Trends in Cancer

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Review

NAD^+ Deficits in Age-Related Diseases and Cancer

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The phenomenon of aging has gained widespread attention in recent times. Although significant advances have been made to better understand aging and its related pathologies including cancer, there is not yet a clear mechanism explaining why diseases and cancer are inherent parts of the aging process. Finding a unifying equation that could bridge aging and its related diseases would allow therapeutic development and solve an immense human health problem to live longer and better. In this review, we discuss NAD^+ reduction as the central mechanism that may connect aging to its related pathologies and cancer. NAD^+ boosters would ensure and ameliorate health quality during aging.

Aging, Age-Related Diseases and Cancer

Most Western societies saw significant increase in mean life expectancy during the past decades, and a growing elderly population. People live longer due to environmental factors, changes in lifestyle and improvements in medical care and public health. According to WHO, the world's population over 60 years will nearly double between 2015 and 2050[i]. Yet, living longer is not equivalent to living healthier.

Aging (see Glossary) is a multifactorial process that causes a progressive dilapidation of physiological functions, leading to vulnerability and death [1]. Even though we can expect longer lives, quality of life is affected by **age-related diseases**. Metabolic syndrome including cardiovascular disorders, type 2 diabetes and obesity, neurodegenerative diseases such as Alzheimer and Parkinson diseases, arthritis, and osteoporosis, and cancer are some of the diseases that commonly affect human health during aging. The increasing incidence of these diseases in a population that is living longer demands a better understanding of the aging process and its consequences. Yet, there is a fundamental question which has not been thoroughly answered: why does the risk of diseases and particularly cancer increase as we age?

There is not a unique answer to explain the mechanisms of aging leading to cancer, and various molecular players have been reported, but no cellular pathway has yet been able to explain why disease is an inherent part of the aging process. Aging *per se* may not be responsible for the development of age-related pathologies, but rather the disruption of a critical pathway may drive aging, and in parallel facilitate the development of diverse pathologies including cancer. Connecting aging and its related diseases through a unifying equation would disentangle a convoluted human health problem and would facilitate the search and development of novel therapeutics to live longer and better.

In this review, we discuss, argue, and speculate that a decrease in nicotinamide adenine dinucleotide (NAD^+) is at the heart of the aging process and its associated diseases, including cancer (Figure 1, Key Figure and Box 1). If so, NAD^+ levels may gradually decline over a lifespan,

Trends

The increase in life expectancy during the last decades was accompanied by a rise in the incidence of diseases related to aging, including cancer.

Diseases and cancer are inherent parts of the aging process and aging can be considered as a disease among other diseases while we age.

NAD^+ levels are described to decrease during aging, likely through changes in metabolic reactions leading to NAD^+ synthesis.

Models for age-related diseases and cancer show reductions in NAD^+ pools.

Boosting NAD^+ through precursors such as NAM, NMN or NR may increase longevity and prevent age-related diseases and cancer in animal models.

Beneficial effects of dietary restriction on lifespan and cancer may converge to increases in NAD^+ levels.

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Key Figure

Decrease in NAD+ Levels May Be at the Heart of Age-Related Diseases and Cancer

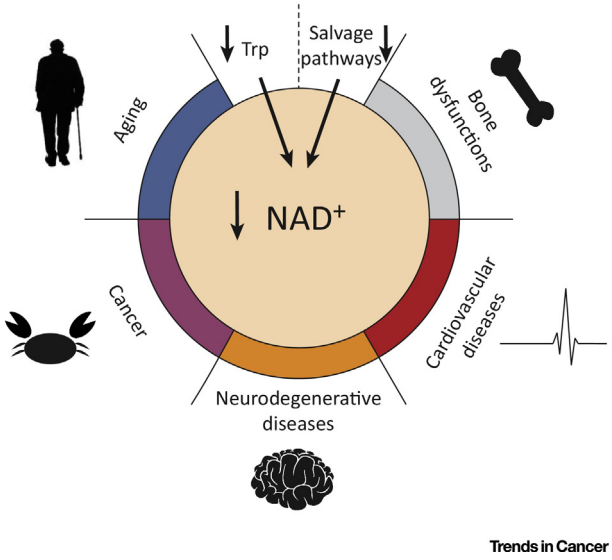

Figure 1. Age-related diseases and cancer could be the consequence of a decline in NAD+ concentrations [alterations or reductions in de novo NAD+ synthesis and/or in the salvage pathways (Box 1)]. Depletion in NAD+ levels during aging may lead to the appearance of different diseases including cancer affecting quality of life in the elderly population. Replenishing NAD+ may thus extend lifespan and prevent age-related diseases and cancer. Abbreviations: NAD+, nicotinamide adenine dinucleotide; Trp, tryptophan.

linking both aging and the incidence, or severity, of age-related diseases. Aging is thus a disease among others that appears temporally and NAD+ reduction may bridge aging to its related pathologies. Because the phenomenon of aging has gained widespread attention in recent times, we try to shed light through the published literature on why NAD+ levels may decline during aging and how this decline is linked to age-related diseases and cancer. We propose that NAD+ precursors such as **nicotinamide (NAM), nicotinamide riboside (NR)** or **nicotinamide mononucleotide (NMN)** (see Figure IA in Box 1) may have potential therapeutic values against age-related diseases and cancer. Finally, we discuss the beneficial health effects of **dietary restriction (DR)** that may converge to NAD+ increases. NAD+ depletion may be the unifying equation that could connect aging and its related diseases, including cancer.

Decline of NAD+ during Aging, Age-Related Diseases, and Cancer

NAD+ Degradation Increases during Aging

Several evidences suggest a decline in NAD+ levels while we age, connecting NAD+ deficits to age-related diseases and cancer. Inflammation increases during the aging process, possibly due to the presence of senescent cells [1]. CD38 and bone marrow stromal cell antigen-1 (BST-1) may provide explanations to NAD+ decline during aging. CD38 is a membrane-bound hydrolase implicated in immune responses and metabolism. NAD+ can be degraded through its hydrolysis, deacetylation, or by NAD+ nucleosidases (also called NAD+ hydrolases or NADases) such as CD38. Expression and activity of CD38 increase in older mice, promoting NMN degradation *in vivo*, responsible for NAD+ decline and mitochondrial dysfunctions [2].

