Nicotinamide riboside, a trace nutrient in foods, is a Vitamin B3 with effects on energy metabolism and neuroprotection

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Purpose of review
This review focuses upon the biology and metabolism of a trace component in foods called nicotinamide riboside. Nicotinamide riboside is a precursor of nicotinamide adenine dinucleotide (NAD^+), and is a source of Vitamin B3. Evidence indicates that nicotinamide riboside has unique properties as a Vitamin B3. We review knowledge of the metabolism of this substance, as well as recent work suggesting novel health benefits that might be associated with nicotinamide riboside taken in larger quantities than is found naturally in foods.

Recent findings
Recent work investigating the effects of nicotinamide riboside in yeast and mammals established that it is metabolized by at least two types of metabolic pathways. The first of these is degradative and produces nicotinamide. The second pathway involves kinases called nicotinamide riboside kinases (Nrk1 and Nrk2, in humans). The likely involvement of the kinase pathway is implicated in the unique effects of nicotinamide riboside in raising tissue NAD^+ concentrations in rodents and for potent effects in eliciting insulin sensitivity, mitochondrial biogenesis, and enhancement of sirtuin functions. Additional studies with nicotinamide riboside in models of Alzheimer's disease indicate bioavailability to brain and protective effects, likely by stimulation of brain NAD^+ synthesis.

Summary
Initial studies have clarified the potential for a lesser-known Vitamin B3 called nicotinamide riboside that is available in selected foods, and possibly available to humans by supplements. It has properties that are insulin sensitizing, enhancing to exercise, resisting to negative effects of high-fat diet, and neuroprotecting.

Keywords
Alzheimer's, metabolic syndrome, mitochondria, nicotinamide adenine dinucleotide, nicotinamide riboside

INTRODUCTION

Nicotinamide riboside is a nucleoside, which incorporates nicotinamide and ribose into a single chemical moiety (Fig. 1). Nicotinamide riboside naturally occurs in yeast, bacteria, and mammals. The foods most enriched in nicotinamide riboside are not well identified, although yeast-containing food products are presumed natural sources of the compound [1], and milk-derived products such as whey fractions have been reported to contain the nutrient. The quantities in foods are quite low, and probably do not exceed the low micromolar range. The mechanisms by which nicotinamide riboside is produced in the biological setting are barely studied, especially in mammals. In baker's yeast, a phosphatase is implicated in the dephosphorylation of nicotinamide mononucleotide to produce nicotinamide riboside [2]. Nicotinamide riboside is secreted by yeast, suggesting it may be a natural product of yeast fermentative actions in foods [3].

The relatively small quantities of nicotinamide riboside in foods (there are few quantitative studies available) and relative difficulty in obtaining large

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KEY POINTS

- Nicotinamide riboside enhances nicotinamide adenine dinucleotide (NAD) levels in animal tissues including muscle and brain.
- Nicotinamide riboside increases insulin sensitivity and induces mitochondrial biogenesis.
- Nicotinamide riboside exhibits neuroprotective effects in a mouse model of Alzheimer's.
- Nicotinamide riboside is found in small amounts in foods.
- Nicotinamide riboside is a newly appreciated form of Vitamin B3, with unique properties.

amounts in purified form has limited investigations into the effects of nicotinamide riboside on cells and tissues. However, in the last few years new and reliable synthetic methods for producing nicotinamide riboside have been developed [4], thereby enabling larger amounts of this compound to be made available for cell-based studies [4] and for animal feeding experiments [5▪,6▪]. In July 2013, nicotinamide riboside became available in supplement form with the brand name NIAGEN (Chromadex Incorporated, Irvine, California, USA).

