East Tennessee State University

Digital Commons @ East Tennessee State University

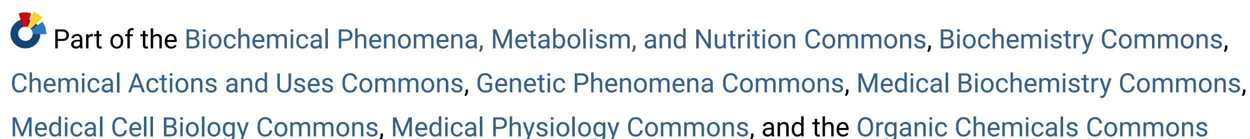
Undergraduate Honors Theses | Student Works

5-2023

Nicotinamide Riboside and Beta-hydroxybutyrate Activate Parallel Pathways for C. elegans Lifespan Extension

McKenzie Peters
East Tennessee State University

Follow this and additional works at: https://dc.etsu.edu/honors



Part of the Biochemical Phenomena, Metabolism, and Nutrition Commons, Biochemistry Commons, Chemical Actions and Uses Commons, Genetic Phenomena Commons, Medical Biochemistry Commons, Medical Cell Biology Commons, Medical Physiology Commons, and the Organic Chemicals Commons

Recommended Citation

Peters, McKenzie, "Nicotinamide Riboside and Beta-hydroxybutyrate Activate Parallel Pathways for C. elegans Lifespan Extension" (2023). *Undergraduate Honors Theses.* Paper 776. https://dc.etsu.edu/honors/776

This Honors Thesis - Open Access is brought to you for free and open access by the Student Works at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Undergraduate Honors Theses by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact digilib@etsu.edu.

Nicotinamide Riboside and Beta-hydroxybutyrate Activate Parallel Pathways for *C. elegans* Lifespan Extension

By

McKenzie Peters

An Undergraduate Thesis Submitted in Partial Fulfillment

of the Requirements for the

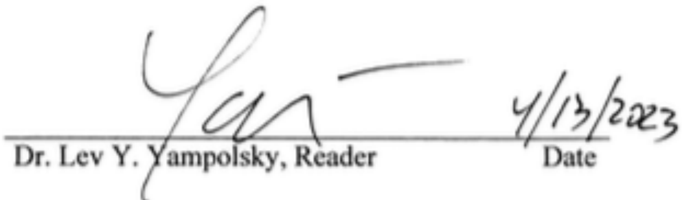
Midway Honors Scholars Program

Honors College

East Tennessee State University

McKenzie P. Peters   Date

Dr. Patrick C. Bradshaw, Thesis Mentor   Date



Dr. Lev Y. Yampolsky, Reader   Date

Dr. Hugh A. Miller, Reader   Date

Abstract

Supplementation with nicotinamide riboside (NR), a form of vitamin B3 and a precursor of nicotinamide adenine dinucleotide (NAD^+) extends lifespan in the nematode *C. elegans* and delays aging-related pathologies in mammals. During aging, levels of NAD^+ decline causing metabolic dysfunction and oxidative damage. Studies in *C. elegans* found that when NR was administered during larval development it induced the mitochondrial unfolded protein response (UPR^{mt}), which is frequently associated with lifespan extension. Both calorie restriction (CR) and ketogenic diets (KD) have been shown to extend lifespan, in part through increasing NAD^+ and through increasing levels of the pro-longevity ketone body beta-hydroxybutyrate (BHB). In a previous study from my lab, NR increased *C. elegans* lifespan to a much larger extent when administered starting at the L1 larval stage as compared to when started at the L4 larval stage. Conversely, a high dose of BHB greatly increased lifespan when administered starting at the L4 stage. But this same dose decreased lifespan when it was first administered at the L1 stage. I further found that NR greatly extended lifespan when only administered during larval development and that the combination of both individual pro-longevity NR and BHB treatments significantly decreased lifespan. These chemical epistasis experiments suggest that NR and BHB function in parallel pathways to extend *C. elegans* lifespan through a common downstream target with hormesis playing a role. Therefore, human subjects who supplement with both NAD^+ precursors and ketone esters should be aware of possible negative interactions when high doses of both are administered.

Table of Contents

Abstract	2
Table of Contents	3
Introduction	4
Materials and Methods	8
Results and Discussion	10
Conclusions and Suggestions for Future References	20
References	21

Introduction

Nicotinamide adenine dinucleotide (NAD^+) is a coenzyme that functions in roughly 300 human enzymatic reactions [1]. NAD^+ is commonly used as an electron acceptor in redox reactions, but it can also be used as a non-redox cofactor and hydrolyzed by sirtuin protein deacetylase enzymes and poly-ADP-ribose polymerase (PARP) enzymes for cellular signaling [2]. Levels of NAD^+ steadily decline with aging leading to metabolic inefficiencies such as reduced rates of glycolysis, fatty acid beta-oxidation, and the citric acid cycle that rely on NAD^+ as an electron acceptor to break down the carbohydrate, fat, and protein in food [3-7]. Declining levels of NAD^+ during aging have also been found in studies using mice [8], fruit flies [9], and the nematode *C. elegans* [10]. So, the aging-induced loss of NAD^+ occurs in many tissues [8] and appears to be conserved during evolution, perhaps to attempt to decrease mitochondrial metabolism and the potentially damaging mitochondrial reactive oxygen species (ROS) production. Adding NAD^+ or its precursors to invertebrates can extend their lifespan on a normal diet [11] and extend the health span [12], but not the lifespan [13] of mice. NAD^+ precursors such as nicotinamide riboside (NR) or nicotinamide mononucleotide (NMN), the phosphorylated form of NR, can be consumed to delay age-related disease in mice [14]. There are several dietary interventions or supplements that can increase NAD^+ levels to delay aging and age-related disease. For example, a calorie restricted (CR) diet can be consumed [15] or time-restricted feeding (intermittent fasting) can be performed [16] to extend rodent lifespan and increase NAD^+ in skeletal muscle and white adipose tissue [17]. To obtain the health and longevity benefits of CR, one must maintain adequate nutrition by consuming appropriate amounts of vitamins and nutrients, as well as reduce dietary calorie intake below energy requirements. When performed correctly, CR induces gene expression changes and metabolic adaptations that can delay the

intrinsic processes of aging, and therefore reduce the risk of developing many aging-induced diseases [18]. Studies have shown that time-restricted feeding can limit mitochondrial oxidative damage as well as delay tissue aging [19]. Additionally, due to the low level of carbohydrates in a CR diet, many tissues in the body switch from primarily metabolizing glucose to primarily metabolizing fatty acids and ketone bodies [20].

A healthy, sustainable way for human adults to prevent aging-induced loss of cellular NAD^+ levels is through dietary supplementation with forms of vitamin B3. There are currently four forms of vitamin B3 available commercially including nicotinamide (NAM), nicotinic acid (NA), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN) [21]. NAM and NA are the main source of vitamin B3 in food. But expression of the rate limiting enzyme, nicotinamide phosphoribosyl-transferase (NAMPT) in the mammalian pathway that converts NAM to NAD^+ decreases with aging [22, 23], which contributes to the loss of NAD^+ with age. The primary cause for the loss of NAD^+ in mammals with age appears to be increased inflammation that increases the expression of the CD38 enzyme in macrophages that hydrolyzes NAD^+ to NAM [24]. Consumption of high doses of NA causes painful skin flushing [25]. NMN is the product of the NAMPT reaction, and so therefore does not rely on NAMPT activity to be converted to NAD^+. NR is phosphorylated to NMN by NR kinase enzymes that bypass the NAMPT reaction as well. Therefore, NR and NMN are the most promising NAD^+ precursors for dietary supplementation. However, they are largely broken down to NAM and NA by gut microbiota and first pass hepatic metabolism [26]. So, non-oral delivery routes of NMN and NR, such as injection, intranasal sprays, or sublingual (under the tongue) powders or films may become the preferred delivery methods for NMN and NR, although clinical trials have yet to be performed to show the efficacy of any of these alternative routes of delivery.