Glossary

Aging: multifactorial process that causes a progressive dilapidation of several physiological functions, leading to vulnerability and death.
Age-related diseases: diseases considered as inherent parts of the aging process and thus, that appear gradually during aging representing a health deterioration of elderly.
Dietary restriction (DR): reduction of macro- and micro-nutrients intake without causing malnourishment. Dietary restriction is different from caloric restriction in which total calorie consumption is diminished independent of nutrient ratios or proportions.
L-Tryptophan/kynurenine pathway/*de novo* NAD+ synthesis: a metabolic pathway leading to the production of NAD+ from the degradation of the essential amino acid L-tryptophan into quinolinic acid in the kynurenine pathway.
Nicotinamide (NAM): vitamin B3 derivative also known as niacinamide and NAD+ biochemical precursor. NAM is converted into nicotinamide mononucleotide (NMN) by the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAmPRTase or NAMPT), enabling NAD+ biosynthesis in mammals.
Nicotinamide mononucleotide (NMN): nucleotide and NAD+ booster derived from ribose and nicotinamide. As a phosphorylated compound, NMN is considered as a biosynthetic intermediate rather than a vitamin B3 precursor of NAD+.
Nicotinamide riboside (NR): vitamin B3 derivative converted to NAD+ in a two-step reaction, first in NMN by **nicotinamide riboside kinase (NRK)-dependent phosphorylation** and second, by adenylylation of NMN by **nicotinamide mononucleotide adenylyltransferase (NMNAT).**
Nicotinic acid (NA) or niacin: organic compound and essential nutrient that build in presence of nicotinamide (NAM) the vitamin B3 complex.
Salvage reactions or pathways: intermediates from nucleotide degradation that can be used as substrates to reenter the synthetic metabolic pathway during nucleotide synthesis. NAD+ is generated through salvage pathways from nicotinic acid (NA), nicotinamide (NAM), and nicotinamide riboside

(NR) which represent alternative routes to *de novo* synthesis of NAD^+.

Interestingly, loss of CD38 inhibits glioma progression and extends the survival of glioma-bearing mice. Targeting CD38 in the tumor microenvironment may clearly serve as a novel therapeutic approach to treat glioma [3]. Daratumumab, a CD38 monoclonal antibody, represents a first-in-class drug for the treatment of multiple myeloma. It promotes T cell expansion through inhibition of $CD38^+$ immunosuppressive cells, improving patients' responses [4]. These findings suggest that NAD^+ boosters should be combined with CD38 inhibitors for a more efficient antiaging therapy (Figure 2).

BST-1 is a glycosylphosphatidylinositol-anchored molecule and signaling receptor, a key regulator of leukocyte trafficking [5]. Importantly it has some NAD^+ nucleosidase activity [6]. It is enriched in bone marrow stromal cell lines derived from patients with rheumatoid arthritis and is known to facilitate pre-B cell growth [7,8]. The polyclonal B cell abnormalities in rheumatoid arthritis in elderly individuals can be partly attributed to BST-1 overexpression in the stromal cell population but also to its NADase activity reducing NAD^+ levels [6–8]. Evidences obtained by *in vitro* experiments and from correlative clinical studies in ovarian cancer patients suggest a functional link between high expression of BST-1 and tumor cell malignancy, including their increased motility, adhesion, migration, and invasion of surrounding tissues [5,9]. However, whether these cancer phenotypes are associated to the BST-1 NAD^+ hydrolase activity and thus, NAD^+ degradation remains to be determined.

NAD^+ Biosynthesis Decreases during Aging, Age-Related Diseases, and Cancer

As we age, our bodies undergo changes in metabolism, and a key part of these processes may affect ***de novo* NAD^+ synthesis,** also called the **L-tryptophan/kynurenine pathway** (see Figure IB in Box 1). In mammals, the use of the *de novo* NAD^+ biosynthetic pathway is limited to a few specific organs. By checking the expression of L-tryptophan 2,3-dioxygenase (TDO2), the rate-limiting enzyme of the L-tryptophan-kynurenine pathway, publicly available datasets

Box 1. Synthesis and Role of NAD^+

NAD^+ is synthesized through different routes, either **salvage reactions** implicating the utilization of **nicotinic acid or niacin (NA), nicotinamide (NAM) and nicotinamide riboside (NR)**, or ***de novo* NAD^+ synthesis (L-tryptophan/kynurenine pathway)** [50,152,153] (Figure IA). In 1958, Preiss and Handler identified the intermediates and enzymes involved in the salvage reactions in which conversion of NA leads to the generation of NAD^+ in yeast and erythrocytes, familiarly called the Preiss–Handler route. During a phosphoribosylation step, NA is converted into NA mononucleotide (NAMN), which afterwards produces NA adenine dinucleotide (NAAD) during an adenyltransferase reaction; ultimately, NAAD generates NAD^+ [36,154].

NAD^+ increases can also occur independently of the Preiss–Handler route. NAM and NR are important NAD^+ precursors first converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT) and NR kinase (NRK), respectively. NMN is then transformed into NAD^+ by NMN adenylyltransferase [36].

The major pathway sustaining NAD^+ levels is the L-tryptophan/kynurenine pathway leading to *de novo* NAD^+ synthesis (Figure IB). Degradation of L-tryptophan in the kynurenine pathway converging to NAD^+ biosynthesis happens in all vertebrates and almost all eukaryotic cells. L-Tryptophan is first oxidized into N-formylkynurenine (NFK) leading to the accumulation of kynurenine. Kynurenine is then converted into either 3-hydroxykynurenine (3-HK) in peripheral organs or in anthranilic acid (ANA), mainly in the brain. 3-HK or ANA are then oxidized into 3-hydroxyanthranilic acid (3-HANA) to generate 2-amino-3-carboxymuconic-6-semialdehyde (ACMS) which is spontaneously converted into quinolinic acid (QA) (Figure IB). QA is decarboxylated to generate NAMN, which is then converted into NAD^+ through the Preiss–Handler route.

NAD^+ is a well-known oxidizing agent that can be reduced to form NADH, a reducing agent and electrons donor. It participates in different cellular processes, as a signaling molecule ensuring cell survival and regulating energy metabolism, cellular repair, and circadian rhythms. Various enzymes including the sirtuins deacetylases (SIRT1–SIRT7) and poly-ADP-ribose polymerases (PARPs) use NAD^+ as substrate for their reactions [155]. Some members of this family of enzymes can sense physiological NAD^+ fluctuations powering their enzymatic reactions. If NAD^+ levels decline over aging, this fall may affect enzyme activities and hence, several cellular processes leading to an overall metabolic defect that can accelerate age-associated diseases and cancer development.

