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SYSTEMATIC REVIEW

Evaluation of safety and effectiveness of NAD in different clinical conditions: a systematic review

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Abstract

Nicotinamide adenine dinucleotide (NAD^+) is an essential pyridine nucleotide cofactor that is present in cells and in several important biological processes, including oxidative phosphorylation and production of adenosine triphosphate, DNA repair, calcium-dependent secondary messenger and gene expression. The purpose of this systematic review is to examine whether the coenzyme formulae NAD^+ and NADH are safe and effective when acting as a supplement to humans. This systematic review of randomized clinical trials performed a search in six electronic databases: PubMed, MEDLINE (*ovid*), Embase, Cochrane CENTRAL (clinical trials), Web of Science, and Scopus. Secondary search included the databases (e.g., Clinical trials.gov, Rebec, Google Scholar - advance). Two reviewers assessed and extracted the studies independently. The risk of bias in studies was performed using version 2 of the Cochrane risk of bias tool for randomized trials. This review includes 10 studies, with a total of 489 participants. The studies included different clinical conditions, such as chronic fatigue syndrome (CFS), older adults, Parkinson's disease, overweight, postmenopausal prediabetes, and Alzheimer's disease. Based on studies, the supplementation with NADH and precursors was well tolerated and observed clinical results such as, a decrease in anxiety conditions and maximum heart rate was observed after a stress test, increased muscle insulin sensitivity, insulin signaling. Quality of life, fatigue intensity, and sleep quality among others were evaluated on patients with CFS. All studies showed some side effects, thus, the most common associated with NADs use are muscle pain, nervous disorders, fatigue, sleep disturbance, and headaches. All adverse events cataloged by the studies did not present a serious risk to the health of the participants. Overall, these findings support that the oral administration of NADH can be associated to an increase in general quality of life and improvement on health parameters (e.g., a decrease in anxiety, maximum heart rate, inflammatory cytokines in serum, and cerebrospinal fluid). NADH supplementation is safe and has a low incidence of side effects. Future investigations are needed to evidence the clinical benefits regarding specific diseases and doses administered.

effectiveness; NAD; NAD^+; NADH; safety

BACKGROUND

Nicotinamide adenine dinucleotide (NAD) is a coenzyme that was identified for its role in regulating metabolic rates as a major hydride acceptor in redox reactions (1). The nicotinamide adenine dinucleotide presents itself in the body as NAD^+ (an oxidized form of nicotinamide adenine) and NADH (a reduced form of a nicotinamide adenine found in every cell of the human body). Generally, when humans ingest carbohydrates, these are metabolized as sugars and later absorbed by the cells in many reactions on the Krebs cycle. When these carbohydrates are broken, they generate electrons that are collected by the NAD^+. With the presence of electrons, the NAD^+ is then reduced and forms NADH (2). NADH carries the electrons and donates them to the enzyme chain that is involved in the production of ATP, which is an indispensable molecule that stores energy and releases it when necessary, being a supply of energy to the body.

Furthermore, NADH from external sources is capable of entering a cell through the membrane and since it carries electrons inside the cell, it helps in the production of ATP and available energy for the body (3). There are two discrete routes where NAD^+ is synthesized in humans: the deamidated pathway synthesis from tryptophan generates quinolinic acid which is converted to nicotinic acid mononucleotide (NAMN); second, the amidated pathway precursors such as vitamin B3 compounds nicotinamide (NAM) or nicotinamide riboside (NR) generate nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyl

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transferase (NAMPT) or nicotinamide riboside kinase (NRK), respectively (4).

Physiological NAD/NADH levels decline with increasing age, as the activities of both the salvage pathways and synthesis are reduced, the result of altered levels of rate-limiting enzymes and precursors (5). The decrease of NAD^+ could be associated with a higher risk for diseases and/or disabilities, cognitive dysfunction, auto-immunity, and dysregulation of the immune response (6). In addition, studies showed several problems based on the decline of NAD^+. During an intervention, NAD^+ boosters in mouse models were shown to prevent or to treat a variety of different diseases. Commercially and scientifically, a search for NAD boosters that are safe and effective as drugs to treat both rare and common diseases is being conducted, especially because of the potentially anti-aging effect itself (7). Based on these evidence, studies showed a drop in NAD levels can be prevented with the supplementation of NAD precursors, such as nicotinamide, nicotinic acid, nicotinamide mononucleotide (NMN), and nicotinamide riboside (NR) (8).

Clinical evidence suggests that NAD is a promising substance to treat age-related conditions and has a favorable side effect profile for human use. Studies assess NAD pharmacology in the context of aging and metabolic and age-related disease (9). NAD has been discussed as a therapeutic intervention for immunity decline and chronic inflammation. The available NAD supplementation found commercially can be found in various forms, such as NADH, NAD^+, NAM, and NR, with dosages ranging from 4 mg/day to 1,000 mg/day administered orally. The effect of each supplementation and the differences between them is still unclear. Many studies indicate benefits such as increases in blood NAD^+ levels following supplementation with NAM and NR, favorable outcomes on several age-related disorders associated with the accumulation of chronic oxidative stress, inflammation and impaired mitochondrial function in animal models and humans (10, 11). However, this evidence is limited, so this systematic review is aimed to examine if the coenzyme formula NAD^+ and NADH are safe and effective as a supplementation to humans in different conditions.

METHODS

This systematic review followed the guidelines of Preferred Reporting Items for systematic reviews and meta-analysis (PRISMA) (12). This systematic review was registered on PROSPERO (CRD42022349043), and updates to the protocol were provided when needed. The following PICO strategy was used for the search: Population: humans; Intervention: NAD, NAD^+ NADH, NR, NMN; Comparator: usual care, other treatments, and/or placebo; Outcomes: safety and effectiveness/adverse events.

