Pharmacodynamics Study Summary of RiaGev™

RiaGev™ is a proprietary compound that has been shown to enter the pathway directly and more efficiently increase NAD^+.

A third-party pharmacodynamics study has demonstrated that RiaGev enters the NAD+ biosynthetic pathway directly and increases NAD^+ efficiently throughout the body, including muscle and brain.

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Introduction

Nicotinamide adenine dinucleotide (NAD) is a co-factor in the metabolic process and is a crucial element for life and every day health. Levels of NAD in the body decrease naturally as we age. It declines faster when we are under stress, in an obese state or with any type of disease [7]. Evidence accumulated in the last decade has demonstrated that decreased levels of NAD are correlated with health issues including cognitive decline, neurological disorder, mitochondrial dysfunction, metabolic imbalance and possibly a shortened healthy lifespan [3,4,6,8].

NAD^+ is pivotal for cell life—first as a reusable coenzyme for redox reaction and energy production, second as a consumable substrate in enzymatic reactions regulating crucial biological processes including gene expression, DNA repair, cell death and lifespan, calcium signaling, glucose homeostasis, and circadian rhythms [1–5]. As coenzymes, NAD^+ and its related metabolites NAD^+H, NAD^+P^+, and NAD^+PH, participate in over 60% of the reactions in cellular metabolism. And their homeostasis is the determinant for oxidation versus reduction and anabolism versus catabolism balances [1,2]. As a consumable substrate, NAD^+ concentration is directly linked with aging [6] and fat composition [7]. And NAD^+-consuming enzymes, including PARPs, sirtuins, and CD38, have wide-spread ramification for health and disease [8].

There are four NAD^+ biosynthetic pathways operating in mammals [9,10], including a *de novo* pathway starting from amino acid tryptophan, and three alternative routes of pyridine salvage. These pyridines are nicotinic acid (NA), nicotinamide (NAM), and nicotinamide riboside (NR), collectively referred to as niacin or vitamin B3 [11]. They may arise from dietary supply and/or intracellular NAD^+ catabolism. The starting material for the *de novo* pathway, tryptophan, is also from dietary protein sources such as egg, meat, and cheese.

Although all four NAD^+ biosynthetic routes increase NAD^+ levels in the body, the NAD^+ generated via different routes are distributed differently in organs and tissues. In liver, all enzymes of the four pathways are known to be present, allowing conversion to NAD^+ from all NAD^+ precursors, and to refuel the whole organism with NAD^+ through the bloodstream circulation [12, 13]. In other tissues, conversely, different enzyme levels reflect particular and intrinsic metabolic needs, also depending on the availability of exogenous pyridine source(s). Tryptophan is the only recognized source for *de novo*

NAD^+ synthesis, but generally considered insufficient to sustain normal NAD^+ homeostasis [14]. Most NAD^+ in mammals is synthesized from NAM via the amidated salvage route. Liver again, with its elevated NAD^+ turnover, represents a crucial tissue where NAM recycling prevails and NAD^+ resynthesis is regulated by nicotinamide phosphoribosyltransferase (NAMPT) [15], also based upon a circadian transcriptional control by the clock machinery [5]. Thus, it is understandable that NAD^+ from NAM and NR has a distinctive local and temporal distribution than that from *de novo* synthesis.

Organ and tissue distribution of NAD^+ metabolite is important for their biological function. A recent publication on NAD^+PH variation between liver and muscle in exercising mouse is a prime example [16]. While NAD^+ deficiency has been linked with various pathological conditions [17], NMNAT gene alterations have been recently linked to cancer [18], Leber's congenital amaurosis [19], and axon protection in several neurodegeneration and acute injury models, including Wallerian degeneration models [20].

Purpose of the Study

The aim of this study is the pharmacodynamics and tissue distribution of RiaGev™ metabolites, specifically NAD^+.

Study Design

RiaGev is a compound product containing nicotinamide and Bioenergy Ribose®. Metabolic analysis predicts that RiaGev would increase NAD^+, NMN, and NR levels via nicotinamide salvage pathway (Figure 1). Therefore, the experiment is designed to measure NAD^+, NMN, and NR levels in the blood over a 5-day period. Also, contents of these metabolites are measured in liver, muscle, brain, and adipose tissues for their tissue distribution.