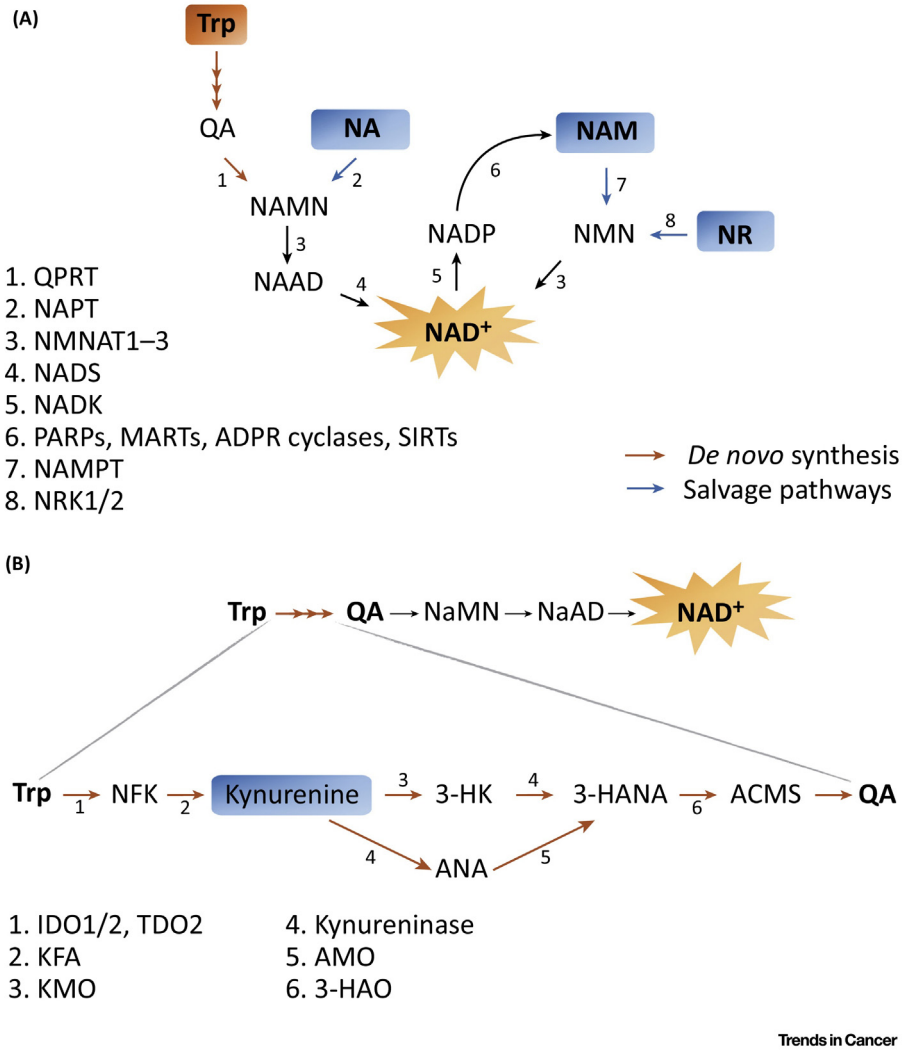

Figure I. Routes of NAD^+ Synthesis. (A) NAD^+ can be synthesized either from L-tryptophan (Trp) degradation through the *de novo* pathway, or through salvage reactions by reusing metabolites such as nicotinic acid (NA), nicotinamide (NAM), or nicotinamide riboside (NR). Trp is metabolized through a multi-step reaction process to quinolinic acid (QA), which is after decarboxylated to NA adenine mononucleotide (NAMN). In parallel, NA is phosphorybosylated to form NAMN. Both the *de novo* NAD^+ synthesis and the salvage pathways through NA converge into NAMN. NAMN is transferred an adenyl group, producing NA adenine dinucleotide (NAAD). Finally, NAAD generates NAD^+. NAM and NR are first converted to nicotinamide mononucleotide (NMN), NMN is then transformed into NAD^+. After NAD^+ utilization by enzymatic reactions, it can be converted into NAM to reenter the biosynthetic pathway. (B) Trp is the initial substrate of the *de novo* NAD^+ synthesis. Trp is converted to QA through oxidation reactions. Tryptophan is first oxidized into N-formylkynurenine (NFK), which is converted into either 3-hydroxykynurenine (3-HK) in peripheral organs or in anthranilic acid (ANA), mainly in the brain. 3-HK or ANA are oxidized into 3-hydroxyanthranilic acid (3-HANA) to generate 2-amino-3-carboxymuconic-6-semialdehyde (ACMS). ACMS is spontaneously converted into QA. QA is transformed to NAMN, which enters in the Preiss–Handler route to form NAD^+.

Abbreviations: NAD^+, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; QPRT, quinolinate phosphoribosyl transferase; NAPT, nicotinic acid phosphoribosyl transferase; NMNAT1–3, nicotinamide mononucleotide adenylyl transferases 1 to 3; NADS, NAD synthetase; NADK, NAD kinase; PARPs, poly-ADP-ribose polymerases; MARTs, mono-ADP ribosyltransferases; ADPR cyclases, ADP ribosylcyclases; SIRTs, sirtuins; NAMPT, nicotinamide phosphoribosyl transferase; NRK1,2, nicotinamide riboside kinase 1 and 2; IDO1/2, indoleamine 2,3-dioxygenase 1/2; TDO2, tryptophan 2,3-dioxygenase 2; KFA, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; AMO, anthranilate 3-monooxygenase; 3-HAO, 3-hydroxyanthranilate 3,4 dioxygenase.

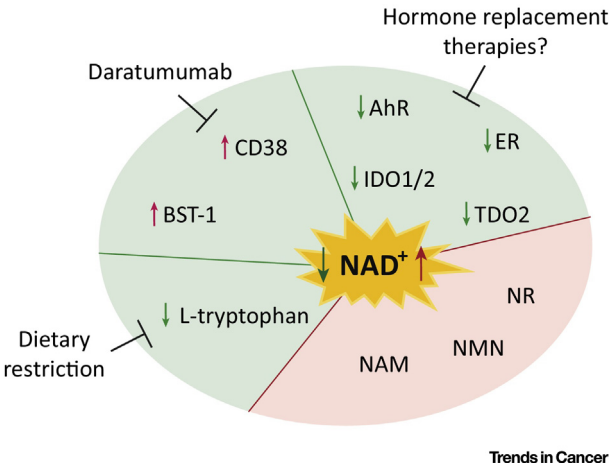

Figure 2. Modulation of NAD^+ Levels. Several mechanisms can explain the decline of NAD^+ levels during aging. First, activity of NAD^+-degrading enzymes, such as CD38 and bone marrow stromal cell antigen-1 (BST-1) can increase during the aging process. Second, enzymes (IDO1/2 and TDO2) degrading L-tryptophan to NAD^+ can be affected. Levels of ER and AhR, transcription factors of TDO2, may also decrease during aging. Finally, decreased levels of L-tryptophan due to a reduction in protein consumption or inefficient metabolism may reduce *de novo* NAD^+ synthesis. Several strategies can replenish NAD^+ pools. Inhibition of CD38 by the monoclonal antibody daratumumab can prevent NAD^+ degradation by this enzyme. ER and AhR activation by hormone replacement therapies may boost NAD^+. Dietary restriction may also cause muscle mass waste- or autophagy-mediated release of essential amino acids, including L-tryptophan, and therefore its degradation to NAD^+. Boosting NAD^+ directly through salvage reactions by providing precursors – nicotinamide (NAM), nicotinamide mononucleotide (NMN) or nicotinamide riboside (NR) – may ensure NAD^+ replenishment.

argue that this pathway is predominantly active in bone marrow/immune system, muscle tissues, liver/gall bladder, gastrointestinal tract, and kidney[ii]. Other reports also describe the liver, brain, and kidney as the main organs expressing TDO2 [10,11]. However, these studies are conducted by checking mRNA or protein quantities and metabolite production should be measured in different organs to have conclusive results on the activity of this pathway.

