Depleted nicotinamide adenine dinucleotide (NAD+) is a common hallmark of metabolic disorders. Therefore, NAD+-increasing strategies have evolved as a potential therapeutic venue to combat cardiometabolic diseases. Several forms of vitamin B3, i.e., nicotinamide and nicotinamide mononucleotide, and especially nicotinamide riboside, have attracted most interest as potentially safe and efficacious candidates for NAD+ restoration. Herein, we dissected the characteristics of the latest clinical trials testing the therapeutic potential of different vitamin B3 molecules to improve cardiometabolic health, with a special focus on randomized, placebo-controlled clinical trials performed in the context of obesity or other pathologies, mainly linked to cardiovascular system and skeletal muscle functionality. The favorable outcomes via NAD+-increasing strategies found in the different studies were quite heterogeneous. NAD+-increasing interventions improved capacity to exercise, decreased blood pressure, increased the anti-inflammatory profile and insulin-stimulated glucose disposal, and reduced the fat-free mass. Except for the decreased blood pressure, the significant results did not include many hard clinical endpoints, such as decreases in weight, BMI, fasting glucose, or HbA1c percentage. However, the analyzed trials were short-term interventions. Overall, the accumulated clinical data can be interpreted as moderately promising. Additional and long-term studies will be needed to directly compare the doses and duration of treatments among different vitamin B3 regimes, as well as to define the type of patients, if any, that could benefit from these treatments. In this context, a major point of advancement in delineating future clinical trials would be to identify subjects with a recognized NAD+ deficiency using novel, appropriate biomarkers. Also, confirmation of gender-specific effect of NAD+-increasing treatments would be needed.

Keywords: vitamin B3, clinical trials, obesity, diabetes mellitus, nicotinamide
INTRODUCTION

Nicotinamide adenine dinucleotide (NAD+) is a dual molecule. Apart from its well-established role as a cofactor for redox reactions and, in its reduced form, as an electron donor to the mitochondrial oxidative phosphorylation system for the synthesis of ATP, NAD+ has also been described as a signaling molecule. Indeed, NAD+ is also a substrate for NAD+-consuming enzymes involved in the metabolic adaptations that govern cell metabolism and survival.

It is known that low levels of NAD+ result from altered NAD+ homoeostasis. Impaired NAD+-mediated signaling and concurrent alterations in dysfunctional mitochondria commonly underlie cardiometabolic disorders, such as type 2 diabetes, non-alcoholic fatty liver, and aging.

NAD+ precursors are naturally found in food, and their use has emerged as a strategy for NAD+ replenishment and hence to favorably influence NAD+ dependent pathways and rescue tissues from the adverse consequences of aging and metabolic diseases (1). NAD+ content in animal and human tissues can be increased via the supplementation of different precursors (2–4). Although NAD+ can be synthesized directly from tryptophan (de novo), it is more efficiently generated from other precursors, such as nicotinic acid (NA) (Preiss-Handler pathway) and nicotinamide (NAM) (Salvage pathway). Nicotinamide phosphoribosyltransferase (NAMPT), which converts NAM into nicotinamide mononucleotide (NMN), is the key step for NAD+ synthesis (5). Another source of NAD+ comes from nicotinamide riboside (NR).

NAD+ precursors that are used to elevate NAD+ availability in target tissues have demonstrated efficiency in improving insulin sensitivity and reducing diabetes burden and associated metabolic derangements in preclinical models. Actually, the growing number of studies have successfully used different vitamin B3 forms to boost NAD+ production and positively influence aging and cardiometabolic diseases in experimental in vivo models, including our studies performed in the context of accelerated atherosclerosis or obesity (4, 6, 7). However, less consistent results have been obtained in clinical studies.

This review analyzes the accumulating evidence from the latest clinical studies that used different NAD+-increasing strategies to improve cardiometabolic health, with a special focus on randomized, double-blind, placebo-controlled clinical trials, since they are considered the “gold standard” for testing clinical intervention-based studies (8). Particularly, a PubMed search on clinical reports published in the last 5 years on this topic using “nicotinamide” and “randomized clinical trials” as keywords was performed.

RESULTS OF THERAPEUTIC INTERVENTIONS WITH DIFFERENT FORMS OF VITAMIN B3

A plethora of pathways require NAD+ as a coenzyme. The easiest strategy to increase NAD+ in vivo is via the provision of NAD+ precursors, which include NA (also termed niacin), NAM, NR, or NMN (5, 6). However, these NAD+ intermediates can exhibit a distinct behavior due to the different tissue distribution and relative abundance of enzymes or transporters involved in NAD+ metabolism.

