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ROLE OF NAD^+ CONSUMERS IN LIPID HOMEOSTASIS

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ABSTRACT

This thesis delves into the intricate relationship between consumer activity, NAD^+ biosynthesis, and lipid metabolism using Caenorhabditis elegans as a model organism. By examining the function of the PNC-1 enzyme and its mutations, the study aims to comprehend how reducing consumer activity affects fat formation. The mechanisms involved in NAD^+ biosynthesis, which are crucial for energy metabolism and cellular redox functions, are thoroughly examined. This helps to clarify how different pathways related to NAD^+ production and recycling are interconnected.

The research demonstrates the critical role of NAD^+ in maintaining cellular homeostasis and highlights how dysregulation of NAD^+ metabolism can lead to conditions such as obesity and age-related illnesses. In particular, the impact of gene alterations on lipid accumulation and NAD^+ levels are investigated for *tir-1*, *parp-1*, and *parp-2*. Experiments utilizing Oil Red O staining and NA supplementation provide important new understandings of how these mutations affect lipid accumulation and how NA supplementation may be used therapeutically to reduce lipid accumulation in some mutant strains. The research also sheds light on the possibility that NAM inhibits NAD^+ consumers, which adds to our understanding of the complex regulatory processes regulating lipid metabolism.

These findings not only deepen our understanding of the role of NAD^+ in lipid homeostasis but also offer potential avenues for therapeutic interventions targeting NAD^+ metabolism in the context of obesity, metabolic diseases, aging, and neurodegenerative disorders. Future directions include exploring the specific mechanisms underlying the observed effects and investigating additional therapeutic targets within the NAD^+ metabolic pathway.

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LIST OF ABBREVIATIONS

C.elegans Caenorhabditis elegans

NAD^+ nicotinamide adenine dinucleotide

PARP poly (ADP-ribose) polymerase

QA quinolinic acid

UMPS-1 uridine monophosphate synthetase 1

KYNU-1 kynurenine 1

NAM nicotinamide

NA nicotinic acid

PNC-1 pyrazinamidase and nicotinamidase 1

NR nicotinamide riboside

NMRK-1 nicotinamide riboside kinase 1

NMN nicotinamide mononucleotide

NMAT nicotinamide mononucleotide adenylyltransferase

NaMN nicotinic acid mononucleotide

Trp tryptophan

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CHAPTER 1

Introduction

My thesis is focused on the effect of knocking down consumers' activity in decreasing fat accumulation. My research aims to delve into the intricate relationship between NAD+ biosynthesis, consumer activities, and fat accumulation, particularly focusing on the role of PNC-1 and PARPs in this process. Specifically, I aim to understand how knocking down consumer activity impacts fat accumulation, with a particular emphasis on investigating the role of the PNC-1 mutation in NAD+ biosynthesis and its downstream effects on lipid metabolism. To this end, I utilized the model organism C. elegans to dissect the pathways involved in NAD+ metabolism and their impact on lipid homeostasis.

To do this, I will be utilizing *Caenorhabditis elegans* as a model organism to aid in investigating the role consumers play in the salvage pathway and how they maintain pathways for NAD^+. *C.elegans* is a model organism that I will use to analyze NAD^+ consumer activities to regulate lipid metabolism. It has been demonstrated that sirtuins, which are NAD^+-dependent enzymes, are involved in the metabolism of fat in mammals. Sirtuins are controlled by the route that salvages NAD^+ (Imai S, 2014). A pnc-1 mutation results in an accumulation of NAM and a reduction in NAD^+ production, which in turn lowers sirtuin function (Imai S, 2014). I hypothesize that parp-1 (RB1042), a mutant of *C. elegans,* will have a significant decrease in lipid accumulation in relation to the other mutants being tested. Showcasing lipid accumulation

will be done by Oil Red O staining method and MATLAB. These findings can later provide more information on obesity related health conditions.

