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NAD+ precursor role in modulating NAD level in human beings and their therapeutic effects

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Abstract

Background: A vital component in cellular bioenergetics and adaptive responses to stress is NAD. NAD deficiency is linked to age-related illnesses, malignancy, and neurological disorders, it has been the focus of numerous studies. NNM is an orally accessible precursor of NAD that has been shown to have positive impacts on the body's levels of NAD, which helps prevent aging and several disorders. Thus, to assess NMN's potential as a treatment, we carried out the study to determine its role in increasing NAD levels.

Keywords: *NAD+, Therapeutics, NAD+ precursor, NMN, Nicotinamide Mononucleotide, NAD, Aging.*

Material and Method: A total of 86 individuals were selected for the oral administration of NMN and its efficiency in increasing NAD level and its health consequences were evaluated. Healthy volunteers received placeblo and 250mg NMN, 350mg NMN, and 850mg NMN up to 60 days. During this period the blood NAD concentration, homeostatic model assessment for

insulin resistance, 6-minute walk test, and 36-item short-form survey (SF-36) were assessed at baseline and after the supplement.

Result: A total of 80 individuals were chosen to receive NMN orally, and their effectiveness in raising NAD levels as well as any potential health effects were assessed. Placeblo and 250 mg, 550 mg, and 850 mg of NMN were given to healthy participants for up to 60 days. At initial and after providing the supplement, the following measurements were made: the blood NAD quantity, blood biological age, 6-minute walk test, HOMAR-IR, and the 36-item short-form survey (SF-36).

Conclusion: NMN enhances quality of life, enhances physical performance, and slows down aging by increasing NAD. The effects become more pronounced at greater dosages, but there is a potential it will not address insulin resistance, which necessitates further study.

1. Introduction

The oxidized form of NAD, known as NAD+, is a chemical necessary for the overall state of cellular health in living things [1]. One of the most prevalent metabolites in the human body, NAD+, is in a homeostatic state that involves cellular and systemic biosynthesis, use, recycling, and breakdown [2]. Numerous NAD+ precursors have been found in the foods we naturally eat. Among these include the amino acid tryptophan and the three vitamin B-3 forms nicotinamide riboside (NR), NAM, and NA [3].

A nucleoside containing nicotinamide and ribose combines with a phosphate group to create NMN, a bioactive nucleotide [4]. It is one of the least harmful biosynthetic precursors of nicotinamide adenine dinucleotide (NAD+) [5, 6]. It often comes with 2 anomeric types: beta and alpha [7]. The functional form of NMN is called β NMN [5].In contrast, it may be detected in placenta tissue and bodily fluids like urine and blood in humans, and it typically occurs in a wide range of plants and food sources like cabbage, broccoli, cauliflower, tomato, and mushroom [4].

NAD, or nicotinamide adenine nucleotide, is a widely distributed hydrophilic component that is engaged in several facets of cellular metabolism. It is made up of nucleotides of nicotinamide and adenine connected by their 5′-phosphate ends. NAD+ and NADH, the acronyms for their oxidized and reduced forms, respectively, are used to refer to them [8]. It is known that NAD

takes part in more than 300 oxidation-reduction processes that are mediated by enzymes. Furthermore, a variety of processes utilizing NAD as a catalyst have been identified [9].

Numerous pathologic disorders, such as malignancy, diabetes, obesity, neurodegenerative disorders, and digestive disorder, have been linked to deficiencies in NAD or its production . On the other hand, it seems that higher NAD availability is advantageous for tissues and cellular function. Several studies investigating the medicinal potential of targeting NAD biosynthetic pathways have been spurred by the vital function of NAD production in diseases [10].

Three different pathways (Figure 1) can be used to produce NAD: the Preiss-Handler pathway, the de novo synthesis pathway, which is sometimes referred to as the kynurenine pathway, or KP, and the salvage pathway [11].

