State of the Science: Preclinical & Clinical NAD+ Research (Gut Health)

QUOTE ON DESIGNED COVER: *"Recent research suggests that NAD+ precursors play a crucial role in maintaining the integrity of the gut barrier. Indeed, its deficiency has been associated with enhanced gut inflammation and leakage, and dysbiosis. (Niño-Narvión et al., 2023) [1]"*

Executive Summary

Nicotinamide adenine dinucleotide (NAD+) is an essential molecule found in all living cells, playing a crucial role in various cellular processes involving energy production and cellular repair. As research on NAD+ continues to unfold, its significance for overall cellular health becomes increasingly clear, with a growing recognition of its influence on gut health.

The health of the gut is largely determined by the composition of the gut microbiota, where a diverse population of beneficial bacteria is essential for maintaining a healthy gut environment. Age is one of the most inevitable factors affecting gut microbiota composition, with the aging process often leading to dysbiosis—a condition characterized by an imbalance in bacterial populations [2,3].

Aging is linked to both dysbiosis and a decline in NAD+ levels. Maintaining adequate NAD+ levels is critical for preserving gut health and combating the age-related deterioration of the microbiome [1].

Emerging research has shown that that supplementation with **nicotinamide riboside (NR)**, a precursor of NAD+, can safely elevate NAD+ levels in the blood while enhancing the diversity of the gut microbiota, highlighting NR's beneficial effects on gut health [4]. In addition, a separate investigation revealed a trend toward an increase in beneficial bacteria, although no significant changes in overall gut microbiota composition were observed [5]. Despite these varying outcomes, the collective evidence suggests that NR supplementation can positively influence the gut microbiome, positioning it as a promising strategy for supporting gut health and mitigating age-related decline.

Research in animal models has provided valuable insights into the effects of NR and gut health, demonstrating that NR supplementation can modify the gut microbiome in healthy chickens, mice, and even in an Alzheimer's disease mouse model. These findings suggest potential homeostatic and therapeutic effects of NR [6–9]. NR treatment has also been shown to protect against ethanol-induced intestinal dysfunction in both humans and mice [10]. Beyond these protective effects, NR offers various benefits for intestinal health. For instance, in mice and rats, NR helps prevent age-induced intestinal stem cell dysfunction, as well as damage from ischemia-reperfusion injury, radiation exposure, and inflammation associated with conditions like ulcerative colitis and necrotizing enterocolitis–two inflammatory conditions that affect the bowel [9,11–15]. In diabetic rats, NR has been found to improve digestion [16].

NAD+ plays a crucial role in mitigating the age-related decline in gut health. Emerging research suggests that NAD+ may help maintain gut health by influencing the diversity of beneficial gut bacteria in both humans and animal models. Animal studies reveal that NR may help restore the gut microbiome, particularly under stressors like alcohol consumption or bowel disease, paving the way for future research to explore these effects in humans. As the research progresses, NR may emerge as a promising and effective approach to combating the age-related decline in gut health.

Ex Vivo/Clinical Studies

Publication	Intervention	Objective	Key Outcomes
Lapatto et al., 2023 [4] Nicotinamide Riboside Improves Muscle Mitochondrial Biogenesis, Satellite Cell Differentiation and Gut Microbiota Composition in a Twin Study CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the role of NR supplementation on muscle mitochondrial biogenesis and metabolic health in BMI-discordant (one normal weight, one obese) identical twin pairs.	• In both cotwins, NR increased whole blood NAD+ levels and muscle mitochondrial biogenesis, and improved gut microbiota composition, as seen through an increase in the abundance of *Faecalibacterium prausnitzii*—one of the most beneficial bacteria found in the microbiome of healthy humans.

Peluso et al., 2023 [5] Oral Supplementation of Nicotinamide Riboside Alters Intestinal Microbial Composition in Rats and Mice, But Not Humans CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To explore the effects of NR supplementation on gut microbial diversity linked to obesity and metabolic syndrome in a rat model of high-fat-diet-induced obesity and in mice. Additionally, an analysis of gut microbiota composition was conducted in humans.	• In humans, NR did not affect the diversity or abundance of gut bacteria. However, NR increased—albeit not significantly—the ratio of *Firmicutes* and *Proteobacteria*, suggesting a potential positive effect.

Ongoing Clinical Trials		
Trial Registry	**Intervention**	**Objective**
NCT05561738 Nicotinamide Riboside in Ulcerative Colitis CERP ChromaDex External Research Program	**Nicotinamide Riboside**	• To investigate the effects of NR on mitochondrial function and metabolic homeostasis in the intestinal cells of pediatric patients with ulcerative colitis.