NA

NA has been the most widely used form of vitamin B3 in clinical practice due to its hypolipemic properties, i.e., triglyceride-reducing and high-density lipoprotein cholesterol (HDL-C)-raising effects (5). Besides such lipid effects, NA also interacts with the GPR109A receptor expressed in immune cells, thus blunting immune activation (5). Although such NA properties are beneficial to treat hyperlipidemia, NA administration is frequently associated with some serious adverse effects, i.e., flushing, deterioration of insulin resistance, and hepatic and gastrointestinal toxicity (5), which reduce medication adherence. Moreover, early reports supporting efficacy of NA in secondary cardiovascular prevention were not confirmed by more recent, large prospective trials, including 29,087 subjects, the AIM-HIGH (9), and the HPS2-THRIVE (10), which used extended-released NA and laropiprant, a selective antagonist of prostaglandin D2 receptors, to control adverse effects (5). Since NA also ameliorated wound healing and cardiac function after myocardial infarction via prostaglandin D2 receptor subtype 1-mediated M2 macrophage polarization, the inhibition of prostaglandin D2 signaling could have attenuated NA cardiovascular benefits (11). The latter finding would be consistent with data from earlier clinical trials using NA, showing a reduction of mortality in subjects nine years after stopping NA therapy (12). It is also possible that the protective effect of NA is no longer significant in the context of previous statin treatments (9). However, NA may also be a potential source for NAD+ synthesis (13), and could present beneficial effects raising tissue NAD+ levels in case of deficiency. In this regard, the effect of NA in 5 subjects (4 females) with adult-onset mitochondrial myopathy subjects and systemic NAD+ deficiency and 10 healthy controls (8 females) was recently assessed (14). NA (up to 750–1,000 mg/day) or placebos were administered in a non-randomized, open-able parallel assignment to subjects and their matched controls for 10 or 4 months, respectively. The NA administration resulted in blood elevations of NAD+ in all subjects, up to eightfold, also restoring the muscle NAD+ levels of the subjects (14). Noteworthy was that muscle strength and mitochondrial biogenesis increased in all subjects. Furthermore, the muscle metabolome in the subjects with adult-onset mitochondrial myopathy subjects and systemic NAD+ deficiency and 10 healthy controls (8 females) was recently assessed (14). Adiponectin concomitantly increased, a finding consistent with previous studies in subjects treated with a high dose of NA (i.e., 1,500 mg/day) (15, 16). Important, the blood analysis was revealed as a useful sample to identify NAD+ deficiency in subjects with myopathy (14).

NAM

Unlike NA, NAM is not considered a GPR109A agonist, thus avoiding prostaglandin-related vasodilatory side effects. NAM is a widely available dietary supplement, which, at doses of no more
than 3 g/day, has been proven to be safe (17). In the ENDIT study, 276 subjects with type 1 diabetes mellitus were administered with NAM in daily doses of 1.2 g/m² (25–50 mg/kg), up to a maximum of 3 g/day for 5 years, with minimal side effects (17). However, this clinical trial with NAM failed to prevent the progression to overt of autoimmune type 1 diabetes (18). Importantly, higher doses of NAM have been reported to produce severe but reversible hepatotoxicity (19). Effects on glucose kinetics and insulin sensitivity are inconsistent, but minor degrees of insulin resistance have also been reported (17).

NAD+ deficiency is also a risk factor for acute kidney injury (20). Recently, a phase 1 placebo-controlled study of oral NAM demonstrated that a dose-related increase in circulating NAD+ was associated with less acute kidney injury (20). Similarly, although minimal, NAD+ biosynthesis from tryptophan, at the expense of the quinoline phosphoribosyltransferase, also prevents renal NAD+ depletion and mediates resistance to acute kidney injury. Overall, these data support the concept that increasing NAD+ levels could be beneficial for the treatment of some forms of kidney disease.

Consistent experimental observations have suggested similar NAD+-increasing benefits by NAM for the treatment of heart failure with preserved ejection fraction (21). In the former study, it was found that the dietary intake of NAD+ precursors was negatively associated with decreased blood pressure and cardiovascular mortality in a 20-year follow-up of the Brunec Study, and persisted after adjusting for caloric intake, age, BMI, sex, smoking, diabetes, alcohol intake, and categories of food items.

