Newsletter for the ChromaDex External Research Program Investigators (CERPI)

CERPI Communiqué

Volume 3, Issue 4, December 2022

In This Issue

Have you completed the 2022 CERP Investigator Survey?

We are preparing to make significant changes to our program, and we need your input. Please complete the 15-minute survey by clicking this link:

CERP SURVEY

When a Headline Spreads Like Wildfire but is not Reflective of the Peer-Reviewed Science

The safety of our consumers and CERP study participants is our top priority, and we continue to stand by the safety of our Niagen® ingredient and product.

A recent publication describing the development of a bioluminescent-based probe for *in vivo* monitoring of nicotinamide riboside (NR) uptake made news and media headlines following the press release from the University of Missouri. In the study, the proof of principle assessment evaluating the effects of human triple negative breast cancer (TNBC) cells in immunocompromised mice produced surprising results when the number of mice given NR in the study developed a higher, but not statistically significant increase in breast cancer when compared to mice on the control diet. In a second experiment, different animals were injected in the heart with MDA-MB-231 TNBC cells, a cell line that specifically relies on the NAD+ salvage pathway precursors to evaluate metastasis in NR supplemented and control mice.

Though neither of the experiments were reflective of typical human carcinogenesis, the press release shared globally failed to indicate that the study was not conducted in humans and used rodent models that are not directly translatable to humans. The press release did not present a balanced view, as it omitted studies that have demonstrated how NR reduced the rate of tumor growth, supported the immune system to reduce differentiation of cells into a carcinogenic phenotype, enhanced tumor-infiltrating lymphocytes, and induced "additive antitumor immunity in conjunction with immune checkpoint blockade treatments." Overall, the study generated conclusions and headlines, that one of the co-authors indicated on social media was unwarranted attention.

For those who are concerned about the outcomes from this study, we recommend that your team read through and discuss the entire article. This may serve as a good article for a journal club, as there are many limitations in both the article and the press release. Our team and Scientific Advisory Board members have noted several limitations in the study design, models used, and translatability to human cancer outcomes including, 1) the study was not designed to truly evaluate cancer outcomes; 2) the rate of tumor development in the NR fed animals was not statistically significant when compared to control diet animals; and 3) the cancer cells that were used to model breast metastasis where injected into the heart, not the breast tissue, and were not from the tumors that developed in the animals, thus not modeling cancer seen in humans; 4) separate animals were used for the tumor development assay (which was not statistically significant) and the metastasis models. Other limitations include a lack of validation of NR in the feed once prepared (NR can degrade in water and high heat settings, and should always be validated and quantitated following diet preparation and at the end of the study), the vendor listed for supplying NR-Cl does not list the product on its website catalogue preventing others from testing the material to replicate the findings, and normal "non-cancerous" cell lines were not used as controls in the study.

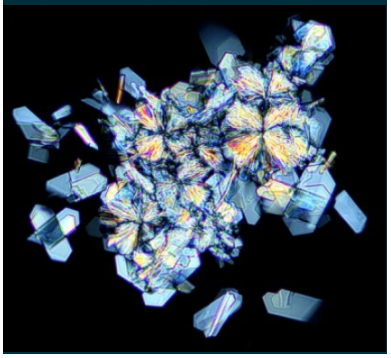
Do You Want Your Voice Heard?

As always, the CERP team is immensely interested in hearing the opinions and viewpoints of our investigators.

Therefore, we are happy to report that The CERPI Communiqué will now accept Letters to the Editor.

CERPIs who wish to write a letter may email cerp@chromadex.com. In the email, please be sure to clearly indicate your title, institution, and which article the letter is referring to.

The letter should preferably contain no more than 500 words We look forward to hearing from you!



We have been contacted by clinical NR researchers who have indicated that their protocols include tests for cancer biomarkers in their study participants, and to date, they have not had any cancer-related adverse events or positive tests. NR is being tested in cancer patients and survivors (clinicaltrials.gov), however, with the exception of the case study in a patient with Li-Fraumeni Syndrome, results are not available, as the studies are still listed as recruiting. We hope the results from one or more of the studies will be available in early 2023.