Several biological systems are affected by changes in the L-tryptophan/kynurenine pathway during aging. A reduction in the concentration of the essential amino acid, L-tryptophan, with age has also been revealed in aged Wistar rats [11] and humans [12]. This decrease in L-tryptophan while we age can be caused by the loss of the metabolic efficiency of the *de novo* NAD^+ synthesis pathway, or can be a consequence of increased NAD^+ demands in certain tissues over time (Figure 2). This can also be explained by a reduction of protein consumption in the elderly population decreasing the uptake of L-tryptophan. Indeed, high protein intake has health beneficial effects in the elderly population [13]. Moreover, a reduction with age of TDO2 or indoleamine 2,3-dioxygenase 1/2 (IDO1/2) activities, the rate-limiting step enzymes implicated in L-tryptophan degradation in the kynurenine pathway, occurs in several rat tissues [11]. Additionally, the activity of quinolinate phosphoribosyl transferase (QPRT), the enzyme mediating the conversion of quinolinic acid (QA) into NAD^+ (see Figure IB in Box 1), is reportedly reduced in liver and brain tissues of aged Wistar rats [11]. Finally, dysregulation of the kynurenine pathway is also linked to genetic disorders and age-related diseases such as obesity and cancer [14,15]. These age-associated changes in *de novo* NAD^+ biosynthesis may have the potential to impact several biological processes, and thus contribute to age-related diseases and cancer in the elderly. Animal models mimicking downregulation of NAD^+ biosynthesis are needed to modulate its activity and understand its pathophysiological relevance in age-related pathologies and cancer.

Conversely to the above findings, depletion of TDO2 in *Caenorhabditis elegans* suppresses toxicity of aggregation-prone proteins such as amyloid-β and polyglutamine proteins that play an important role in age-related neurological diseases like Parkinson and Alzheimer diseases [16]. Importantly, depletion of TDO2 extends lifespan in these worms and increases L-tryptophan concentrations. Feeding worms with L-tryptophan also suppresses toxicity, suggesting that TDO2 regulates proteotoxicity through L-tryptophan [16]. These findings may be explained by the fact that decreased TDO2 levels can lead to less production of QA (see Figure IB in Box 1), a selective N-methyl-D-aspartate receptor agonist found in mammalian brain which contributes to the pathophysiology of neurological disorders [17]. In support of this, pharmacological inhibition of kynurenine 3-monooxygenase (KMO), another enzyme of the kynurenine/*de novo* NAD^+ synthesis pathway (see Figure IB in Box 1), increases kynurenic acid levels and reduces extracellular glutamate in the murine brain, preventing disease progression in Alzheimer and Huntington disease mouse models [18]. Thus, activation of the kynurenine pathway may account for the production of neurotoxic intermediates (e.g., QA) and favor the development of neurological disorders (Figure 2). Consistently, brain IDO2 activity and metabolites from the L-tryptophan/kynurenine pathway increased in aged Wistar rats [11]. Although more studies related to the inhibition of *de novo* NAD^+ synthesis should be conducted in organs other than brain to have conclusive results, replenishment of NAD^+ stores through **salvage reactions** may circumvent neurotoxicity (Figure 2). If so, relative levels of NAD^+ may serve as an indicator to predict longevity and the development of age-related diseases and cancer.

A major question still remains to be answered: how and why does the *de novo* NAD^+ biosynthetic pathway decrease over age? The inhibition of the nuclear transcription factors estrogen receptor (ER) and the aryl hydrocarbon receptor (AhR) has been shown to inactivate *de novo* NAD^+ synthesis [19] (Figure 2). Interestingly, the activity and expression levels of these receptors decline during aging, as well as in different models of carcinogenesis [20,21]. It is reported that the co-chaperone unconventional prefoldin RPB5 interactor (URI), which has oncogenic activities, acts in a complex with the heat shock protein 90 (HSP90) to repress nuclear translocation and hence activity of AhR and ER-mediated transcription of enzymes of the kynurenine pathway implicated in *de novo* NAD^+ synthesis [19]. This inactivation leads to reduction of NAD^+ levels, increased DNA damage, and liver tumorigenesis [19]. Several studies demonstrate the protective role of estrogen and decreased ER activity can lead to the development of age-related diseases and cancer [15,22,23]. ER activity may also explain the gender disparity in aging, as female life expectancy is significantly higher than that of men. Clearly, studies aiming to determine differences in NAD^+ levels between men and woman, or in individuals receiving hormone replacement therapy, would shed light on this question.

Experiments have also shown that mice with an AhR-null allele experience premature hematopoietic stem cell exhaustion and develop myeloproliferative disorders during aging [24]. Moreover, decreases in AhR activity and protein levels over the human lifespan are associated with macular degeneration, which is the most common cause of blindness and visual impairment among the elderly [25]. This is supported by the observation that AhR homozygous null mice present age-related macular degeneration-like pathology [25]. Additionally, these mice exhibit different age-related lesions in several other organs, including liver tumors [26]. Although direct evidences on the causal relationship between ER/AhR and NAD^+ reductions still need to be provided, mechanisms linking inactivation of these nuclear receptors, inhibition of *de novo* NAD^+ synthesis, and liver cancer have been recently reported [19].

Boosting NAD^+ in Age-Related Diseases and Cancer

Boosting NAD^+ with Niacin in Age-Related Diseases and Cancer

In humans, a lack of **nicotinic acid (NA**, also called **niacin)** in the diet causes the vitamin B3 deficiency disease pellagra, characterized by changes in the skin with very characteristic

pigmented sunburn-like rashes developing in areas that are exposed to sunlight. Likewise, people with chronic L-tryptophan-poor diets or malnutrition develop pellagra. Furthermore, several epidemiologic studies in human reported an association between incidence of certain types of cancers and niacin deficiency [27]. In this regard, low dietary niacin has also been associated with an increased frequency of oral, gastric, and colon cancers, as well as esophageal dysplasia. In some populations, it was shown that daily supplementation of niacin decreased esophageal cancer incidence and mortality. Although the molecular mechanisms of niacin deprivation and cancer incidence are not well understood, it has been recently reported that NAD^+ depletion leads to DNA damage and increased tumorigenesis, and boosting NAD^+ levels is shown to play a role in the prevention of liver and pancreatic cancers in mice [19,28,29].

Thus, malnutrition through inadequate amounts and/or diversity of food may affect the intracellular pools of nicotinamide and NAD^+ thereby influencing cellular responses to genotoxic damage, which can lead to mutagenesis and cancer formation [19,27]. NAD^+ boosters are therefore essential in patients at risk of exposure to genotoxic and mutagenic agents, including ionizing or UV radiations or, DNA damaging chemicals. In addition, niacin deficiency in combination with carcinogenic agents was described to induce and increase tumorigenesis in rats and mice. For instance, in rats, the lack of niacin together with carcinogen treatment increased tumorigenesis and death of rats [30,31]. Additionally, in mice, the incidence of skin tumours induced by UV was significantly reduced by local application of NAM or by niacin supplementation in the diet [32].

Boosting NAD^+ with NAM in Age-Related Diseases and Cancer

Recent research has focused on uncovering the consequences of a decrease in NAD^+ during aging using age-related disease models. In PGC1α knockout mouse, a model of kidney failure, NAD^+ levels are reportedly decreased, and boosting NAD^+ by NAM improves kidney function [33]. NAM injections during four days re-establish local NAD^+ levels via **nicotinamide phosphoribosyltransferase (NAmPRTase or NAMPT)** activation and improve renal function in postischaemic PGC1α knockout mice [33]. Surgical resection of small renal tumors can induce kidney ischemia severely affecting the renal function. Therefore, NAD^+ boosters can be beneficial to protect the organ from severe injury. Moreover, in a model of muscular dystrophy in zebrafish, NAD^+ increases, which functions as an agonist of muscle fiber–extracellular matrix adhesion, and corrects dystrophic phenotype recovering muscle architecture [34].

