State of the Science: Preclinical & Clinical NR Research (Heart Health)

QUOTE ON DESIGNED COVER: *"NAD+ plays an important role directly or in-directly in protecting against various cardiovascular diseases, including heart failure, occlusion, ischemia-reperfusion injury, arrhythmia, myocardial infarction, rhythmic disorder, and a higher order of cardiovascular complexity." (Kibria et al., 2024)* [1]

Executive Summary

Nicotinamide adenine dinucleotide (NAD+) is an essential molecule found in all living cells, crucial for various cellular processes, including energy production, mitochondrial function, and cellular repair. As research into NAD+ continues to expand, its significance in supporting overall cellular health has become increasingly evident, particularly in the context of cardiovascular health.

The heart relies on a continuous supply of energy and oxygen to maintain its rhythm and effectively pump blood throughout the body. Disruptions in either mitochondrial function or blood flow can impair the heart's ability to function properly, leading to conditions like heart failure, which is characterized by the heart's inability to pump blood effectively [2]. If blood flow is severely restricted, a myocardial infarction (MI), or heart attack, can occur, often resulting in cardiac cell death [3].

Since age is a primary risk factor for cardiovascular diseases, and NAD+ levels naturally decline with age, maintaining adequate NAD+ levels becomes increasingly important to support heart health, especially in the face of age-related challenges to mitochondrial function and cellular repair [4,5].

Studies show that **nicotinamide riboside (NR),** a precursor to NAD+, can safely increase NAD+ levels in individuals with cardiovascular dysfunction, eliciting promising results [6–8]. For instance, supplementation with NR in individuals with peripheral artery disease (PAD) resulted in meaningful improvements in exercise capacity [9]. Additionally, NR supplementation has been shown to enhance mitochondrial function and reduce inflammation in individuals with heart failure [6,7]. One study suggests that NR treatment may help promote healthy blood pressure and reduce aortic stiffness, though the results were not conclusive, indicating that further research is needed [8].

Animal studies have laid the groundwork for human research by demonstrating that NR supplementation increases NAD+ levels in the hearts of rats and mice, reduces inflammation, and limits cell death following ischemic events, therefore supporting recovery and improving post-MI survival [10–12]. Furthermore, intravenous NR has been shown to protect heart function in mice undergoing chemotherapy with doxorubicin, a drug often linked to heart failure. Interestingly, NR has been identified as the preferred NAD+ precursor in both failing human and mouse heart cells, illuminating its potential superiority over other NAD+ precursors in supporting heart health [13,14]. In light of the research, ongoing clinical trials aim to further investigate the link between NAD+ and cardiovascular function.

In summary, NAD+ is crucial for healthy heart function, and emerging evidence suggests that boosting its levels through NR supplementation could benefit cardiovascular health. As research advances, and the link between NAD+ and heart function becomes clearer, boosting NAD+ levels may emerge as a novel and effective approach to improve the health status of individuals experiencing cardiovascular dysfunction.

Ex Vivo/Clinical Studies

Publication	Intervention	Objective	Key Outcomes
Martens et al., 2018 [8] Chronic Nicotinamide Riboside Supplementation is Well-tolerated and Elevates NAD⁺ in Healthy Middle-aged and Older Adults CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To establish insight into the efficacy of NR supplementation in improving cardiovascular and physiological functions in healthy humans.	• NR supplementation significantly increased average NAD+ levels in peripheral blood mononuclear cells by 60% compared to placebo. • NR tended to lower blood pressure, especially in subjects with elevated blood pressure (in the stage I hypertension range.

			• NR also tended to decrease aortic stiffness.
Zhou et al., 2020 [7] Boosting NAD Level Suppresses Inflammatory Activation of PBMCs in Heart Failure CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate how boosting NAD+ levels affects mitochondrial dysfunction and inflammatory activation in the peripheral blood mononuclear cells of heart failure patients.	• NR supplementation increased whole blood NAD+ levels and mitochondrial respiration rate of the heart failure patients' peripheral blood mononuclear cells (PBMC) • NR reduced the production and gene expression of proinflammatory cytokines. • The systemic inflammation in heart failure patients was causally linked to mitochondrial function of the PBMC.
Wang et al., 2022 [6] Safety and Tolerability of Nicotinamide Riboside in Heart Failure with Reduced Ejection Fraction CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the safety, tolerability, and therapeutic potential of oral NR in clinically stable individuals with heart failure with reduced ejection fraction.	• High-dose NR supplementation was safe and well-tolerated, significantly, and dose dependently (nearly) doubled whole blood NAD+ levels, and increased PBMC mitochondrial respiration. • NR also decreased expression of inflammatory markers, such as NLRP3.
McDermott et al., 2024 [9] Nicotinamide Riboside for Peripheral Artery Disease: The NICE Randomized Clinical Trial CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To assess the effects of six months of supplementation with NR alone and NR + resveratrol on the six-minute walk distance in individuals with lower extremity peripheral artery disease (PAD).	• NR supplementation significantly improved treadmill walking time and the six-minute walking distance (by 17.6 meters), a clinically meaningful change. • NR effectively improved walking performance in individuals with PAD, while resveratrol did not enhance the benefits of NR. • NR combined with resveratrol did not significantly improve the six-min