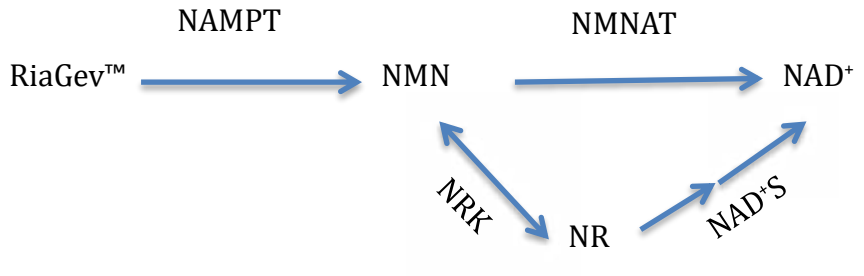

Figure 1: Proposed Pathway for RiaGev and Its Metabolites. RiaGev is primarily converted to NMN by nicotinamide phosphoribosyltransferase (NAMPT), which then is converted to NAD^+ by NMN adenylyltranferase (NMNAT). An overflow of NMN may lose its phosphate moiety, generating NR which is then converted into NAD^+ in a series of steps. NRK = NR kinase. NADS = NAD^+ synthase.

Sprague-Dawley intact male rats of same age and weight are selected as test animals in the study because the animal has been widely used in NAD^+ boosting experiments, including NR, nicotinamide, and NMN studies with reliable results. The animals are grouped into six groups, each supplemented with test articles including water, nicotinamide (108mg/kg), 100mg/kg RiaGev, 300mg/kg RiaGev, 900mg/kg RiaGev, and 2700mg/kg RiaGev. The test article is gavaged to the animal twice daily starting Day 0 to

Day 4. The first dose is given at 8 a.m. and second dose at 5 p.m. each day. Blood samples (500nl) are collected from each animal at 6 p.m. and immediately extracted and analyzed afterward in the evening. At the end of the 5th day, animals are anaesthetized and their tissues are harvested for analysis. The analysis is conducted using LC-MS following established protocol [21], with internal standard compounds (NAD^+, NMN, and NR) for quantification.

All the experiments and data analyses have been conducted by American Preclinical Services, a professional third-party laboratory located in Minneapolis, Minnesota.

Study Results

1) **Pharmacodynamics of RiaGev**. The RiaGev metabolites, including NAD^+, NMN, and NR, were measured in the blood daily over a five-day period while the animals were supplemented twice daily with test articles orally. The NAD^+ levels in the blood over time are presented in Figure 2. After RiaGev ingestion, NAD^+ levels increase steadily at all dosages in a dose-dependent manner. The NAD^+ levels reach their plateau after four days (8 doses) of supplementation.