**NMN**

The safety of NMN administration (100, 250, and 500 mg) was recently investigated in 10 healthy men, aged from 40 to 60, in a single-arm, non-randomized intervention clinical trial (22). In this study, single oral administrations of NMN did not cause any significant clinical symptoms or changes in heart rate, blood pressure, oxygen saturation, or body temperature 5 h after NMN administration. The plasma concentrations of NMN metabolites (N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-5-carboxamide) were dose-dependently increased in treated subjects.

In relation to the impact of NMN intervention on dynamic metabolic adaptations, NMN supplementation enhanced aerobic capacity in amateur runners in a 6-week randomized, double-blind, placebo-controlled, four-arm clinical trial (Table 1) (23). In this study, up to 48 young and middle-aged recreational runners were included (40 males and 8 females, aged between 27–50 years, with a previous history of 1–5 years of regular exercise). The participants were randomized into three groups (with a ratio of 10 males to 2 females per group), taking a low (300 mg/day), medium (600 mg/day), and a high dosage group (1,200 mg/day). A control group (placebos) followed the same male to female ratio. The runners trained 5–6 times/week in sessions of 40–60 min. The participants did not show any obvious side effects. Exercise combined with NMN intake did not change the body composition or BMI, but analysis of the change from baseline over the 6-week treatment showed that the oxygen uptake (VO₂), the percentage of maximum oxygen uptake (VO₂ max), and the power at the first and second ventilatory thresholds increased in the medium- and high-dose NMN groups compared with the placebo.

In a very recent report (24), 25 overweight or obese women (BMI ranged from 25.3 to 39.1 kg/m², mean age of 61.5) and prediabetes completed a randomized, double-blind, parallel-assigned treatment with 250 mg of NMN or placebos over 10 consecutive weeks (Table 1). There were no major adverse side effects. Plasma concentrations of N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-5-carboxamide metabolites of NMN and NAD+ content in peripheral blood mononuclear cells (PBMC) increased in subjects with NMN supplementation, but not in those taking placebo. Neither body weight nor body composition differed on NMN treatment. In contrast, insulin-stimulated glucose disposal, as assessed by hyperinsulinemic-euglycemic clamp, and skeletal muscle insulin signaling (AKT and mTOR total protein and the phosphorylated forms) increased with NMN, thus revealing improved insulin signaling in treated subjects. In line with this, NMN also upregulated the expression of platelet-derived growth factor receptor β (which enhances insulin-stimulated AKT phosphorylation and glucose transport) and other genes in skeletal muscle that are involved in tissue remodeling. However, no changes in muscle mitochondrial oxidative capacity or muscle function were observed. NMN could

| Country, NCT code | Dose, duration of treatment, number, and main clinical characteristics | Main results | Reference |
|------------------|-----------------------------------------------------------------|-------------|-----------|
| China 2000035138 | NMN or placebos (300, 600, and 1,200 mg) were administered during 6 weeks to 48 subjects (8 women), aged 27–50. The recreational runners, training 5–6 times a week, were divided into four groups. | No major adverse effects. Evidence of increased plasma metabolites. Medium and high doses presented increased exercise capacity (i.e., increased oxygen uptake). No effect on cardiac function, BMI, or fat-free mass. | (23) |
| USA 03151239   | NMN or placebo per day (250 mg) over 10 weeks, given to 25 women with mean age of 61, BMI of 33.5 kg/m², plasma glucose of 5.65 mmol/L, and HbA1c of 5.55%, | No major adverse effects. Increased insulin-stimulated glucose disposal and skeletal muscle insulin signaling. Up-regulated expression of platelet-derived growth factor receptor β and other genes related to muscle remodeling. No change in body weight or composition. | (24) |

**BMI**, body mass index; **NMN**, nicotinamide mononucleotide; **NCT**, clinical trial identifier (ClinicalTrials.gov).

**TABLE 1** Recently (from 2018) published randomized, double-blind, placebo-controlled clinical trials that tested NMN administration on healthy individuals or in individuals with overweight and obesity.
therefore be a useful intervention approach in obese, prediabetic subjects, which often present muscle loss with impaired glucose metabolism (25). Further studies are warranted to clarify the potential contribution of NMN in the latter condition.