NAD^+ Biosynthesis pathways

Energy metabolism depends on nicotinamide adenine dinucleotide (NAD^+), a crucial coenzyme for redox processes (Covarrubias et al; 2021). For non-redox NAD^+-dependent enzymes, such as sirtuins and poly (ADP-ribose) polymerases (PARPs), NAD^+ is also a necessary cofactor (Sahar et al; 2011). Yeast extracts' ability to regulate metabolic rates led to the discovery of NAD^+, which was later discovered to be the main hydride acceptor in redox processes (Imai et al; 2000). The capacity of NAD^+ to take a hydride ion and produce its reduced form, NADH, is essential for metabolic reactions in all living things (Imai et al; 2000). It also controls the activity of dehydrogenases engaged in a variety of catabolic pathways, such as glycolysis, glutaminolysis, and fatty acid oxidation. To create ATP in eukaryotes, the accepted electrons from these processes are subsequently provided to the electron transport chain (Covarrubias et al; 2021).

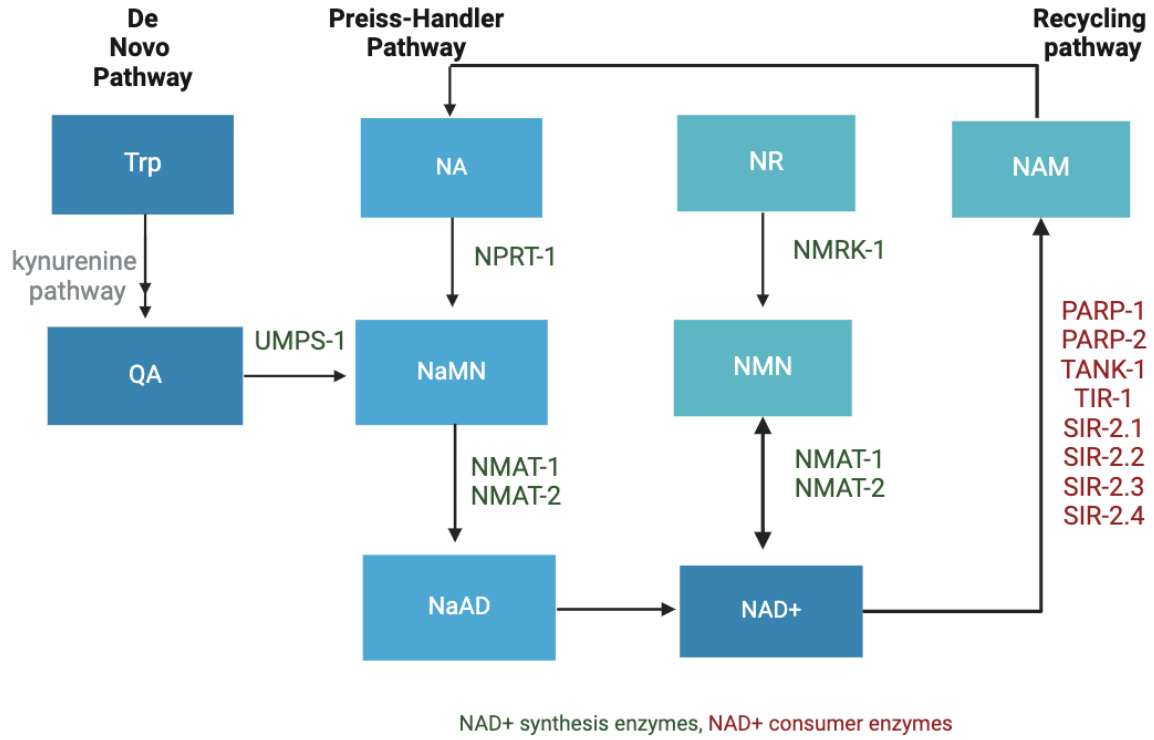

Figure 1. Interconnected Pathways in NAD$^+$ Biosynthesis: *De Novo,* Preiss-Handler, and Recycling Pathways. Diagram illustrating the *de novo*, Preiss-Handler, and recycling routes involved in NAD$^+$ production. The kynurenine pathway in the *de novo* pathway converts tryptophan to quinolinic acid (QA), which then uses UMPS-1 to create nicotinic acid mononucleotide (NaMN). Nicotinic acid (NA) is converted to nicotinic acid monomeric nucleotide (NaMN) via the Preiss-Handler pathway, which is aided by the actions of NMAT-1 and NMAT-2 enzymes. NaAD is subsequently changed into NAD$^+$. The recycling process entails using consumption enzymes to change NAD$^+$ back into nicotinamide (NAM) and finish the cycle. This diagram highlights the use of precursor molecules and enzymatic processes essential for the production and recycling of NAD$^+$ in cellular metabolism, demonstrating the connectivity of various pathways in NAD$^+$ biosynthesis.

NAD^+ reduction is necessary to keep a cell's redox status and energy balance in check (Covarrubias et al; 2021). In addition, three groups of NAD^+-consuming enzymes—NAD^+glycohydrolases, also known as NADases, CD38, CD157, and SARM1—as well as the protein deacylase family of sirtuins and PARPs—constantly produce NAD^+ by consuming it (Covarrubias et al; 2021). These enzymes serve a variety of crucial cellular activities (Covarrubias et al; 2021). These produce nicotinamide (NAM) as a byproduct and use NAD^+ as a substrate or cofactor. NAD^+, which is constantly in need, thereby mediates numerous important cellular activities (Covarrubias et al; 2021). The NAM salvage route allows NAM to be recycled back into NAD^+ to maintain NAD^+ levels (Covarrubias et al; 2021). Additionally, NAD^+ can be synthesized from various food sources by some cells, namely those in the liver (Chi et al; 2013).