ELIGIBILITY CRITERIA

We included only randomized controlled trials and randomized clinical trials (both referred to RCTs) in this review. Studies that were other than RCTs with humans with age ranging from 18 to 70 yr old who participated in interventions with NAD, NAD^+, NADH, NR, and NMN administered in different dosages (e.g., 100, 300, or 1,000 mg) through the oral route. Outcomes such as safety, effectiveness, and adverse events were analyzed. Studies with animals, and articles that associated NAD, NAD^+, NADH, NR, or NMN with other hormones or drugs. Studies without an appropriate control group were also excluded.

Types of Studies

We included only randomized controlled trials and randomized clinical trials in this review. Studies that were other than RCTs, observational studies, single abstracts, or incomplete texts were excluded.

Types of Participants

The types of participants were not criteria for inclusion or exclusion of articles. The trials had to report outcome data on the administration of NAD, NAD^+ NADH, NR, and NMN; however, the population and its characteristics were not predefined.

Types of Interventions

We included interventions with NAD, NAD^+ NADH, NR, and NMN. Interventions that use different milligrams of NAD and its precursors (e.g., 5 mg or 250 mg) were included, as well as different ways of administration (e.g., oral), duration, and frequency (e.g., weeks or months). We excluded interventions with the association of NAD with other drugs or treatments.

Types of Outcomes

The reported outcomes were not predefined in the search period and specific outcomes were not a reason for inclusion or exclusion. Secondary outcomes included the adverse events/side effects.

Search Methods for Identification of Studies

The literature search was conducted in the following databases: PubMed, MEDLINE (ovid), Embase, Cochrane CENTRAL (clinical trials), Web of Science, and Scopus. Other possible databases (e.g., Clinical trials.gov, Rebec, Google Scholar – advance). The reference lists of papers included in the review were searched for additional relevant publications. Gray literature searching was performed by using web-based, gray literature catalogs, Ethos and Open Grey. The search strategy was developed using a combination of subject headings (i.e., Medical Subject Headings) and author keywords for the following concepts: "NADH," "NAD^+," and "human." The full search strategy is reported in Table 1. There were no restrictions by language or date. The first search was performed in September 2022 and updated in February 2023.

DATA COLLECTION AND ANALYSIS

The process of selection and extraction was conducted by two reviewers independently using Rayyan QRCI (13), any conflicts were resolved by a third independent reviewer. First, we removed all duplicates, analyzed the title and abstract, and then finally analyzed full texts. All eligibility criteria were respected based on population, intervention, and outcomes. The data collected included: *1*) primary

Table 1. *MEDLINE (Ovid) search strategy*

Search Strategy
1. NAD +
2. NADH
3. "Nicotinamide adenine dinucleotide"
4. "coenzyme"
5. "nicotinamide mononucleotide"
6. "Nicotinamide riboside"
7. Nadide
8. "Dihydronicotinamide Adenine Dinucleotide"
9. humans
10. human
11. clinical trials
12. "clinical trial"
13."randomized controlled trial"
14. RCT
15. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8
16. #9 OR #10
17. #11 OR #12 OR 13 OR 14
18.#15 AND #16 AND #17

NAD, nicotinamide adenine dinucleotide; RCT, randomized clinical trial.

characteristics of the study (author's name, publication year, country, study design); *2*) participants characteristics (diagnostic, mean age, sex percentage of gender, weight, height, and body mass index); *3*) characteristics of the intervention and comparison (type, dose, administration route and frequency, duration, adherence, dropouts); *4*) characteristics of our main outcomes (adverse events, safety, effectiveness).

Assessment of Methodological Quality

The methodologic quality of studies was assessed using Cochrane Risk of Bias tool 2.0 (14). There are domains such as random sequence generation, blinding, allocation concealment, incomplete outcome data and selective reporting (14). All studies were classified as low risk of bias, high risk, and unclear. This process was managed by two reviewers (I.K.d.S. and G.F.) independently, and all conflicts were observed and discussed between reviewers. A third independent reviewer (I.d.M.G.) resolved any conflicts.

RESULTS

Our search identified 1,902 articles. After title and abstract screening, 57 articles were assessed for inclusion as full text, of which 10 met the inclusion criteria for the review. The flow of information and the breakdown of included and excluded studies are shown in Fig. 1.

Included Studies Characteristics

This review includes 10 studies, with a total of 489 participants. Trials were conducted in the USA (3 studies) (15–17), Spain (18), Norway (19), Netherlands (20), Croatia (21), Denmark (22), Austria (23), and Japan (24) (1 study each). The median sample size was around 50 but ranged from 13 to 108 participants. Participants were selected considering the diagnosis of chronic fatigue syndrome (CFS) in the studies of Alegre et al. and Forsyth et al. (18, 23). Brakedal et al. (19) included participants with diagnosed Parkinson's disease, while Demarin et al. (21) included participants with diagnosed Alzheimer's disease (AD). Middle-aged obese males with sedentary habits were selected by Dollerup et al. (22) and physically compromised older adults were selected by Connel et al. (20). The remaining studies included healthy men and women (20). Only the study by Yoshino et al. (15) included postmenopausal women with prediabetes who were overweight or obese. Chronic fatigue syndrome was diagnosed according to the criteria and review of the Centers for Disease Control and Prevention (CDC) (25). Further details regarding reasons for exclusion in phase 2 are presented in APPENDIX A and the complete list of references to included articles is available in APPENDIX B.