**NR**

NR is also a source for NAD+ synthesis. Consistently, human blood NAD+ levels rose 2.7-fold in a pilot study of one healthy 52-year-old male individual, receiving an oral daily dose of NR (1,000 mg/day) for 7 consecutive days (26). More recently, the different daily doses of NR, administered orally (i.e., 100, 300, and 1,000 mg) in 12 healthy subjects (6 male and 6 female) with ages from 30–55 and BMI 18.5–29.9 kg/m², were investigated in the context of a randomized, double-blind, three-arm crossover pharmacokinetic study design. The NR produced concomitant dose-dependent elevations in the blood NAD+ metabolome. The rise in nicotinic acid adenine dinucleotide (NAAD) was a highly sensitive biomarker of effective NAD+ replenishment (26).

In an independent, non-randomized, open-label pharmacokinetic study made in 8 healthy volunteers (6 female, 2 male, age range 21–50 years), 250 mg of NR was orally administered on days 1 and 2, and then increased to 1,000 mg twice daily on days 7 and 8. On the morning of day 9, subjects completed a 24-h pharmacokinetics study after receiving 1,000 mg of NR (27). The treatment was well tolerated with no apparent adverse side events. Significant increases from baseline to mean NR blood concentrations at a steady state were observed for both NR and NAD+, with a 100% increase in the NAD+ levels. Absolute changes from baseline to day 9 in NR and NAD+ levels were highly correlated (27).

In another study, a 2- × 6-week randomized, double-blind, placebo-controlled, crossover clinical trial, 24 healthy lean (average BMI = 24 ± 4 kg/m²) men (n = 11) and women (n = 13), aged 55 to 79, received 500 mg of NR twice a day (28). NR supplementation was well tolerated by all participants. The NR increased the NAD+ and related metabolite concentrations in PBMC. Importantly, treatment with NR significantly decreased blood pressure, aortic pulse wave velocity, and carotid artery compliance. There were no changes in total energy expenditure or energy expenditure from fat oxidation under resting conditions, blood glucose control, insulin sensitivity, aerobic exercise capacity, or motor function. It is worth noting that this study was conducted in non-obese, healthy middle-aged, and older adults without baseline metabolic dysfunction (28), and this could have limited the improved metabolic outcomes.

The effect of different doses of NR (100, 300, and 1,000 mg/day) on NAD+ metabolite concentration in urine and blood was investigated in 133 healthy males and females (54%–66%, depending on the group), aged 40–60, and overweight (BMI 25–30 kg/m²), in an independent 8-week randomized, double-blind, placebo-controlled parallel clinical trial (29). The NR increased, dose-dependently and significantly, whole blood NAD+ (from 22% to 142%) and other NAD+ metabolites within the first 2 weeks. The NAD+ elevations were maintained thereafter, without any adverse effects (29).

In another study, 12 healthy old men, aged from 70 to 80, and BMI 20–30 kg/m² (able to discontinue aspirin for 3 days prior to a muscle biopsy, statins and vitamin D one week before the beginning of the study) were supplemented with 500 mg of NR, twice a day, for 21 days, in a placebo-controlled, randomized, double-blind, crossover trial (Table 2) (30). NR supplementation decreased levels of circulating inflammatory cytokines (30) but did not produce favorable changes in body weight, blood pressure, lipid profile, fasting glucose and insulin, or homeostatic model assessment of insulin resistance (HOMA-IR). Although the supplementation of NR elevated the muscle NAD+ metabolome, it did not influence mitochondrial bioenergetics or whole-body glucose homeostasis. Despite the hand-grip strength values in these subjects being consistent with muscle aging, targeted NAD+ metabolome analysis likely revealed NAD+ sufficiency. Overall, these findings suggest that chronological age per se may not be a major factor impairing NAD+ metabolism.

In an early phase I study of 5 patients admitted with a class IV New York Heart Failure Classification to demonstrate NR safety and feasibility, blood samples were obtained before and after 5–9 days of oral NR administration (NR was up-titrated over 3 days to a final NR dose of 1,000 mg twice daily) (31). The treatment enhanced PBMC respiration and reduced pro-inflammatory cytokine gene expression in 4 male subjects, as one of the subjects did not complete the study.