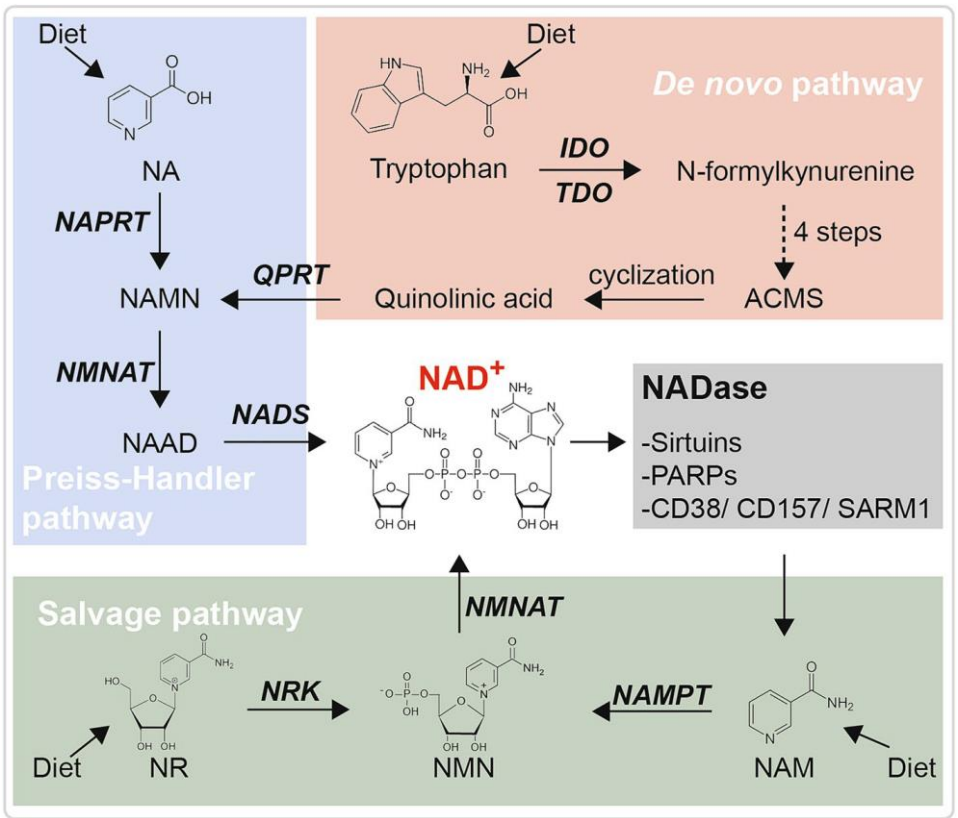


Figure 1: Three distinct pathways for NAD biosynthesis [11]

Numerous biochemical investigations have demonstrated that cellular damage may result from inadequate NAD+ synthesis, where catabolism outpaces anabolism. Reduction in NAD+ levels may be an important variable in stress resistance [2] and aging [3], since NAD+ levels are increased in the conditions of enhanced life span or health span and drop in conditions of rapid

aging and/or lower health span. NAD + has a significant impact on the breakdown of energy and mitochondrial processes, as well as inflammatory and calcium balance. However, it is most crucial in reducing the risk of several neurological disorders such as ischemic brain injury, Parkinson's condition, and Alzheimer's disorder. Moreover, increased NAD levels have a preventive effect on tumor development [12].

As a result, the field of aging research has focused a lot of emphasis on the preventive and therapeutic interventions using essential NAD+ intermediates that can restore cellular NAD levels, such as nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR) [13].

NMN may provide new opportunities for contemporary therapy. In a number of preclinical models of disease, such as those for Alzheimer's disease, diabetes, MI and cerebral ischemia, and neurological disorders, this biomolecule has shown a wide range of advantageous medicinal properties [7]. When taken orally, NMN has been shown to protect against diet- and age-related obesity and diabetes [29]. Although NMN human clinical studies began later than NR trials, several papers have been published lately [15]. We carried out the study to investigate the therapeutic effects of the NAD+ precursor NMN impact at different doses to increase NAD in humans.

2. MATERIAL AND METHODS

The study comprised healthy people with body mass indices (BMIs) ranging from 18.5 to 35 kg/m2 who provided consent in writing. The individuals were aged 35 to 65. Throughout the trial, all individuals were given instructions to stick to their regular daily routines and food, and not consume any caffeine [16]. The NMN or placebo groups were assigned individuals randomly [29]. Oral NMN supplements of 250 mg, 550 mg, and 850 mg were delivered for 60 days [17], while patients in the placebo group received the same regimen but without NMN [29].

2.1.Efficacy Evaluation:

To assess the effectiveness of NMN in stimulating NAD a number of parameters, including blood cellular NAD+/NADH levels in blood, a 6-minute walking endurance test, systolic and diastolic blood pressure, and the SF-36 questionnaire, were evaluated as primary endpoints in the study [18]. The colorimetric total NAD/NADH assay was used to determine the concentrations of NAD [19]. When volunteers visited trial locations on days 0, 30, and 60,

blood samples were obtained from each of them [20]. Plasma samples were loaded on 10 kDa PierceTM protein concentrators, and centrifuged at 13000×g for 30 minutes at 4°C, to extract proteins. This separation phase is crucial for NAD identification, as mentioned by the manufacturer. A portion of the filtrate was placed on ice and another portion was raised to 60°C for 30 minutes. In a transparent 348-well microplate, 20 µl of the warmed or cooled filtrates and 0.1–5.0 µM of soluble NADH were added. Using an endless M1000 plate reader (Tecan, Männedorf, Switzerland), the colour of the formazan generated was monitored at 460 nm for 120 minutes, with 1-min periods, following the addition of 20 µl of the enzyme reaction mixture. Every sample underwent at least four measurements. While NAD+ is only measurable at 260 nm, NADH can be measured at 260 and 340 nm [19].