Dose, Administration Route, Frequency, and Setting Interventions

All the included articles reported the oral administration of NAD and its precursors:

Nicotinamide Adenine Dinucleotide

Three studies evaluated the effectiveness of NADH in participants with chronic fatigue syndrome (CFS) and Alzheimer's disease. Alegre et al. (18), Demarin et al. (21), and Forsyth et al. (23) administered 5, 10, and 100 mg of NADH per day orally for a 3- and 6-mo period. Based on results Forsyth et al. (23) observed NADH may be a valuable adjunctive therapy in the management of chronic fatigue syndrome. This response was characterized by improvement in fatigue, decrease of symptoms, and improvement in quality of life. Alegre et al. (18) related supplementation with oral NADH in patients with CFS was able to reduce the level of anxiety, maximum HR, and maximum HR/theoretical HR, but did not significantly modify other clinical and quality of life variables related to this syndrome. Furthermore, Alegre et al. (18) showed NADH is a coenzyme that plays a key role in cellular energy production and stimulates dopamine

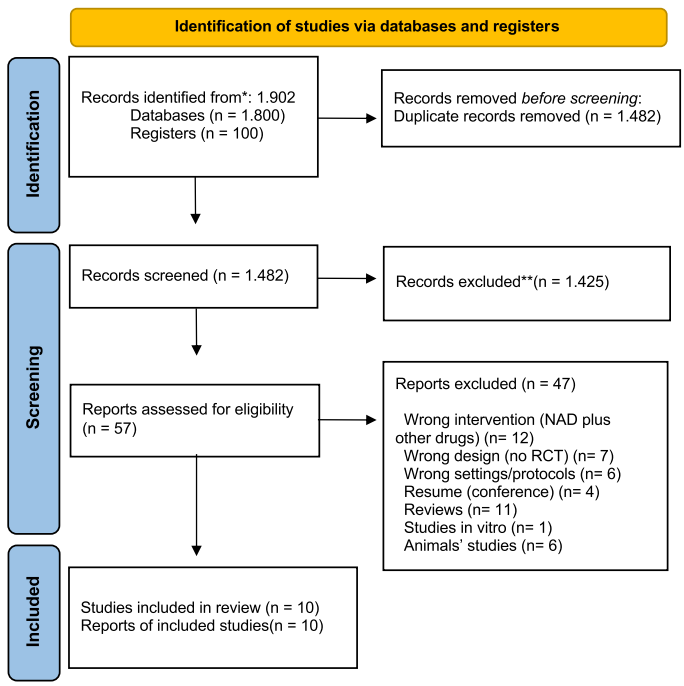

Figure 1. Breakdown of included and excluded articles. NAD, nicotinamide adenine dinucleotide; RCT, randomized clinical trial.

production. After 6 mo of treatment, subjects treated with NADH showed no evidence of progressive cognitive deterioration and had significantly higher total scores on the Mattis Dementia Rating Scale (MDRS) compared with subjects treated with placebo (18).

Nicotinamide Riboside

Four studies investigated the potential of nicotinamide riboside (NR) for different clinical populations (16, 17, 19, 22). The trials of Dollerup et al. (22) and Brakedal et al. (19) administered NR of 1,000 mg/day orally. Martens et al. (17) compared the administration of 500 mg/day for 48 days and Conze et al. (16) reported the NR by oral administration of 100 mg/day orally. The duration of NR was between 30, 48, and 58 days. In addition, Dollerup et al. (22) reported that the precursor NR had no changes in circulating adipsin or bile acids in nondiabetic males with obesity. Moreover, bile acid levels in plasma did not change in response to NR supplementation. Conze et al. (16) found NR dose-dependently and significantly increased whole blood NAD^+ and other NAD^+ metabolites within 2 wk.

Brakedal et al. (19) and Martens et al. (17) showed NR treatment was well tolerated and led to a significant, but variable, increase in cerebral NAD levels. Furthermore, NR decreases the levels of inflammatory cytokines in serum and cerebrospinal fluid and effectively stimulates NAD^+ metabolism in healthy middle-aged and older adults.

Nicotinamide Mononucleotide

Two studies used NMN supplementation (15, 24). Kim et al. (24) and Yoshino et al. (15) included an administration of 250 mg/day of nicotinamide mononucleotide for 48 and 56 days. This study observed NMN intake in the afternoon effectively improved lower limb function and reduced drowsiness in older adults. The potential of NMN is related to preventing loss of physical performance, improving fatigue, increases in muscle insulin sensitivity, insulin signaling.

Nicotinic Acid + Nicotinamide

Connell et al. (20) compared the administration of 4 mg/day and 200 mg/day for 32 days. The NAD^+ precursors L-Trp, NA, and NAM improved mitochondrial oxidative capacity in community-dwelling older adults with impaired physical function, but no improvement in mitochondrial respiration.

Comparator

In general, comparator groups were described as receiving placebo treatment, which consisted of lactose tablets, whey protein or maltitol, crystalline cellulose, silicon dioxide, and magnesium stearate. The articles reported tablets were similar to the intervention, except for Connell et al. (20) who administered the dose through a powder dissolved in 200 mL of water. The adherence of the treatments with both intervention and placebo ranged from 95 to 98%. Table 2 summarizes the population and intervention characteristics of the included studies.

Effects of Intervention

Table 2 summarizes the results of the included studies and their main characteristics considering the participants profiles. The articles reported outcomes such as fatigue intensity, mood functional impact of fatigue, quality of life, sleep quality, adherence, general safety, plasma concentration and urinary metabolites, clinical dementia scale, verbal recognition memory, blood hormones, adverse events, metabolic response, and physical performance tests. It is worth mentioning the articles presented a broad spectrum of outcomes with many nonhomogeneous variables. Each article presented outcomes specific to the investigated disease. For example, Demarin et al. (21) included participants with diagnosed Alzheimer's disease, thus, the reported outcomes were: clinical dementia scale and verbal recognition memory. On trials in which the nicotinamide riboside supplementation for a healthy population was evaluated, or fatigue was being evaluated, the reported outcomes were regarding tolerability, adverse events, and adherence of patients. Thus, we sorted the analysis of the intervention in some clinical conditions mentioned in the articles.

Alzheimer's disease.

Considering the measure of the validated and generally accepted measure of dementia, the MDRS, patients with Alzheimer's disease receiving stabilized orally absorbable NADH showed significantly better performance after 6 mo of double-blind treatment than patients receiving placebo. Authors state that it is difficult to directly compare the findings on NADH with other therapies and outcome measures; however, adverse or side effects were not reported during the 6-mo treatment period. Statistically significant improvements in certain cognitive functions were observed, and the authors are positive that the treatment could also improve certain cognitive functions in patients with AD. The analysis of MDRS subscales revealed significantly better performance by NADH subjects on measures of verbal fluency, visual constructional ability and a tendency to better performance on a measure of abstract verbal reasoning (21).