Boosting NAD^+ with NR in Age-Related Diseases and Cancer

Further research has extensively used NR to ameliorate the effects of NAD^+ deficits in pleiotropic disorders. NR naturally occurs in milk [35,36]. NR is converted to NAD^+ in two step reactions by **nicotinamide riboside kinases (NRKs)**-dependent phosphorylation and adenylylation by **nicotinamide mononucleotide adenylyl transferases (NMNATs)** [36]. It is considered to be a relevant NAD^+ precursor *in vivo*. Evidences demonstrate the beneficial effect of NR in skeletal muscle aging [37,38] and mitochondrial-associated disorders, such as myopathies [39,40] or those characterized by impaired cytochrome c oxidase biogenesis affecting the respiratory chain [41]. In line of these findings, a mouse model of Duchenne muscular dystrophy present significant reductions in muscle NAD^+ levels accompanied with increased poly-ADP-ribose polymerases (PARP) activity, and reduced expression of NAMPT [42]. Replenishing NAD^+ stores with dietary NR supplementation improved muscle function in these mice through better mitochondrial function [42]. Additionally, enhanced NAD^+ concentrations by NR are apparently beneficial for some neurodegenerative diseases [43], as well as in noise-induced hearing loss [44]. NR-mediated NAD^+ repletion is also protective, and even therapeutic, in certain metabolic disorders associated with cancer, such as fatty liver disease [28,45] and type 2 diabetes [28,46]. Metabolic disorders characterized by defective mitochondrial function could also benefit from an increase in NAD^+ levels. Indeed, stimulation of the

oxidative metabolism in liver, muscle, and brown adipose tissue potentially protects against obesity [47]. Interestingly, NAMPT protein levels are not affected in chow- and high fat diet (HFD)-treated mice fed with NR, arguing that in models of obesity, NR directly increases NAD^+ levels without affecting other salvage reactions [47]. Recently, diabetic mice with insulin resistance and sensory neuropathy treated with NR reportedly show a better glucose tolerance, reduced weight gain and liver damage, and protection against hepatic steatosis and sensory and diabetic neuropathy [48]. However, further evidences suggest that hepatic $NADP^+$ and NADPH were significantly degraded in these mice and NAD^+ levels were not significantly increased after treatment. Moreover, in this model, the antidiabetic and neuroprotective effects of NR may be due to reactive oxygen species (ROS) detoxification rather than NAD^+ boost [48]. Further research to investigate the mechanisms of ROS detoxification by NR will be of valuable importance to understand additional functions of increased NAD^+. Finally, NR is recently reported to reduce the proliferation and activation of hepatic progenitor cells which participate in liver tumor heterogeneity [29].

Boosting NAD^+ with NMN in Age-Related Diseases and Cancer

NMN is also a key biosynthetic intermediate enhancing NAD^+ synthesis and ameliorates various pathologies in mouse disease models [49,50]. Very recent research demonstrate that a 12-month-long NMN administration to regular chow-fed wild-type C57BL/6 mice during normal aging rapidly increases NAD^+ levels in numerous tissues and blunts age-associated physiological decline in treated mice without any toxic effects [49]. NMN is also beneficial in treating age- and diet-induced diabetes, and vascular dysfunction associated with aging in mice [51,52]. Administration of NMN also protects the heart of mice from ischemia-reperfusion injury [53] and restores mitochondrial function in muscles of aged mice [37,54].

It has been speculated that NMN is a circulating NAD^+ precursor, due to the extracellular activity of NAMPT [55]. However, the mechanisms by which extracellular NMN is converted to cellular NAD^+ still remain elusive. On the one hand, it is reported that NMN is directly transported into hepatocytes [51]. On the other hand, NMN can be dephosphorylated to NR to support elevated NAD^+ synthesis [56–59].

It is recently shown that NAM can be metabolized extracellularly into NMN by extracellular NAMPT. NMN is then converted into NR by CD73 [60]. Hence, NR is taken up by the cells and intracellularly phosphorylated firstly into NMN by NRKs and then, converted into NAD^+ by NMNATs [60] (Figure 3). Thus, mammalian cells require conversion of extracellular NMN to NR for cellular uptake and NAD^+ synthesis. Consistent with these findings, in murine skeletal muscle specifically depleted for NAMPT, administration of NR rapidly restored muscle mass by entering the muscles and replenishing the pools of NAD^+ through its conversion to NMN [38].

Interestingly, mice injected with NMN had increased NAM in their plasma that may come after initial conversion of NMN into NR [60]. However, degradation of NR into NAM could only be observed when cells were cultured in media supplementing with 10% FBS [60]. Finally, it is important to note that NR is stably associated with protein fractions in milk with a lifetime of weeks [35]. Notably, as reported above, NMN may be degraded by CD38 in older mice promoting NAD^+ decline and mitochondrial dysfunctions [2], suggesting that NR may be more efficient than NMN in elderly. Yet, the beneficial synergistic activation of sirtuins and metabolic pathways to replenish NAD^+ pools cannot be excluded. However, given its efficient assimilation and high tolerance, NR represents still the most convenient and efficient NAD^+ booster.

Overall, these findings suggest that NAD^+ decrease in disease models and NAD^+ precursors (NAM, NR or NMN) may circumvent NAD^+ decline to generate adequate levels of NAD^+ during aging and thus be used as preventive and therapeutic antiaging supplements. NMN and NR

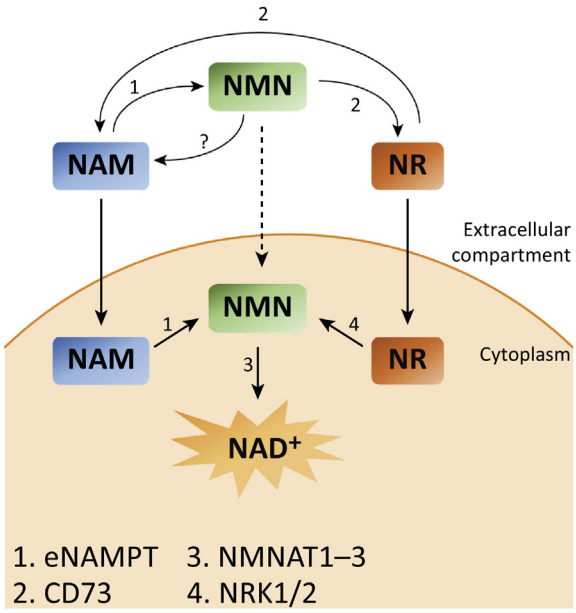

Figure 3. Metabolism of NAD^+ Precursors. Nicotinamide (NAM), nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR) can replenish NAD^+ levels. NAM, NMN, and NR are interconvertible precursors. NAM can be converted in NMN by extracellular nicotinamide phosphoribosyl transferase (eNAMPT). CD73 can dephosphorylate NMN to NR, and convert NR to NAM. NAM and NR can directly enter the cells, while NMN needs first to be dephosphorylated to NR. NAM and NR are intracellularly converted to NMN by nicotinamide phosphoribosyl transferase (NAMPT) and nicotinamide riboside kinase 1 and 2 (NRK1/2), respectively. NMN is then intracellularly adenylated to form NAD^+ by nicotinamide mononucleotide adenylyl transferases 1 to 3 (NMNAT1–3).

supplementations may be equivalent strategies to enhance NAD^+ biosynthesis with their own limitations. However, evidences argue that NR may be the most efficient compound to boost NAD^+. Clearly, finding new NAD^+ boosters would provide alternatives to NAM, NR or NMN as a therapeutic approach for age-related diseases and cancer.

