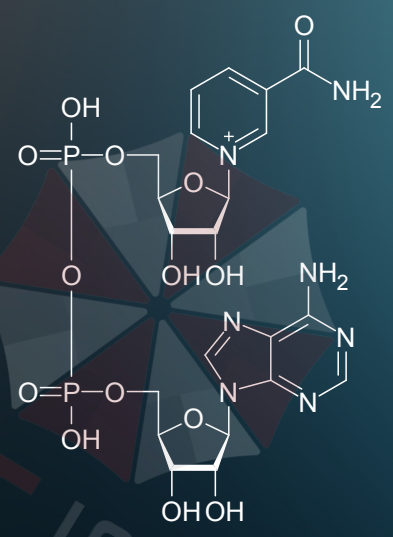


Exploring NAD+ Potential for Aging, Metabolic, and Neurodegenerative Diseases

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Nicotinamide adenine dinucleotide (NAD) is a coenzyme central to metabolism, which is found in all living cells. NAD exists in an oxidized and a reduced form, abbreviated as NAD^+ and NADH respectively. If one form is administered from the outside, the body will convert NAD^+ and NADH into each other depending on the internal metabolic need. Usually, the substance is referred to as NAD^+. The superscript $^+$ refers to the positive charge of the molecule in that state.

In cellular metabolism, NAD^+ is involved in redox reactions, carrying electrons from one reaction to another. It is used as a Cofactor for a variety of redox enzymes involved in core metabolic processes like the citric acid cycle. However, it is also used in a variety of other cellular processes.

Humans can produce NAD^+ from essential building blocks from vitamin B3 (niacin). Hence in some studies niacin supplementation is used as a way to increase NAD^+ levels in humans. Since NAD has attracted a lot of attention in animal and human studies, alternative compounds have been suggested to increase NAD^+ concentrations more efficiently compared to niacin have been suggested. The most common forms, are nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) which are essentially intermediate products in the conversion of niacin to NAD^+.

Animal Studies

There are a lot of animal studies on the subject of supplementing one of the various forms of NAD^+. Due to the large amount of clinical trials in humans, the some animal studies are references in the “further reading” section at the end of this document.

Human Trials

There have been a lot of clinical studies with human participants supplementing one of the various precursors for NAD^+. There are some main indications for which NAD^+ supplementation is currently discussed and was previously tested in animal models.

NAD^+ is a fundamental molecule in human life and health as it participates in energy metabolism, cell signaling, mitochondrial homeostasis, and in dictating cell survival or death. Emerging evidence from preclinical and human studies indicates an age-dependent reduction of cellular NAD^+, possibly due to reduced synthesis and increased consumption. In preclinical models, NAD^+ repletion extends health span and/or lifespan and mitigates several conditions, such as premature ageing diseases and

neurodegenerative diseases.

These findings suggest that NAD^+ replenishment through NAD^+ precursors has great potential as a therapeutic target for ageing and age-predisposed diseases, such as Alzheimer's disease.

Aged Individuals

It has been suggested that supplementation of redox agents, such as NAD^+, would raise subjective energy levels only in individuals suffering from a deficiency of NAD^+, which is often seen in elders. Hence, the authors of a study hypothesized that old individuals would more likely benefit from NR supplementation. 12 young and 12 old men received nicotinamide riboside (NR) or placebo in a double-blind cross-over study. Before and 2h after NR or placebo supplementation, blood and urine samples were collected, while physical performance was assessed after the second blood sample collection. At rest, old individuals exhibited lower erythrocyte NAD(P)H levels, higher urine F2-isoprostanes and lower erythrocyte glutathione levels compared to young study participants. NR supplementation increased NADH (51% young; 59% old) and NADPH (32% young; 38% old) levels in both groups, decreased F2-isoprostanes by 18%, and tended to increase glutathione only in the old. NR supplementation did improve muscle contraction strength by 8% and the fatigue in the old. In contrast, NR supplementation did not exert any redox or physiological effect in the young. Therefore, NR supplementation increased NAD(P)H levels, decreased oxidative stress, and improved physical performance only in old subjects, substantiating that redox supplementation may be beneficial only in individuals with antioxidant deficiencies.[1]