Though there were many limitations to the Maric et al., 2022 study, we acknowledge that every cancer is different and everyone with cancer is unique. Many clinical studies in our program have exclusion criteria for active cancer diagnosis, and I would recommend that such an exclusion is continued for most clinical studies until results from the studies of NR in cancer patients and survivors is made public.

Early safety studies demonstrated that NR was not a carcinogen. NAD, however, can be elevated in some progressive and aggressive cancer cell lines, and Chowdhry et al. 2019 started to reveal some of these potential mechanisms, as well as the preferred pathways associated with elevating NAD+ in specific cell lines.

Based upon the current scientific literature, from our perspective, the risks of using NR and developing cancer are very low. However, for our CERPIs conducting clinical research, we recommend that your team and IRB evaluate the potential risks in your study population, which may include the incorporation of blood tests to monitor for various cancer biomarkers.

If you would like to share any comments or discuss this further, please reach out to us at cerp@chromadex.com. ■

Nicotinamide Riboside Clinically Reduces Levels of Inflammatory Markers

Inflammation represents a defense mechanism mounted by the immune system to protect the body against harmful stimuli, such as infection and injury. The inflammatory response, characterized by a release of chemical signals followed by increased white blood cell activity, is typically acute and transient, resolving in a matter of days upon neutralization of the threat. Although acute inflammation is clearly protective, prolonged exposure to inflammation, i.e., low-grade inflammation lasting weeks, months, or even years in duration, can adversely alter tissue and organ function, and confers increased risk for multiple diseases and conditions.

Chronic inflammation is heavily modified by environmental and lifestyle factors, including diet, physical activity, and sleep [1]. Moreover, a growing body of research suggests an association between chronic low-grade inflammation and aging, in a phenomenon commonly referred to as inflammaging [2,3]. Inflammaging is marked by an increased influx of proinflammatory immune cells, elevated IL-6 and TNF cytokine levels, and activation of the NLRP3 inflammasome, a multiprotein complex that can respond to a variety of cellular signals and initiate an inflammatory form of cell death [4]. It is thought to play a prominent role in the development and progression of several age-associated conditions, including diabetes, cardiovascular disease, Alzheimer's and other neurodegenerative diseases, and certain cancers [2,5,6]. Nonetheless, because chronic inflammation so intimately accompanies these conditions, it has been difficult to determine to what extent inflammation is a cause versus a manifestation of disease. Further, sources of inflammaging are likely multifactorial and remain incompletely understood. Emerging research suggests that age-associated chronic inflammation is a potential contributor of NAD+ decline and modulation of NAD+ with precursors may mitigate this decline [7,8].

NAD+ supports critical cellular functions by serving as a co-substrate for several classes of proteins involved in the inflammatory response: CD38 glycohydrolases, poly (adenosine diphosphate ribose—ADPribose) polymerases (PARPs), and sirtuins (SIRTs) [9, 10, 11, 12]. Expression of these NAD+ consuming enzymes are activated during various types of metabolic stress such as poor diet, excess sun exposure, and a sedentary lifestyle [13, 14, 15,16]. These enzymes break down NAD+ into its component parts, using it as a source of ADP-ribose during inflammatory macrophage activation, which leads to NAD+ depletion. When NAD+ is depleted, less is available for energy production, resulting in a decline in cellular health [7]. Therefore, maintaining a sufficient supply of NAD+ becomes critical for optimal cellular health and function.

While the NAD+ system is dysregulated by repeated exposure to metabolic stress and various health conditions, nicotinamide riboside (NR) augments NAD+ levels and may contribute to the reduction of inflammation. Oral supplementation of NR has been shown to be safe, well-tolerated, and bioavailable. Specifically, eight clinical studies have demonstrated NR's anti-inflammatory effect either alone or in combination with other ingredients [13, 17, 18, 19, 20, 21, 22, 23]. A summary of these eight studies is provided in Table 1.