Ongoing Clinical Trials		
Trial Registry	**Intervention**	**Objective**
NCT04528004	**Nicotinamide Riboside**	To investigate the mechanisms by which increasing human blood and myocardial NAD+ levels affects mitochondrial function, protein and

Mechanistic Studies of Nicotinamide Riboside in Human Heart Failure (NRII) 		epigenetic modifications, and inflammation.
NCT05732051 Nicotinamide Riboside and Prevention of Cancer Therapy Related Cardiac Dysfunction in Breast Cancer Patients (NARNIA) 	**Nicotinamide Riboside**	To evaluate the effects of NR supplementation on cancer-related cardiac dysfunction, skeletal muscle injury, and functional capacity in metastatic breast cancer patients scheduled for anthracycline therapy.
NCT03821623 Nicotinamide Riboside for Treating Elevated Systolic Blood Pressure and Arterial Stiffness in Middle-aged and Older Adults 	**Nicotinamide Riboside**	To assess the safety and efficacy of NR supplementation for improving systolic blood pressure and arterial stiffness in middle-aged and older men and women with elevated to high blood pressure.

Preclinical Studies			
Publication	**Intervention**	**Objective**	**Key Outcomes**
Diguet et al., 2018 [13] Nicotinamide Riboside Preserves Cardiac Function in a Mouse Model of Dilated Cardiomyopathy 	**Nicotinamide Riboside**	To investigate the role of NAD+ in heart failure in humans, rats, and mice.	• In the failing mouse heart, NAD+ levels decreased by 30%. • In failing mice and human hearts, the conversion of NAM to NAD+ decreased, while the conversion of NR to NAD+ increased. • NR rescues NAD+ biosynthesis when the conversion of NAM to NAD+ is inhibited and stimulates the use of carbohydrates to generate cellular energy in rats.
Mateuszuk et al., 2020 [12] Reversal of Endothelial Dysfunction by Nicotinamide Mononucleotide *via* Extracellular Conversion to Nicotinamide Riboside	**Nicotinamide Riboside** **Nicotinamide Mononucleotide**	To investigate the effects of NR and NMN treatment on endothelial inflammation and dysfunction and the	• Both NR and NMN increased NAD+ levels and activated anti-inflammatory effects in human aortic endothelial

CERP ChromaDex External Research Program		role of CD73 human cells and mice.	cells (HAECs) stimulated by proteins linked to chronic inflammation. • The conversion of NMN into NAD+ in HAECs was diminished by a synthetic compound that inhibits the consumption of ADP, while NR was unaffected. • In mice, both NR and NMN protected against endothelial dysfunction in the aorta of the heart. In mice deficient in CD73, however, the effects of NMN were lost, while that of NR was preserved.
Podyacheva et al., 2022 [14] Intravenous Nicotinamide Riboside Administration Has a Cardioprotective Effect in Chronic Doxorubicin-Induced Cardiomyopathy	**Nicotinamide Riboside**	To investigate the cardioprotective effects of intravenous NR treatment in a mouse model of cardiomyopathy treated with doxorubicin – a common chemotherapy drug linked to heart failure.	• Intravenous NR treatment improved antioxidant system performance and mitigated the doxorubicin-induced fibrous tissue formation and reduction in fractional shortening in mice.
Tannous et al., 2023 [11] Nicotinamide Riboside Supplementation Restores Myocardial Nicotinamide Adenine Dinucleotide Levels, Improves Survival, and Promotes Protective Environment Post Myocardial Infarction CERP ChromaDex External Research Program	**Nicotinamide Riboside**	To investigate the cardioprotective effects of NR in male mice post-myocardial infarction (MI).	• NR restored NAD+ levels in the heart and increased survivability of mice from 61% to 92%. • NR protected against MI-induced mitochondrial dysfunction and activated anti-inflammatory and pro-fibrotic responses.
Xiao et al., 2024 [10] Insulin and Glycolysis Dependency of Cardioprotection by Nicotinamide Riboside	**Nicotinamide Riboside**	To investigate the effects of NR on the heart and determine its link to glycolysis and insulin in the	• NR treatment reduced infarct size by about 40%, decreased total lactate

		isolated mouse heart.	dehydrogenase (LDH) release, improved post-ischemic recovery in the heart, increased NAD+ content in the heart by nearly two times, and increased the heart's ability to use carbohydrates as fuel to generate cellular energy by about 42%. • In the presence of insulin, NR's protective effect was gone as indicated by similar levels of infarct size, LDH release, and rate pressure product recovery between NR and control groups. Rate pressure product recovery is a calculated value that reflects workload of the heart.