In studies of human adults, it was found that supplementation with NR improved markers of mitochondrial function and inflammation in blood cells [27]. It has been shown that supplementation with NR is well tolerated, and it effectively restored NAD^+ levels in some cell types, but not others [28]. Additionally, depletion of NAD^+ occurs in many neurodegenerative disorders. So, NR supplementation to restore neural NAD^+ levels is a promising potential therapy to prevent neurodegeneration and protect against neuronal excitotoxicity. NR has also been shown to decrease axonal degeneration [29].

The ketone body D-beta-hydroxybutyrate (BHB) has also shown some anti-aging effects to delay age-related diseases [30]. Both endogenous hepatic ketogenesis and exogenous supplementation of BHB salts or more effective ketone esters can be utilized to induce a metabolic state of nutritional ketosis [31]. Ketosis is the process in which BHB rises to levels of roughly 1 mM or greater from its normal level around 50 μM [32]. Fatty acids are broken down into the ketone bodies acetoacetate and BHB in the liver and then the ketone bodies are distributed throughout the body through the bloodstream in order to fulfil energetic needs and signal the state of energy deficiency. The use of ketone bodies as a fuel is especially important for neurons that have limited capacity to break down fatty acids for energy [33, 34]. Studies have also linked BHB with inhibiting age-related and oxidative stress-induced changes within cells of *Drosophila* [35] to extend the lifespan of the flies [36]. Additionally, studies conducted with *C. elegans* [37] and mice [38, 39] have shown that increasing BHB levels increases lifespan in a way similar to decreased signaling through insulin and insulin-like growth factor receptors. Decreased insulin signaling occurs during fasting when insulin levels are low [40], so increased BHB levels during ketosis partially mimics fasting.

C. elegans is a commonly used model organism with facile genetics frequently used for aging studies due to the ability to conduct lifespan assays in a relatively short amount of time compared to vertebrate models [41]. Past studies have shown that NR [11] or BHB [37] when administered to *C. elegans* starting at the beginning of larval development (L1 stage) extended lifespan. A recent unpublished study performed in our lab compared the effects of either NR or BHB supplementation on *C. elegans* lifespan when one or the other was administered starting from the end of larval development (L4) with when one or the other was administered starting at the beginning of larval development (L1). The rationale behind this set of experiments was that many of the protective effects of NR administration to *C. elegans* have been associated with the mitochondrial unfolded protein response (UPR^{mt}) [11], which is not robustly activated in *C. elegans* following the L3 stage of larval development [42]. The unpublished study showed that when given at the first larval stage of development, 0.5 mM NR increased lifespan, while 20 mM DL-BHB robustly decreased it. Conversely, when administered at the final L4 larval stage, BHB increased lifespan robustly, while NR only increased lifespan very slightly [43]. Due to the different temporal requirements of NR and BHB for lifespan extension, there is interest in determining whether these compounds would produce an additive effect when administered in combination. Therefore, this thesis describes the results of experiments determining *C. elegans* lifespan when NR was present only during larval development or BHB was present only during adulthood or the combined effect of these treatments.

Materials and Methods

A mixed age population of wild-type N2 *C. elegans* was cultured on several nematode growth media (NGM) agar plates according to standard practice [44]. Once a sufficient amount of *C. elegans* was obtained, an age synchronization protocol was performed as described in [44]. Briefly, this entailed washing the NGM agar plates containing *C. elegans* with a chilled 0.1 M NaCl solution. The suspension of *C. elegans* nematodes in 0.1 M NaCl was then transferred to 50 mL conical tubes. Following this step, the worms were suspended in a 1% bleach and 0.5 M NaOH solution. This solution killed any larval or adult *C. elegans* contained in the suspension, but not the eggs, which are protected by their eggshell [41]. This procedure ensured that the eggs collected and utilized in the lifespan assay were age-synchronized within 9 hours. Following this procedure, the eggs were placed in M9 minimal growth media overnight. In this solution, the nematodes were able to hatch into the L1 larval stage, but they could not develop further until they were fed. The next day the nematodes were spun down and resuspended in liquid S-medium and 25-60 nematodes in a volume of 1.35 mL were transferred into an 8-micron cell culture insert (VWR product # 10769-218), which was placed into a well of a 12-well microplate containing 0.15 mL of 9×10^9 (10 mg protein/mL) HT115(DE3) *E. coli* cells/mL. To provide complete nutrition during the larval stages, the worms were fed live *E. coli* through the first two days of the experiment. The microplates, with a total volume of 1.5 mL each, were incubated at 20°C and shaken at 60 rpm to allow aeration. On the third day, and every other day following, the cell culture media was replaced with media containing heat-killed *E. coli* of the same concentration and strain. The *E. coli* were heat-killed at 80 °C for two hours, which prevented them from metabolizing the treatment compounds. To change out the culture media the inserts were first raised out of the wells. The media in the wells was then aspirated off and the media

remaining in the cell culture inserts was removed by capillary action by touching a chemical wipe tissue to the bottom side of the insert. On the third day when the nematodes reached the L4 stage, 0.4 mM fluorodeoxyuridine (FUdR) was added to each well. FUdR is a DNA synthesis inhibitor, which kills the actively dividing germ cells sterilizing the nematodes, allowing for a synchronous nematode population throughout the duration of the experiment. Every Monday, Wednesday, and Friday when the *C. elegans* were adults, the number of live worms in each insert was counted under a stereomicroscope, and the culture media containing bacteria and FUdR was replaced.

One cohort of worms was treated with 0.5 mM nicotinamide riboside (NR), while the second cohort was treated with 20 mM DL-BHB (BHB), while the third cohort was treated with both 0.5 mM NR and 20 mM BHB. In each experiment, the first cohort of worms was administered 0.5 mM NR only on the first day of the experiment when the worms were in the L1 larval stage and the NR was removed two days later when the nematodes were at the L4 larval stage. The second cohort of worms was given BHB each Monday, Wednesday and Friday beginning on the third day of the experiment when the nematodes were at the L4 larval stage continuing for the entirety of adulthood. The third cohort of worms was administered 0.5 mM nicotinamide riboside only on day one of the experiment, so it was present only during the L1 to L4 larval stages in addition to BHB every Monday, Wednesday, and Friday beginning from the L4 larval stage continuing through the entirety of adulthood. For each individual experiment performed, there was an untreated control group. Upon the death of all *C. elegans* in each trial, the mean lifespans and percent change in mean survival compared to the untreated controls over the course of each experiment were calculated using Log-rank statistical analysis and graphed using Kaplan-Meier survival curves. This experiment consisted of four independent trials.