Several clinical trials have focused on searching for the beneficial effects of NR in individuals with overweight or obesity. Data from the first of these clinical trials were published in three different reports (32–34). This clinical trial consisted of a 12-week randomized, double-blind, placebo-controlled, parallel-group trial conducted in 40 non-smoking, medication-free middle-aged males (40–70 years) with obesity (BMI mean of 32.85 kg/m²), sedentarism (< 30 min of daily exercise), with a mean fasting glucose of 5.6 mmol/L and HbA1c of 5.7% (Table 2) (32). They were administered NR at 1,000 mg, twice a day (n = 20) or a placebo (n = 20). After 12 weeks of treatment, increased concentrations of NAD-derived metabolites were detected in the urine of NR-treated subjects, thereby showing that the oral NR was readily absorbed, metabolized, and excreted. No serious adverse events were observed upon NR supplementation, and the safety blood tests were normal. HbA1c, insulin sensitivity, endogenouse glucose production, glucose disposal and oxidation, resting energy expenditure, lipolysis, oxidation of lipids, and body composition did not change with NR supplementation (32). The NR supplementation did not affect fasting, the post-glucose challenge concentrations of glucose, insulin, C-peptide, glucagon, glucagon-like peptide-1 or gastric inhibior polypeptide, or the beta-cell function (as revealed by the oral glucose test tolerance testing) (33). Protein levels of NAMPT in skeletal muscle decreased by 14% with NR, while NAD+ levels, as well as gene expression and protein abundance of other NAD+ biosynthetic enzymes, remained unchanged between the groups. The respiratory capacity of skeletal muscle mitochondria, abundance of mitochondrial associated proteins, mitochondrial fractional area, or network morphology in the skeletal muscle of NR-treated participants did
TABLE 2 | Recently (from 2018) published randomized, double-blind, placebo-controlled clinical trials that tested NR administration on individuals either healthy, aged, or overweight or obese.

| Country, NCT code | Dose, duration of treatment, number, and main clinical characteristics | Main results | Reference |
|------------------|---------------------------------------------------------------|--------------|-----------|
| USA, 02921659    | Administered 1,000 mg per day, 6 weeks in a crossover design, n = 24 (13 women), described as 55–79-year-old healthy, aged, lean subjects (BMI 24 ± 4 kg/m²). | No major adverse effects. Increased NAD+ and related metabolites in peripheral blood mononuclear cells. Treatment decreased blood pressure, aortic pulse wave velocity, and carotid compliance. No change in total energy intake and expenditure, BMI, % body fat, glucose and insulin metabolism, or exercise capacity. | (28) |
| USA, 0271593     | Administered 100 mg, 300 mg, 1,000 mg per day, 8 weeks parallel study, 133 (85 women): 40–60 years old, healthy overweight (BMI 25–30 kg/m²) in a parallel study. | No major adverse effects. Dose-dependent increase of NAD+ whole blood and urine metabolome. No changes in blood pressure, mean heart rate, weight, or resting energy expenditure. | (29) |
| UK, USA, Australia, 02950441 | Single center, double blind, placebo-controlled, and crossover study on 12 male aged volunteers, recruited from the Birmingham 1,000 Elders group (https://www.birmingham.ac.uk/research/activity/mids/centres/healthy-ageing/eiders.aspx), age 70–80 years, BMI 20–30 kg/m² receiving 1,000 mg of NR or placebos during 21 days | Muscle RNA sequencing revealed that NR down-regulated energy metabolism and mitochondria pathways without altering mitochondrial bioenergetics or whole-body glucose homeostasis, decreasing circulatory cytokines (especially IL-6, IL-5 and IL-2). NR did not alter hand-grip strength. | (30) |
| Denmark, 2303483 | 2,000 mg per day, 12 weeks: 40 men, 40–70-year-old, insulin resistant, with mean BMI of 38.5 kg/m², glucose of 5.6 mmol/L, and HbA1c of 5.7%. | No major adverse effects. No changes in carbohydrate metabolism, resting energy expenditure, lipolysis, lipid oxidation, or body composition. No impact on glucose tolerance, β-cell secretory capacity, α-cell function or incretin secretion, or bile acid levels. Moreover, there were no changes in NAD+ metabolite concentration in skeletal muscle. | (32–34) |
| Netherlands 02835664 | 1,000 mg per day, 6 weeks, 13 (7 women), age 59 ± 5, healthy overweight or obese (BMI 30.2 ± 2.6 kg/m²) | No major adverse effects. Increased markers of NAD+ synthesis in skeletal muscle. Minor increase in fat free mass, especially in women (6 out of 7). Minor increase in sleeping metabolic rate that could be related to the change in fat free mass. Acetylcarnitine concentrations in skeletal muscle and the capacity to form acylcarnitine upon exercise were increased in NR in respect to placebo. 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. | (37) |

BMI, body mass index; NAD, nicotinamide-adenine dinucleotide; NR, nicotinamide riboside; NCT, clinical trial identifier (ClinicalTrials.gov).

not differ from the placebo group (34). Overall, these data do not support the hypothesis that dietary NR supplementation has a significant impact on skeletal muscle mitochondria in obese and insulin-resistant men. This could at least partly be explained by the fact that, despite having elevated urine levels of NAD+-derived metabolites, the tissue content of NAD+, NADH, NADP+, or NADPH in the skeletal muscle in men receiving NR did not differ from those receiving placebos (35, 36).