The HOMA IR Index was calculated using the HOMA2 IR. The calculator makes use of blood insulin concentrations and fasting blood glucose concentrations. After an overnight fast, subjects' samples of blood were taken for HOMA [18]. The American Thoracic Society's 6-minute walking test was performed in accordance with its protocol to assess the amount of energy and capacity. It was carried out on days 0 through 60. Respondents in the six-minute walking test had to walk on a physically driven treadmill machine that replicated a walking field. A computerized treadmill clock was used to record the distance travelled in meters [20].

The individual was asked to respond to 36 items about energy, emotions, social interactions, and physical wellness as part of the SF-36 questionnaire, which was used to gauge improvements in the individual's overall well-being [18].Ageing.AI 3.0 was utilized to compute the biological age [17].

2.2.Assessment of Adherence, Tolerability, and Safety:

Individuals documented their subjective symptoms in a diary, which were then evaluated at the 4-weekly visits. Additionally, the quantity of pills skipped was noted in a diary. It was requested of the participants to promptly record any noteworthy negative occurrences. Every four weeks after the treatment began, laboratory results were assessed to keep an eye out for any possible side effects [29].

2.3.Data Analysis:

With SPSS 22.0, the statistical analyses were performed. The mean and standard deviation are used to convey baseline data [16]. The difference between the blood NAD concentration at day 30 or day 60 and the baseline NAD level was denoted as NADΔ [17]. Statistical significance

was indicated by $p< 0.05$ [16]. The CI is 95% as there may be a chance of a 5% error in the study.

3. Results

Following the screening of 86 volunteers, the experiment comprised 80 healthy participants of either gender, with a mean age of 50 years. Not a single individual exceeded the prescribed dosage by 100%. All subject took the NMN or placebo as directed during the intervention period, and not a single person reported a side effect. Table 1 show respondents characteristics at baseline for the placebo and three NMN-treated groups.

Based on parameters including SF-36 score, HOMAR-IR, Blood biological age, and Six Minute Walking, the study's investigation of the NMN supplement's impact on blood NAD concentration revealed a considerable difference in the NMN-treated and placebo groups over the course of 30 and 60 days (Table 2).

At baseline, in the case of placebo, the HOMAR-IR was 1.40, and it ascended to 2.09 on Day 60. A similar outcome was seen when 250 mg, 550 mg, and 850 mg of NMN were used, as the HOMAR-IR increased on Day 60. At Day 60, NMN 850 mg group showed a significant increase to 2.66 whereas there was a moderate increase in the HOMAR-IR in the case of NMN 250 mg and NMN 550mg cohort (Figure 2). When the NMN-treated cohort was compared to the placebo, there was no statistically substantial difference in the HOMA-IR score.

Walking distances were considerably greater for individuals in the 250 mg, 550 mg, and 850 mg NMN-treated cohort after 30 and 60 days as compared with baseline. In comparison to the baseline, the walking performance of the placebo group decreased at 30 days and then increased at 60 days. On days 30 and 60, all three NMN-treated groups had considerably higher walking distances in the six-minute walking test when compared to the placebo group. At days 30 and 60, individuals in the 850 mg NMN treatment group walked a statistically greater distance than those in the 250 mg NMN and 550 mg NMN treatment groups (Figure 3). The walking distance did not change qualitatively between the cohort administered with 550mg and 850 mg of NMN (both $p > 0.05$).

The blood biological age from baseline and at day 60 was compared for the 4 cohorts in the study (Figure 4). On average, the biological age was 38.79 years for the placebo cohort, 43.8 years for NMN 250mg, 43.9 years for 550mg, and 44.3 for NMN 850 mg. By day 60 there was

a considerable rise in the blood biological age up to 45.1 years in the case of placebo groups indicating an aging effect. There was a decline in an NMN treatment group. The NMN 250 mg saw a decline to 42.7 years, in case of NMN 550 mg biological blood age was reduced to 41.9 years and in NMN 850 mg group it was reduced more significantly to 40.79 years.

In case of SF-36 score, at the baseline the scores were similar across all the groups. By Day 30, the Placebo group increased to 124, NMN 250 mg to 130, NMN 550 mg to 128, and NMN 850 mg to 135. At day 60, there was a further elevation in the SF-36 score, with the Placebo group at 127, NMN 250 mg at 132, NMN 550 mg at 135, and NMN 850 mg at 137. On day 60, the SF-36 scores of all three NMN-treated groups were considerably higher than those of the placebo (Figure 5).

There was no statistically considerable variation between the cohorts at baseline ($P = 0.588$), and the NAD concentrations (Figure 6) were similar across all groups, with median values ranging from 6.1 nmol/L in the placebo group to 7.6 nmol/L in the NMN 550 mg (first occurrence) group. This suggests that before the intervention, the starting levels of NAD were identical. After supplementing for 30 days, the subjects treated with NMN showed a substantial rise in NAD level (NADΔ30) relative to the cohort receiving the placebo. The NMN 250 mg cohort showed a substantial rise (17 nmol/L) while the placebo group showed no change at all (1.2 nmol/L). Even higher increases were seen in the NMN 550 mg groups, with increments of 30.1 and 33.6 nmol/L, respectively. As of day 30, there had been a substantial difference between the groups ($P < 0.0001$). After 60 days, the groups treated with NMN showed significant increases in the change in NAD concentration (NADΔ60). The change in the NMN 250 mg group was 19.8 nmol/L, while the change in the placebo group stayed very small at 2.7 nmol/L. With changes of 36.4 nmol/L and 37.1 nmol/L, respectively, the NMN 550 mg groups showed an increase in the NAD level. These differences remained statistically relevant ($P < 0.0001$).