Results and Discussion

Lifespan experiments were performed when administering *C. elegans* 1) 0.5 mM NR only during larval development, 2) 20 mM BHB administered only during adulthood, 3) the combination of 0.5 mM administered only during larval development and 20 mM BHB administered only during adulthood, and using 4) untreated controls. The results are summarized in Table 1. Kaplan-Meier survival curves for the four replicates that were performed for all four groups are shown as Figure 1, Figure 2, Figure 3, and Figure 4, while the survival curve for all four trials combined is shown in Figure 5. Significant changes in lifespan were defined as Log-rank $p < 0.05$. The mean lifespans for all treatment groups were significantly different than the untreated control. The data shows that treatment with NR during the larval stages increased the mean lifespan of *C. elegans* by 28%, while treatment with BHB only during adulthood increased the mean lifespan by 16% compared to the untreated control group. Lastly, the combined treatment of NR only present during the larval stages and BHB only present during adulthood decreased the mean lifespan of the nematodes by 46%.

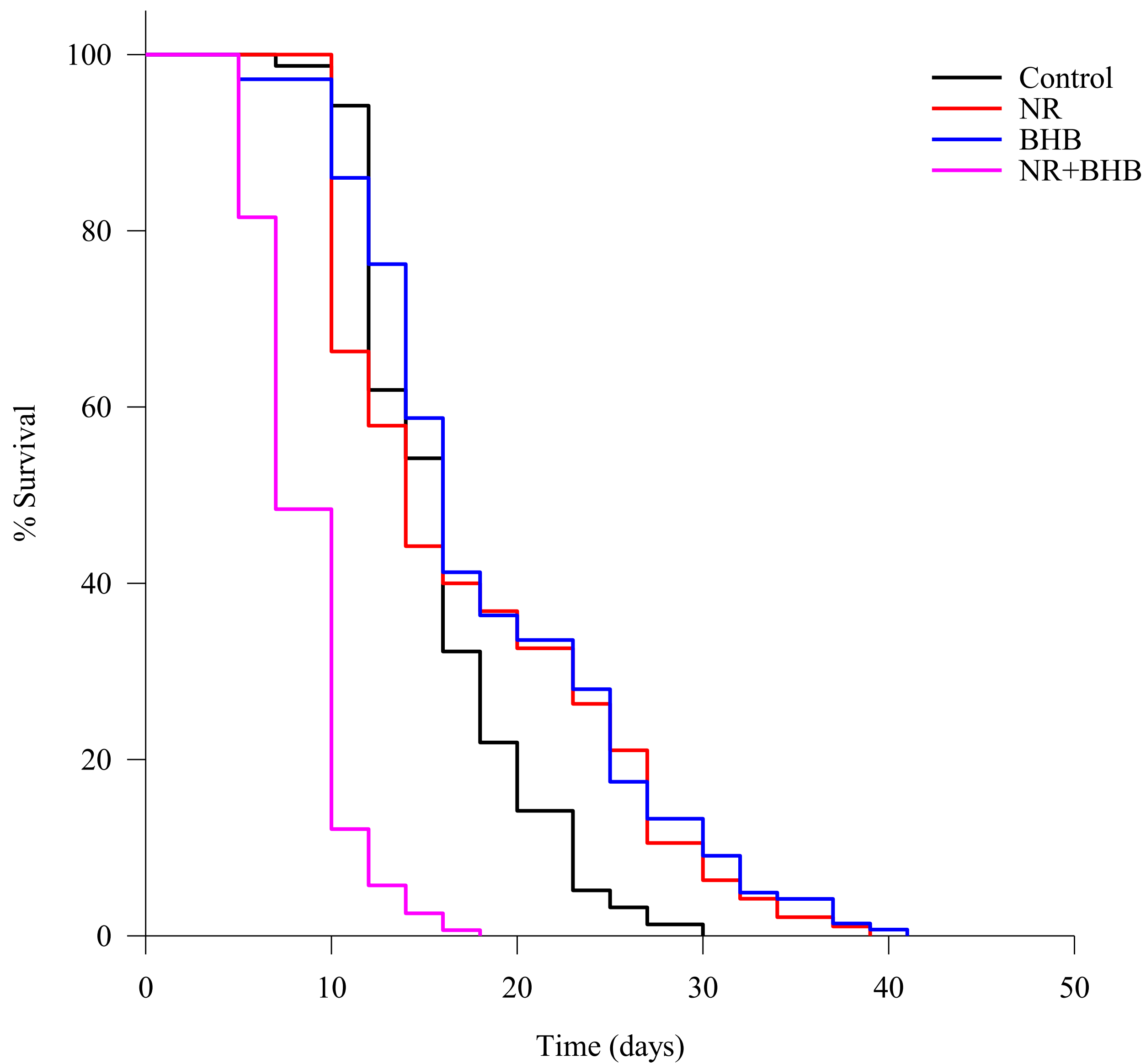


Figure 1: Trial 1 *C. elegans* lifespan analysis when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or both treatments were combined, or no treatment was added.