In a clinical trial on chronic supplementation with NR it was found that NR is well tolerated and effectively stimulates NAD^+ metabolism in healthy middle-aged and older adults. For this study, 60 healthy middle-aged and older men and women between the ages of 55 and 79 years were consented. NR was well tolerated at the dose tested, and no serious adverse events occurred. A total of 14 treatment-emergent adverse events (AEs) were reported by 7 of the 30 participants enrolled in the study, with the other 23 subjects reporting no AEs.[2]

A study aimed to investigate the influence of a oral 12-week nicotinamide mononucleotide (NMN) supplementation on biochemical and metabolic health parameters. A total of 36 healthy middle-aged participants received either 125 mg NMN or placebo. The levels of nicotinamide in the serum were significantly higher in the NMN intake group than in the placebo group. Arterial stiffness tended to decrease in the NMN intake group. However, no significant difference was found between the two groups. Long-term NMN supplementation at 250 mg/day was well tolerated and did not cause adverse events. NMN safely and effectively elevated NAD^+ metabolism in healthy middle-aged adults.[3]

A Japanese study from 2022 investigated the effects of the time-dependent intake of nicotinamide mononucleotide (NMN) on sleep quality, fatigue, and physical performance in older adults. 108 participants divided into four groups were enrolled. NMN (250 mg) or placebo was administered once a day for 12 weeks. Overall, NMN intake in the afternoon effectively improved lower limb function and reduced drowsiness in older adults.[4]

NAD^+ depletion of which has been proposed to promote aging and degenerative diseases in rodents. However, whether NAD^+ depletion occurs in humans with degenerative disorders and whether NAD^+ repletion improves their symptoms had remained open until the first human studies had been conducted in the 1990's.

Obesity & Fatty Liver Disease

While oral supplementation of NR suppresses diet-induced weight gain and enhances calorie expenditure in mice, most studies in humans report no effect on body composition in aged humans. Aging and fatty liver, along with obesity, both affect the hepatic NAD^+ levels. Aging tends to decline the hepatic NAD^+ levels in both mice and humans. Hence, some studies have focused in reducing body weight and treating liver disease with NAD^+ precursors.

A randomized placebo-controlled clinical trial of nicotinamide riboside (NR) in 40 obese men evaluated safety, insulin-sensitivity and lipid-mobilizing effects in the study participants.

Resting energy expenditure and respiration efficiency were not affected by NR supplementation. NR at 2000 mg/d was well tolerated. There were reports of minor adverse reactions from 4 participants in the NR group and from 2 participants in the placebo group. Adverse reactions in the NR group included pruritus, excessive sweating, bloating, and transient changes in stool, and in the placebo group acid reflux and periodic loose stools.[5,6] No treatment effects on oxidation rates of glucose, lipid, or protein were observed. 12 weeks of NR supplementation did also not affect body composition. Another study in healthy older males also did not report any effects on body composition.[7]

Preclinical findings suggested that targeting NAD^+ metabolism could be a viable therapeutic strategy for treating non-alcoholic fatty liver disease (NAFLD). NR slightly diminish body fat- free mass but failed to improve insulin sensitivity, mitochondrial function, and hepatic lipid content in healthy overweight patients. NR did not show a desirable result in clinical studies. However, NMN single dose intravenously considerably lessens blood triglycerides in healthy people.[8] Similarly, another trial mentioned NAM at a dose of (1000 mg/day) decreases the low-density lipoprotein, cholesterol, and liver enzyme alanine transaminase, improved quality of life in diabetic NAFLD patients while no significant effects have seen in liver fibrosis or steatosis. The effect of each NAD^+ precursor is variable, and further studies with different NAD^+ precursors are required to analyze their efficacy against NAFLD.