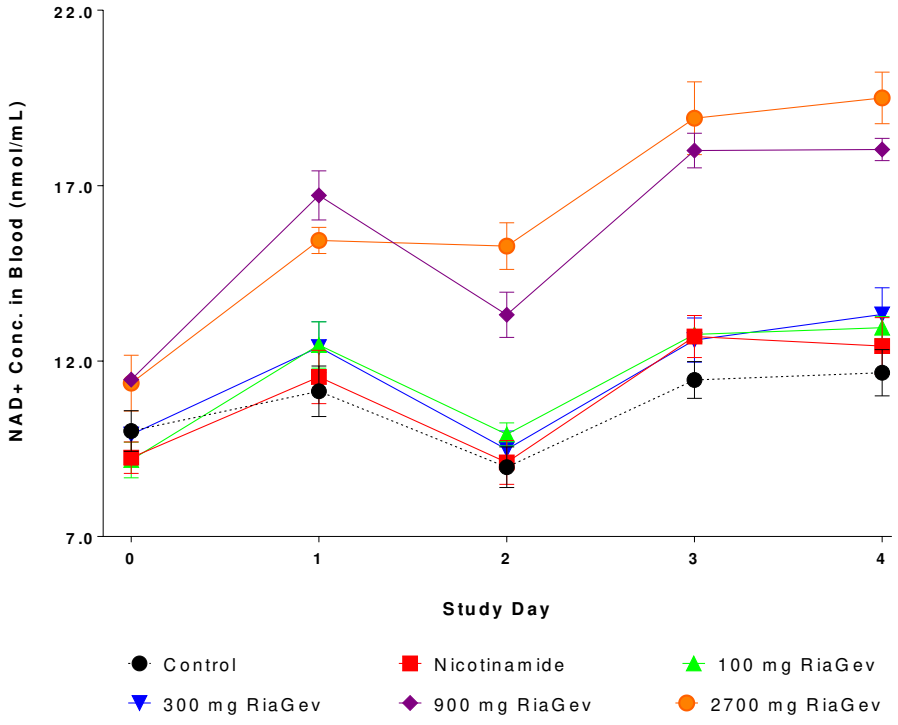

Figure 2. NAD^+ Concentration in Blood After Oral RiaGev Supplementation.

The NR measurement in blood also increased over time, similar to NAD^+, although on a smaller scale (data is not shown). This is consistent with the fact the NR is an overflow shunt product in NAD^+ biosynthesis (Fig 1). The NMN level in the blood does not change (data not shown), consistent with other publications [22].

2) **Tissue Distribution of RiaGev Metabolites.** At the end of the five-day supplementation, tissues are harvested and analyzed for their NAD^+ metabolites content**.** The NAD^+ contents are presented in

Figure 3. The highest levels of NAD^+ derived from RiaGev are detected in the liver. Liver also has the highest level of background NAD^+. The brain has the second largest NAD^+ pools, then muscle follow closely behind (Figure 3).

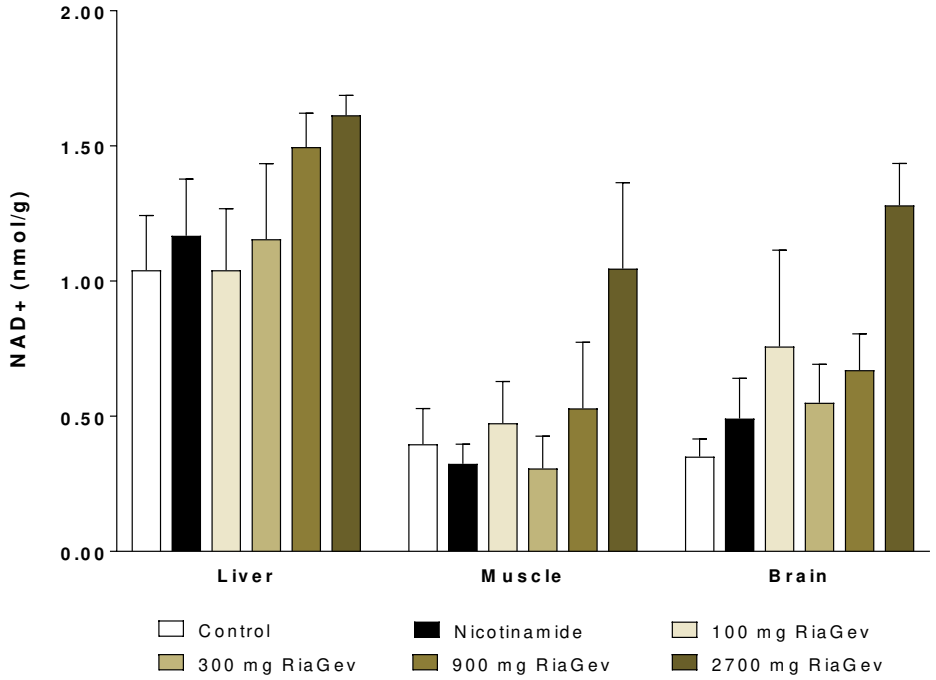

Figure 3. Organ Distribution of RiaGev Metabolite NAD^+. A similar distribution pattern is also found for metabolites NMN and NR (not shown).

The tissue distribution of NMN and NR are similar to that of NAD^+ [23]. However, their concentration difference between liver and other organs are much larger than NAD^+. (Data is not shown.)