Postmenopausal women.

Based on the study, using NMN supplementation increases muscle insulin sensitivity, insulin signaling, and remodeling in women with prediabetes who are overweight or obese (15).

Chronic fatigue syndrome.

Based on two studies using the supplementation with NADH, a decrease in anxiety conditions and maximum heart rate was observed after a stress test. Quality of life, fatigue intensity, and sleep quality among others were evaluated in patients with CFS. General mood (states of anxiety or depression) showed no difference after the treatment with NAD and placebo, ranging stated as 10% (16). Quality of life was separated into many variables for Alegre et al. (18); however, there is a significant increase in body pain using NADH when compared with placebo (from 17.6 to 18.4% after treatment). However, Alegre et al. (18) reported that the clinical variables and quality of life related to the CFS did not change significantly after the administration of NADH. Forsyth et al. (23) reported four times as many patients responded to NADH in contrast to placebo. This response was characterized by improvement in fatigue, decrease of symptoms, and improvement in quality of life. The authors also state NADH

Table 2. *Population, intervention characteristics of the included studies (n = 10)*

Author Study	Country	Population	Study Design	Population Characteristics	Intervention/Comparator	Duration of Intervention (days)	Administration Route and Frequency
Alegre et al. (18)	Spain	88 patients (5 men and 77 women) > 18–65 yr	RCT double blind	Chronic fatigue syndrome (CFS)	NADH – (Vitanadhs) (20 mg)/Placebo	90	4 tablets/day, orally
Demarin et al. (21)	Croatia	26 patients > 52–79 yr	RCT double blind	Alzheimer's disease	NADH (Enada) (5 mg)/ Placebo	180	2 tablets
Forsyth et al. (23)	Austria	35 patients > 20–70 yr	RCT double blind	Chronic fatigue syndrome (CFS)	NADH (Enada) (10 mg)/ Placebo	48	2 tablets, orally
Brakedal et al. (19)	Norway	30 patients (25 men and 5 women) > 18–45 yr	RCT double blind phase 1	Parkinson's disease	Nicotinamide riboside (NR) (1,000 mg)/Placebo	30	Orally
Dollerup et al. (22)	Denmark	40 males > 40–70 yr	RCT double blind	Males, obese (BMI.30 kg/m^2), sedentary (30 min of exercise per day) and middle-aged	Nicotinamide riboside (NR) (Niagen) (1,000 mg)/ Placebo	48	2 tablets, orally
Martens et al. (17)	USA	24 patients> 55–79 yr	RCT, placebo-controlled, crossover clinical trial	Healthy middle-aged and older men and women	Nicotinamide riboside (NR) (Niagen) (500 mg)/ Placebo	48	Orally
Conze et al. (16)	USA	100 patients > 40–60 yr	RCT double blind	Healthy men and non-pregnant, nonbreast-feeding women	Nicotinamide riboside (NR) (Niagen) doses (100, 300, 1,000 mg)/ Placebo	56	4 capsules/day, orally
Connell et al. (20)	Netherlands	13 patients > 18–45 yr	RCT double blind	Physically compromised older adults	Nicotinic acid (NA) and Nicotinamide (NAM) NA (4 mg) NAM (200 mg)/ Placebo	32	Whey protein powder in 200 mL, orally
Kim et al. (24)	Japan	109 patients > 65–75 yr	RCT double blind	Independent mobility	Nicotinamide Mononucleotide (NMN) (250 mg)/Placebo	48	1 capsule, orally
Yoshino et al. (15)	USA	25 patients > 55–75 yr	RCT double blind	Postmenopausal prediabetes who was overweight or obese	Nicotinamide Mononucleotide (NMN) (250 mg)/Placebo	56	2 capsules orally

RCT, randomized clinical trials.

as a valuable adjunctive therapy in the management of this condition (18, 23). Within the cohort of 26 patients performed by Forsyth et al. (23), 8 of 26 (31%) responded favorably to NADH in contrast to 2 of 26 (8%) to placebo. Based on these encouraging results, the authors decided to conduct an open-label study in a larger cohort of patients. The results of the pilot study indicate NADH may be a valuable adjunctive therapy in the management of the CFS.

Older adults.

NR may be effective and well tolerated. Conze et al. (16) and Dollerup et al. (22) state the agent as precursor supplementation through L-tryptophan, nicotinic acid; however, nicotinamide does not improve mitochondrial or skeletal muscle function. Articles observed an improvement in lower limb function and reduced drowsiness in older adults. In addition, there is a potential for the supplementation to prevent loss of physical performance, reducing blood pressure and improving fatigue in older adults. Conze et al. (16) verified that blood levels increase significantly after an administration of 100, 300, and 1,000 mg/day compared with the placebo. Metabolites in the liver are dose-dependent and an increase in those metabolites was also seen; however, authors state that the oral Niagen is safe and well-tolerated up to 1,000 mg/day for 8 wk, as there were no dose-dependent adverse events or side effects. There were no serious side effects or reports of flushing, and all side effects were resolved by the end of the study (16).

Parkinson's disease.

Brakedal et al. (19) observed that the oral ingestion of nicotinamide riboside (NR) is safe, leads to an increase in brain levels of NAD, and alters brain metabolism in these patients. Furthermore, nicotinamide riboside may be well tolerated and it decreased the levels of inflammatory cytokines in serum and cerebrospinal fluid, which can act as a potential neuroprotective therapy. Authors mention that higher NAD availability could lead to increased acetyl-CoA synthesis by promoting glucose and fatty acid catabolism. This could, in turn, exacerbate the histone hyperacetylation state observed in the brain, further dysregulating gene expression (26). However, their analyses detected no significant changes in acetyl-CoA or CoA levels upon NR supplementation (19). Generally, Brakedal et al. (19) stated the administration of NR is positive and shows mild clinical improvement because it shows an increase of NAD levels in the brain, then exhibiting an altered cerebral metabolism. This induced transcriptional upregulation of processes related to mitochondrial, lysosomal, and proteasomal function in the blood cells and/or skeletal muscle. NR also decreased the levels of inflammatory cytokines in serum and cerebrospinal fluid, nominating NR as a potential neuroprotective therapy for Parkinson's disease.