NAD^+ has been reported to protect against liver steatosis and metabolic imbalances in NAFLD in animal models. 61 diabetic patients with suffering NAFLD received nicotinamide 1000 mg once daily for 12 weeks in addition to their antidiabetic therapy or the control group who received their antidiabetic therapy only. Significant reduction was observed in liver enzymes with a median decrease in liver damage markers of 26.6%. After 12 weeks of treatment, the NAD^+ group showed significantly lower levels of LDL, total cholesterol, and insulin resistance marker compared with control.[9]

Type 2 Diabetes Mellitus

Several groups reported NAD^+ precursors NR or NMN raise NAD^+ levels and demonstrated their ability to reduce obesity-induced insulin resistance in mice. Rodent studies demonstrate that NAD^+ metabolism is a suitable target for insulin resistance.

A randomized, double-blinded, placebo-controlled study was conducted in 13 healthy overweight or obese men and women. Participants received NR (1000 mg/d) for 6 weeks and placebo supplementation. Markers of increased NAD^+ synthesis—nicotinic acid adenine dinucleotide and methyl nicotinamide—were elevated in muscle after NR compared with placebo. Here, NR increased body fat-free mass (change: 1.34 %) and increased sleeping metabolic rate. Interestingly, acetylcarnitine (a molecule most commonly known of shuttling fats into the mitochondrion) concentrations in skeletal muscle were increased upon NR and the capacity to form acetylcarnitine upon exercise was higher in NR than in placebo. However, no effects of NR were found on insulin sensitivity, mitochondrial function, hepatic and intramyocellular lipid accumulation, cardiac energy status, cardiac ejection fraction, ambulatory blood pressure, plasma markers of inflammation, or energy metabolism.[10]

The clinical translation of NAD^+ precursor niacin for diabetes treatment was conducted in patients with type 2 diabetes. In this study, they administered the niacin with insulin and sulphonylureas, a class of anti-diabetic medication. The finding of this study shows that niacin administration increases the release of insulin in both groups. This means that niacin can increase insulin production in type 2 diabetes. Because it can be taken orally and is much cheaper, niacin is a more attractive treatment option for diabetes type 2 than insulin.[11]

Later, NR has also been translated into more clinical studies. However, NR did not show the anticipated results. These studies showed that single doses of 100, 300 and 1,000 mg of NR produce dose-dependent increases in the blood NAD^+ levels, but did not report any significant insulin sensitivity.12 An additional study in 40 obese non-diabetic men did also not find any effect of NR supplementation (2000 mg/d) to

impact glucose tolerance or endocrine pancreas function.[7]

In 14 elder males with diabetes, NMN in a dose of 250 mg/day was well-tolerated, but produced no significant change in grip-strength, walking speed or any other observed health indicators. [13] A different study did however find significant effects of NMN on muscle insulin sensitivity in overweight or obese postmenopausal women with prediabetes.[14]

Overall, the studies on diabetic patients are mixed. Some studies report small benefits, other report no effects. There is also no clear subgroup that profits more than others. More studies with a larger patient population are needed.

Cardiovascular Diseases

A recent study reported that healthy middle-aged and older persons who supplement with the NR for six weeks experience a decrease in blood pressure and rigorousness of the aorta 2. The other clinical study also reported that using other NAD^+ metabolite NMN (125–250 mg) for 12 weeks improved arterial stiffness.[3] Overall, this indicates NAD^+ supplementation may be useful to combat vascular degeneration in older individuals.

A study with 148 participants aimed to evaluate the effects of NR on average daytime systolic blood pressure. Patients received 1,000 mg/day of NR combined with three days/week of supervised, center-based walking exercise. The results showed no statistical difference in mean blood pressure or arterial stiffens between the groups. (NCT04112043)

Neurodegenerative Diseases

A team of researchers found vitamin B3, nicotinamide (NA), to significantly reduce improve symptom burden in transgenic mice suffering from Alzheimer's disease. The team afterwards conducted a clinical study with 29 subjects thereafter to investigate of the mouse results were transferrable to humans. Subjects received either niacin (1500 mg twice daily) or placebo for 24 weeks. However, there were no significant effects of NA on the symptoms of Alzheimer's disease. [15]