Table 1: Respondents characteristics at baseline for the placebo and three NMN-treated groups

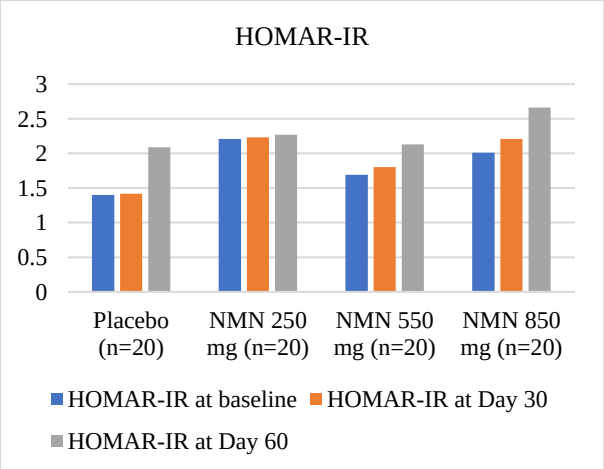
	Placebo (n=20)	**NMN 250 mg, n=20**	**NMN 550 mg, n=20**	**NMN 850 mg, n=20**	**p value**
Age (Year)	47.5 ± 5.7	51.2 ± 6.9	48.4 ± 6.9	49.9 ± 6.0	0.03

BMI (Kg/m^2)	33 ± 5.6	27.2 ± 6.6	30.1 ± 3.9	26.9 ± 4.9	0.05
Blood Biological age (years)	38.8 ± 6.2	41.2 ± 5.0	44.2 ± 6.4	44.3 ±7.2	0.27
Systolic Blood Pressure	128.8 ± 14.9	127.2 ± 14.9	128.2 ± 14.9	129.2 ± 13.9	0.01
Diastolic Blood Pressure	80.8 ± 11.1	79.4 ± 11.9	82.3 ± 11.9	81.4 ± 11.8	0.02
NAD (nM)	6.1 ± 6.16	7.4 ± 11.6	7.6 ± 3.30	8.0± 5.8	0.20
Six-minute walking (m)	324 ± 140	306 ± 107	289 ± 91	323 ± 112	0.27
HOMAR-IR	1.40 ± 0.77	2.21 ± 1.42	1.69 ± 1.02	2.01 ± 1.22	0.25
SF-36 (score)	122 ± 12	121 ± 13	117 ± 15	123 ± 16	0.40

Table 2: The effectiveness of three NMN-treated groups and the placebo, as well as comparisons of treatment over baseline within the same cohort

	Baseline	**Day 30**	**Day 60**	**p Value**
Placebo (n=20)				
Six-minute walking (m)	324 ± 140	310 ± 125	330 ± 100	0.05
HOMAR-IR	1.40 ± 0.77	1.42 ± 0.80	2.09 ± 1.40	0.048
SF-36 (score)	122 ± 12	124 ± 11	127 ± 13	0.2
Blood Biological age (years)	38.8 ± 6.2	-	45.1 ± 8.3	0.029
NMN 250 mg (n=20)				

Six-minute walking (m)	306±107	349 ± 115	380 ± 143	0.23
HOMAR-IR	2.21 ± 1.42	2.23 ± 1.41	2.27 ± 1.42	0.003
SF-36 (score)	121 ± 13	130 ± 11	132 ± 10	0.058
Blood Biological age (years)	43.8 ± 5.0	-	42.7 ± 6.7	0.46
NMN 550 mg (n=20)				
Six-minute walking (m)	289 ± 91	399 ± 87	436 ± 103	0.57
HOMAR-IR	1.69 ± 1.02	1.80 ± 1.01	2.13 ± 1.03	0.037
SF-36 (score)	117 ± 15	128±13	135 ± 13	0.015
Blood Biological age (years)	43.9 ± 6.4	-	41.9 ± 6.4	0.57
NMN 850 mg (n=20)				
Six-minute walking (m)	323 ± 112	424 ± 142	481 ± 127	0.016
HOMAR-IR	2.01 ± 1.22	2.21 ± 1.00	2.66 ± 1.32	0.006
SF-36 (score)	123 ± 16	135 ± 11	137 ± 10	0.005
Blood Biological age (years)	44.3 ± 7.2	-	40.8 ± 5.9	0.04



