efficacy of NMN should be conducted focusing on toxicological parameters and safe metabolite levels in the human body [8].

Increased attention on potential pro-longevity effects of NAD^{+} precursors over the recent years has led to increased consumption of nicotinamide either as a clinical treatment or a dietary supplement, raising concerns on the safety of their long-term usage. Nicotinamide has been found to cause adverse effects on several organs in the human body such as kidney, liver, pancreatic β-cells and cells in plasma and induce nausea, stomach discomfort and headaches [63,64]. In addition, high dose of nicotinamide supplementation is associated with decreased insulin sensitivity and increased oxidative stress [64]. However, NMN supplementation has been found to have significant recovering effects on hepatocyte functions and liver pathologies in early-stage of ethanol toxicity,

instead of causing adverse effects to the liver [65]. It has also been found to improve insulin sensitivity and oxidative stress in animal models [23,29].

Although, nicotinamide supplementation induced DNA damage and oxidative DNA damages in various tissues of the body such as renal and hepatic tissues [66], NMN has been proven to reduce DNA damage and accumulation of ROS [32,42]. Supplementation of nicotinamide caused neurodegeneration of dopaminergic neurons and structural brain changes and behavioural deficits in rats [67]. Supplementation of NMN has been demonstrated to stimulate neuroprotective effects and ameliorate cognitive decline and behavioural dysfunctions [35,36]. Rolfe [68] reported that administration of nicotinamide and niacin may cause development of PD, whereas supplementation of NMN has been found to be a promising therapeutic remedy for PD [51].

NR is also a precursor of NAD^{+} and administration of NR has the capability to elevate body NAD^{+} levels. According to the results of clinical trials that have been performed to assess the safety of NR administration, NR supplementation increased LDL cholesterol levels in the body [69] and enhanced fatty liver conditions [70] as adverse effects. Nevertheless, NMN administration was observed to improve plasma lipid profile, ameliorating free fatty acids, triglycerides and cholesterol levels and lower intrahepatic triglyceride levels in mice [10]. Shi et al. [71] have shown that excessive dose of NR generated white adipose tissue dysfunction and heightened insulin resistance in mice. However, contradictory results have been reported with NMN supplementation as it reduced adiposity and enhanced insulin sensitivity [24,72].

NMN as another precursor of NAD^{+}, has the potential to encounter similar safety concerns and challenges as other NAD^{+} precursors. Thus, further clinical studies on the safety and toxicology of NMN are urgently needed. Mehmel et al. [60] argued that NR is a more suitable NAD^{+} precursor as an anti-aging treatment. This highlights the needs to study adverse effects of nicotinamide and NMN, even though NMN appears to have considerable beneficial pharmacological actions in preclinical studies. Yoshino et al. [57] conducted a clinical trial, supplementing 250 mg/day of NMN for 10 weeks to 25 prediabetes women who were overweight or obese in order to identify effect on body composition, gene expression profile, insulin signalling and insulin sensitivity and observed potential metabolic benefits without any adverse effects.

A toxicological study for NR chloride has been done in a clinical setting, and the no observed adverse effect level (NOAEL) was 300 mg/kg/day and the lowest observed adverse effect level was 1000 mg/kg/day [73]. No such data is available for human administration of NMN yet. However, Cros et al. [74] investigated sub-chronic oral toxicity, acute oral toxicity, genotoxicity and mutagenicity of high purity synthetic form of NMN (NMN-C®) using female Sprague-Dawley rats (7 weeks old). According to the results, NOAEL of NMN-C® was ≥1500 mg/kg/d. At an oral administration dose of 2666 mg/kg of NMN-C®, no treatment-associated adverse effects or mortality were nor resulted. NMN-C® did not show toxic effects and appeared to be safe over a 90-day sub-chronic period of repeated oral administration at doses of 375, 750 and 1500 mg/kg/d.

Conze, Crespo-Barreto, and Kruger [75] determined safety of a synthetic form of NR namely, Niagen™ using *in vitro* and rat toxicological study, and found that it was not genotoxic. The lowest observed adverse effect level for Niagen™ was 1000 mg/kg body weight/day and NOAEL was 300 mg/kg body weight/day. Furthermore, NR has been examined in six clinical trials, where it has been established that it is safe for short-term (8 days) and long-term (6 weeks) supplementation in compliance with confirmed oral availability [60]. These values have not been established regarding NMN supplementation. Furthermore, recently, NR has been granted Generally Recognised as Safe (GRAS) status by the US Food

and Drug Administration (FDA), which is yet to be achieved for NMN.

Mills et al. [10] reported that *in vivo* long-term administration of NMN mitigated age-associated physiological decline, effectively without generating noticeable toxicity, deleterious side effects or raised mortality. Tsubota [76] reported that the first phase I human clinical study (UMIN000021309) has been initiated aiming at evaluating the bioavailability, mechanism of action and the safety of NMN in the human body. This has been a collaborative study between Keio University School of Medicine in Tokyo and Washington University School of Medicine in St. Louis. Using ten healthy volunteers, the time course of blood NMN concentration and the safety of NMN administration were assessed, which was intended for developing anti-aging nutraceutical. However, the research team has been unsuccessful in detecting NMN in plasma samples.