Conclusions and Discussion

The experiment demonstrated that RiaGev effectively increases NAD^+, including NAD^+, MNN, and NR, levels in the body. For all the organs and tissue analyzed, a positive dose-response occurs between RiaGev and NAD^+ metabolites content. The five-day time course experiment indicates that RiaGev increased NAD^+ levels and it reached its plateau in blood after four days (8 doses) of oral supplementation. The plateau concentration of NAD^+ in blood versus dose is summarized in Figure 4.

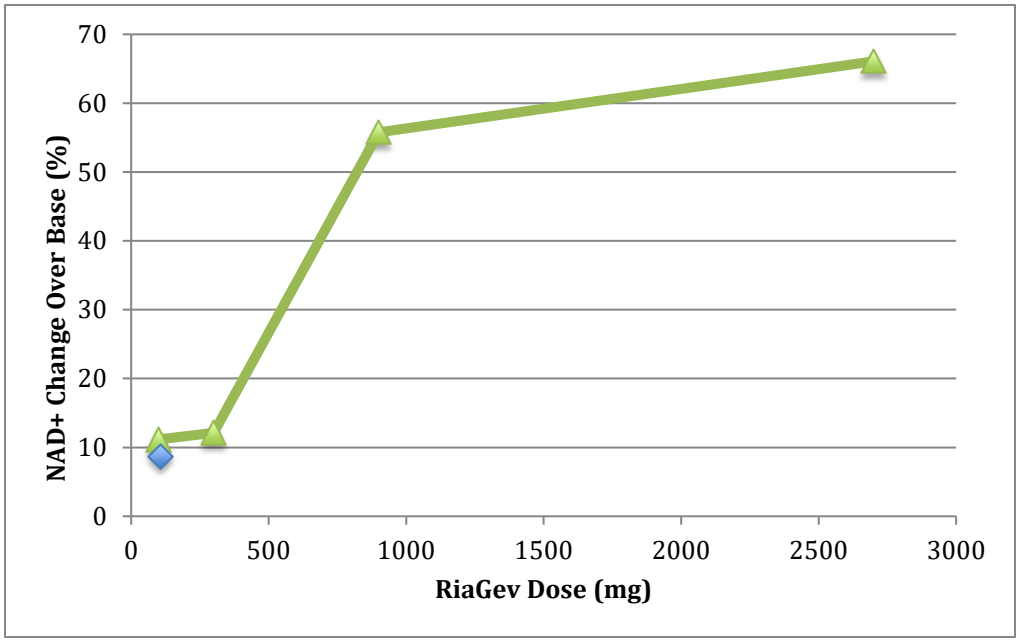

Figure 4: Dose-Response Relationship of RiaGev and NAD^+ in Blood. The x-axis is doses and the y-axis is blood NAD^+ concentration change over its base (water control) level. The line connects RiaGev doses and Nicotinamide control is outside of the line.

Comparing the final (5^{th} day) NAD^+ concentration in the blood with concurrent NAD^+ contents in liver, muscle, and brain, it is interesting to note that blood and liver seems to be saturated with high dose RiaGev, while brain and muscle do not. This may reflect the fact that liver and blood are sites of NAD^+ production and transfer, while muscle and brains are organs of NAD^+ usage.

The pharmacodynamics of RiaGev is distinctly different from that of NR. NR reaches its peak at about eight hours after consumption [23]. However, our 24-hour analysis indicated that RiaGev produces a broad elevation in NAD^+ level without a significant peak (data not shown). This implies a much more complex metabolism of RiaGev than other NAD^+ boosting compounds which rely on a single enzyme such as NRK or NAMPT to get into a NAD^+ synthetic pathway. Indeed, a closer look at the metabolic fate of the RiaGev main component, Bioenergy Ribose, reveals that it may enter the NAD^+ synthetic pathway via multiple entrances.

Bioenergy Ribose enhances the efficiency of pyridine salvage via PRPP which means a lesser amount of pyridine is needed to achieve a response. One concern of large dose of vitamin B3, including niacin, nicotinamide, or nicotinamide riboside (NR), is their possible over taxation on detoxing capacity of the body, particularly methylation and hydroxylation capacities of the liver [24]. The presence of Bioenergy Ribose in RiaGev alleviates this concern.

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