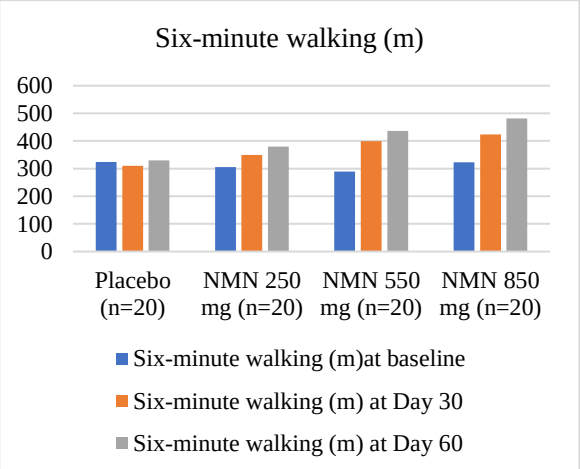


Figure 2: Comparisons between the HOMAR-IR at baseline, day 30 & day

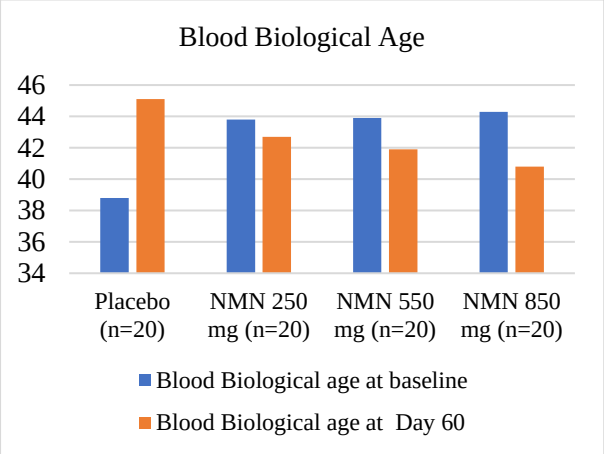


Figure 4: Comparisons between the blood blood biological age at baseline and day 60

Figure 3: Comparisons between the Six minute walk(m) at baseline, day 30 & day 60

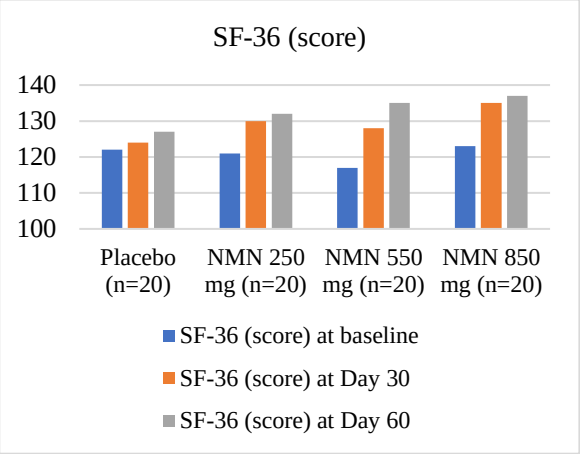


Figure 5: Comparisons between the SF -36 (score) at baseline, day 30 & day 60

Table 3: NAD concentration in the blood of the subject for placebo and NMN three groups at baseline, at day 30 and day 60

	Placebo (n=20)	**NMN 250 mg (n=20)**	**NMN 550 mg (n=20)**	**NMN 850 mg (n=20)**	**P Value**
NAD baseline (nmol/L	6.1(4.6,9.4)	7.4(5.7,15)	7.6(5.7,8.9)	8(6.0,12.2)	0.588
NADΔ30 (nmol/L)	1.2(8.1)	17(20.4)	30.1(13.1)	33.6(15.3)	<0.0001
NADΔ60 (nmol/L	2.7(9.1)	19.8(23.2)	36.4(11.9)	37.1(19.8)	<0.0001

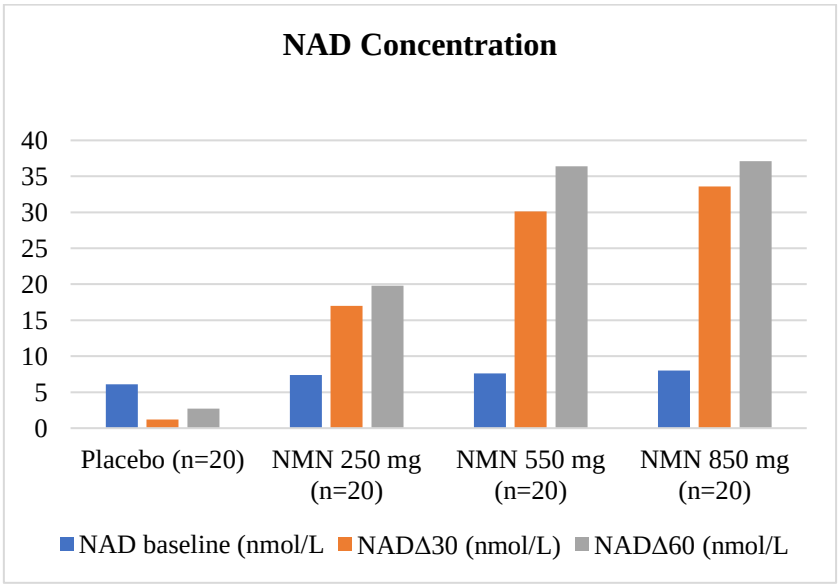


Figure 6: NAD concentration at baseline, at day 30, & day 60 in Placebo and three NMN-treatment groups

4. Discussion

The research assesses the impact of NMN supplements on blood NAD levels in addition to evaluating their effects on many health outcomes utilizing measures like the HOMAR-IR, six-minute walking distance, SF-36 score, and biological age.