NICOTINAMIDE RIBOSIDE AS A NICOTINAMIDE ADENINE DINUCLEOTIDE PRECURSOR

Nicotinamide riboside is a formal precursor of NAD^+, and in yeast nicotinamide riboside added to growth media leads to augmentation of NAD^+ level [7]. The metabolic fate of nicotinamide riboside in mammalian tissues was first investigated by Rowen and Kornberg in 1951. These authors speculated that mammalian cells might synthesize NAD from this metabolic precursor, but concluded from work with liver lysates that phosphorolytic degradation to nicotinamide (Pathway 1, Fig. 2) was the most likely fate of nicotinamide riboside in cells, thereby discouraging further investigation of nicotinamide riboside fate in mammalian tissues. Although Kornberg also described an enzymatic activity capable of converting nicotinamide riboside to nicotinamide mononucleotide (NMN), in an adenosine triphosphate (ATP)-dependent manner, it was only when Brenner *et al.* described the human nicotinamide riboside kinases (Nrk1 and Nrk2) in 2004, and provided characterization of their enzymatic properties that it became relevant to reconsider this fate of nicotinamide riboside in mammalian cells. Specifically, nicotinamide riboside might be processed by an Nrk-dependent pathway (Pathway 2, Fig. 2). Yang *et al.* [4] provided additional key data to highlight the possibility that nicotinamide riboside might behave differently than nicotinamide, by showing that nicotinamide riboside is a potent stimulator of NAD^+ production in several cultured mammalian cell types, including mouse and human cells. Increases in NAD^+ were in some cases as high as 270% of controls [4], levels unprecedented for nicotinamide or nicotinic acid as NAD^+ sources. These data have collectively suggested that nicotinamide riboside has a unique metabolic pathway to NAD^+ [8] independent of other studied Vitamin B3 compounds [1], and with remarkable potency in enhancing NAD^+ level [4].

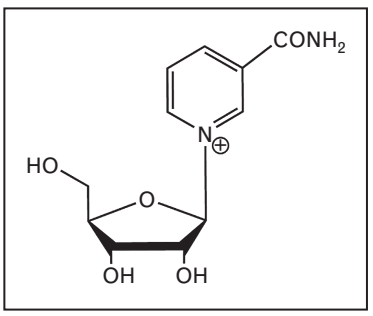

FIGURE 1. The molecular structure of nicotinamide riboside.

NAD^+ is a versatile acceptor of hydride equivalents to form the reduced dinucleotide nicotinamide adenine dinucleotide (NADH), chemistry shared by its phosphorylated derivatives NADP and NADPH. NAD^+ and its derivatives function as coenzymes for oxidoreductases and dehydrogenases and play integral roles in basic energy metabolism such as glycolysis, citric acid cycle, and mitochondrial electron transport. NAD^+ is also a key substrate for signaling enzymes such as polyADPribosyl-polymerases, sirtuins, and ADPribosyltransferases, which are dubbed 'NAD^+ consumers' (See Fig. 2) [9]. NAD^+ is, therefore, a fundamental and abundant metabolite in all mammalian cells, involved in numerous cellular processes such as metabolism as well as cell signaling that are vital for survival.

Synthetic processes of nicotinamide riboside to NAD^+ occur by intracellular mechanisms. Nicotinamide riboside is presumed to be first translocated from the extracellular compartment to the intracellular compartment by a nicotinamide riboside transporter (Nrt). This transporter has been identified in *Haemophilus influenzae* and in *Saccharomyces cerevisceae* [3,10]. The corresponding mammalian nicotinamide riboside transporter(s) are currently unidentified. Nevertheless, stable derivatives of nicotinamide riboside, such as benzamide riboside