Table 1. Summary of the clinical peer-reviewed, publications evaluating the anti-inflammatory effect of nicotinamide riboside in humans.

NICOTINAMIDE RIBOSIDE ONLY			
Publication	**Dose/Duration**	**Study Population**	**Key Results**
Elhassan et al., 2019	1,000mg/day for 21 days	Marginally overweight, but otherwise healthy older adult men	NR reduced levels of circulating inflammatory cytokines IL-6, IL-5, IL-2, and TNF-α
Zhou et al., 2020	1,000mg/day for 5-9 days	Hospitalized patients with stage D heart failure undergoing advanced heart failure therapy evaluations	NR reduced gene expression of NLRP3 and inflammatory cytokines (IL-1B, IL-6, and IL-18)
Remie et al., 2020	1,000mg/day for 6 weeks	Healthy overweight and obese men and postmenopausal women	NR resulted in a statistical trend toward a reduction in plasma IL-1α levels
Wu et al., 2022	1,000mg/day for one week	Young healthy subjects and patients with systemic lupus erythematosus (SLE)	NR reduced relative mRNA expressions of inflammatory cytokines IFN-β and CXCL10
Brakedal et al., 2022	1,000mg/day for 4 weeks	Newly diagnosed dopaminergic therapy-naïve Parkinson's disease patients	NR reduced levels of inflammatory cytokines in the serum: VEGF and GDF15, as well as in cerebrospinal fluid: G-CSF, IL-7, IL-1RA, CCL4
Wang et al., 2022	2,000mg/day for 12 weeks	Stage C heart failure with reduced ejection fraction patients and age-matched healthy subjects	NR reduced expression of NLRP3 and resulted in directionally similar, though nonsignificant, changes in expression of other inflammatory markers (IL-1B, IL-6, IL-18, and TNF-α)
NICOTINAMIDE RIBOSIDE IN COMBINATION WITH OTHER INGREDIENTS			
Altay et al., 2022	CMA*	Ambulatory COVID-19 patients	CMA decreased levels of inflammatory cytokines CSF-1, IL-15RA, IL-18, MCP-1, and TNF-α
Zeybel et al., 2022	CMA*	Nonalcoholic fatty liver disease (NAFLD) patients	CMA decreased levels of inflammatory cytokines CD-8A, CCL23, FGF-21, and oncostatin-M (OSM)

**Nutritional cocktail termed "Combined Metabolic Activators," comprised of 1,000mg NR, 12.35g L-serine, 3.73g L-carnitine tartrate, 2.55g N-acetylcysteine (NAC).*

Reminder

Do not forget to submit your progress report every six (6) months, or as stipulated in your MTA. An updated progress report is required when requesting additional material or submitting an MTA amendment. We will provide you with a progress report form to simplify the process.

Request forms at cerp@chromadex.com for:

- **Abstract, manuscript, poster, or presentation slides submissions**
- **Bulk or clinical material requests**
- **Requesting an amendment**

CHROMADEX, EMPOWERED BY:

Did we miss your publication?

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Preclinical and clinical studies have linked metabolic stress-induced NAD+ depletion to cellular and tissue dysfunction as well as a host of other diseases. Disruptions in NAD metabolism are increasingly associated with impaired inflammatory responses, while utilization of NAD+ precursors has been shown to decrease expression of inflammatory markers. Research suggests increasing NAD+ levels may better equip cells and tissues to cope with metabolic stress such as inflammation, over time benefiting healthspan and mitigating functional decline.