In the study, giving NMN supplements to the participants did not have any negative consequences. According to research by Irie et al., giving NMN orally once up to 500 mg was safe, well-tolerated, and did not significantly harm healthy men [13]. In another study conducted by Mills et al shows that throughout the 12-month treatment period, NMN administration did not result in any visible toxicity, severe adverse reactions, or elevated death rates, indicating NMN's long-term safety [22]. The result of the study was contradictory with the study conducted by Okabe et al in which they observed gastrointestinal symptoms as a side effect [29] also in another study conducted by Yi et al there were some AEs were found that were mild or severe and none was associated to the NMN treatment [20].

All groups, including the placebo group, had increases in the HOMAR-IR index, a marker of insulin resistance, irrespective the beneficial gains in NAD levels. When comparing the three NMN concentration levels to the placebo, the HOMAR-IR index increased. Yet, no statistically considerable variations were observed in HOMAR-IR between the groups who received NMN treatment and the placebo group, suggesting that NMN supplementation had no discernible effect on insulin resistance. Following NMN consumption, postprandial blood insulin levels increased considerably, according to research by Yamane et al. The lack of prior observations of a rise in insulin levels in clinical trials including NMN intake is likely because the earlier investigations assessed insulin concentrations in fasting blood [6]. The outcome was opposed by Kuerec et al. who found that using NMN supplements raises blood NAD concentrations, which are linked to improvements in insulin resistance [17].

On days 30 and 60 of the six-minute walking test, every NMN-treated cohort in the research showed substantial improvements over the baseline.The outcome of the placebo group decreased on day 30, but by day 60, it had improved. At both time points, the 850 mg NMN group showed the most improvement, walking noticeably farther than the 250 mg and 550 mg groups. The research indicates that using NMN supplements enhances physical capabilities. In

people with chronic illnesses, NMN supplementation may be a useful strategy for improving physical function and general well-being by restoring NAD+ levels and supporting cellular function, according to research by Loreto et al. and Sharma et al. However, more studies are required to fully determine its beneficial effects on health [23,14]. Blood NAD concentrations were observed to rise statistically considerably over baseline in all NMN-treated cohorts in research by Yi et al. at days 30 and 60 [20]. A study by Kuerec et al. discovered an association between a rise in blood NAD concentration and an improvement in the 6-minute walking distance, which is a measure of improved physical performance. The link between blood NAD concertation and a functional outcome indicator is described for the first time in their work [17].

The study evaluates the blood biological age at baseline and day 60. It was observed that there was an increase in blood biological age among the placebo groups. This highlights the aging process among the group and emphasizes that without intervention, blood biological age tends to rise. In contrast, there is a decrease in the blood biological age among the groups that received NMN treatment. The maximum reduction was observed at 850 mg, indicating that NMN may result in slowing or reversing the aging process. The study also found that as the concentration of the NMN supplement increased there was a considerable reduction in the aging process. Thus, the study highlights the dose-dependent response, indicating that there might be an optimal dosage range where the anti-aging benefits of NMN are maximized. According to a study by Nadeeshani et al., this breakdown of interaction involving the nucleus and mitochondria causes a sharp drop in the function of mitochondria, which in turn contributes to age-related problems and illnesses. On the other hand, certain routes of communication can be reestablished and mitochondrial function can be enhanced by giving NMN as a precursor to NAD+ [4]. In a different study, Niu et al. discovered that giving NMN to pre-aging mice and human volunteers can increase their biological age by lengthening their telomeres, a molecular indicator of ageing [26]. Furthermore, a study by Bhasin et al. demonstrates that NMN is a precursor that can increase NAD levels, which decrease with age and are associated with age-related diseases. This suggests that augmenting NAD+ may be a useful tactic to prevent and cure age-related ailments and metabolic disorders in humans [21]. Song et al. highlighted in their study that longer-term research with large populations have not yet been carried out, so it is simply too early to say with assurance whether NAD+ supplementation will delay age-related functional loss and lower the chance of disease in the wider population [25].

SF-36 significantly improved in the study's NMN-treated group as well. The concentration of NAD increased somewhat in the placebo group. All NMN-treated groups outperformed the placebo group on day 60 in terms of SF-36 scores, suggesting that NMN supplementation improves the quality of life connected to health. The study also suggests that a higher dose of NMN may be more effective as there was the highest improvement in health-related quality of life in the case of 850mg. In a study conducted by Kuerec et al found that the increase in the blood NAD concentration was linked with a higher overall SF-36 score [17]. In a study conducted by Yi et al there was no substantial difference in SF-36 ratings between the NMN-treated individuals [20].