Overweight.

The results support data that NR supplementation during 12 wk did not affect fasting or post glucose challenge concentrations of glucose, insulin, C-peptide, glucagon, GLP-1, or GIP, low-density lipoprotein cholesterol, and b-cell function did not respond to the intervention (22).

Age and gender.

In accordance with Schwarzmann et al. (27) who performed a study including 91 men and 114 women, neither sex nor increasing age influenced the total amount of plasma NAD in a normal human population having no severe diseases. The groups of men and women were nearly equally distributed according to their chronological age and showed expected sex-dependent differences. The study showed the amounts of NAD^+ and NADH are nearly balanced in human plasma and the total plasma NAD concentration was no different between men and women. In contrast, the ratio of plasma NAD^+/NADH was significantly higher in women than in men (higher values in NAD^+ and lower values for NADH). For both genders, the plasma concentration of its oxidized form, NAD^+, was somewhat higher than that of its reduced form, NADH. To achieve a more in-depth discussion, the authors divided all men and women according to the sex-specific median values for the plasma NAD^+/NADH ratio in subgroups, followed by the statistical evaluation of the parameters collected. It identified lower total NAD concentrations in the plasma samples of the low NAD^+/NADH ratio subgroup, an effect that was much more pronounced in men than women. Thus, there is a difference of NAD^+/NADH ratio in men and women; however, the use of a supplementation should not consider only gender factors, because there are age-related and dietary differences as well, which could potentially contribute to different plasma ratios. Neither sex nor increasing age influenced the total amount of plasma NAD in a normal human population having no severe diseases. Furthermore, NAD^+/NADH regulating mechanisms must depend on age as the sex-related difference in plasma was abolished in old probands. There is enough evidence that support the sex hormones and differences between pre- and postmenopausal phase in women contribute to the difference in the plasma NAD^+/NADH ratio between men and women and its loss with higher age. However, experimental evidence are still missing. In addition to hormonal differences, also sex- and age-related differences in the dietary intake of NAD^+ precursors eventually contribute to the different plasma NAD^+/NADH ratios observed (2).

Side Effects

From the 10 studies included in this review, only three articles reported side effects (16, 23). These included one or more side effects, thus, the most common associated with NAD's use are: muscle pain (24%), nervous disorders (22%), fatigue (20%), sleep disturbance (20%), headaches (18%), gastrointestinal disorders (9%), infections (7%), cardiac disorders (2%), immune system disorders (1%), nausea (1%), and skin rash (1%). Figure 2 displays the medium percentage of a reported side effect using NAD and placebo. It is worth noting muscle pain was reported by 92% of patients in the study of Forsyth et al. (23) with an oral administration of 10 mg/day. However, muscle pain was reported by 8.6% of patients in the study of Conze et al. (16), where a dose of 1,000 mg/day was administered. In the intervention of Forsyth et al. (23), 100% of patients also reported nervous disorders, fatigue, and sleep disturbances and 92% reported headaches. In contrast, the patients in Conze et al. (16) reported only 5%

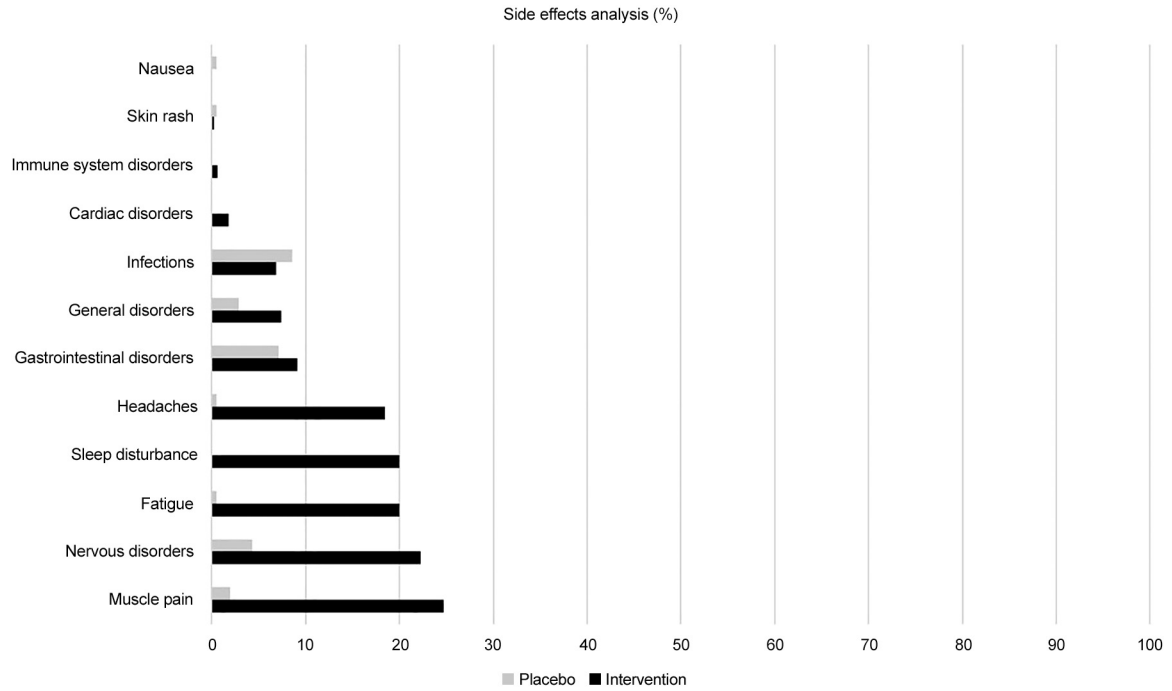

Figure 2. Medium percentage of side effects observed for NAD compared with the placebo. NAD, nicotinamide adenine dinucleotide.