Side-Effects of Some NAD^+ Boosters

Clearly, several intermediates of the salvage pathway can be considered to boost NAD^+ levels but some have contraindications. High doses of NA given to rats are needed to robustly increase NAD^+ levels [61]. Additionally, relevant and unpleasant side effects through NA-induced prostaglandin-mediated cutaneous vasodilation (flushing) affecting patient compliance are due to the activation of the G-protein-coupled receptor GPR109A (HM74A) and represent a limitation in the pharmacological use of NA [62]. NAM is much less efficient than NA as a lipid lowering agent and has also several side effects; in particular, it causes hepatic toxicity through NAM-mediated inhibition of sirtuins [63]. The metabolism of these conventional compounds to NAD^+ is also different, as NA is converted via the three-step Preiss–Handler pathway, whereas NAM is metabolized into NMN via NAMPT and then to NAD^+ by NMNATs [64] (see Figure IA in Box 1). Also, as discussed above, QA, intermediate from the kynurenine pathway (see Figure IB in Box 1), can be neurologically toxic, acting on NMDA receptors in the brain [18]. Contrary to NR, which in a two-step reaction increases NAD^+ concentrations, QA has to react first with phosphoribosyl pyrophosphate to produce NAMN, making it less efficient than NR in enhancing NAD^+ concentrations.

Manipulating NAD^+ by Manipulating Enzyme Activity of Salvage Reactions

Enhancing the activity of enzymes that participate in salvage reactions can also be a strategic intervention to increase NAD^+ concentrations. Different studies have addressed the importance

of regulating the activity of NAMPT during disease, including metabolic disorders and cancer. NAMPT is implicated in boosting NAD^+ pools via the salvage pathway (see Figure IA in Box 1). In this context, NAMPT expression optimizes oxidative stress response, conferring resistance to DNA damage in cancer and preventing senescence in vascular smooth muscle cells [65,66]. Consequently, NAMPT deletion provokes obesity-related insulin resistance, a phenotype rescued by boosting NAD^+ levels in the white adipose tissue by giving NMN in drinking water [67]. Conversely, in a mouse model for atherosclerosis, NAMPT depletion promotes macrophage reversal cholesterol transport, a key process for peripheral cholesterol efflux during atherosclerosis reversion [68]. Other recent reports suggest that NAMPT downregulation could be beneficial in treating pancreatic ductal adenocarcinoma [69,70] and colorectal cancer [71].

Recent findings show that Duchenne muscular dystrophy was accompanied by reduced levels of NAMPT in mice [42]. Moreover, NAMPT knockout mice exhibit a dramatic decline in intramuscular NAD^+ content, accompanied by fiber degeneration and progressive loss of both muscle strength and treadmill endurance. NR treatment induced a modest increase in intramuscular NAD^+ pools but sufficient to rapidly restore muscle mass. Importantly, overexpression of NAMPT preserves muscle NAD^+ levels and exercise capacity in aged mice [38]. Inhibitors against NAMPT are being used in several phase II clinical trials as anticancer therapy. Given that NAMPT activation is important to boost NAD^+ levels, therapy involving NAMPT inhibition should be considered with caution. Although levels of NAD^+ remain to be determined in models with NAMPT depletion, further investigation on the effects of NAMPT modulation is clearly required. The specific mechanisms and actual benefits of regulation of NAMPT activity remain elusive, evidencing the need of more specific disease models.

NR is converted to NAD^+ in two steps by NRK-dependent phosphorylation and adenylylation by NMNATs. NRKs, encoded by the Nmrk genes, are highly conserved enzymes in all eukaryotes [36]. In mammals there are two NRK enzymes, NRK1 and NRK2, but little is known about their physiological roles. NRK1-depleted mice do not show significant abnormalities, but they are unable to utilize either NR or NMN to increase NAD^+ pools [60], suggesting that NRK1 is necessary and rate-limiting for the exogenous use of NR and NMN in NAD^+ synthesis. However, NRK1 is dispensable for other NAD^+ precursors such as NAM and NA [60]. More research using NRK1/2 knockout mice is needed to elucidate the potential benefits of NRK modulation. NAM, NR, and NMN have thus recently emerged as efficient NAD^+ precursors in several model organisms, providing beneficial effects, such as extending lifespan, protecting against metabolic abnormalities, and preventing and ameliorating cancer and age-related diseases. Yet, the beneficial effects through the manipulation of NAD^+ concentrations still remain elusive.

Mechanisms of NAD^+ Action in Age-Related Diseases and Cancer

PARP Activity in Age-Related Diseases and Cancer

DNA damage associated to DNA double strand breaks (DSBs) is more and more recognized as a driving force of the aging process possibly due to a defect in the repair machinery that may happen during aging, accelerating aging [72,73]. Active PARPs poly-ADP-ribosylate proteins in the nucleus by transferring the ADP-ribose group from NAD^+, and participate in DNA repair of single-stranded DNA (ssDNA) breaks [74,75]. Depletion of PARP1 protein using siRNA or inhibiting PARP1 activity reduces repair of ssDNA breaks. During replication, replication forks stall leading to the accumulation of dsDNA breaks. These dsDNA breaks are repaired via the homologous recombination repair process, a potentially error-free repair mechanism. Therefore, NAD^+ depletion may affect DNA repair though inhibition of PARP activity. Consistent with this, growing mouse leukemia lymphoblasts are unable to carry out excision repair when cellular NAD^+ content is lowered by nutritional nicotinamide deprivation, causing PARP inactivation and

high levels of DNA damage. Importantly, these effects are alleviated when cells were re-incubated in the presence of nicotinamide [74].