To keep steady intracellular NAD^+ levels, NAD^+ is continuously produced, catabolized, and recycled in the cell (Covarrubias et al; 2021). However, as humans age, the ratio of catabolic to anabolic activities can change, and the rate of NAD^+ degradation may outrun the rate at which cells can synthesize NAD^+ from scratch or efficiently recycle or salvage NAM (Covarrubias et al; 2021). Furthermore, excess NAM may be catabolized via alternate metabolic routes, thereby diverting it away from the NAM salvage pathway and negatively influencing NAD^+ levels (Covarrubias et al; 2021). In addition to their shared function as NAD^+-consuming enzymes, NAD^+ glycohydrolases, sirtuins, and PARPs each play a unique role in aging and age-related illnesses (Covarrubias et al; 2021).

The poly (ADP-ribose) polymerases (PARPs), sirtuins, NAD^+ glycohydrolase, and cyclic ADP-ribose synthases CD38, CD157, and SARM1 are examples of NAD^+-consuming enzymes (Carty et al; 2006). The NAD+ salvage route recycles the nicotinamide (NAM) produced as a by-product of their enzymatic activities (Covarrubias et al; 2021). NAM is initially recycled into nicotinamide mononucleotide (NMN) via the intracellular nicotinamide phosphoribosyl transferase (iNAMPT), which is subsequently changed into NAD^+ by the various NMNATs (Carty et al; 2006). Nicotinamide N-methyltransferase (NNMT) is an enzyme that can alternatively methylate NAM, allowing it to be excreted through the urine (Kraus et al; 2014).

NAD^+ consumer activities

In addition, NAD^+ serves as a co-substrate for enzymes known as NAD^+ consumers, which affect important biological functions like stress reactions and lifetime (Sauve, 2008). NAD^+ biosynthesis is thought to be a universal biological activity that is essential to every cell's metabolism based on the significance of NAD^+ in oxidative phosphorylation. NAD^+ biosynthesis pathways may, however, trigger biological reactions as evidenced by the regulatory activity of NAD^+ and its metabolite nicotinamide on NAD^+ consumers. In the context of the growth of a multicellular organism, we are interested in examining the biological activities of the NAD^+ salvage pathway and differentiating between them.