of nervous disorders with a 1,000 mg/day dose and there were no reports of fatigue, sleep disturbances, or headaches. Gastrointestinal disorders were reported by Conze et al. (16) in 14.3% using placebo, 20% using a 100 mg/day dose, 14% using a 300 mg/day dose, and 11% using a 1,000 mg/day dose. When analyzing the infections, the doses ranging from 100 to 1,000 mg/day and the placebo had the same reported percentage (11.4%). It is important to state that there was minimal difference between the intervention and placebo regarding the side effects. Also, the interventions were through the oral route which is well known for a higher incidence of side effects when compared with the subcutaneous route, for example. However, there was no evidence to state this affirmation with NAD and its precursors. No patient had to interrupt or to be removed from the treatments due to side effects. The side effect with a higher percentage of reports was muscle pain and it was reported in only two studies. The side effects presented for the use of NAD are much milder when compared with the use of medications to treat specific diseases. Therefore, the benefits of NAD^+/NADH supplementation overcome the risks and severity of side effects. For instance, chemotherapy for the treatment of cancer can cause fatigue, diarrhea, constipation, vomiting, loss of appetite, nausea, and in a few cases, a patient can suffer from severe side effects with reactions that could be life-threatening such as liver failure. However, when compared with the use of other supplementation that are used for the same purpose, the side effects are similar. Hormonal supplementation like testosterone can cause chest and overall pain, dizziness, throat tightening, and hair loss (16).

Risk of Bias in Included Studies

Based on the Cochrane risk-of-bias tool for randomized trials (Fig. 3), three studies reported information about the randomization process as sequential, allocated, and generated random numbers; however, seven studies described a few details about this process. In the majority of studies, all participants, investigators, and assessors were blinded. All studies reported doubled-blind, but only five specified all the processes of blindage. Seven studies reported participants who withdrew from the intervention. The withdrawal rate in Alegre et al. (18) was 10%. Nine participants withdrew at the point of randomization for their own reasons. The withdrawal rate in Connell et al. (20) was 1%, where only one participant reported cold sores (herpes labialis), which resolved itself without intervention. The withdrawal rate in Conze et al. (16) was 5% (7 participants). Reasons for withdrawal included: one subject dropped out of the placebo group due to nausea, one subject was withdrawn due to noncompliance with the study protocol, four subjects withdrew without consent and lost to follow-up. The withdrawal rate in Demarin et al. (21) was 3% where only one participant dropped due to psychiatric hospitalization before initiating treatment. The withdrawal rate in Forsyth et al. (23) was 25%. Nine participants withdrew at the point of randomization after an initial due receiving psychotropic drugs. The withdrawal rate in Kim et al. (24) was 2%. The withdrawal rate in Martens et al. (17) was 20%. Three subjects withdrew due to failed catheterization ($n = 1$), administrative error ($n = 1$), or due to nurse study error ($n = 1$). Only three studies did not report withdrawals (15, 19, 22). The majority of studies used appropriate methods for measuring the outcomes. Only two studies did not report a protocol in accordance with a prespecified plan (23, 24).

DISCUSSION

Our systematic review identified the studies reporting NAD and its precursors in clinical trials with different

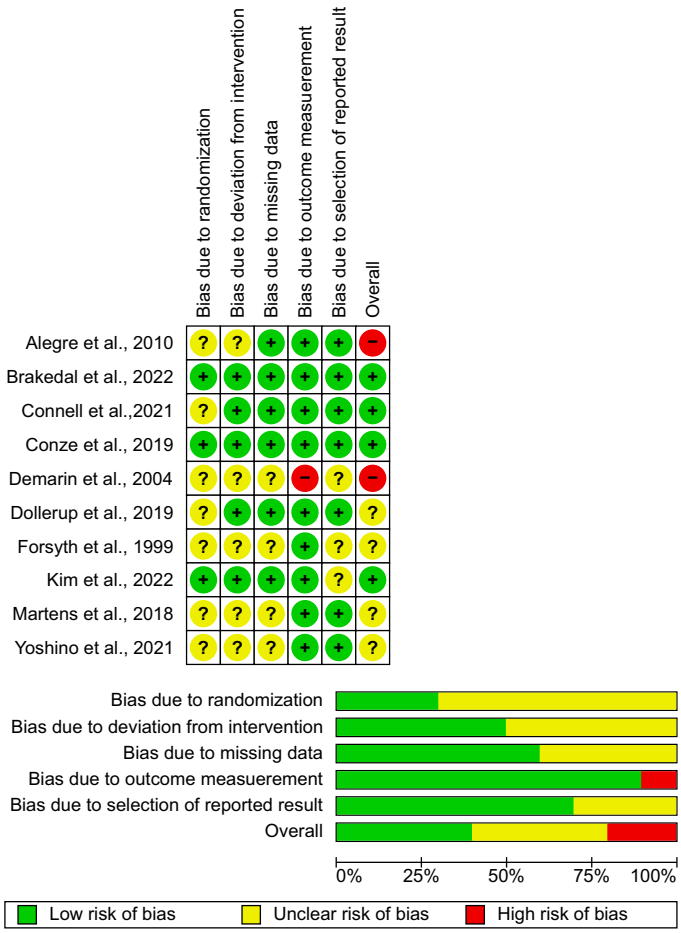

Figure 3. Risk of bias summary table. D1 – randomization process; D2 – deviations from the intended interventions; D3 – missing outcome data; D4 – measurement of the outcome; D5 – selection of the reported result.

populations. Since the reported outcomes on treatments were broad, we were not able to identify a pattern in the evaluation and the criteria of studies. However, in the aspect of AD and Parkinson's, the NAD therapy showed positive results. For Parkinson's, the NAD administration shows an increase of NAD levels in the brain, then exhibiting an altered cerebral metabolism. This induced transcriptional upregulation of processes related to mitochondrial, lysosomal, and proteasomal function in the blood cells and/or skeletal muscle. NR also decreased the levels of inflammatory cytokines in serum and cerebrospinal fluid, nominating NR as a potential neuroprotective therapy (19). For Alzheimer's, the analysis of MDRS subscales revealed significantly better performance by NADH subjects on measures of verbal fluency, visual constructional ability, and a trend to better performance on a measure of abstract verbal reasoning (21).

