Age-Dependent Decline of NAD+—Universal Truth or Confounded Consensus?

Subjects: Biology

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Nicotinamide adenine dinucleotide (NAD+) is an essential molecule involved in various metabolic reactions, acting as an electron donor in the electron transport chain and as a co-factor for NAD+-dependent enzymes. Despite systematic claims of overall decline in NAD+ levels with aging in multiple species, including humans, the evidence to support such claims is very limited and often restricted to a single tissue or cell type. The literature on the topic has been reviewed and it is found that there is a need for much larger, preferably longitudinal, studies to assess how NAD+ levels develop with aging.

NAD+ aging yeast C. elegans rat monkey human

1. Introduction

Nicotinamide adenine dinucleotide (NAD^+) can be generated from tryptophan or micronutrient precursors from the Vitamin B_3 family, which consist of nicotinamide (NAM), nicotinic acid (NA), and nicotinamide riboside (NR). NAD^+ precursors are micronutrients naturally found in the diet and can be obtained from different vegetal and animal food sources, found in high levels in, for example, cucumber, cabbage, soybeans, broccoli, avocado, tomato, whole wheat, yeast, eggs, milk, meat, and liver.

NAD^+ is an important cofactor for adenosine triphosphate (ATP) production in glycolysis and oxidative phosphorylation as well as in cellular redox reactions by oxidoreductase enzymes [1]. NAD^+ also functions as an essential co-substrate in pathways that regulate a wide variety of cellular processes such as DNA repair [2], cellular senescence [3][4], and mitochondrial respiratory function [5]. NAD^+ is synthesized through distinct pathways in mammalian cells, including the following pathways: the kynurenine pathway, the Preiss–Handler pathway, and the salvage pathway (**Figure 1**).

Although the pathways controlling cellular NAD^+ content are tightly regulated, decreased levels of intracellular NAD^+, as well as the NAD^+/NADH ratio, have been observed during aging and aging-related pathophysiological conditions, but while there is certainly evidence of a decline in NAD^+ levels in age-related diseases in both humans and animal models [6][7][8][9][10], reductions in NAD^+ as part of physiological aging are also commonly being touted as a universal truth for all tissues in all organisms. However, the actual literature on the topic is limited and somewhat discrepant.