NAD concentrations at baseline were nearly identical in all groups, indicating that each person's starting NAD levels prior to the intervention were comparable. When compared to the placebo, the NAD concentration increased at all NMN values after 30 days. At 60 days, the NMN-treated groups' NAD concentration changes (NADΔ60) were still significantly higher. The study also showed that, by day 30, all groups aside from the placebo group saw an increase in NAD concentration in response to an increase in NMN concentration. This shows that NMN may be a powerful therapeutic intervention to improve NAD production, which may improve cellular metabolism and general health in several ways. Permatasari et al.'s investigation revealed that an increase in NMN concentration can increase NAD concentration. Additionally, they stressed the need to utilize NMN supplements sparingly because overdosing could lead to unfavorable effects [5]. Additionally, a study by Liu et al. discovered that NMN consumption increases the body's levels of both NMN and NAD+, demonstrating a clear correlation between NMN concentration and NAD+ levels [27]. According to a study by Katayoshi et al., supplementing with 250 mg of NMN for 12 weeks was secure and successful in promoting NAD metabolism in middle-aged, apparently healthy people [28]. In another study conducted by Okabe et al, the group treated with NMN, there was a considerable increase in the levels of NAD+ in whole blood. These findings imply that oral NMN treatment is safe and may be a useful method for raising human NAD+ levels [29].

5. Conclusion

Supplementing with NMN raises blood NAD concentrations dramatically, promotes physical performance, and improves quality of life without having any negative side effects. It is possible to enhance cellular NAD+ levels with oral administration of NMN, which may be

used as a therapeutic technique to improve human physical performance. The lack of negative side effects provides more evidence for the safety of NMN supplementation in healthy people. However, the rise in HOMAR-IR points to a possible effect on insulin resistance that needs more research. Biological age decreased among NMN treated, indicating potential anti-aging benefits. This study suggests that NMN helps in improving physical performance, and quality of life and slowing the aging process by increasing NAD, with effects becoming more noticeable at higher dosages. Future research must look at the pharmacokinetics of NMN and NAD+ in the plasma and tissues, optimal dosages, and long-term benefits of NMN on human health.

REFERENCES:

1. Alegre GF, Pastore GM. Nad+ precursors nicotinamide mononucleotide (nmn) and nicotinamide riboside (nr): Potential dietary contribution to health. Current nutrition reports. 2023 Sep;12(3):445-64.
2. Xie N, Zhang L, Gao W, Huang C, Huber PE, Zhou X, Li C, Shen G, Zou B. NAD+ metabolism: pathophysiologic mechanisms and therapeutic potential. Signal transduction and targeted therapy. 2020 Oct 7;5(1):227.
3. Braidy N, Berg J, Clement J, Khorshidi F, Poljak A, Jayasena T, Grant R, Sachdev P. Role of nicotinamide adenine dinucleotide and related precursors as therapeutic targets for age-related degenerative diseases: rationale, biochemistry, pharmacokinetics, and outcomes. Antioxidants & redox signaling. 2019 Jan 10;30(2):251-94.
4. Nadeeshani H, Li J, Ying T, Zhang B, Lu J. Nicotinamide mononucleotide (NMN) as an anti-aging health product–promises and safety concerns. Journal of advanced research. 2022 Mar 1;37:267-78.
5. Permatasari NK, Widiantara IG, Putra B, Purnamasidhi CA, Aryana IG. Nicotinamide Mononucleotide (NMN) as a Solution of Decrease Nicotinamide Adenine Dinucleotide (NAD+) in Aging.
6. Yamane T, Imai M, Bamba T, Uchiyama S. Nicotinamide mononucleotide (NMN) intake increases plasma NMN and insulin levels in healthy subjects. Clinical Nutrition ESPEN. 2023 Aug 1;56:83-6.
7. Poddar SK, Sifat AE, Haque S, Nahid NA, Chowdhury S, Mehedi I. Nicotinamide mononucleotide: exploration of diverse therapeutic applications of a potential molecule. Biomolecules. 2019 Jan 21;9(1):34.