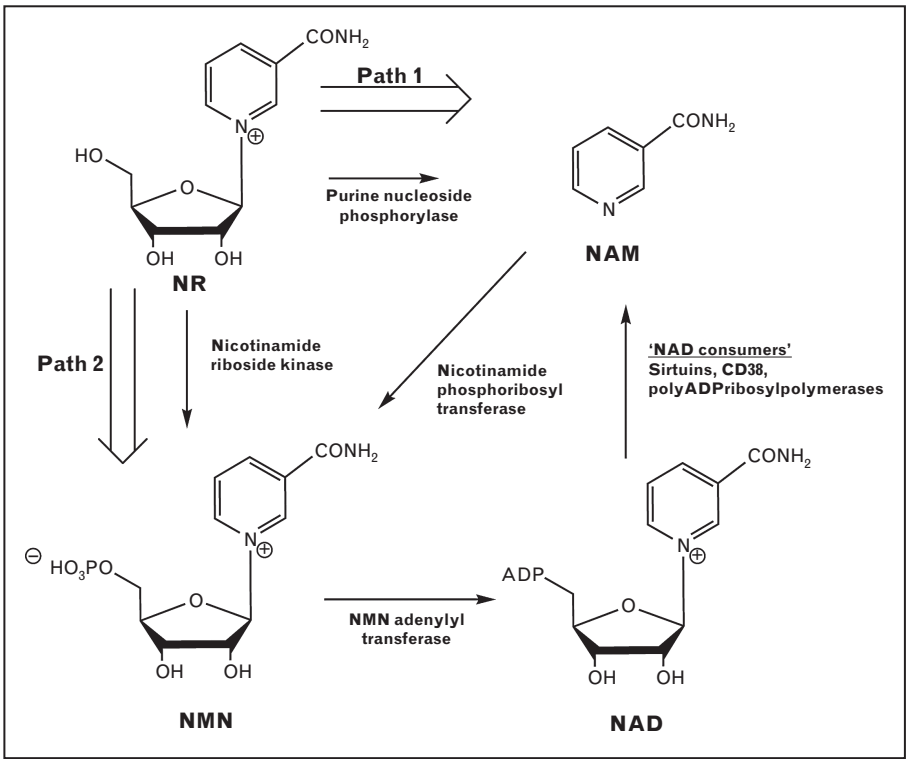

FIGURE 2. Pathways to NAD^+ from nicotinamide riboside. Pathway 1 depicts a degradative fate of NR to nicotinamide followed by conversion of nicotinamide to NMN via nicotinamide phosphoribosyltransferase. In Pathway 2, NR is phosphorylated by Nicotinamide riboside kinases (Nrk1 and Nrk2) to NMN. Both pathways lead to NMN as a penultimate precursor to NAD^+, which is made via NMN adenylyl transferases. NAD^+ is subject to degradation back to NAM via NAD^+ consumers such as sirtuins, PARPs and $CD38^+$. NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside.

and tiazofurin, which are not cleaved by phosphorolytic processes, are known to be transported into mammalian cells.

After nicotinamide riboside is transported into cells, it can be made to NAD^+ via two pathways, as already described. One pathway requires the nicotinamide riboside kinases (Nrks), wherein ATP-dependent phosphorylation of nicotinamide riboside to NMN occurs, followed by adenylylation by NMN adenylyltransferase (NMNAT) to NAD^+. In humans, two isoforms of Nrk (Nrk1 and Nrk2) [11], and three isoforms of NMNAT (Nmnat1, 2, 3) have been characterized. The second pathway is initiated by purine nucleoside phosphorylase-mediated nicotinamide riboside degradation to nicotinamide, as first described by Rowen and Kornberg [12], followed by action of nicotinamide phosphoribosyltransferase (Nampt) which converts nicotinamide to NMN (See Fig. 2) [13]. The Nmnat enzymes would then act upon NMN, as above. In yeast, the degradative enzymes are uridine hydrolase (Urh1), purine nucleoside phosphorylase (Pnp1), and methylthioadenosine phosphorylase (Meu1) [7]. Nicotinamide salvage in yeast is an important pathway for nicotinamide riboside metabolism, although it does not involve the same pathway as humans as yeast do not encode a Nampt equivalent, and degrade nicotinamide to nicotinic acid. This suggests human and yeast metabolic handling of nicotinamide riboside are likely to be quite distinct. Further, research in this area is clearly needed to clarify the relative importance of the two distinct metabolic pathways from nicotinamide riboside in mammalian cells.

BIOLOGICAL EFFECTS OF NICOTINAMIDE RIBOSIDE

In yeast, assimilation of endogenous nicotinamide riboside has been shown to be essential for calorie restriction-mediated life span extension [14]. 10 μmol/l of exogenously added nicotinamide riboside doubles intracellular NAD^+ in *S. cerevisceae* in a nicotinic acid depleted medium, thereby doubling replicative longevity [7]. This effect was shown to be dependent upon yeast Sir2, suggesting

that nicotinamide riboside could provide sirtuin activation, at least in yeast. The possibility that increasing nicotinamide riboside could stimulate sirtuins in mammalian systems was further investigated.

To evaluate for nicotinamide riboside effects in live mammals, Canto *et al.* [5▪] treated mice with synthetically derived nicotinamide riboside (at a dose of 400 mg compound/kg animal weight per day), and showed that this intervention caused increase of NAD^+ levels in muscle and liver. Animals challenged with high-fat diet were protected from body weight gain, and had enhanced endurance and improved oxidation of fatty acids as a fuel source. Nicotinamide riboside also markedly improved insulin sensitivity in weight-matched animals [5▪]. Nicotinamide riboside treatment caused increased mitochondrial biogenesis as measured by higher cristae content in muscle tissue. These data suggest that nicotinamide riboside could be a novel agent for treating metabolic disorders and reactive oxygen syndromes associated with mitochondrial dysfunction. Consistent with increases in tissue NAD^+ levels, sirtuins SIRT1 and SIRT3 appeared to be upregulated, as measured by FOXO1 and SOD2 acetylation levels [5▪]. In these studies, nicotinamide riboside was also shown to have a greater ability to increase NAD^+ level than other NAD^+ precursors such as NMN, nicotinamide, and nicotinic acid [5▪]. These proof-of-concept studies illustrated the potent biological effects of nicotinamide riboside in mitigating negative consequences of fat-rich diets, and has increased interest in nicotinamide riboside as a possible new therapeutic in several disease states, in which NAD^+ level could be of importance for outcomes.