There are many ways that NAD^+ biosynthesis is carried out, including "salvage" pathways from preformed substrates such nicotinamide (NAM), nicotinic acid (NA), and

nicotinamide riboside and *de novo* synthesis from amino acid substrates (Bieganowski and Brenner, 2004). All eukaryotes have the Preiss-Handler route, which converts NA to NAD^+ (Preiss and Handler, 1958a; Preiss and Handler, 1958b). For recycling NAM to NAD^+, there are two different enzymatic pathways that exist. The first one passes via a single intermediate and another that is longer and converges with the Preiss-Handler pathway (Magni et al., 1999). The type of the salvage pathway in vertebrate species is determined by the nicotinamide phosphoribosyl transferase (Nampt) enzyme that is encoded in their genomes. Nampt converts NAM to nicotinamide mononucleotide (NMN) to start the shorter pathway (Kraus et al; 2014).

NAMPT and nicotinamidases perform the same tasks while converting NAM to NAD+ and influence the level of NAD^+ and NAM activity (Kraus et al; 2014). NAM is a byproduct of NAD^+ consumers as well as a non-competitive feedback inhibitor of those consumers (Vrablik TL et al. 2009). These enzymes are expected to have biological regulatory action and regulate the NAD^+-dependent NAD^+ consumers, such as sirtuins and PARPs, due to the predicted effects of nicotinamidases and Nampt on the levels of NAD^+ and NAM (Vrablik TL et al. 2009).

PNC-1 in the NAD^+ biosynthesis pathway

Pnc-1 holds great significance in the NAD^+ biosynthesis pathway. Converting nicotinamide to nicotinic acid, a key precursor in NAD^+ production. This enzyme helps regulate NAD^+ levels, ensuring the cell has an adequate supply of NAD^+ to support essential cellular processes. PNC-1 exhibits nicotinamidase activity, which means it can cleave nicotinamide

(NAM) into nicotinic acid (NA). This reaction is a crucial step in the salvage pathway because it provides the precursor, nicotinic acid, for NAD^+ synthesis. NAD^+ is consumed in numerous metabolic reactions, and its levels need to be maintained for various cellular processes. PNC-1 helps in recycling NAM, which is generated when NAD^+ is consumed. By converting NAM back to NA, it enables the cell to regenerate NAD^+ efficiently. The activity of PNC-1 can be regulated to control NAD^+ levels in response to cellular needs. When NAD^+ levels drop, PNC-1 activity can increase, promoting the conversion of NAM to NA and, ultimately, NAD+ synthesis.

The role of PNC-1 in the NAD+ biosynthesis pathway is essential for maintaining cellular NAD+ levels and has implications for various cellular processes such as energy metabolism, DNA repair, and longevity regulation. Studies in *C. elegans* have shown that modulating the activity of PNC-1 can impact NAD+ levels and influence lifespan and health span in these organisms. One study by Mouchiroud et al. (2013) demonstrated that overexpression of PNC-1 in *C. elegans* led to increased NAD+ levels and extended lifespan, while knockdown of PNC-1 resulted in reduced NAD+ levels and shortened lifespan. This research highlighted the importance of PNC-1 in regulating NAD+ levels and its potential role in modulating aging processes.

Chapter 2. Role of *C.elegans*

C.elegans as a model organism

In 1963, Sydney Brenner, a molecular biologist at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, started constructing *Caenorhabditis elegans* as a model organism for basic biological research (Strange, 2006.). The preferred organism for studies into the genetics of longevity and aging is *Caenorhabditis elegans* (Zhang S. et al. 2020). A tiny, translucent, free-living nematode called *Caenorhabditis elegan*s inhabits dirt (Zhang S. et al. 2020). The organism has drawn interest for its usage as a different model for in vivo (Ha et al., 2022). *C. elegans* can be easily viewed by a microscope due to their tiny size, about 1 mm for adults, and behavior, including their movement, feeding, and reproduction (Ha et al., 2022).