Taken together, there is accumulating clinical evidence that NR may reduce levels of inflammatory cytokines in individuals, with potential for more robust effects among the elderly or diseased populations who tend to have compromised NAD+ and higher inflammation status. However, more research is needed to clarify which inflammatory markers are targeted by NR and the extent to which they are modified by dose and demographics. ■

NAD+ Science & News We Are Talking About

Q4 Publications

- The HF-AF ENERGY Trial: Nicotinamide Riboside for the Treatment of Atrial Fibrillation in Heart Failure Patients
- Low Nephron Number Induced by Maternal Protein Restriction Is Prevented by Nicotinamide Riboside Supplementation Depending on Sirtuin 3 Activation
- A comparative study of metformin and nicotinamide riboside in alleviating tissue aging in rats.
- Long-term NAD+ supplementation prevents the progression of age-related hearing loss in mice
- Safety and Tolerability of Nicotinamide Riboside in Heart Failure with Reduced Ejection Fraction
- The Use of Progeroid DNA Repair-Deficient Mice for Assessing Anti-Aging Compounds, Illustrating the Benefits of Nicotinamide Riboside
- Microbiota Dysbiosis and Gut Barrier Dysfunction Associated with Non-Alcoholic Fatty Liver Disease Are Modulated by a Specific Metabolic Cofactors' Combination
- Nicotinamide Riboside for Ataxia Telangiectasia: Report of an Early Treated Individual
- Reduction of Obesity and Insulin Resistance through Dual Targeting of VAT and BAT by a Novel Combination of Metabolic Cofactors
- Sirt3 deficiency induced down regulation of insulin degrading enzyme in comorbid Alzheimer's disease with metabolic syndrome
- Oral nicotinamide riboside raises NAD+ and lowers biomarkers of neurodegenerative pathology in plasma extracellular vesicles enriched for neuronal origin
- Autophagy promotes cell survival by maintaining NAD levels

Newly Registered Clinical Trials

- N-DOSE AD: A Dose Optimization Trial of Nicotinamide Riboside in Alzheimer's Disease
- NOPARK Open Label Extension Study
- N-DOSE: A Dose Optimization Trial of Nicotinamide Riboside in Parkinson's Disease
- Nicotinamide Riboside in Ulcerative Colitis
- A Study to Evaluate Vitamin B3 Derivative to Treat Mitochondrial Myopathy

Regulatory Updates

On November 4, 2022, a series of letters was sent by the United States Food and Drug Administration stating that the NAD+ precursor nicotinamide mononucleotide (NMN) is now *"excluded from the definition of a dietary supplement."* Therefore, NMN is no longer allowed to be sold in the United States as a dietary supplement or food product. This article provides the regulatory details, and you can access one of the FDA letters here.

Conferences and Funding Corner

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NUTRITION 2023

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USA Funding Opportunity for Dietary Supplement Research

Did you know that your research evaluating Nicotinamide Riboside and other NAD+ precursors may qualify for funding through the United States National Institutes of Health, Office of Dietary Supplements (ODS) Grants Program and Research Support? ODS research funding priorities include "nutrition across the lifespan; nutrition and chronic and noncommunicable diseases; micronutrient status assessment, bioavailability, biomarkers, and nutrient levels and their role in health and disease; transporter proteins; the microbiome; and the developmental origins of health and disease." For more information, please visit the ODS Grants and Funding Website or review their video.

Happy Holidays!

Wishing you a Scientifically Inspiring 2023

Interested in learning more about how to develop intellectual property that industry would want to license or how to commercialize your ideas?

If so, ChromaDex has a dynamic Business Development Team that would love to talk with you. For more information, send an email to cerp@chromadex.com with the subject line: Business Development, and we will get you connected.

Expand Your NAD+ Research Portfolio by including rarely studied NAD+ precursors. For more information send an email to cerp@chromadex.com with the subject line: Novel NAD+ Precursors.

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CERP
ChromaDex External Research Program

ChromaDex, Inc.
10900 Wilshire Boulevard, Suite 600
Los Angeles, California, USA 90024
+1 (310) 388-6706
cerp@chromadex.com
http://www.chromadex.com/cerp

Special thanks to our CERPI Communiqué content contributors, editors, and reviewers:

Rebecca Idoine
Jun Kwon
Yasmeen Nkrumah-Elie, PhD
Andrew Shao, PhD
Heather Van Blarcom, JD

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