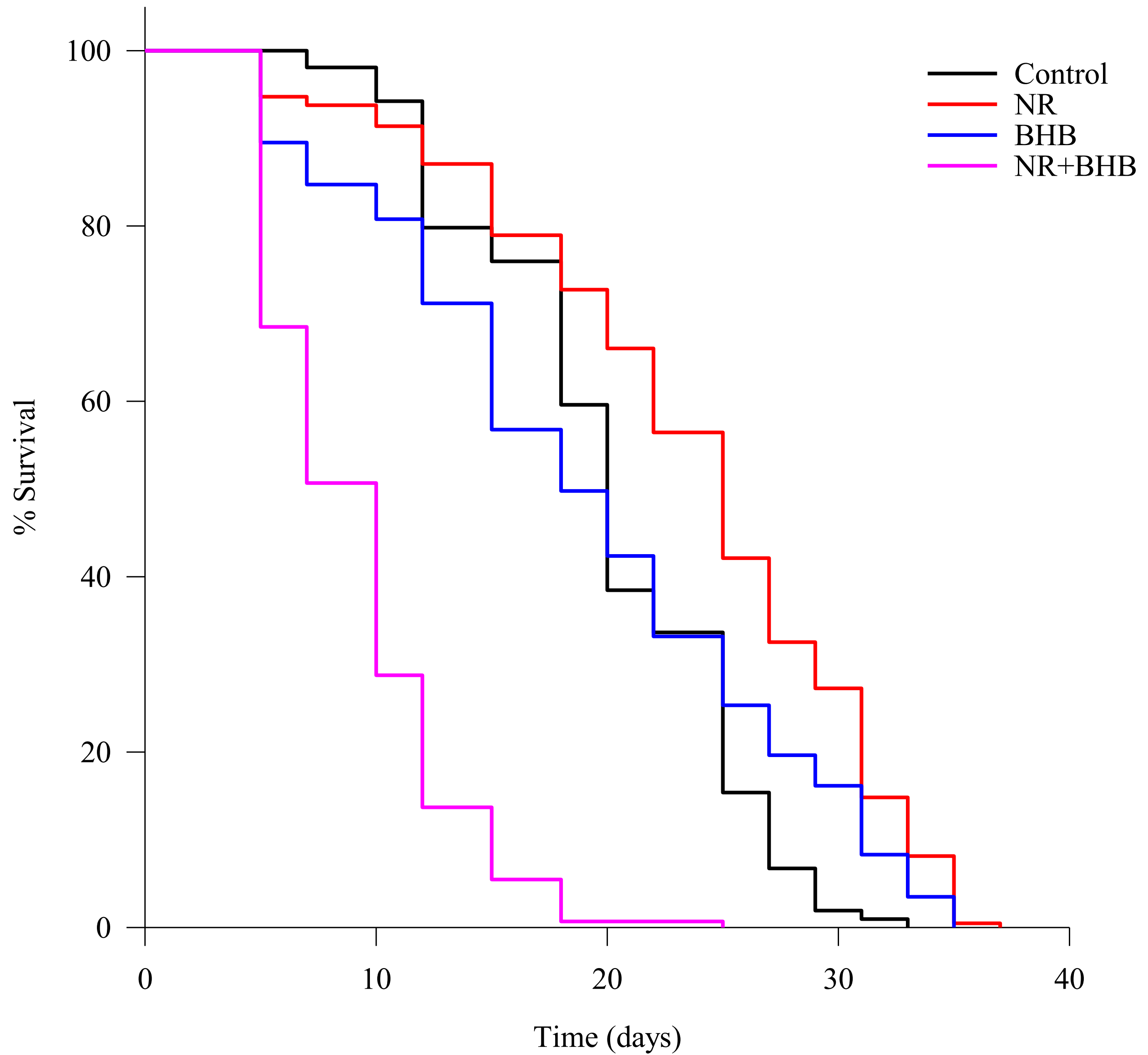


Figure 2: Trial 2 *C. elegans* lifespan analysis when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or both treatments were combined, or no treatment was added.

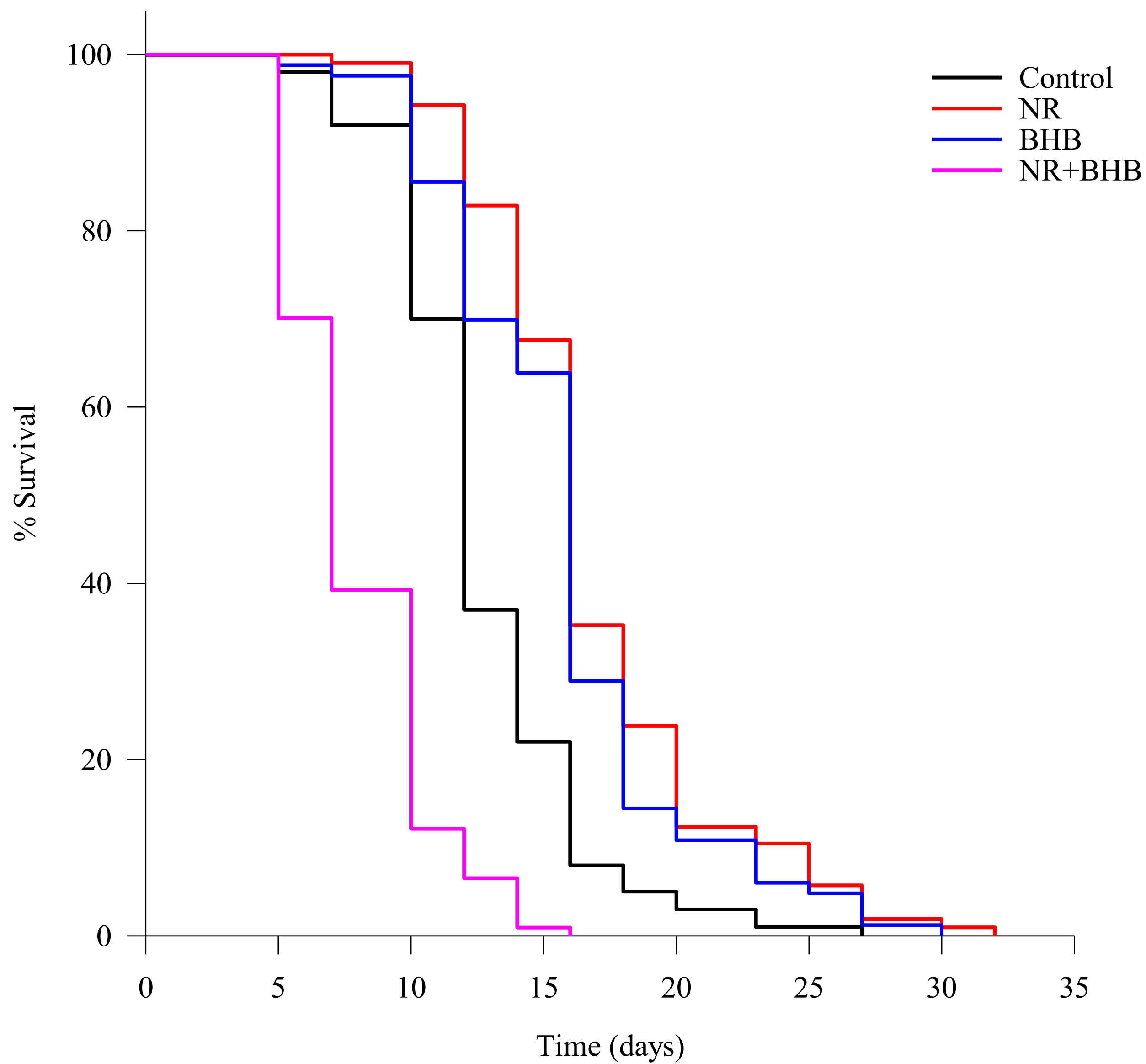


Figure 3: Trial 3 *C. elegans* lifespan analysis when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or both treatments were combined, or no treatment was added.