In another randomized, double-blinded, placebo-controlled, crossover intervention study, 13 healthy overweight or obese men and women (age: 59 ± 5; BMI: 30.2 ± 2.6; n = 7 women) receiving either NR (1,000 mg/day) or placebo supplementation during 6 consecutive weeks were followed by broad metabolic phenotyping, including hyperinsulinemic-euglycemic clamps, magnetic resonance spectroscopy, muscle biopsies, ex vivo mitochondrial function, and in vivo energy metabolism assessment (Table 2) (37). NR supplementation resulted in elevations of NAD+ synthesis, such as NAAD and methyl-nicotinamide (me-NAM), in skeletal muscle. In particular, the percentage of fat-free mass (62.65% ± 2.49% compared with 61.32% ± 2.58%), the skeletal muscle acetyl carnitine (4,558 ± 749 compared with 3,025 ± 316 pmol/mg dry weight), and the capacity to form acyl carnitine upon exercise were increased upon NR supplementation (37). To our knowledge, such an effect of NR on body composition in humans has not been reported before. Consistently, the sleeping metabolic rate was also affected by NR supplementation. The NR-treated subjects also showed concomitantly higher metabolic rates than the placebo subjects. In contrast, insulin sensitivity was not improved in NR-treated men. However, NR did increase the fat-free mass in 6 out of 7 women, whereas such an increase was only observed in 1 out of 6 men; thus, there might be a gender-specific response pattern in the effect of NR supplementation.

DISCUSSION

Thanks to the extensive research already performed with some vitamin B3 forms, its considerable safety has been confirmed. The analyzed clinical trials were built on this concept. However, special consideration should be given to the fact that some enzymes determining NAD+ levels have been related to cancer progression, at least at an experimental level (38).

Data from different clinical trials are somewhat difficult to compare and interpret, mainly due to differences in the vitamin B3 forms, doses, duration of treatment, gender, and cardiometabolic characteristics of the studied subjects.

One of the emerging interpretations is that gender could be a determinant of positive response to treatment, since most reports that obtained significant cardiometabolic differences in vitamin B3 treatment comprised a higher proportion of women (24, 28, 37) than those in which no such differences were found (32–34).
However, this was not without exceptions. Elhassaen et al. (30) found some differences in men only, and Conze et al. (2019) did not find such differences, even though the study featured a majority of women (29). Interestingly, the percentage of men in the AIM-HIGH (9) and HPS-THRIVE (10) trials was around 85%.

Apart from gender-specific differences, cardiometabolic status may also differ among studies. In this regard, subjects with obesity were excluded from these two latter studies (29, 30), whereas the analysis of the effect of NR was conducted in subjects with obesity and prediabetes in other clinical trials (24, 37). In the study of Remie et al. in 2020, basically only the women in the study showed a response to the NR (37). In contrast, the main difference in the clinical trials that failed to find response to vitamin B3 treatment was directed only to men (32–34), even though it should also be considered that it also used twice the dose of the other studies. Furthermore, circadian rhythms influence the action of at least some of the key enzymes involved in the bioavailability of the currently used NAD+ precursors, and this could also be considered to be minimizing the variability among future clinical trials (39). Last, in the clinical trial of Dollerup et al. (32–34) there were some indications of limited NR tissue bioavailability.

As mentioned, the favorable outcomes in response to NAD+ precursor supplementation found in the different studies were quite heterogeneous. Thus, NAD+-increasing interventions increased capacity to exercise (23), decreased blood pressure (28), decreased anti-inflammatory circulating cytokines (30, 31), increased insulin-stimulated glucose disposal (24), or decreased fat-free mass (37). Most of these concrete findings were not reproduced in the rest of the clinical trials. Most of the positive findings were not very hard clinical end points (except the decreased blood pressure). No significant reductions in body weight or its surrogate, BMI, fasting glucose, or %HbA1c were observed in response to NAD+ precursors. This could be due to the short duration of the clinical trials analyzed, which obviously impacted the probability of the change in HbA1c percentage.