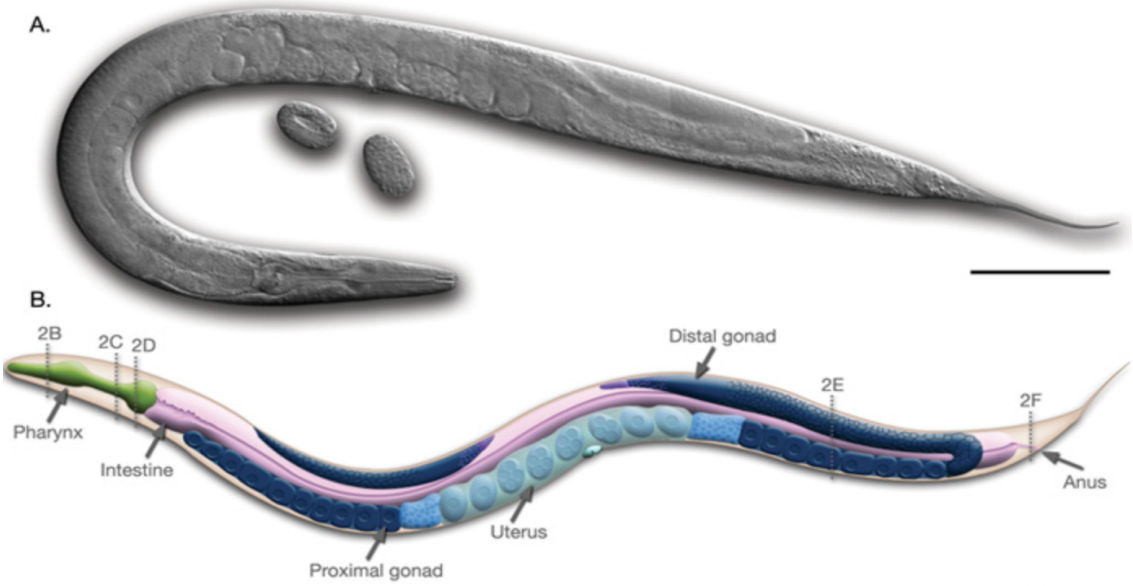

Figure 2. C.elegans structure of adult nematode from Wormatlas (Hall 2009)