These studies *in vitro* are supported by the fact that PARP knockout mice are predisposed to genomic instability in response to alkylating agents and ionizing radiation [76]. Surprisingly, PARP1 knockout mice do not show any increase in tumor incidence, possibly due to the hyper-recombinagenic phenotype observed in cells lacking PARP1 [77–79]. Indeed, together with high levels of DNA damage, hyper-recombination can trigger apoptosis of transformed cells, thus inhibiting tumorigenesis. Additionally, pharmacological PARP inhibition in mice prevents hepatocarcinogenesis induced by the carcinogen diethylnitrosamine (DEN) and points out that PARP inhibition may be effective for treatment of hepatocellular carcinoma (HCC) [80]. Clearly, as pointed out for sirtuins, NAD^+ levels are critical to be measured since either PARP activation or inhibition may influence NAD^+ concentrations. In this regard, PARP1 inhibition increases mitochondrial metabolism through SIRT1 activation that can be beneficial during tumorigenesis [81] and PARP2 acts as a direct negative regulator of the SIRT1 promoter [82] and thus, may also influence SIRT1 activity. Increased NAD^+ pools may benefit DEN-induced tumorigenesis through SIRT1 stimulation, supporting previous observations in which boosting NAD^+ prevents DNA damage and HCC formation possibly via activation ofSIRT1 [19,83]. Pharmacological inhibition of PARP, which raises NAD^+ levels, and stimulation of NAD^+ production by precursors, can extend the lifespan of worms and rejuvenate tissue functions in mice [40,51,54,82,84–86]. Recently, an *in vitro* study revealed that direct PARP1 inhibition deleted in breast cancer protein 1 (DBC1) occurs when NAD^+ is reduced to maintain NAD^+ homeostasis [87]. This supports the idea that PARP1 inhibition may be a part of a sophisticated mechanism maintaining NAD^+ pools during aging, despite loss of DNA repair capacity. In addition, in a mouse model of Duchenne muscular dystrophy, reduction in muscle NAD^+ is accompanied by increased PARP activity [42], suggesting that decreased NAD^+ stores are due to high PARP activation or PARylation.

Other studies demonstrate the susceptibility of PARP knockout mice to skin disease and epidermal hyperplasia, suggesting a role of environmental stress in skin lesions. Clearly, the role of environmental and intrinsic factors preventing tumorigenesis cannot be excluded. However, *in vitro* PARP1 inhibition is shown to prevent NF-κB signalling and suppress LPS-induced TNF-α production in mice [88]. Therefore, indirect inhibition of NF-κB may prevent inflammation and tumour progression [89]. PARP knockout mice treated with nitrosamine are also more susceptible to hemangiomas and hemangiosarcomas in the liver [90]. Furthermore, azoxymethane (AOM)-induced tumorigenesis in colon and liver is enhanced in PARP1 knockout mice [91]. Notably, the mouse strain (ICR) used in these last studies is reported to be highly susceptible to tumorigenesis upon various carcinogen treatments [92]. Therefore, inherent characteristics of the mouse strain may cause different vulnerabilities to carcinogens upon PARP depletion.

Sirtuins Activity in Age-Related Diseases and Cancer

NAD^+ is also a substrate of the sirtuins deacetylase family (SIRT1-SIRT7) and NAD^+ declines may affect their activities during aging. Sir2, the SIRT1 homolog in yeast, is the first sirtuin discovered. It is shown to contribute to caloric restriction-mediated lifespan extension in *Saccharomyces cerevisiae* [93], possibly due to a role in maintaining genomic stability by limiting the recombination of ribosomal DNA (rDNA) [94]. Sir2 normally controls rDNA chromatin silencing through lysine deacetylation, but when its function is jeopardized, a deregulation of rDNA silencing causes the formation of toxic rDNA circles from excessive recombination [95]. Activation of Sir2 is also reported to extend lifespan in worms and flies [96,97].

The evidence of the positive effects of increased NAD^+ concentrations on sirtuin activation in mammalian cells comes from data in mice showing that sirtuin stimulation by NAD^+ boosters

protects against neurodegenerative disorders and improves longevity and muscular performances [41,44,85]. Additional informative research conducted in various model organisms also suggests the antiaging potential of sirtuins [98–102]. Mammalian SIRT1 limits replicative lifespan in response to chronic genotoxic stress [103]. SIRT1 has also a critical role in glucose and lipid metabolism, in which deregulation can cause liver diseases and cancer [104]. SIRT1-deficient mice show defects in cholesterol metabolism to a similar level observed in patients with atherosclerosis [105,106]. Pharmacological and genetic activation of SIRT1 improves insulin resistance and reduces the liver cancer risk factor, steatosis, in HFD-treated mice [107–112]. Additionally, mice overexpressing SIRT1 showed a reduced susceptibility to DEN/HFD-induced liver cancer and were protected from both DNA and metabolic damage [113]. Consistently, boosting NAD^+ reduces HFD-induced DNA damage and liver tumorigenesis [28].

Recent findings demonstrate a paradox action of SIRT1. Using liver cancer stem cells and xenograft studies, it is shown that SIRT1 stabilization by MEK1 promotes tumorigenesis, and synergistic inhibition of MEK1 and SIRT1 may be a valuable liver cancer therapy [114]. We may speculate that NAD^+ levels may fluctuate depending on SIRT1 activity. It is thus critical to measure levels of NAD^+ in disease models in which enzymes consuming NAD^+ have been either depleted or overexpressed. For example, when SIRT1 is overexpressed, it may consume and deplete NAD^+ concentrations but, when it is downregulated, it may increase NAD^+ levels. Additionally, SIRT1 expression may have protective functions in absence of MAPK pathway activation but these effects might be diluted when MAPK are overactivated. Finally, these experiments have been conducted using cell lines and effects of NAD^+ on the whole body physiology may impact tumorigenesis differently [114]. In this regard, it is proposed that increased NAD^+ concentrations may have prophylactic effects on hepatocarcinogenesis, possibly through SIRT1 activation [19].

SIRT2 is mainly localized in the cytoplasm, but it can also regulate gene expression by deacetylation of transcription factors that circulate from the cytoplasm to the nucleus [115]. SIRT3, SIRT4, and SIRT5 are generally considered as mitochondrial proteins [115]. SIRT3 is a deacetylase which has been recently shown to limit the activity of the NLRP3 inflammasome, which is linked to metabolic dysfunction and cancer, by maintaining mitochondrial homeostasis. This deacetylase-dependent inflammasome attenuation highlights another part of metabolic dysfunctions that can be amended by sustaining adequate levels of NAD^+ [116]. Boosting NAD^+ levels may also activate the mitochondria SIRT3, a proapoptotic tumor suppressor [117], enhancing cancer cell apoptosis and tumor regression [19].

SIRT6 and SIRT7 are nuclear proteins [115]. SIRT6 is the mammalian homolog of the yeast Sir2 deacetylase, although activity of SIRT6 seems to be less dependent on NAD^+ than SIRT1 [115]. Emerging evidence suggests that SIRT6 activation extends lifespan by promoting genomic stability, regulating metabolic processes, and decreasing inflammation [118]. Under oxidative stress, SIRT6 stimulates DSB repair through mono-ADP-ribosylation and activation of PARP1 in mammalian cells [119]. SIRT6-deficient cells are also characterized by dysfunctional telomeres and hyperactive NF-κB signaling [118]. Moreover, SIRT6 knockout mice develop normally but reassemble features of premature aging. Tissues of these mice have particularly high genomic instability [120]. Therefore SIRT6 activation may ensure the quality control of the genome during aging [119,121] decreasing age-related diseases. In this regard, loss of SIRT6 induces aerobic glycolysis, ribosomal biogenesis, and anabolic glutamine metabolism independently of mutations in growth factor signaling pathways, but essential to transform cells [122]. Additionally, SIRT6 overexpression induces cell death in several cancer cell lines but not in nontransformed cells [118]. It also suppresses cancer stem-like capacity in tumors with PI3K activation independently of its histone deacetylase activity [123]. Thus, SIRT6 seems to act as a tumor suppressor. However, it remains to determine whether expression of SIRT6 rewires