The study of Dizdar et al. (28) performed an intravenous and an intramuscular administration of NADH for the treatment of Parkinson's and there were improvements in the Unified Parkinson's Disease Rating Scale. However, the authors state the changes were not statistically significant and no changes occurred during the following weeks. The lack of standardization regarding the therapy's name and the low knowledge on the several NAD forms in the body contribute negatively to the interpretation of results. Although there are promising results for the use of this substance, the interpretation of results must be cautious. The reported side effects were not critical, and no dropouts were seen because of the use of the substance. In addition, Castro-Marrero et al. (29) pointed out that NAD is safe, approved and an interesting compound for the formulation of food supplements and medicinal products in other populations.

A few aspects of the reported outcomes should be further analyzed, as we have seen that quality of life and fatigue can improve in a few cases, there are authors who are evaluating the NAD therapy for healthy people in comparison with authors who use the therapy for people with serious diseases. Obviously, the response is different for healthy and unhealthy cases. A lot of commercial effort is being uplifted towards NAD and its use in healthy people because it can improve fatigue, improve sleep, and general quality of life; however, the commercial approach is lacking in scientific studies, so any indication of use must be further investigated, as well as many aspects of the individual's health must be taken into consideration (30, 31).

Furthermore, we found that there is no consensus on dosage application, which indicates a need for further studies on this substance. Rainer et al. (32) showed that high doses of NADH (certainly much more than 10 mg/day used in the available studies) externally administered might inhibit the acquisition of ascorbate by the brain by inhibiting its oxidation to dehydroascorbate. However, in healthy middle-aged and older humans, the chronic oral supplementation with 1,000 mg per day of NR is a well-tolerated and effective strategy for stimulating NAD^+ metabolism (17). According to Poljsak et al. (33), there are many alternative ways to increase NAD^+ and NADH concentration, such as regular exercise practice, regulation of circadian rhythms, sleeping habits, caloric restriction, and improvement of eating habits. Regarding food and eating habits, the daily requirements for NAD^+ synthesis can be obtained with around 15 mg of niacin, a collective term for nicotinic acid and nicotinamide, which can be found in meat and fish (33). However, the amount of specific food eaten for the purpose of increasing NAD concentration would have to be enormous, thus the supplementation. Also, for instance, a high-fat/sugar diet causes energy overload, culminating in reduced NAD^+/NADH ratio and decreased NAD^+ levels. The literature states that NAD^+ level is not only nutritionally controlled, but it depends also on the sports activities and other lifestyle factors as mentioned before, thus supplementation can act to stimulate NAD^+ metabolism (33).

It is worth noting that all the forms of NAD present as therapies (NAD^+, NADH, NA, NAM, NR, NMN) were used in doses ranging from 5 to 1,000 mg/day. The studies were conducted in several kinds of people (e.g., healthy, with Alzheimer's, etc.) (34).

A review article from Radenkovic et al. (9) separated all the NAD forms and dosages into a perspective and identified 289 studies on NAD pharmacology and related interventions. The gap of knowledge is noticeable because NAD levels decrease with age in tissues throughout the body. While numerous mechanisms have been proposed to explain this observation, the understanding of this phenomenon is still incomplete. Investigations of NAD biochemistry have largely focused on proximate causes, meaning changes in the expression of

proteins that are rate-limiting in NAD synthesis and salvage pathways (9). The broad spectrum of the therapies, dosages and patients makes one think we are still in the beginning stages of this research. NAD^+ seems to be effective, well tolerated and able to prevent loss of physical performance, reducing blood pressure and improving fatigue in older adults. In order to conduct safety and effectiveness studies towards NAD, future studies must have a well-defined population, at least, and outcomes must be less qualitative and more measurable. The theme could also be enriched if there were data and explanations on the molecular mechanisms that justify the functional changes (e.g., possible molecular pathways that could be described for the improvement in cognitive function caused by NAD or its precursors supplementation). There are numerous pathways known to lead to brain aging, which includes increased oxidative stress, inflammation, disturbances in energy metabolism such as deregulated autophagy, mitochondrial dysfunction, and IGF-1, mTOR, ROS, AMPK, SIRTs, and p53 as central modulators of the metabolic control, connecting aging to the pathways, which lead to neurodegenerative disorders. However, it is also known that calorie restriction (CR), physical exercise, and mental activities can extend lifespan and increase nervous system resistance to age-associated neurodegenerative diseases, thus interfering in the mentioned pathways (35). Also, there are only publications studying NAD's administration through oral routes, however, there are commercially available and FDA-approved implants to deliver substances subcutaneously. The delivery of drugs without crossing through the metabolism in the liver can significantly improve the bioavailability and reduce side effects; thus, understanding the alternative routes can give us a glimpse of the commercial aspect of NAD therapy (36).

To conclude, a benefit versus risk analysis was conducted by Braidy and Liu (10) through reviewing the literature and the authors state NAD metabolism represents a promising therapeutic target for the treatment of metabolic and age-related disorders, such as obesity, diabetes, cardiovascular, and neurodegenerative diseases. Liu et al. (37) performed a complete study on the metabolization, adsorption, and distribution of NAD to several tissues, such as the skeletal muscle, heart, liver, lung, brain, and so on. In vivo, NAD was made from tryptophan selectively in the liver, which then excreted nicotinamide. NAD concentration varied widely across tissues, presenting a high flux in the small intestine and spleen and low flux in the skeletal muscle. Intravenous administration of nicotinamide riboside or mononucleotide delivered intact molecules to multiple tissues, but the same agents given orally were metabolized to nicotinamide in the liver.