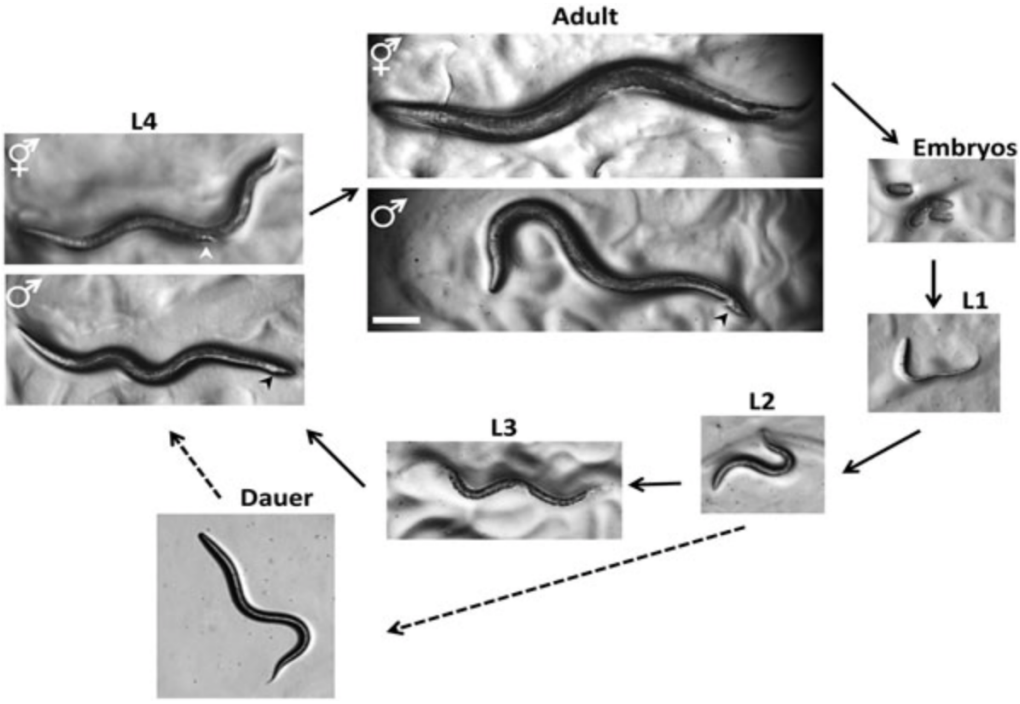

Figure 3. ***C.elegans*** **life stages from Wormbook (Corsi 2015)**

Many human illness genes and disease pathways are present in *C. elegans* when comparing the genomes of the two species (Ha et al., 2022). Human genes have about 40-80% orthologs that are found in the *C. elegans* genome, and 40–50% of human disease-related genes as well (Ha et al., 2022). *C. elegans* has substantially conserved human disease-related pathways and genes (Ha et al., 2022). Such as lipid metabolism genes and signaling pathways, including the insulin signaling pathway (Ha et al., 2022). It has a short life cycle and a high reproductive rate and is relatively simple to maintain in a lab. *C. elegans* is a valuable model for examining the molecular causes of human diseases because its entire genome sequence is readily accessible (Ha et al., 2022)

Lipid Metabolism in *C. elegans.*

Fatty acid synthesis occurs *de novo* in *C. elegans* via a conserved biosynthesis route. Acetyl-CoA is the substrate used in the synthesis of fatty acids. It is produced via glycolysis and other metabolic activities. Acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and elongated complexes are important enzymes in this process because they catalyze the progressive elongation of fatty acid chains. The significance of this route in lipid metabolism has been highlighted by genetic studies that have clarified the involvement of several genes in controlling fatty acid production in *C. elegans* (Watts & Browse, 2006).

In *C. elegans*, the majority of lipid storage takes place in specialized cells known as adipocytes or fat storage cells. In times of nutritional constraint, these cells store energy in the form of lipid droplets made up of triglycerides and other neutral lipids. Conserved proteins that are involved in lipid mobilization and storage, such as perilipins and lipid droplet-associated proteins, control the formation and dynamics of lipid droplets (O'Rourke et al., 2009).

When there is a need for energy, stored lipids are released and go through β-oxidation to produce ATP. Acetyl-CoA is produced by the successive oxidation of fatty acids in mitochondria and peroxisomes, and it then enters the citric acid cycle to provide more energy. This process is known as β-oxidation. NAD+ plays a crucial role in the process of β-oxidation by acting as a cofactor for several dehydrogenase enzymes involved in fatty acid oxidation. These enzymes catalyze the oxidation steps in the breakdown of fatty acids, leading to NADH production, which

is then used in the electron transport chain to generate ATP. Thus, NAD+ acts as a vital mediator in the energy-producing pathways, including β-oxidation, ensuring efficient energy metabolism and cellular function. Mutants of *C. elegans* missing functional β-oxidation enzymes display abnormalities in energy homeostasis and lipid metabolism (Van Gilst et al., 2005).

In *C. elegans*, lipids also function as signaling molecules that control several physiological functions. Certain lipid species, such phosphoinositides and ceramides, for instance, are involved in signaling pathways linked to development, lifespan, and the stress response. According to Park et al. (2012), lipid signaling pathways frequently cross paths with other signaling cascades to generate complex regulatory networks that alter cellular functions. Lipid metabolism consists of several interrelated processes that are involved in the synthesis, storage, mobilization, and signaling of fatty acids. Research on *C. elegans* has shed important light on the molecular mechanisms governing lipid metabolism and its control, emphasizing the evolutionary conservation of lipid metabolic pathways across a wide range of animals.

Visualizing Lipid Accumulation in *C. elegans.*

Triglycerides and cholesterol esters, which make up a large portion of lipid droplets—the main form in which lipids are stored in cells—are examples of neutral lipids that Oil Red O specifically stains (Soukas et al., 2009). This selectivity enables the labeling of lipid-rich structures clearly and distinctly by enabling the observation of lipid droplets without staining other cellular components. Oil Red O gives lipid droplets a striking reddish-orange stain that makes them stand

out from surrounding tissues in bright-field microscopy. Even at low magnifications, the strong staining makes it easier to identify and measure lipid buildup.