Irie et al. [77] conducted a non-blinded clinical trial using 10 healthy men to investigate the safety of oral NMN administration and the pharmacokinetics of nicotinamide metabolites at the Keio University School Medicine, Japan. They found that single oral administration of 100, 250 and 500 mg of NMN doses was well-tolerated and safe since it did not cause any observable clinical symptoms or changes in body temperature, oxygen saturation, blood pressure and heart rate. In addition, significant changes in ophthalmic, ocular fundus and neurological system parameters were not observed after NMN administration. There were no changes in the results of laboratory analysis of urine and blood as well as sleep quality and score before and after the NMN administration. Oral administration of NMN increased serum bilirubin contents and decreased blood glucose, chloride and serum creatinine levels, but within the normal range. NMN administration did not increase nicotinamide in blood to the level which causes adverse effects associated with high dose of nicotinamide. Since the safety of single oral administration of NMN has only been considered in this study, further clinical investigations are essential to be performed to assess the safety and efficacy of long-term administration of NMN. The organs and the tissue NMN levels have not been analysed in this study, which should be considered in future clinical studies.

As stated by Hong et al. [15], three more human clinical studies are being carried out to evaluate the safety concerns of NMN administration. One study is a phase II study (UMIN000030609), which has been initiated by the Keio University School of Medicine, Japan to evaluate the safety of long-term administration of NMN, pharmacokinetics and metabolites of NMN, and its effect on glucose metabolism in healthy adults. Another study is being performed to assess the effect of long-term intake of NMN on different hormones in healthy individuals (UMIN000025739) by the Hiroshima University, Institute of Biomedical and Health Sciences. The third clinical study (UMIN000036321) has been designed to assess the consequences of oral administration of NMN on the body composition in elderly people at the University of Tokyo Hospital. These clinical trials are still ongoing and there is no result published yet. Yoshino et al. [23] have also highlighted the importance of more comprehensively assessing potential adverse effects of NMN by conducting preclinical and clinical studies, considering different dietary conditions as well.

According to Di Stefano et al. [78], inhibitors of NMN synthesising enzyme (NAMPT), provided robust functional and morphological protection of injured synapses and axons, regardless of reducing NAD. But, exogenous NMN eliminated this protection with the accumulation of NMN after nerve injury and NMNAT2 degradation advanced axon degeneration. They suggested that the relationship between the increase of NMN and axon degeneration could be a long-hypothesised toxic and deleterious factor. Poljsak and Milisav [79] reported that both NR and NMN, as vitamin B_3 forms and NAD$^+$ precursors were not detected to be

cancerogenic. Radenkovic and Verdin [80] also have stated that since no study has reported rigorous side effects and they have been in use for many years, therapeutic interventions including NMN, NR, vitamin B_3 and niacin supplementation, which increase NAD$^+$ levels, are likely to be fairly safe for the human use.

Radenkovic and Verdin [80] further explained that side effects of long-term NAD upregulation are complicated to identify and quantify, but they still can exist. In addition, it was illustrated that prolonged high dose of vitamin B_3 compounds, including NMN may possibly have long-term side effects. According to Rolfe [68], NAD upregulation has the possibility to make worse the senescence-associated secretory phenotype (SASP) generated by senescent cells in aged tissues. A suggested mechanism to cause this side effect was the suppression of 5′ adenosine monophosphate-activated protein kinase and tumor protein p53, directing to stimulate nuclear factor kappa B protein transcription factor through p38 mitogen-activated protein kinases and raised expression of inflammatory cytokines. The cellular senescence burden enlarged with aging, while the produced ASAP was a responsible factor for different age-related pathologies [81].

However, evidences to support these potential side effects of long-term NMN usage and NAD upregulation are lacking. In fact, the usage of anti-aging interventions is expected to be initiated at comparatively younger and healthier age than very old age. Therefore, identification and quantification of adverse effects and challenges of long-term utilisation of anti-aging health products is extremely essential and critical since these anti-aging products are apparently used for a long time. According to Yu et al. [82], NMN treatment (400 mg/kg) obstructed the exercise-induced benefits of a mouse model of diet-induced obesity such as reduced hepatic triglyceride accumulation, glucose stimulated insulin secretion from islets and glucose tolerance. This finding should be further investigated thoroughly since the exercise is one of the key components for maintaining health and wellbeing of the elderly.

Conclusions

NMN is a precursor of NAD$^+$ and an intermediate of NAD$^+$ biosynthesis, which is achieved through three pathways. NMN is an intermediate by-product in two of them. NAD$^+$ levels in the body are depleted with aging as a result of activities of NAD$^+$ consuming enzymes. Depletion of NAD$^+$ level is associated with down-regulation of energy production in mitochondria, increasing oxidative stress, DNA damage, cognitive impairments and inflammatory diseases. NMN, as the precursor of NAD$^+$, has been seen to likely reverse these age-related complications and slow down the rate of aging by enhancing NAD$^+$ levels in the body.

Many studies have been done to explore NMN's anti-aging effects in various cells and tissues. Most of the works have been done *in vitro* or in animal models. However, published reports about NMN's long-term safety and clinical efficacy of anti-aging effects in humans are scarce. From the above review, it can be seen that only very few pre-clinical and clinical studies have been conducted to investigate the safety of long-term administration of NMN. A few more human clinical trials are being conducted to evaluate the safety concerns of NMN supplementation and the outcomes are yet to be available.

However, many NMN anti-aging health products are already available in the market and manufacturers are aggressively marketing the products using *in vitro* and *in vivo* results from the literature. Therefore, the first priority should be to establish toxicology, pharmacology and safety profiles of NMN in humans, including healthy and diseased. For NMN's anti-aging efficacy, the most feasible route to obtain data will probably through long-term follow-

ups of people who consume NMN regularly. Such research should be supported by NMN manufacturers as they have the moral responsibility to provide efficacy results of their products.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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