cancer metabolism as deletion did and to evaluate NAD^+ pools, even though SIRT6 expression may not have a consequent effects on NAD^+ concentrations due to its high K_m [115]. SIRT6 is recently shown to suppress pancreatic cancer through the control of Lin28B [124]. Consistent with this, Tummala *et al*. demonstrates that boosting NAD^+ levels by NR decreases pancreatic cancer in mouse model expressing c-MYC [19]. SIRT6 also functions as a histone H3K9 deacetylase and corepresses the transcription factor HIF1α and hence, the expression of multiple glycolytic genes in response to nutrient stress [125]. Consequently, SIRT6-deficient cells display high HIF1α, increased glucose uptake, and glycolysis causing a lethal hypoglycemia in SIRT6-deficient mice which die early in life [125]. Notably, during aerobic glycolysis, activation of GAPDH causes the consumption of NAD^+ which may affect sirtuins activity due to the limiting substrate, delineating a critical mechanistic feedback loop to further repress SIRT6 activity. Finally, SIRT6 regulates dendritic cell differentiation, maturation, and function [126], and brain specific expression of SIRT6 in mice significantly increases lifespan [127].

The enzymatic activity, molecular targets, and physiological functions of SIRT7 are poorly defined. A detailed and comprehensive knowledge of the mechanisms underlying sirtuins activity is critical to understand their broad action and their possible beneficial effects in cancer and age-related diseases through their regulation [128].

Can Dietary Restriction and Protein Catabolism Maintain NAD^+ Levels?

Among the questions that still remain not well understood is why DR profoundly increases lifespan? Can DR affect NAD^+ levels? It is well established that overfeeding and obesity are important risk factors for cancer in humans [129] and obesity-induced liver and colorectal cancer, among others, can shorten lifespan. Earlier research has also shown that both increased physical activity and reduction in caloric intake (without suffering malnourishment) can extend lifespan in yeasts, flies, worms, fish, rodents, and primates [3–8]. Furthermore, a recent study pointed to the importance of the ratio of macronutrients more than the caloric intake as the determinant factor in nutrition-mediated health status and lifespan extension [9]. Although in humans it is difficult to measure the beneficial effects of DR and currently there is no reliable data that describe the consequences of significantly limiting food intake, some studies have assessed how DR affects health status. People practicing DR seem to be healthier, at least based on risk parameters such as LDL cholesterol, triglycerides, and blood pressure [130].

Activation of the salvage pathways during DR could be turned on and glucose restriction can stimulate SIRT1 through activation of the AMPK-NAMPT pathway resulting in inhibition of skeletal myoblast differentiation [131]. Interestingly, effects of NMN supplementation and exercise on glucose tolerance in HFD-treated mice are very similar [132]. Even though these effects are tissue-specific since exercise predominantly affects muscle, whereas NMN shows major effects in liver, and that mechanism of action can be different, exercise and NMN predominantly affect mitochondrial functions and may both contribute to the boost of NAD^+. In yeast, intracellular NAD^+ levels do not change during glucose restriction, suggesting that DR may have a role in maintaining NAD^+ concentrations [133,134]. However, NAD^+ is a necessary part of DR-mediated lifespan extension in yeast [93,135], and in mice DR increases NAD^+ levels [136]. Importantly, research in budding yeast has shown that during glucose restriction, NAD^+ levels affect the local environment in a non-cell-autonomous manner to promote longevity [133].

It is thus tempting to speculate that L-tryptophan concentrations and thus the *de novo* NAD^+ biosynthesis could fluctuate during DR ameliorating the aging process. Recent studies in humans and mice suggest that moderate exercise can increase blood NAD^+ levels and decrease L-tryptophan levels [137]. A possible explanation for this phenomenon is that DR,

and/or exercise, can induce autophagy and promote the release of several metabolites and essential amino acids [138]. The long-term health effects of moderate DR or exercise can also cause muscle mass waste, possibly increasing the intracellular levels of amino acids and L-tryptophan. During DR and exercise, the products of catabolism to build new proteins can be utilized, promoting the turnover of molecules that are important for cell function. For example, DR increases the expression of enzymes responsible for gluconeogenesis and uses the nitrogen derived from muscle protein catabolism for energy production [139–141]. Thus, one process involved in DR-increased longevity appears to be the replacement and increased turnover of extrahepatic proteins [142–146]. In this way, in humans, supposing that enzymes implicated in *de novo* NAD^+ synthesis are fully functional, essential amino acids such as L-tryptophan released from muscle mass and protein catabolism during DR could be degraded into NAD^+ powering NAD^+-dependent enzymes that maintain vital cellular processes (Figure 2). It will be interesting to measure L-tryptophan levels in various tissues of rodents or human submitted to DR. Increased L-tryptophan degradation may also be implicated in cachexic patients who experience catabolism of muscle protein coupled with downregulated protein synthesis resulting in overall muscle loss. Thus, cachexia may be rather a surviving process providing fuels to the cells than a process causing death.

Other studies have shown that branched-chain amino acids, through an increase in mitochondrial biogenesis and upregulation of ROS defense genes, are important players in extending the lifespan of middle-aged mice [147]. In addition, the hydrogen sulfide generated from restriction of sulfur amino acids has been proposed to improve longevity [148,149]. Therefore DR, through muscle mass waste, can ensure that the L-tryptophan resulting from protein catabolism is transformed into NAD^+ to be disposable to benefit neighboring cells.

Concluding Remarks

Aging is proposed to be responsible for diverse pathologies, however, it should be considered as a disease among other diseases that appear in time while individuals age. Although some questions still remain unclear (see Outstanding Questions), NAD^+ deficits may be part of the answer unifying the aging process to its associated pathologies. NAD^+ precursors may present possible therapeutic solutions for the maintenance of NAD^+ levels during aging and thus may provide prophylaxis to live longer and better. Because NR is the most efficient and inexpensive NAD^+ booster *in vivo*, NR dietary supplementation could be a natural elixir to extend life expectancy and decrease the risk of developing disease. Although more research is needed to understand the efficacy as well as potential adverse side effects of NR therapy in humans, recent studies already provided some pharmacological properties, showing its low toxicity and high effectiveness [150,151]. An increased human lifespan without any health problems still remains a fundamental challenge in our society and, finding new NAD^+ boosters with better efficacy and stability would be an interesting perspective in the future.

Outstanding Questions

Do NAD^+ levels decline during aging?

How and why NAD^+ levels decrease while we age?

Do NAD^+ deficits cause age-related diseases and cancer?

How could NAD^+ concentrations be efficiently replenished *in vivo*?

How and why could boosting NAD^+ protect against and prevent age-related diseases and cancer?

Why is dietary restriction beneficial for age-related diseases and cancer?

Can dietary restriction and protein catabolism maintain NAD^+ levels?

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Resources

[i] www.who.int/ageing/events/world-report-2015-launch

[ii] www.proteinatlas.org/ENSG00000151790-TDO2/tissue

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