Also, a comprehensive review of emerging findings from Wang et al. (34) states that the data has shown that supplementation with NAD^+ precursors appeared to be an effective and safe anti-AD strategy with suitable bioavailability for preventing neuropathological and behavioral symptoms. Currently, the stage is set to test whether these exciting preclinical trials are precursors for success in large randomized clinical trials and whether the results can be translated into the clinic to improve AD patient phenotypes.

We acknowledge the strong evidence of this review; however, there are some limitations such as: first, we included studies with different clinical conditions, supplementation, and NADH precursors. Second, it was difficult to conduct a meta-analysis due to a variety of outcomes and dosages adopted. Third, the sample size was small and the risk of bias of some appraised studies was critical. We suggest that future research should expand on and study the effects of supplementation NAD^+ and NADH, to determine the safety and effectiveness of specific groups of humans using health parameters and outcomes.

CONCLUSIONS

This systematic review investigated the safety and effectiveness of the use of NAD as therapy for several purposes. The broad spectrum of NAD's use is evidence that this substance must be further investigated. Authors do not compare this substance with others, only with placebo and the reported outcomes suggest NAD is safe and has a low incidence of side effects. The reported side effects were also mild, in comparison with other critical substances, such as chemotherapy. There are promising results indicating the use of NAD to treat neurological disorders such as Parkinson's disease and Alzheimer's disease; however, further clinical trials must be performed. Although there is data suggesting that it is safe to use and can improve fatigue, sleep, and quality of life, more evidence should be searched to firm robust conclusions about the safety and effectiveness of this drug. High-quality trials exploring NAD's use are important to professionals and patients because they can be useful in informing future guidelines.

APPENDIX A: ARTICLES EXCLUDED AND THE REASONS FOR EXCLUSION (n = 47)

No	Reference	Reason for Exclusion
01	Abreu et al., 2016	Wrong study type/conference
02	Adams et al., 2005	Wrong intervention/NAD associated with another drug
03	Altay et al., 2021	Wrong intervention/ NAD associated with another drug
04	Birkmayer et al., 1993	Wrong study type/no RCT
05	Blum et al., 2022	Wrong study type/no RCT
06	Bussières et al., 2016	Wrong study type/review
07	Castaño et al., 2014	Wrong population/mice
08	Castro-Marrero et al., 2016	Wrong intervention/NAD associated with another drug
09	Chan et al., 2016	Wrong intervention/NAD associated with another intervention
10	Chen et al., 2014	Wrong study type/conference
11	Chen et al., 2018	Wrong study type/review
12	Chien et al., 2020	Wrong intervention/NAD associated with another drug
13	Cleroux et al., 1989	Wrong intervention/NAD associated with another drug
14	Colombi, Tholen, Huber, 1969	Wrong study type/no RCT
15	Covarrubias et al., 2020	Wrong study type/review
16	Craps et al., 1978	Wrong study type/review
17	De Livera et al., 2020	Wrong study type/protocol
18	Desnoyers et al., 2022	Wrong study type/review
19	Dolopikou et al., 2019	Wrong intervention/NAD associated with another intervention
20	Gibson et al., 2021	Wrong study type/conference
21	Hoffmann et al., 2013	Wrong study type/no RCT
22	Inagi, 2010	Wrong study type/review

Continued

— *Continued*

No	Reference	Reason for Exclusion
23	Jeyaraj et al., 2019	Wrong study type/review
24	Jun Tao et al., 2022	Wrong study type/protocol
25	Kim et al., 2017	Wrong intervention/NAD associated with another drug
26	Koleti et al., 2021	Wrong study type/conference
27	Kriebs, 2022	Wrong study type/protocol
28	Leung et al., 2022	Wrong study type/protocol
29	Liao et al., 2021	Wrong intervention/NAD associated with another intervention
30	Nakamura et al., 2015	Wrong population/mice
31	Natali, 2015	Wrong study type/review
32	Nieto, 1997	Wrong intervention/NAD associated with another drug
33	Onal et al., 2009	Wrong study type/no RCT
34	Pencina et al., 2022	Wrong intervention/NAD associated with another drug
35	Pires et al., 2022	Wrong study type/review
36	Rappou et al., 2016	Wrong study type/no RCT
37	Sharma et al., 2022	Wrong study type/protocol
38	Sims et al., 2018	Wrong population/mice
39	Sorensen et al., 2005	Wrong study type/no RCT
40	Tang, Zhou, Wen, 2019	Wrong study type/in vitro
41	Toth et al., 2016	Wrong population/horses
42	VA office	Wrong study type/protocol
43	Wang et al., 2016	Wrong population/mice
44	Wang et al., 2019	Wrong intervention/NAD associated with another drug
45	Xiong et al., 2010	Wrong population/mice
46	Yang and Sauve, 2016	Wrong study type/review
47	Yu et al., 2016	Wrong study type/review

NAD, nicotinamide adenine dinucleotide; RCT, randomized clinical trial.

APPENDIX B

Authors	Final Decision
Alegre et al., 2010	Include
Brakedal et al., 2022	Include
Connell et al., 2021	Include
Conze et al., 2019	Include
Demarin et al., 2004	Include
Dollerup et al., 2019	Include
Forsyth et al., 1999	Include
Kim et al., 2022	Include
Martens et al., 2018	Include

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

I.d.M.G., C.R.d.M.R., and D.D. conceived and designed research; G.F. and I.K.d.S. analyzed data; G.F. and I.K.d.S. prepared figures; G.F. and I.K.d.S. drafted manuscript; I.d.M.G., G.F., L.P.S.P., J.B., I.K.d.S., D.D., and C.R.d.M.R. edited and revised manuscript; I.d.M.G., G.F., L.P.S.P., J.B., I.K.d.S., D.D., and C.R.d.M.R. approved final version of manuscript.

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