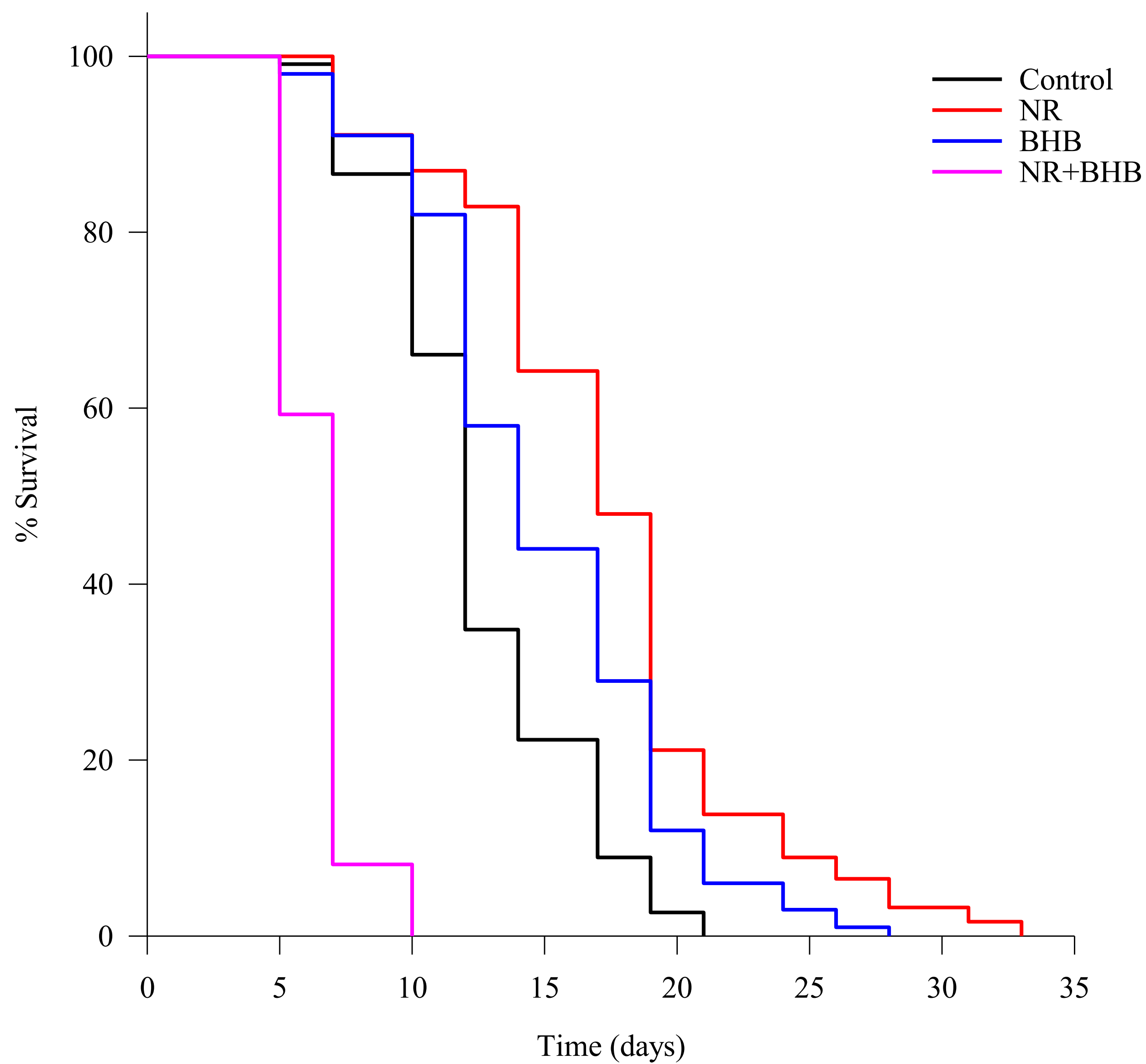


Figure 4: Trial 4 *C. elegans* lifespan analysis when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or both treatments were combined, or no treatment was added.

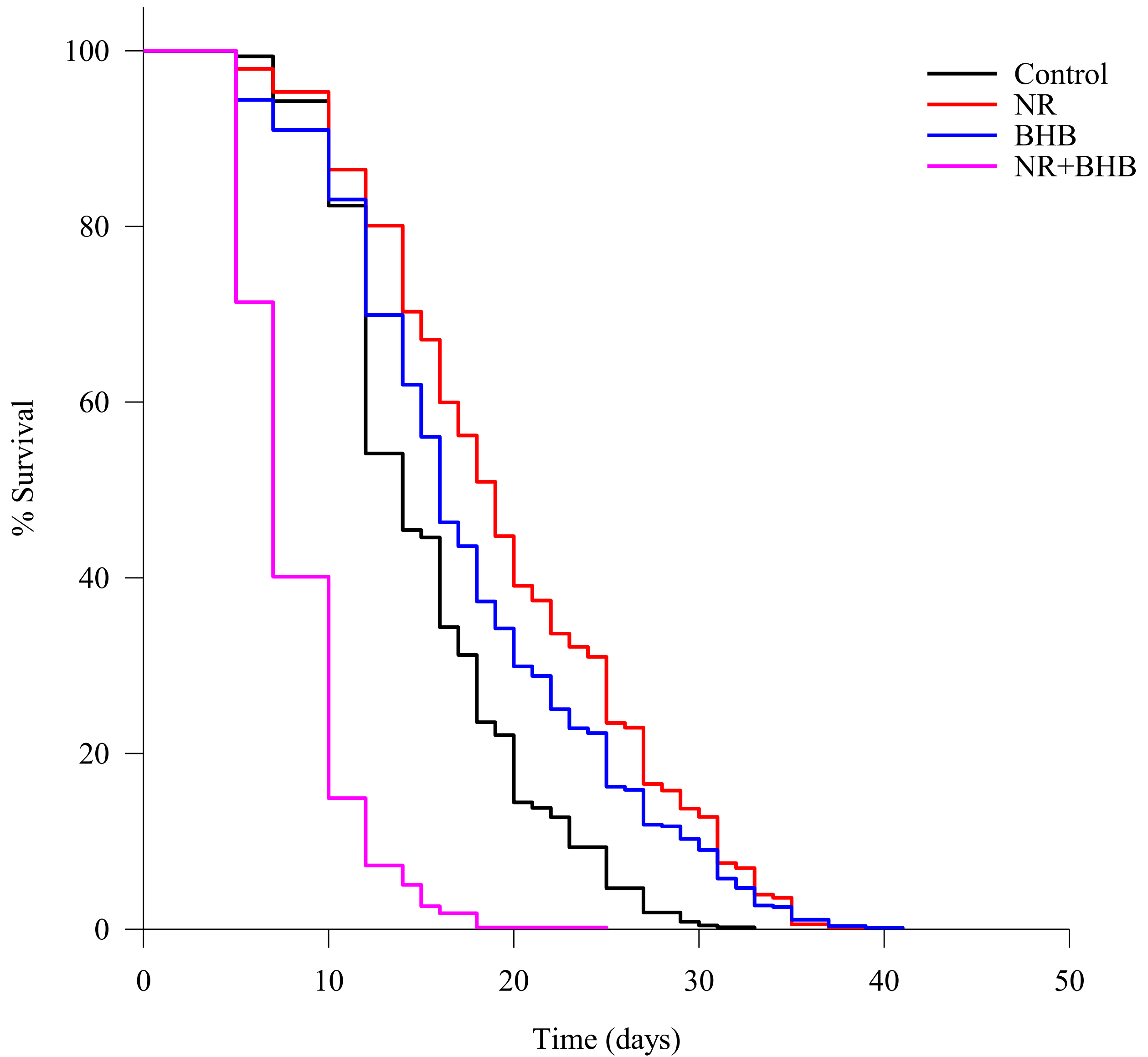


Figure 5: Combined lifespan analysis of each of the four trials shown in Figures 1-4 when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or both treatments were combined, or no treatment was added.

Table 1: The mean lifespan of untreated control worms or when 0.5 mM NR was present only during the larval stages, 20 mM BHB was present only during adulthood, or the combination of both treatments.

Treatment	Mean Survival Time (Days)
Control	15.304
NR	19.662
BHB	17.701
NR + BHB	8.202

Table 2: The percent change in *C. elegans* lifespan when treated with 0.5 mM NR only during development, 20 mM BHB only during adulthood, or both treatments combined.

Treatment	Percent Change in Lifespan
NR	+28%
BHB	+16%
NR + BHB	-46%

To determine the molecular mechanisms through which compounds extend lifespan in *C. elegans*, chemical epistasis experiments are routinely performed where both lifespan-extending compounds are added together, and the mean lifespan is determined. If the compounds have an additive effect on the mean lifespan, it is most likely that they extend lifespan through independent molecular pathways. Non-additive effects on lifespan indicate that the compounds extend lifespan either through the same pathway or through parallel pathways with a common downstream effector. Since NR extends lifespan by acting during larval development and BHB

extends lifespan by acting during adulthood, the compounds cannot be extending lifespan through the same pathway. So, it is concluded that NR and BHB extend lifespan through activating parallel pathways that then converge on a common downstream target or targets. The common targets activated by both NR and BHB are likely the DAF-16/FOXO and SKN-1/Nrf2 longevity-inducing transcriptional regulators. NR [11], NAD^+ [45], or BHB [37] were all shown to require DAF-16 for lifespan extension. BHB was also shown to require SKN-1/Nrf2 for lifespan extension [37], while the UPR^{mt} was shown to lead to SKN-1/Nrf2 activation as well [46].