A double-blinded phase I clinical trial to was conducted to establish whether oral intake of nicotinamide riboside (NR), is safe, augments cerebral NAD^+ levels, and impacts cerebral metabolism in Parkinson's disease (PD). 30 newly diagnosed, treatment-naive patients received 1,000 mg NR or placebo for 30 days. NR treatment was well tolerated and led to a significant, but variable, increase in cerebral NAD^+ levels. NR recipients showing increased brain NAD levels exhibited altered cerebral metabolism. Furthermore, NR decreased the levels of inflammatory cytokines in serum and cerebrospinal fluid. These findings nominate NR as a potential neuroprotective therapy for Parkinson's disease.[16] A further clinical study with NR in muscle showed that NR also reduces the levels of inflammatory cytokines in muscle tissue.[17]

In a 12-month study with 46 participants with Parkinson's disease which received either placebo, 100 mg of niacin or 250 mg of niacin. After the full year, a significant alleviation of Parkinson's symptoms was observed in the niacin groups. The authors concluded that niacin augmentation has the potential to maintain or improve symptoms.[18]

A small study with 26 participants examined the degree of loss of intraepidermal nerve fibers in the skin after experimental denervation by capsaicin. Experiment participants received Nicotinamide riboside at a dose of 900 mg twice a day orally. There was no difference between the patients who received NR and those who received placebo. (NCT03912220).

Similarly, 3 g of NMN (1500 mg twice per day) for a period of 24 weeks did not improve the cognitive function of patients with Alzheimer's disease.[15]

Skeletal Muscle Diseases

Muscular mass and strength often decline with age, leading to a condition known as sarcopenia, which is often thought to be best targeted by SARMs. Several studies have mentioned the potential association between NAD^+ and sarcopenia and reported that mammals' NAD^+ levels decrease in skeletal muscle with age.

NMN, also showed beneficial results in exercise training. One study reported that NMN enhanced oxygen consumption in healthy runners. A study has shown that administering 250 mg of NMN can lessen drowsiness,

enhance physical performance, and increase grip strength and gait speed in older men during exercise. 4,19 However, another group reported 250 mg of NMN does not affect muscle strength.[13]

The authors of a study from Finland report systemic NAD^+ deficiency in adult with beginning mitochondrial diseases. They were administered an increasing dose of NAD^+-booster niacin, a vitamin B3 form (to 750–1,000 mg/day; NCT03973203) for patients and their matched controls for 10 or 4 months, respectively. Blood NAD^+ increased in all subjects, up to 8-fold, and muscle NAD^+ of patients reached the level of their controls. Some patients showed anemia tendency, while muscle strength and mitochondrial biogenesis increased in all subjects. In patients, muscle metabolic parameters shifted toward controls and liver fat decreased even 50%. Our evidence indicates that blood analysis is useful in identifying NAD^+ deficiency and points niacin to be an efficient NAD+ booster for treating mitochondrial myopathy.[20]

An additional small study on middle aged obese men aimed to determine of dietary NR supplementation affects mitochondrial respiration, content or morphology in skeletal muscle. The study found that NR did not affect respiratory capacity of mitochondria or the levels of various mitochondrial associated proteins.[6]

Conclusion

The various NAD^+ precursors have been extensively studied in humans and animals for a variety of conditions of metabolic origin, such as diabetes and muscular diseases. There is often conflicting evidence between the studies. However, a general trend of the results is that older people and those suffering from certain conditions profit more from NAD+ supplementation compared to younger and healthy individuals. There seems to be also a slight general benefit for young and healthy adults, although the effect strength of NAD^+ supplementation seems to lower compared to elders, probably due to the reason that these individuals already have sufficient NAD in their tissues and further supplementation only elevates those levels marginally.

There is considerable evidence for the usefulness of NAD^+ supplementation for obesity and fatty liver diseases, as well for diabetes type II and Parkinson's disease. The evidence for cardiovascular diseases, Alzheimer's and for weight loss is not sufficient though.

All forms of NAD^+ precursors, including niacin, NMN and NR are generally safe and produce only limited side effects, even in doses exceeding 2g/day. In summary, NAD^+ precursors could have an exciting therapeutic potential for ageing, metabolic and neurodegenerative diseases.[21]

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