Preclinical Studies			
Publication	**Intervention**	**Objective**	**Key Outcomes**
Toropova et al., 2018 [13] Nicotinamide Riboside has Protective Effects in a Rat Model of Mesenteric Ischaemia-Reperfusion	**Nicotinamide Riboside**	To investigate the effect of NR on endothelial function, microcirculation, and intestinal morphology in acute mesenteric ischemia and reperfusion in rats.	• NR protected the intestinal wall from ischemia-reperfusion injury and improved the relaxation function of mesenteric vessels, highlighting its potential in preserving intestinal grafts until transplant.
Igarashi et al., 2019 [11] NAD+ Supplementation Rejuvenates Aged Gut Adult Stem Cells CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the age-induced decline in intestinal stem cell function and assess the potential protective effects of NR in mice.	• In aged mice, NR treatment improved intestinal stem cell function and rescued damage-repair defects in the gut.
Jiang et al., 2019 [12] Nicotinamide Riboside Alleviates Alcohol-Induced Depression-Like Behaviours in C57BL/6J Mice by Altering the Intestinal Microbiota Associated with Microglial Activation and BDNF Expression	**Nicotinamide Riboside**	To examine the effects of NR on a mouse model of alcohol-induced depression and determine whether these effects are linked to changes in the gut microbiome composition.	• NR protected against alcohol-induced depression by improving the gut microbiota composition, effectively overcoming the bacterial dysbiosis caused by alcohol use.
Yu et al., 2021 [9] Effect of Nicotinamide Riboside on Lipid Metabolism	**Nicotinamide Riboside**	To investigate the effects of NR on lipid metabolism and gut microflora-bile acid axis in alcohol-exposed mice.	• In alcohol-exposed mice, NR altered the microbial community at the phylum, family and genus levels, and returned species abundances to

and Gut Microflora-Bile Acid Axis in Alcohol-Exposed Mice			levels seen in the normal mice, indicating a potential therapeutic effect of NR.
Chu et al., 2022 [6] Nicotinamide Adenine Dinucleotide Supplementation Drives Gut Microbiota Variation in Alzheimer's Mouse Model CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the impact of NR supplementation on the gut microbiota in mice with Alzheimer's disease (AD).	• NR normalized the diversity and richness of the gut microbiota in the AD mice. More specifically, NR restored levels of the following gut microbiota species: *Oscillospira, Butyricicoccus, Desulfovibrio, Bifidobacterium, Olsenella, Adlercreutzia, Bacteroides, Akkermansia,* and *Lactobacillus*. • Most effects of NR on the AD gut microbiome were linked to inflammatory functions.
Kolba et al., 2022 [8] Alterations in Intestinal Brush Border Membrane Functionality and Bacterial Populations Following Intra-Amniotic Administration (Gallus gallus) of Nicotinamide Riboside and Its Derivatives CERP ChromaDex External Research Program	**Nicotinamide Riboside derivatives: Chloride, Tributyrate Chloride, and Trioleate Chloride**	To assess the effects of intra-amniotic administration of nicotinamide riboside chloride (NRCl) and two new derivatives, a water-soluble form called nicotinamide riboside tributyrate chloride (NRTBCl) and an oil-soluble form called nicotinamide riboside trioleate chloride (NRTOCl) on intestinal brush border membrane (BBM) functionality in chickens. The BBM is the innermost layer of the small intestine, which is responsible for absorbing nutrients from food.	• NRCl treatment increased certain bacterial species in the gut microbiome such as *Clostridium, Bifidobacterium, Lactobacillus,* and *E. coli*. • NRCl and its derivatives altered the expression of key BBM genes involved in digestion and absorption and affected the intestinal microbiota composition and function, suggesting improved intestinal function.
Li et al., 2022 [10] NAD Supplement Alleviates Intestinal Barrier Injury Induced by Ethanol Via Protecting Epithelial Mitochondrial Function CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate whether NR supplementation could prevent ethanol-induced intestinal barrier injury in mice and human intestinal cells.	• NR mitigated ethanol-induced intestinal barrier dysfunction by maintaining mitochondrial function and promoting mitochondrial biogenesis in mice and human intestinal cells. • NR protected against the ethanol-induced imbalance of NAD+ and energy homeostasis and protected against an increased permeability of the intestinal epithelium—the surface lining of the small and large intestine.
Lozada-Fernández et al., 2022 [7] Nicotinamide Riboside-Conditioned Microbiota Deflects High-Fat Diet-Induced Weight Gain in Mice	**Nicotinamide Riboside**	To determine whether NR supplementation can alter the gut microbiome and impact the deflection of weight gain in a mouse model of high-fat diet-induced obesity.	• NR reduced weight gain caused by a high-fat diet. • NR-supplemented mice had a unique gut microbiota composition compared to control mice Specifically, NR enriched butyrate-producing