The data indicate that while treatment with NR or BHB individually increased lifespan, the combination of the two supplements was toxic. This was likely due to a phenomenon known as hormesis. Hormesis occurs when an organism benefits from a low level of a stressor to activate a stress response, but the stressor is harmful at higher doses that overwhelm the capacity of the stress response to protect. Specifically, in *C. elegans*, there have been many studies that show lifespan extension as a result of the hormetic effects of various stressors [47]. One specific study showed that thermal stress can extend lifespan. In that study, a four-hour exposure to 35 °C, a temperature much higher than the normal 20 °C culture temperature, resulted in an 18% increase in lifespan [48].

There is a related phenomenon known as mitohormesis, in which a small amount of mitochondrial stress leads to increased health, vigor, or lifespan. In order to activate a mitochondrial stress response, mitonuclear communication is essential. Although there are several ways to stimulate mitonuclear communication, one of the most prevalent signals utilized are reactive oxygen species (ROS). Mitochondrial ROS increases as a result of mitohormesis [49]. Studies performed on multiple model organisms have shown that increased ROS as a result

of mitohormesis in young organisms can result in increased lifespan [50-51]. Another common effector of mitohormesis is loss of the mitochondrial membrane potential during larval development, which induces the translocation of the AFTS-1 transcriptional regulator from the mitochondria to the nucleus. This ATFS-1 translocation induces the UPR^{mt} that remodels chromatin by altering histone methylation [52] to alter gene expression to increase mitochondrial protein import [53] leading to stress response pathway activation that can extend lifespan [46].

It is likely that the phenomenon of hormesis and mitohormesis are responsible for the results of this study. Consistent with this idea, a higher concentration of 50 mM BHB than the 20 mM dose used here was previously shown to decrease *C. elegans* lifespan [37]. So, it has already been shown that BHB is a compound that functions through hormesis. In the same report, BHB was also shown to activate the SKN-1 transcriptional regulator, which is commonly activated by ROS. So, mitohormesis is also likely playing a role. It is likely that NR and BHB given at the concentrations used in this study each act as stressors to activate the DAF-16 and SKN-1 stress responses. When given only one of the supplements, the nematodes experienced an extended lifespan. However, when given both supplements, *C. elegans* over-activate the stress responses or disrupt cellular function to a greater degree leading to the decreased lifespan.

BHB has been shown to function as a class I histone deacetylase (HDAC) inhibitor to induce gene expression [54]. Sirtuins, which are activated by NAD^+, are class III HDAC inhibitors that induce other sets of genes. Low dose administration of class I HDAC inhibitors extends lifespan in yeast, worms, and flies [55], while higher doses are toxic and decrease lifespan [56]. Consistent with this, partial knockdown of the expression of the *C. elegans* class I HDACs *hda-2* or *hda-3* extended lifespan, while knockout of *hda-2* or *hda-3* decreased lifespan

[37]. Therefore, too much inhibition of HDACs likely explains why high dose BHB decreased lifespan and why the hormetic effects are observed.

As mentioned above NR activates the UPR^{mt} that activates histone demethylases. There is competition on some histone lysine residues that can either be methylated to repress gene expression or acetylated to activate gene expression. Therefore, NR-induced activation of the UPR^{mt} and induction of the expression of histone demethylases likely increases histone acetylation by decreasing the methylation of lysines that then become available for acetylation. This is consistent with the activation of gene expression to mediate the UPR^{mt}. This increased histone acetylation when combined with the further increased histone acetylation due to BHB-mediated HDAC inhibition likely leads to excessive histone acetylation, such as that which occurs upon addition of a high dose of an HDAC inhibitor or upon HDAC gene knockout, leading to excessive gene expression and decreased lifespan. This proposed mechanism for the observed hormesis could be tested in future experiments.

Conclusions

The data from this study showed that NR administered only during *C. elegans* larval development extended lifespan. In contrast, BHB when administered only during adulthood extended lifespan. The high dose of BHB administered greatly decreased lifespan when administered during larval development. Somewhat surprisingly the combined treatment of NR only during development and BHB only during adulthood greatly decreased lifespan. Future experiments should determine if the addition of lower doses of each compound could lead to an additive effect on lifespan and if excessive histone acetylation is responsible for the decreased lifespan when high doses of both compounds are added in combination.

References

1. Goodman, R. P., Calvo, S. E., & Mootha, V. K. (2018). Spatiotemporal compartmentalization of hepatic NADH and NADPH metabolism. *The Journal of biological chemistry*, *293*(20), 7508–7516. https://doi.org/10.1074/jbc.TM117.000258
2. Ansari, H. R. Raghava, G. PS. Identification of NAD interacting residues in proteins. *BMC Bioinformatics*. 2010. 11, 160.
3. Camacho-Pereira, J. Tarragó, M.G. Chini, C. C.S. Nin, V. Escande, C. Warner, G.M. Puranik, A. S. Schoon, R. A. Reid, J. M. Galina, A. CD38 dictates age-related NAD decline and mitochondrial dysfunction through a SIRT3-dependent mechanism. *Cell Metab.* 2016. 23, 1127-1139.
4. Braidy, N. Guillemin, G.J. Mansour, H. Chan-Ling, T. Poljak, A. Grant, R. Age-related changes in NAD+ metabolism oxidative stress and SIRT1 activity in wistar rats. *PLOS ONE.* 2011. 6,19194.
5. Scheibye-Knudsen, M. Mitchell, S.J. Fang, E.F. Iyama, T. Ward, T. Wang, J. Dunn, C.A. Singh, N. Veith, S. Hasan-Olive, M.M. et al. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metab.* 2014. 20,840–855.
6. Massudi, H. Grant, R. Braidy, N. Guest, J. Farnsworth, B. Guillemin, G.J. Age-associated changes in oxidative stress and Sirt1 activity in wistar rats. *PLOS ONE.* 2012. 7, 42357.
7. Zhu, X.H. Lu, M. Lee, B.Y. Ugurbil, K. Chen, W. In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. *Proc Natl Acad Sci U S A*. 2015. 112(9),2876-2881.
8. McReynolds, M. R., Chellappa, K., Chiles, E., Jankowski, C., Shen, Y., Chen, L., Descamps, H. C., Mukherjee, S., Bhat, Y. R., Lingala, S. R., Chu, Q., Botolin, P., Hayat,

F., Doke, T., Susztak, K., Thaiss, C. A., Lu, W., Migaud, M. E., Su, X., Rabinowitz, J. D., … Baur, J. A. (2021). NAD$^+$ flux is maintained in aged mice despite lower tissue concentrations. *Cell systems*, *12*(12), 1160–1172.e4. https://doi.org/10.1016/j.cels.2021.09.001