Without the need for extensive sample preparation techniques or sectioning, lipid buildup in whole mount *C. elegans* worm samples can be visualized using Oil Red O staining. This lowers the possibility of sample manipulation artifacts and streamlines the staining procedure. With just a few simple procedures and easily accessible materials, it is comparatively easy to do. The staining procedure works well for both qualitative and quantitative studies of lipid accumulation, and it may be modified to fit a variety of experimental configurations. While bright-field microscopy is usually used to examine Oil Red O staining, it can also be used in conjunction with fluorescence microscopy techniques for multiple imaging investigations.

Chapter 3 Results

Parp-2 shows significant decrease in Lipid Accumulation

The experiment began with the 11 strains of consumers and 1 synthesis gene of *C. elegans*. These stains are, N2 (WT), RB1042 (*parp-1*), FX3401 (*parp-2*), VC1171 (*parp-2*), RB684 (*tank-1*), IG685 (*tir-1*), RB1085 (*tir-1),* VC526 *(tir-1*), ZD101 (*tir-1*), FX4924 (*tir-1*), and HV727 (*pnc-1).* Oil Red O staining was used to detect the lipid accumulation in each strain. Three biological replicates of Oil Red O staining was performed on the 11 strains. Significance was identified between RB1042 (*parp-1*), VC1171 (*parp-2*), HV727 (*pnc-1*), HV826 (*pnc-1* and *parp-1*), ZD101(*tir-1*), and N2 (WT).

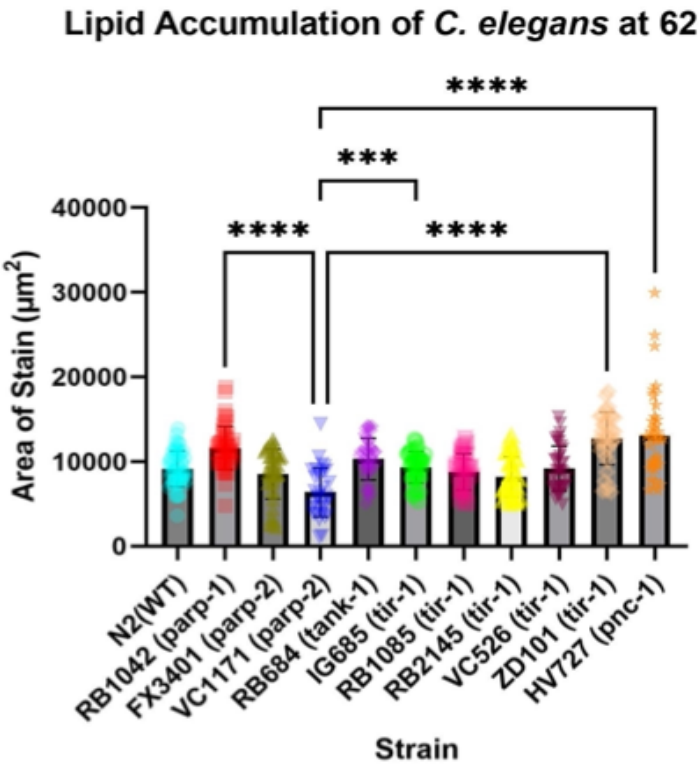

Figure 4. Lipid Accumulation of *C. elegans* at 62 hrs. Oil Red O staining was done on 11 different *C. elegans* strains**.** Each of the 11 strains are shown with their respective areas of lipid accumulation. Each data set for each strain was collected, an average of 40 specimens per strain. When comparing each strain's area to VC1171, there is significance seen between RB1042, ZD101, and HV727. * = $p<.05$ using a t-test; error bars are standard deviation.