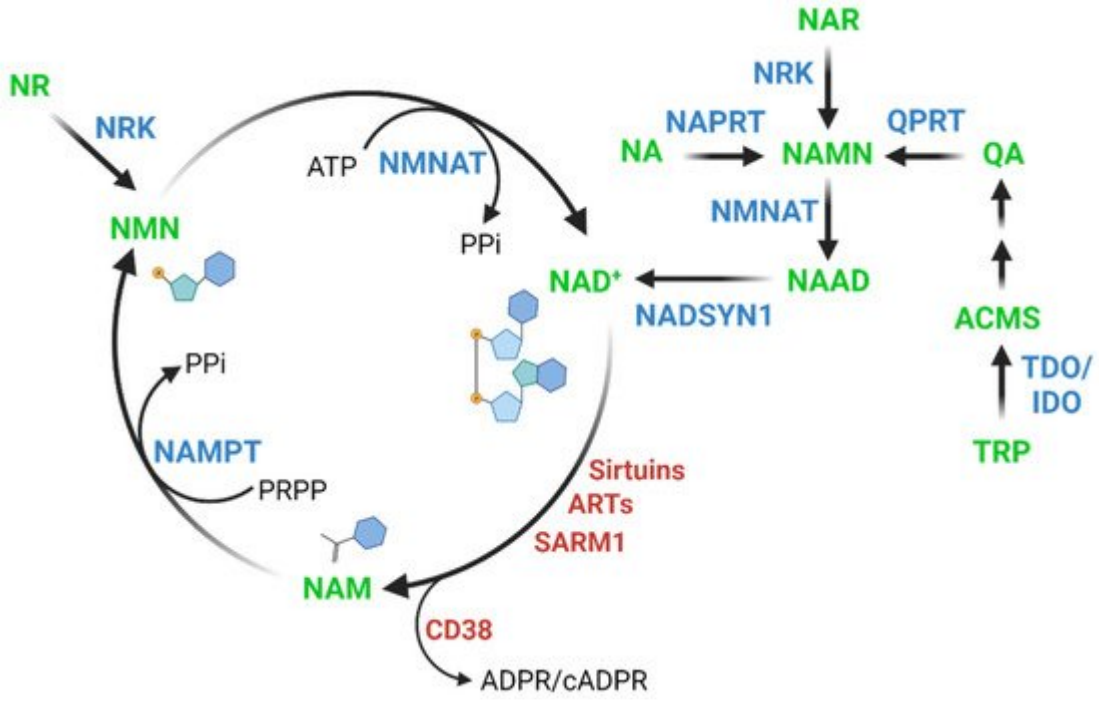

Figure 1. NAD^+ biosynthesis pathways in mammalian cells. ACMS: 2-amino-3-carboxymuconate, NA: nicotinic acid, NAD^+: nicotinamide adenine dinucleotide, NAM: nicotinamide, NAMN: nicotinic acid mononucleotide, NAR: nicotinic acid riboside, NMN: nicotinamide mononucleotide, NAAD: nicotinic acid adenine dinucleotide, NR: nicotinamide riboside, QA: quinolinic acid, TRP: tryptophan, IDO: indoleamine-2,3-dioxygenase, NADSYN1: NAD synthetase, NAMPT: nicotinamide phosphoribosyltransferase, NAPRT: nicotinic acid phosphoribosyltransferase, NMNAT: nicotinamide mononucleotide adenylyl transferase, NRK: NR Kinase, QPRT: quinolinic acid phosphoribosyltransferase, TDO: tryptophan-2,3-dioxygenase, ARTs: ADP-ribosyltransferases, CD38: ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1, SARM1: NAD^+ hydroxylase SARM1, ATP: adenosine triphosphate, PPi: inorganic pyrophosphate, PRPP: 5-phosphoribosyl-1-pyrophosphate.