9. Sohal, R. S., Arnold, L., & Orr, W. C. (1990). Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in Drosophila melanogaster. *Mechanisms of ageing and development*, *56*(3), 223–235. https://doi.org/10.1016/0047-6374(90)90084-s
10. Copes, N., Edwards, C., Chaput, D., Saifee, M., Barjuca, I., Nelson, D., Paraggio, A., Saad, P., Lipps, D., Stevens, S. M., Jr, & Bradshaw, P. C. (2015). Metabolome and proteome changes with aging in Caenorhabditis elegans. *Experimental gerontology*, *72*, 67–84. https://doi.org/10.1016/j.exger.2015.09.013
11. Mouchiroud, L., Houtkooper, R. H., Moullan, N., Katsyuba, E., Ryu, D., Cantó, C., Mottis, A., Jo, Y. S., Viswanathan, M., Schoonjans, K., Guarente, L., & Auwerx, J. (2013). The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. *Cell*, *154*(2), 430–441. https://doi.org/10.1016/j.cell.2013.06.016
12. Mitchell, S. J., Bernier, M., Aon, M. A., Cortassa, S., Kim, E. Y., Fang, E. F., Palacios, H. H., Ali, A., Navas-Enamorado, I., Di Francesco, A., Kaiser, T. A., Waltz, T. B., Zhang, N., Ellis, J. L., Elliott, P. J., Frederick, D. W., Bohr, V. A., Schmidt, M. S., Brenner, C., Sinclair, D. A., … de Cabo, R. (2018). Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice. *Cell metabolism*, *27*(3), 667–676.e4. https://doi.org/10.1016/j.cmet.2018.02.001

13. Harrison, D. E., Strong, R., Reifsnyder, P., Kumar, N., Fernandez, E., Flurkey, K., Javors, M. A., Lopez-Cruzan, M., Macchiarini, F., Nelson, J. F., Markewych, A., Bitto, A., Sindler, A. L., Cortopassi, G., Kavanagh, K., Leng, L., Bucala, R., Rosenthal, N., Salmon, A., Stearns, T. M., … Miller, R. A. (2021). 17-a-estradiol late in life extends lifespan in aging UM-HET3 male mice; nicotinamide riboside and three other drugs do not affect lifespan in either sex. *Aging cell*, *20*(5), e13328. https://doi.org/10.1111/acel.13328
14. Gong, B., Pan, Y., Vempati, P., Zhao, W., Knable, L., Ho, L., Wang, J., Sastre, M., Ono, K., Sauve, A. A., & Pasinetti, G. M. (2013). Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor-γ coactivator 1α regulated β-secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models. *Neurobiology of Aging*, *34*(6), 1581–1588. https://doi.org/10.1016/j.neurobiolaging.2012.12.005
15. McCay, C.M. Crowell, M.F. Maynard, L. A. The effect of retarded growth upon the lengths of life span and upon the ultimate body size. *The Journal of Nutrition*. 1935. 10(1), 63-79.
16. Mitchell, S. J., Bernier, M., Mattison, J. A., Aon, M. A., Kaiser, T. A., Anson, R. M., Ikeno, Y., Anderson, R. M., Ingram, D. K., & de Cabo, R. (2019). Daily Fasting Improves Health and Survival in Male Mice Independent of Diet Composition and Calories. *Cell metabolism*, *29*(1), 221–228.e3. https://doi.org/10.1016/j.cmet.2018.08.011
17. Chen, D., Bruno, J., Easlon, E., Lin, S. J., Cheng, H. L., Alt, F. W., & Guarente, L. (2008). Tissue-specific regulation of SIRT1 by calorie restriction. *Genes & development*, *22*(13), 1753–1757. https://doi.org/10.1101/gad.1650608

18. Flanagan, E. W., Most, J., Mey, J. T., & Redman, L. M. (2020). Calorie Restriction and Aging in Humans. *Annual review of nutrition*, *40*, 105–133. https://doi.org/10.1146/annurev-nutr-122319-034601
19. Lettieri-Barbato, D., Cannata, S. M., Casagrande, V., Ciriolo, M. R., & Aquilano, K. (2018). Time-controlled fasting prevents aging-like mitochondrial changes induced by persistent dietary fat overload in skeletal muscle. *PloS One*, *13*(5), e0195912–e0195912. https://doi.org/10.1371/journal.pone.0195912
20. Bruss, M.D., Khambatta, C.F., Ruby, M.A., Aggarwal, I., and Hellerstein, M.K. Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. *American Journal of Physiology Endocrinology and Metabolism.*2010. 298(1), 108-116.
21. Bieganowski, P., & Brenner, C. (2004). Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD+ in fungi and humans. *Cell*, *117*(4), 495–502. https://doi.org/10.1016/s0092-8674(04)00416-7
22. Khaidizar, F. D., Bessho, Y., & Nakahata, Y. (2021). Nicotinamide Phosphoribosyltransferase as a Key Molecule of the Aging/Senescence Process. *International journal of molecular sciences*, *22*(7), 3709. https://doi.org/10.3390/ijms22073709
23. Stein, L. R., & Imai, S. (2014). Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging. *The EMBO journal*, *33*(12), 1321–1340. https://doi.org/10.1002/embj.201386917
24. Camacho-Pereira, J., Tarragó, M. G., Chini, C. C. S., Nin, V., Escande, C., Warner, G. M., Puranik, A. S., Schoon, R. A., Reid, J. M., Galina, A., & Chini, E. N. (2016). CD38

Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. *Cell metabolism*, *23*(6), 1127–1139. https://doi.org/10.1016/j.cmet.2016.05.006

25. Kei, A., & Elisaf, M. S. (2012). Nicotinic acid: clinical considerations. *Expert opinion on drug safety*, *11*(4), 551–564. https://doi.org/10.1517/14740338.2012.682981
26. Liu, L., Su, X., Quinn, W. J., 3rd, Hui, S., Krukenberg, K., Frederick, D. W., Redpath, P., Zhan, L., Chellappa, K., White, E., Migaud, M., Mitchison, T. J., Baur, J. A., & Rabinowitz, J. D. (2018). Quantitative Analysis of NAD Synthesis-Breakdown Fluxes. *Cell metabolism*, *27*(5), 1067–1080.e5. https://doi.org/10.1016/j.cmet.2018.03.018
27. Wang, D. D., Airhart, S. E., Zhou, B., Shireman, L. M., Jiang, S., Melendez Rodriguez, C., Kirkpatrick, J. N., Shen, D. D., Tian, R., & O'Brien, K. D. (2022). Safety and Tolerability of Nicotinamide Riboside in Heart Failure With Reduced Ejection Fraction. *JACC. Basic to translational science*, *7*(12), 1183–1196. https://doi.org/10.1016/j.jacbts.2022.06.012
28. Martens, C. R., Denman, B. A., Mazzo, M. R., Armstrong, M. L., Reisdorph, N., McQueen, M. B., Chonchol, M., & Seals, D. R. (2018). Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD + in healthy middle-aged and older adults. *Nature Communications*, *9*(1), 1286–11. https://doi.org/10.1038/s41467-018-03421-7
29. Vaur, P., Brugg, B., Mericskay, M., Li, Z., Schmidt, M. S., Vivien, D., Orset, C., Jacotot, E., Brenner, C., & Duplus, E. (2017). Nicotinamide riboside, a form of vitamin B 3 ,

protects against excitotoxicity-induced axonal degeneration. *The FASEB Journal*, *31*(12), 5440–5452. https://doi.org/10.1096/fj.201700221RR