The health potential of targeting NAD+ homeostasis is still an underexplored field for future interventions of non-communicable disorders. Given the contribution of NAD+-sirtuin signaling on mitochondrial and metabolic homeostasis, an interesting proposal for future clinical trials could be the study of the effects of vitamin B3 interventions in combination with moderate exercise (40) to directly assess the metabolic adaptations provided by restored NAD+ homeostasis, concurrent with improved mitochondrial function. Moreover, future clinical trials should focus on subjects with selective biomarkers of recognized NAD+ deficiency (26) and analyzing the gender-effect in order to maximize the chances of success.

**CONCLUSION**

Although NAD+-increasing strategies have the potential to improve overweight, obesity, and cardiometabolic health *in vivo*, additional larger and long-term studies are needed to shed light on its precise clinical indications, preferred vitamin B3 form, doses, and treatment duration.

**AUTHOR CONTRIBUTIONS**

Conceptualization: FB-V, NR, and JJ. Writing-Original Draft Preparation: FB-V and JJ. Writing-Review and Editing: MC, DM, and JE-G. Funding Acquisition: FB-V, NR, JE-G, and JJ. All authors contributed to the article and approved the submitted version.

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**REFERENCES**

1. Abdellatif M, Baur JA. NAD+ Metabolism and Cardiometabolic Health: The Human Evidence. *Cardiovasc Res* (2021) 117:e106–9. doi: 10.1093/cvr/cvab212
2. Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide Mononucleotide, a Key NAD(+) Intermediate, Treats the Pathophysiology of Diet- and Age-Induced Diabetes in Mice. *Cell Metab* (2011) 14:528–36. doi: 10.1016/j.cmet.2011.08.014
3. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, et al. The NAD(+) Precursor Nicotinamide Riboside Enhances Oxidative Metabolism and Protects Against High-Fat Diet-Induced Obesity. *Cell Metab* (2012) 15:838–47. doi: 10.1016/j.cmet.2012.04.022
4. Mendez-Lara KA, Rodriguez-Millan E, Sebastian D, Blanco-Soto R, Camacho M, Nan MN, et al. Nicotinamide Protects Against Diet-Induced Body Weight Gain, Increases Energy Expenditure, and Induces White Adipose Tissue Bingeing. *Mol Nutr Food Res* (2021) 65(11):e2100111. doi: 10.1002/mnfr.202100111
5. Elhassaen YS, Philp AA, Lavery GG. Targeting NAD+ in Metabolic Disease: New Insights Into an Old Molecule. *J Endocr Soc* (2017) 1:816–35. doi: 10.1210/jes.2017-00092
6. Yoshino J, Baur JA, Imai S. NAD(+) Intermediates: The Biology and Therapeutic Potential of NMN and NR. *Cell Metab* (2018) 27:513–28. doi: 10.1016/j.cmet.2017.11.002
7. Mendez-Lara KA, Letelier N, Farre N, Diarte-Anazco EMG, Nieto-Nicolau N, Rodriguez-Millan E, et al. Nicotinamide Prevents Apolipoprotein B-
Containing Lipoprotein Oxidation, Inflammation and Atherosclerosis in Apolipoprotein E-Deficient Mice. *Antioxid (Basel)* (2020) 9(11):1162. doi: 10.3390/antiox9111162

8. Misra S. Randomized Double Blind Placebo Control Studies, the "Gold Standard" in Intervention Based Studies. *Indian J Sex Transm Dis AIDS* (2012) 33:131–4. doi: 10.4103/0253-7184.102130

9. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprivicz K et al. Nicacin in Patients With Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. *N Engl J Med* (2011) 365:2255–67. doi: 10.1056/NEJMoa1107579

10. Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J et al. Effects of Extended-Release Nicacin With Laronigapin in High-Risk Patients. *N Engl J Med* (2014) 371:203–12. doi: 10.1056/NEJMoa1309955

11. Kong D, Li J, Shen Y, Liu G, Zuo S, Tao B, et al. Nicotinamide Promotes Cardiac Healing After Myocardial Infarction Through Activation of the Myeloid Prostaglandin D2 Receptor Subtype 1. *J Pharmacol Exp Ther* (2017) 360:435–44. doi: 10.1122/jpet.11.6.238261

12. Canner PL, Berge KG, Wenger NK, Stamler J, Friedman L, Prineas RJ et al. Fifteen Year Mortality in Coronary Drug Project Patients: Long-Term Benefit With Nicacin. *J Am Coll Cardiol* (1986) 8:1245–55. doi: 10.1016/0735-1097(86)80293-5