An additional effect reported in the Canto study was the ability of nicotinamide riboside to enrich NAD^+ level in mitochondria. In mitochondria, the normal NAD^+/NADH ratio is about 10 : 1, and the normal $NADP^+$/NADPH ratio is about 1 : 4. These ratios regulate metabolic and energy fluxes in the tricarboxylic acid cycle and electron transport chain in mitochondria. Nicotinamide riboside increases NAD^+/NADH [5▪], which could contribute to its ability to enhance mitochondrial oxidative capacity [5▪].

Interestingly, recent data indicate that mitochondrial NAD^+ enables cells to resist genotoxic stress and the mitochondrial permeability transition [15]. The ability of mitochondrial NAD^+ to prevent cell death is linked to a mitochondrial sirtuin, SIRT3, which is required for this protection [15]. SIRT3 represses formation of the mitochondrial permeability pore by deacetylation of Cyclophilin D on Lys 166 [16]. Increased SIRT3 activity stimulates reactive oxygen species detoxifying enzymes such as superoxide dismutase 2 [17]. These data suggest that nicotinamide riboside stimulation of mitochondrial NAD^+ could provide a number of potential benefits in disease states in which cell death and reactive oxygen detoxification are abnormal.

NICOTINAMIDE RIBOSIDE IN NEUROPROTECTION

Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1-α) has been implicated in brain protective effects, in part because PGC1-α is able to stimulate mitochondrial biogenesis, which increases the activity of numerous oxygen detoxification activities [18]. SIRT1 deacetylates peroxisome proliferator-activated receptor gamma-coactivator 1-α (PGC-1α), and thereby activates PGC-1α [19]. Recently, Gong *et al.* [6▪] reported that nicotinamide riboside was able to stimulate increased NAD levels in brains of transgenic Tg2576 AD (a model of Alzheimer's) mice by approximately 70%, when administered nicotinamide riboside for 3 months at a dosage of 250 mg nicotinamide riboside/kg-animal body weight-per day delivered in drinking water. Nicotinamide riboside-treated animals experienced a 50% induction of PGC1α mRNA level, suggesting that increased NAD^+ levels stimulate PGC1α production in the brain. Importantly, reduced PGC-1α levels are found in Alzheimer's brains [20], suggesting that nicotinamide riboside and NAD^+ could provide mitigating effects, if PGC-1α levels are beneficial, as hypothesized. Indeed, in this study, animals treated with nicotinamide riboside experienced reduced Aβ(1-42) burden and performed better in novel object tests used to measure cognition [6▪]. Although this study is promising, and still awaits further confirming studies, there are numerous studies illustrating the neuroprotective value of brain NAD^+, and if nicotinamide riboside proves to be a good means to increase NAD^+ in this tissue, it may have clinical relevance in future neurotherapy approaches.

CONCLUSION

Although nicotinamide riboside has long been known as a possible NAD^+ precursor, it is only recently that its trace quantities in foods as well as its unique biological properties have become appreciated. Evidence is accumulating to suggest nicotinamide riboside could be a third major Vitamin B3 form, different in action from nicotinamide or nicotinic acid (Niacin). Studies indicate that it has a separate and distinct metabolism to

NAD^+ from known Vitamin B3 forms, and has unique properties in small animal models of human disease. Its ability to insulin sensitize and to induce mitochondrial biogenesis, suggests it could find meaningful applications in treatment of metabolic disorders and neurodegenerative diseases.

Acknowledgements

None.

Conflicts of interest

A.A.S. acknowledges that he has intellectual property related to methods to produce NR and possible uses thereof. Royalties on sales of NR are expected to accrue to Cornell University and A.A.S. as inventor. Conflicts of interest for A.A.S. are actively managed by Cornell University to ensure transparency and lack of bias in research and research reporting. No further conflicts of interest are declared.

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- ■■ of outstanding interest

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