30. Bendridi, N., Selmi, A., Balcerczyk, A., & Pirola, L. (2022). Ketone Bodies as Metabolites and Signalling Molecules at the Crossroad between Inflammation and Epigenetic Control of Cardiometabolic Disorders. *International journal of molecular sciences*, *23*(23), 14564. https://doi.org/10.3390/ijms232314564
31. Wang, L., Chen, P., & Xiao, W. (2021). β-hydroxybutyrate as an Anti-Aging Metabolite. *Nutrients*, *13*(10), 3420. https://doi.org/10.3390/nu13103420
32. Poff, A. M., Koutnik, A. P., & Egan, B. (2020). Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. *Current sports medicine reports*, *19*(7), 251–259. https://doi.org/10.1249/JSR.0000000000000732
33. Eraso-Pichot, A., Brasó-Vives, M., Golbano, A., Menacho, C., Claro, E., Galea, E., & Masgrau, R. (2018). GSEA of mouse and human mitochondriomes reveals fatty acid oxidation in astrocytes. *Glia*, *66*(8), 1724–1735. https://doi.org/10.1002/glia.23330
34. Schönfeld, P., & Reiser, G. (2013). Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, *33*(10), 1493–1499. https://doi.org/10.1038/jcbfm.2013.128
35. Park, J. S., & Kim, Y. J. (2020). Anti-Aging Effect of the Ketone Metabolite β-Hydroxybutyrate in *Drosophila* Intestinal Stem Cells. *International journal of molecular sciences*, *21*(10), 3497. https://doi.org/10.3390/ijms21103497

36. Fan, S. Z., Lin, C. S., Wei, Y. W., Yeh, S. R., Tsai, Y. H., Lee, A. C., Lin, W. S., & Wang, P. Y. (2021). Dietary citrate supplementation enhances longevity, metabolic health, and memory performance through promoting ketogenesis. *Aging cell*, *20*(12), e13510. https://doi.org/10.1111/acel.13510
37. Edwards, C., Canfield, J., Copes, N., Rehan, M., Lipps, D., & Bradshaw, P. C. (2014). D-beta-hydroxybutyrate extends lifespan in C. elegans. *Aging*, *6*(8), 621–644. https://doi.org/10.18632/aging.100683
38. Roberts, M. N., Wallace, M. A., Tomilov, A. A., Zhou, Z., Marcotte, G. R., Tran, D., Perez, G., Gutierrez-Casado, E., Koike, S., Knotts, T. A., Imai, D. M., Griffey, S. M., Kim, K., Hagopian, K., McMackin, M. Z., Haj, F. G., Baar, K., Cortopassi, G. A., Ramsey, J. J., & Lopez-Dominguez, J. A. (2017). A Ketogenic Diet Extends Longevity and Healthspan in Adult Mice. *Cell metabolism*, *26*(3), 539–546.e5. https://doi.org/10.1016/j.cmet.2017.08.005
39. Newman, J. C., Covarrubias, A. J., Zhao, M., Yu, X., Gut, P., Ng, C. P., Huang, Y., Haldar, S., & Verdin, E. (2017). Ketogenic Diet Reduces Midlife Mortality and Improves Memory in Aging Mice. *Cell metabolism*, *26*(3), 547–557.e8. https://doi.org/10.1016/j.cmet.2017.08.004
40. Veech, R.L. Bradshaw, P.C. Clarke, K. Curtis, W. Pawlosky, R. King, M.T. Ketone Bodies Mimic the Life Span Extending Properties of Caloric Restriction. IUMB Life. 2017. 69(5), 305-314.
41. Eisenmann, D. M., Wnt signaling (June 25, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.7.1, http://www.wormbook.org.

42. Dillin, A., Hsu, A. L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A. G., Kamath, R. S., Ahringer, J., & Kenyon, C. (2002). Rates of behavior and aging specified by mitochondrial function during development. *Science (New York, N.Y.)*, *298*(5602), 2398–2401. https://doi.org/10.1126/science.1077780

43. Peters, J. (2020, May). *Effects of nicotinamide riboside and beta-hydroxybutyrate on C. elegans lifespan.* Digital Commons at East Tennessee State University. Retrieved October 23, 2021, from https://dc.etsu.edu/honors/531/.

44. Stiernagle T. (2006). Maintenance of C. elegans. *WormBook : the online review of C. elegans biology*, 1–11. https://doi.org/10.1895/wormbook.1.101.1

45. Hashimoto, T., Horikawa, M., Nomura, T., & Sakamoto, K. (2010). Nicotinamide adenine dinucleotide extends the lifespan of Caenorhabditis elegans mediated by sir-2.1 and daf-16. *Biogerontology*, *11*(1), 31–43. https://doi.org/10.1007/s10522-009-9225-3

46. Soo, S. K., Traa, A., Rudich, P. D., Mistry, M., & Van Raamsdonk, J. M. (2021). Activation of mitochondrial unfolded protein response protects against multiple exogenous stressors. *Life science alliance*, *4*(12), e202101182. https://doi.org/10.26508/lsa.202101182

47. Maglioni, S., Schiavi, A., Runci, A., Shaik, A., & Ventura, N. (2014). Mitochondrial stress extends lifespan in C. elegans through neuronal hormesis. *Experimental gerontology*, *56*, 89–98. https://doi.org/10.1016/j.exger.2014.03.026

48. Rodriguez, M., Snoek, L. B., Riksen, J. A., Bevers, R. P., & Kammenga, J. E. (2012). Genetic variation for stress-response hormesis in C. elegans lifespan. *Experimental gerontology*, *47*(8), 581–587. https://doi.org/10.1016/j.exger.2012.05.005

49. Bárcena, C., Mayoral, P., & Quirós, P. M. (2018). Mitohormesis, an Antiaging Paradigm. *International review of cell and molecular biology*, *340*, 35–77. https://doi.org/10.1016/bs.ircmb.2018.05.002

50. Ristow, M., & Zarse, K. (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Experimental gerontology*, *45*(6), 410–418. https://doi.org/10.1016/j.exger.2010.03.014

51. Tauffenberger, A., Vaccaro, A., & Parker, J. A. (2016). Fragile lifespan expansion by dietary mitohormesis in C. elegans. *Aging*, *8*(1), 50–61. https://doi.org/10.18632/aging.100863

52. Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Jordan, S. D., Quiros, P. M., Steffen, K. K., Williams, E. G., Mouchiroud, L., Tronnes, S. U., Murillo, V., Wolff, S. C., Shaw, R. J., Auwerx, J., & Dillin, A. (2016). Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity. *Cell*, *165*(5), 1209–1223. https://doi.org/10.1016/j.cell.2016.04.012

53. Xin, N., Durieux, J., Yang, C., Wolff, S., Kim, H. E., & Dillin, A. (2022). The UPRmt preserves mitochondrial import to extend lifespan. *The Journal of cell biology*, *221*(7), e202201071. https://doi.org/10.1083/jcb.202201071

54. Shimazu, T., Hirschey, M. D., Newman, J., He, W., Shirakawa, K., Le Moan, N., Grueter, C. A., Lim, H., Saunders, L. R., Stevens, R. D., Newgard, C. B., Farese, R. V., Jr, de Cabo, R., Ulrich, S., Akassoglou, K., & Verdin, E. (2013). Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science (New York, N.Y.)*, *339*(6116), 211–214. https://doi.org/10.1126/science.1227166

55. McIntyre, R. L., Daniels, E. G., Molenaars, M., Houtkooper, R. H., & Janssens, G. E. (2019). From molecular promise to preclinical results: HDAC inhibitors in the race for healthy aging drugs. *EMBO molecular medicine*, *11*(9), e9854. https://doi.org/10.15252/emmm.201809854

56. Evason, K., Collins, J. J., Huang, C., Hughes, S., & Kornfeld, K. (2008). Valproic acid extends Caenorhabditis elegans lifespan. *Aging cell*, *7*(3), 305–317. https://doi.org/10.1111/j.1474-9726.2008.00375.x