13. Romani M, Hofer DC, Katsuya E, Auwers J. Nicacin: An Old Lipid Drug in a New NAD(+)- Dosed. *J Lipid Res* (2019) 60:741–6. doi: 10.1194/jlr.S092007

14. Pirinen E, Auranen M, Khan NA, Brilhante V, Urho N, Pessia A et al. Nicotinamide Systemic NAD(+) Deficiency and Improves Muscle Performance in Adult-Onset Mitochondrial Myopathy. *Cell Metab* (2020) 31:1078–1090 e1075. doi: 10.1016/j.celrep.2020.04.008

15. Westphal S, Luley C. Preferential Increase in High-Molecular Weight Adiponectin After Nicacin. *Atherosclerosis* (2008) 198:179–83. doi: 10.1016/j.atherosclerosis.2008.06.028

16. Knip M, Dousek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ et al. Safety of High-Dose Nicotinamide: A Review. *Diabetologia* (2000) 43:1337-45. doi: 10.1007/s001250051536

17. Gale EA, Bingley PJ, Emmett CL, Collier T. European Nicotinamide Diabetes Intervention Trial (ENDIT): A Randomised Controlled Trial of Intervention Before the Onset of Type 1 Diabetes. *Lancet* (2004) 363:925–31. doi: 10.1016/S0140-6736(04)15786-3

18. Winter SL, Boyer JL. Hepatic Toxicity From Large Doses of Vitamin B3. *Atherosclerosis* (2019) 315:162–9. doi: 10.1016/j.atherosclerosis.2019.07.043

19. Hepler C, Bass J. Supplements to Treat Prediabetes. *Am J Clin Nutr* (2021) 104:5703–14. doi: 10.1210/jc.2019-01081

20. Zhuo B, Wang DD, Qiu Y, Aiharto S, Liu Y, Stempien-Otero A et al. Boosting NAD Level Suppresses Inflammatory Activation of PBMCs in Heart Failure. *J Clin Invest* (2020) 130:6054–63. doi: 10.1172/JCI138538

21. Dollerup OL, Christensen B, Svat M, Schmidt MD, Sulek K, Ringgaard S et al. A Randomized Placebo-Controlled Clinical Trial of Nicotinamide Riboside in Obese Men: Safety, Insulin-Sensitivity, and Lipid-Mobilizing Effects. *Am J Clin Nutr* (2018) 108:343–53. doi: 10.1093/ajcn/nxy132

22. Moore MP, Mucinski JM. Impact of Nicotinamide Riboside Supplementation on Skeletal Muscle Mitochondria and Whole-Body Glucose Homeostasis: Challenging the Current Hypothesis. *J Physiol* (2020) 598:3327–8. doi: 10.1113/jph27949

23. Leduc-Gaudet JP, Dulac M, Reynaud O, Arouad MB, Gouspillou G. Nicotinamide Riboside Supplementation to Improve Skeletal Muscle Mitochondrial Health and Whole-Body Glucose Homeostasis Does It Actually Work in Humans? *J Physiol* (2018) 596:619–20. doi: 10.1113/jphysiol.2019.152980

24. Remie CME, Roumans KHM, Moonen MPB, Connell NJ, Havekes B, Mevenkamp J et al. Nicotinamide Riboside Supplementation Alters Body Composition and Skeletal Muscle Acetyl carnitine Concentrations in Healthy Obese Humans. *Am J Clin Nutr* (2021) 112:413–26. doi: 10.1093/ajcn/nqa0672

25. Parsons RB, Facey PD. Nicotinamide N-Methyltransferase: An Emerging Protagonist in Cancer Macroc(R)evolution. *Biomolecules* (2021) 11(10):1418. doi: 10.3390/biom11101418

26. Benedict C, Shostak A, Lange T, Brooks SJ, Schioth HB, Schultes B et al. Diurnal Rhythm of Circulating Nicotinamide Phosphoribosyltransferase (Nampt/visfatin/Protagonist in Cancer Macro(R)Evolution. *Biomolecules* (2021) 11(10):1418. doi: 10.3390/biom11101418

27. Yoshino M, Yoshino J, Kayser BD, Patti GI, Franczyk MP, Mills KE et al. Nicotinamide Mononucleotide Increases Muscle Insulin Sensitivity in Prediabetic Women. *Cell Metab* (2013) 17:263–73. doi: 10.1016/j.cmet.2013.06.007

28. Moore SM, Mucinski JM. Vitamin B3 in Cancer. *J Physiol* (2020) 598:3309–15. doi: 10.1113/jphysiol.2019.152809

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