*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.*

References

[1] Kibria, F., Das, S.K. and Arefin, M.S. (2024) The role of nicotinamide adenine dinucleotide salvage enzymes in cardioprotection. *Kardiochirurgia i Torakochirurgia Polska = Polish Journal of Cardio-Thoracic Surgery*, **21**, 86–95. https://doi.org/10.5114/kitp.2024.141145
[2] Zhou, B. and Tian, R. (2018) Mitochondrial dysfunction in pathophysiology of heart failure. *Journal of Clinical Investigation*, **128**, 3716–26. https://doi.org/10.1172/jci120849
[3] Heusch, G. (2022) Coronary blood flow in heart failure: cause, consequence and bystander. *Basic Research in Cardiology*, **117**, 1. https://doi.org/10.1007/s00395-022-00909-8
[4] Breton, M., Costemale-Lacoste, J.-F., Li, Z., Lafuente-Lafuente, C., Belmin, J. and Mericskay, M. (2020) Blood NAD levels are reduced in very old patients hospitalized for heart failure. *Experimental Gerontology*, **139**, 111051. https://doi.org/10.1016/j.exger.2020.111051
[5] Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B. and Guillemin, G.J. (2012) Age-Associated Changes In Oxidative Stress and NAD+ Metabolism In Human Tissue. *PLoS ONE*, **7**, e42357. https://doi.org/10.1371/journal.pone.0042357
[6] Wang, D.D., Airhart, S.E., Zhou, B., Shireman, L.M., Jiang, S., Rodriguez, C.M. et al. (2022) Safety and Tolerability of Nicotinamide Riboside in Heart Failure With Reduced Ejection Fraction. *JACC: Basic to Translational Science*, **7**, 1183–96. https://doi.org/10.1016/j.jacbts.2022.06.012
[7] Zhou, B., Wang, D.D., Qiu, Y., Airhart, S., Liu, Y., Stempien-Otero, A. et al. (2020) Boosting NAD Level Suppresses Inflammatory Activation of PBMC in Heart Failure. *Journal of Clinical Investigation*, **130**, 6054–63. https://doi.org/10.1172/jci138538
[8] Martens, C.R., Denman, B.A., Mazzo, M.R., Armstrong, M.L., Reisdorph, N., McQueen, M.B. et al. (2018) Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD+ in healthy middle-aged and older adults. *Nature Communications*, **9**, 1286. https://doi.org/10.1038/s41467-018-03421-7
[9] McDermott, M.M., Martens, C.R., Domanchuk, K.J., Zhang, D., Peek, C.B., Criqui, M.H. et al. (2024) Nicotinamide riboside for peripheral artery disease: the NICE randomized clinical trial. *Nature Communications*, **15**, 5046. https://doi.org/10.1038/s41467-024-49092-5
[10] Xiao, Y., Wang, Q., Zhang, H., Nederlof, R., Bakker, D., Siadari, B.A. et al. (2024) Insulin and glycolysis dependency of cardioprotection by nicotinamide riboside. *Basic Research in Cardiology*, **119**, 403–18. https://doi.org/10.1007/s00395-024-01042-4
[11] Tannous, C., Ghali, R., Karoui, A., Habeichi, N.J., Amin, G., Booz, G.W. et al. (2023) Nicotinamide Riboside Supplementation Restores Myocardial Nicotinamide Adenine Dinucleotide Levels, Improves Survival, and Promotes Protective Environment Post Myocardial Infarction. *Cardiovascular Drugs and Therapy*, 1–12. https://doi.org/10.1007/s10557-023-07525-1
[12] Mateuszuk, Ł., Campagna, R., Kutryb-Zając, B., Kuś, K., Słominska, E.M., Smolenski, R.T. et al. (2020) Reversal of endothelial dysfunction by nicotinamide mononucleotide via extracellular conversion to nicotinamide riboside. *Biochemical Pharmacology*, **178**, 114019. https://doi.org/10.1016/j.bcp.2020.114019
[13] Diguet, N., Trammell, S.A.J., Tannous, C., Deloux, R., Piquereau, J., Mougenot, N. et al. (2018) Nicotinamide Riboside Preserves Cardiac Function in a Mouse Model of Dilated Cardiomyopathy. *Circulation*, **137**, 2256–73. https://doi.org/10.1161/circulationaha.116.026099
[14] Podyacheva, E., Semenova, N., Zinserling, V., Mukhametdinova, D., Goncharova, I., Zelinskaya, I. et al. (2022) Intravenous Nicotinamide Riboside Administration Has a Cardioprotective Effect in Chronic Doxorubicin-Induced Cardiomyopathy. *International Journal of Molecular Sciences*, **23**, 13096. https://doi.org/10.3390/ijms232113096