2. Relationship between NAD^+ Levels and Aging across Species

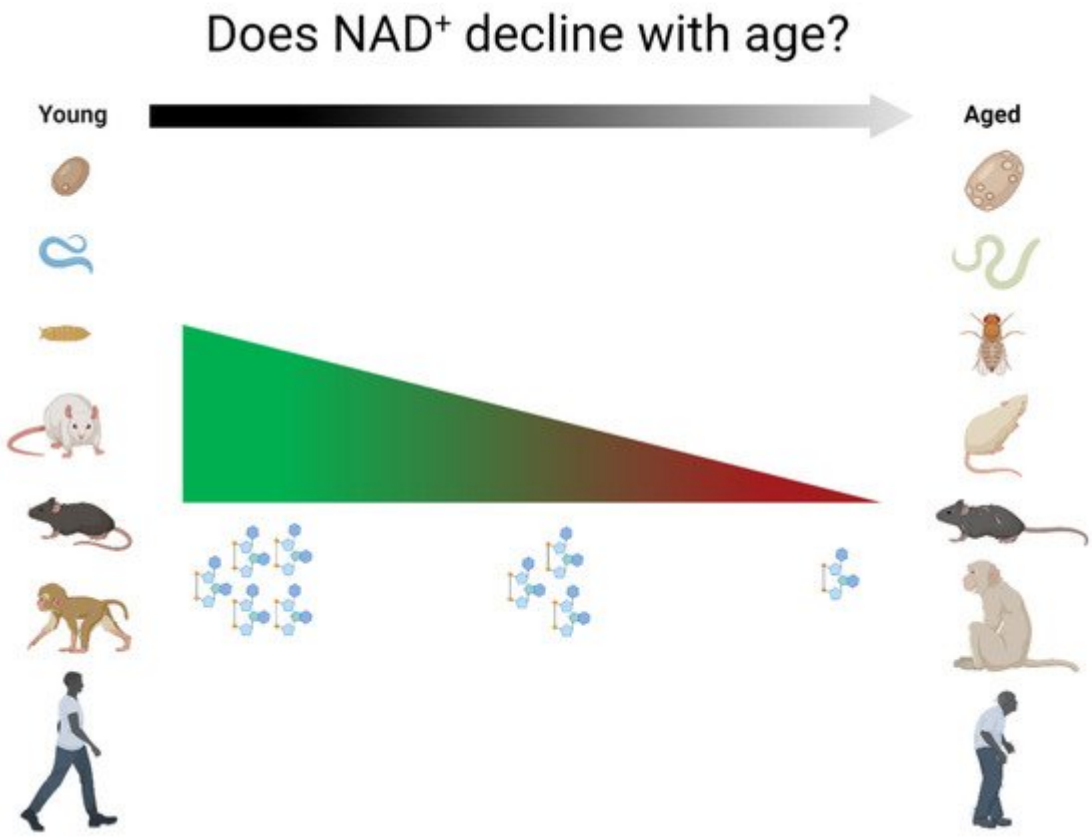

Figure 2. Overview of the organisms included in this entry.

2.1. Non-Mammalian Species

2.1.1. Yeast

Budding yeast presents two different ways to study aging. Replicative aging, the amount of times that a yeast cell divides and chronological aging defined as the time that a single non-proliferating yeast cell survives after the diauxic shift [11]. The diauxic shift is reached when yeast cells switch from glucose fermentation to ethanol respiration and, in that process, most cells stop budding but are still viable. The current literature on replicative aging shows no difference in NAD^+ with aging between age either replicative age 0-1 to 7-8 [12] or replicative age 0 to 16 [13]. It has been demonstrated that NAD^+ levels decline as part of the diauxic shift [14], but there are no reports on chronological aging *per se.* It is unclear whether the reduction during the diauxic shift is simply a part of shifting to a less active metabolic state.

Taken together, there is seemingly no direct evidence of a connection between aging and NAD^+ level decline in yeast cells. Future studies should consider both replicative and chronological models of aging in yeast and assess the whole spectrum of yeast lifespan to fully determine the role of NAD+ in yeast aging.

2.1.2. Caenorhabditis Elegans

Only two studies have reported on NAD^+ levels in aging *C. Elegans*. NAD^+ levels were found to be reduced in aged *C. elegans* (day 17 [15] and day 8 [16], respectively) compared to young controls (day 1). These studies

indicated an association between NAD^+ and lifespan, and many subsequent studies in *C. elegans* have focused on the role of NAD^+ consuming enzymes or supplementation with NAD^+ precursors to affect longevity [16][17][18].

2.1.3. Drosophila Melanogaster

It is impossible to identify any papers that address whether levels of NAD^+ decline with age in this model, despite the ease with which such experiments could be performed.

2.2. Rodents

2.2.1. Rats

Rats have not been studied extensively with regard to NAD^+ levels during aging. In one study, researchers compared female Wistar rats of different ages. They found that NAD^+ was reduced in liver, heart, kidney and lung of 24-month-old rats, compared to younger controls (3 and 12 months old, respectively) [19]. A follow-up study with a similar design revealed the same pattern of NAD^+ decline in four different brain regions: hippocampus, cortex, cerebellum and brainstem [20]. Similarly, isolated mesenchymal stem cells from young (1–2 months of age) and old (15–18 months of age) male Sprague Dawley rats also exhibited a reduction in NAD^+ levels with age [21]. It is noteworthy, however, that the mesenchymal stem cells were kept in culture prior to the assessment, which could, in principle, affect the outcomes. The same group later demonstrated that induced cellular senescence of rat mesenchymal stem cells also resulted in a reduction in NAD^+ [22]. Collectively, our understanding of changes in NAD^+ levels in aging in rats is limited to only a few tissues or cell types, and the information appears to have derived from only two laboratories.