8. Arenas-Jal M, Suñé-Negre JM, García-Montoya E. Therapeutic potential of nicotinamide adenine dinucleotide (NAD). European Journal of Pharmacology. 2020 Jul 15;879:173158.
9. Foster JW, Moat AG. Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbial systems. Microbiological reviews. 1980 Mar;44(1):83-105.
10. Dölle C, Hvidsten Skoge R, R VanLinden M, Ziegler M. NAD biosynthesis in humans-enzymes, metabolites and therapeutic aspects. Current topics in medicinal chemistry. 2013 Dec 1;13(23):2907-17.
11. Li F, Wu C, Wang G. Targeting NAD Metabolism for the Therapy of Age-Related Neurodegenerative Diseases. Neuroscience Bulletin. 2024 Feb;40(2):218-40.
12. Baquero F, Del Campo R, Martínez JL. Interventions in nicotinamide adenine dinucleotide metabolism, the intestinal microbiota and microcin peptide antimicrobials. Frontiers in Molecular Biosciences. 2022 Mar 14;9:861603.
13. Irie J, Inagaki E, Fujita M, Nakaya H, Mitsuishi M, Yamaguchi S, Yamashita K, Shigaki S, Ono T, Yukioka H, Okano H. Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men. Endocrine journal. 2020;67(2):153-60.
14. Sharma A, Chabloz S, Lapides RA, Roider E, Ewald CY. Potential synergistic supplementation of NAD+ promoting compounds as a strategy for increasing healthspan. Nutrients. 2023 Jan 14;15(2):445.
15. Igarashi M, Yamauchi T. Insight into the application of nicotinamide mononucleotide (NMN) to age-related disorders. Journal of Cellular and Molecular. 2023;2(1):9-13.
16. Liao B, Zhao Y, Wang D, Zhang X, Hao X, Hu M. Nicotinamide mononucleotide supplementation enhances aerobic capacity in amateur runners: a randomized, double-blind study. Journal of the International Society of Sports Nutrition. 2021 Dec;18:1-9.
17. Kuerec AH, Wang W, Yi L, Tao R, Lin Z, Vaidya A, Pendse S, Thasma S, Andhalkar N, Avhad G, Kumbhar V. Towards personalized nicotinamide mononucleotide (NMN) supplementation: nicotinamide adenine dinucleotide (NAD) concentration. Mechanisms of Ageing and Development. 2024 Apr 1;218:111917.
18. Huang H. A multicentre, randomised, double blind, parallel design, placebo controlled study to evaluate the efficacy and safety of uthever (NMN supplement), an orally administered supplementation in middle aged and older adults. Frontiers in aging. 2022 May 5;3:851698.

19. Schwarzmann L, Pliquett RU, Simm A, Bartling B. Sex-related differences in human plasma NAD+/NADH levels depend on age. Bioscience Reports. 2021 Jan;41(1):BSR20200340.
20. Yi L, Maier AB, Tao R, Lin Z, Vaidya A, Pendse S, Thasma S, Andhalkar N, Avhad G, Kumbhar V. The efficacy and safety of β-nicotinamide mononucleotide (NMN) supplementation in healthy middle-aged adults: a randomized, multicenter, double-blind, placebo-controlled, parallel-group, dose-dependent clinical trial. Geroscience. 2023 Feb;45(1):29-43.
21. Bhasin S, Seals D, Migaud M, Musi N, Baur JA. Nicotinamide adenine dinucleotide in aging biology: potential applications and many unknowns. Endocrine Reviews. 2023 Dec;44(6):1047-73.
22. Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. Cell metabolism. 2016 Dec 13;24(6):795-806.
23. Loreto A, Antoniou C, Merlini E, Gilley J, Coleman MP. NMN: the NAD precursor at the intersection between axon degeneration and anti-ageing therapies. Neuroscience Research. 2023 Dec 1;197:18-24.
24. Yoshino M, Yoshino J, Kayser BD, Patti GJ, Franczyk MP, Mills KF, Sindelar M, Pietka T, Patterson BW, Imai SI, Klein S. Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science. 2021 Jun 11;372(6547):1224-9.
25. Song Q, Zhou X, Xu K, Liu S, Zhu X, Yang J. The safety and anti-ageing effects of nicotinamide mononucleotide in human clinical trials: an update. Advances in nutrition. 2023 Aug 22.
26. Niu KM, Bao T, Gao L, Ru M, Li Y, Jiang L, Ye C, Wang S, Wu X. The impacts of short-term NMN supplementation on serum metabolism, fecal microbiota, and telomere length in pre-aging phase. Frontiers in nutrition. 2021 Nov 29;8:756243.
27. Liu Y, Chen X, Deng X, Yang F, Zheng J, Zhou T, Xu L, Xie X, Ju Z, Wang B, Zhang C. Association of NAD+ levels with metabolic disease in a community-based study. Frontiers in Endocrinology. 2023 Apr 20;14:1164788.
28. Katayoshi T, Uehata S, Nakashima N, et al. Nicotinamide adenine dinucleotide metabolism and arterial stiffness after long-term nicotinamide mononucleotide supplementation: a randomized, double-blind, placebo-controlled trial. Sci Rep. 2023;13(1). doi:10.1038/s41598-023-29787-3

29. Okabe K, Yaku K, Uchida Y, Fukamizu Y, Sato T, Sakurai T, Tobe K, Nakagawa T. Oral administration of nicotinamide mononucleotide is safe and efficiently increases blood nicotinamide adenine dinucleotide levels in healthy subjects. Frontiers in nutrition. 2022 Apr 11;9:868640.