CERP ChromaDex External Research Program			*Firmicutes*. Butyrate is important for balancing gut microbiota and protecting the intestinal barrier. • Transferal of fecal matter from NR-supplemented mice to naive mice resulted in reduced weight gain and increased energy expenditure, suggesting fecal matter transfer from NR-supplemented mice had the same effect as dietary NR supplementation.
Zhang et al., 2022 [15] Nicotinamide Riboside Relieves the Severity of Experimental Necrotizing Enterocolitis by Regulating Endothelial Function via eNOS Deacetylation	**Nicotinamide Riboside**	To explore whether NR administration can improve the severity of necrotizing enterocolitis (NEC) in mouse pups. NEC is an intestinal disease that typically affects premature babies and occurs when the lining of the intestine becomes inflamed and dies as a result.	• NR prevented the NEC-induced decrease in NAD+ by increasing intestinal NAD+ levels by approximately three-fold. • NR reduced the severity of NEC, improved survival, prevented oxidative stress, and alleviated intestinal injury in the mouse pups.
Costa et al., 2023 [16] Nicotinamide Riboside Improves Enteric Neuropathy in Streptozotocin-Induced Diabetic Rats Through Myenteric Plexus Neuroprotection CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the effects of NR administration on the development of enteric neuropathy in a rat model of diabetes and to test whether fecal microbiota transplant (FMT) could produce the same effects. Enteric neuropathy is a condition where the nerves in the digestive system are damaged, resulting in poor digestion and absorption of nutrients, and is a typical symptom of diabetes.	• NR-treated diabetic rats had improved digestion times and digestive system function comparable to rats without diabetes. • The improved digestion in NR-treated diabetic rats was associated with improved nerve cell density in the small and large intestines, suggesting NR helped prevent nerve damage and support normal digestive functioning. • Rats that received FMT had improved digestion times, however, this was not associated with changes to gut microbiota composition.
Novak et al., 2023 [17] Epithelial NAD+ Depletion Drives Mitochondrial Dysfunction and Contributes to Intestinal Inflammation CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the mechanisms by which peroxisome proliferator activated receptor-gamma (PGC1α) protein expression contributes to the pathogenesis of inflammatory bowel disease, and to further assess the potential protective effects of NR supplementation, using mice models of ulcerative colitis (UC) and intestinal tissues from UC patients. UC is an inflammatory bowel disease that causes chronic	• In UC patients and mice, intestinal NAD+ was significantly lower compared to control groups. In UC patients, this deficit was present regardless of the presence of inflammation in intestinal tissue. • In UC mice, NR reduced weight loss, lowered disease severity scores, improved colon length and tissue health with less intestinal damage, and decreased levels of inflammatory markers. These

		inflammation in the digestive tract.	effects were present both when NR was administered preventatively and during the recovery phase from UC.
Yue et al., 2024 [14] Nicotinamide Riboside Alleviates Ionizing Radiation-Induced Intestinal Senescence by Alleviating Oxidative Damage and Regulating Intestinal Metabolism	**Nicotinamide Riboside**	To investigate the effects of NR supplementation in protecting against radiation-induced intestinal cell senescence and dysregulation in mice.	• NR protected against radiation-induced intestinal damage by preventing hair graying and loss, preserving grip strength, preventing colon shortening, and preventing intestinal structural damage and senescence. • NR rescued intestinal stem cell function, proliferation, and capacity, normalized the gut microbiota, and protected against radiation-induced metabolic dysregulation. • NR prevented oxidative damage in the intestinal crypts—structures that house intestinal stem cells.

CERP ChromaDex External Research Program *The **ChromaDex External Research Program (CERP)** is an essential component of ChromaDex's R&D Program. Through CERP, ChromaDex material, such as the company's patented nicotinamide riboside (NR) ingredient, Niagen®, and technical expertise is freely provided for exceptional preclinical and clinical, investigator-initiated research projects. Additionally, CERP funds research studies supporting ChromaDex's business needs. Please visit https://www.chromadex.com/research/cerp/ for more information.*

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