2.2.2. Mice

The most comprehensive study to date on the development of NAD^+ levels with aging in mice, studied several tissues [23]. It showed that aged mice had a decrease in NAD^+ in only 10 out of 21 tested tissues. In brown and two different white adipose tissues (retroperitoneal and inguinal), as well as in the jejunum, they observed a ~40–50% NAD^+ reduction with age, whereas in gastrocnemius, soleus, quadriceps, liver, kidney, and descending colon NAD^+ levels were reduced by ~10–20%. The NAD^+ levels in the remaining 11 tissues including heart, brain, spleen, lung, pancreas, gonadal white adipose tissue, and multiple parts of the gastrointestinal tract were found to be unchanged by aging. It is clear from this study alone that the apparent consensus that NAD^+ levels universally decline with aging is inaccurate.

Overall, mice have been studied more extensively in this regard and there is quite reliable evidence of NAD^+ decline in aging skeletal muscle [5][15][23][24][25][26][27], some adipose tissues [23][24][25] and hippocampal areas of the brain [28][29][30]. However, there are conflicting reports on liver [15][24][31][32] and effects of age on NAD^+ levels in heart, lung, spleen, pancreas, and intestine remain relatively uninvestigated.

2.3. Primates

2.3.1. Monkeys

Rhesus monkeys (*Macaca mulatta*) are commonly used in aging research due to their social and physiological similarities to humans [33]. No studies appear to have reported on levels of NAD^+ with aging in monkeys. However, one study in rhesus monkeys showed 2.5- and 2-fold increases in NADH levels in vastus lateralis muscle of middle-aged (15–16 years of age) and older (28–32 years of age) animals, respectively, compared to young controls (6–9 years of age). Moreover, the NAD^+/NADH ratio was ~60% lower in middle-aged animals compared to young controls [34]. Although NAD^+ levels were not reported in this entry, they can easily be calculated from the availalable data, revealing an approximate doubling of NAD^+ in old monkeys compared to young. This contrasts the available data from mouse skeletal muscle sharply.

2.3.2. Humans

The literature on the association between human NAD^+ and ageing presents us with very little data on peripheral tissues. There is a single study showing marked reduction in skin NAD^+ levels with age [35], as well as one showing an age-associated 30% reduction in NAD^+ in liver from patients with hepatocellular carcinoma [36]. No study has investigated the effect of age on adipose NAD^+ levels at all, and the data on skeletal muscle is limited to a single report on *BioRxiv* [37] and was removed before peer-reviewed publication [38].
When it comes to plasma NAD^+ and aging in humans, the literature presents us with two discrepant extremes. One study showed no change [39], and the other showed an 80-90% reduction with age [40]. The third and final study on the topic agreed that there is a change in plasma NAD^+ with age but no change in red blood cells and the magnitude of the change in plasma NAD^+ was not disclosed [41]. They did present a figure from whole blood that can be used to estimate a 15-20% reduction of NAD^+ with age. This level of reduction fits well with the observed reductions observed in brain and cerebrospinal fluid [39][42][43] except in the case of the previously mentioned non-peer-reviewed report [37].

Importantly, all of the abovementioned human experiments are cross-sectional studies and had relatively few participants. To some extent, an understanding can be derived from such studies, but to truly investigate NAD+ metabolism in healthy human aging, a much larger group of individuals need to investigated over the span of many years in a longitudinal study.

3. Conclusions and perspective

There are remarkably few studies that assess NAD^+ levels with aging. This is true for most of the commonly used model organisms as well as for humans. Moreover, even within specific tissues, there are discrepancies in the literature, and many tissues in multiple organisms have only been investigated by a single research group or not at all. Thus, there is considerable disagreement between what the field assumes to know on the topic of NAD^+ in aging and what is scientifically supported. This poor-founded perpetuation of the idea that NAD^+ levels universally decrease with age is misleading, and it may lead to the loss of important nuances in our collective understanding of NAD metabolism. There is a need for longitudinal studies investigating the way NAD^+ levels behave in various

tissues during aging in various model organisms, and much larger cross-sectional studies in humans are required to address this specific question.

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