Moreover, to confirm and validate these findings, ORO straining was performed on the strains that showed significance–RB1042 (*parp-1*), VC1171 (*parp-2*), HV727 (*pnc-1*), ZD101 (*tir-1*), and N2 (WT), as well as the HV826 (*pnc-1; parp-1*)–in the first experiment. These were also

performed in three biological replicates and found there to be significance in N2 (WT) between VC1171 (*parp-2*), ZD101(*tir-1*), and HV727 (*pnc-1*). The greatest significance is seen between N2 and HV727 (*pnc-1*) and VC1171 (*parp-2*).

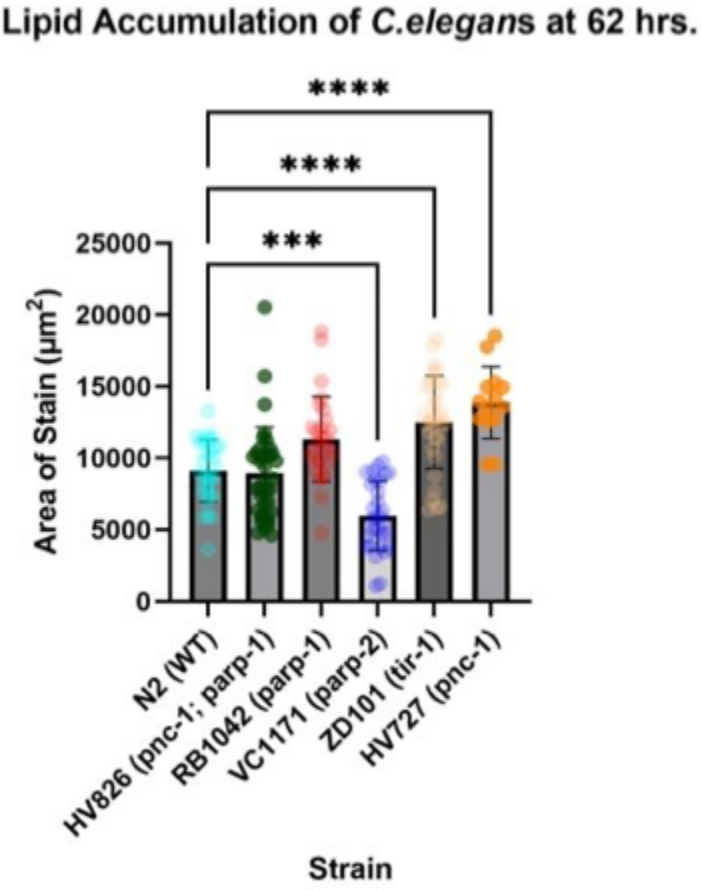

Figure 5. Lipid Accumulation of *C.elegans* strains showed significance at 62 hrs. Continuing the same procedure done in Trial 1, there can be significance seen from N2 between VC1171, ZD101, and

HV727. The greatest significance is seen between N2 and HV727. * = $p<.05$ using a t-test; error bars are standard deviation.

NA Supplementation

The possible effects of nicotinic acid (NA), also referred to as niacin or vitamin B3, on lipid metabolism and accumulation in *C. elegans* have been investigated. Nicotinamide adenine dinucleotide (NAD^+) is a prerequisite for NA and an essential cofactor in several metabolic turn affects *C. elegans* lipid metabolism, by offering an exogenous source of NA. Research has demonstrated that by encouraging fatty acid oxidation and decreasing lipid buildup in worms, NA supplementation can regulate lipid metabolism (Ghazalpour et al., 2010). The activity of NAD^+-dependent enzymes involved in lipid catabolism and storage as well as variations in NAD^+ levels mediate this impact.

Furthermore, it has been discovered that NA administration controls the expression of genes linked to lipid metabolism, resulting in transcriptional modifications that prioritize lipid energy consumption and mobilization over lipid synthesis and storage. Overall, NA supplementation has a complex influence on *C. elegans* lipid metabolism that includes both direct impacts on metabolic pathways and indirect effects on cellular physiology and gene expression (Mitchell et al., 2016). Furthermore, it has been observed that supplementing with NA extends the lifespan and health span of *C. elegans*, implying wider consequences for longevity and metabolic

health. In *C. elegans* and maybe other animals as well, these results demonstrate the potential of NA supplementation as a regulator of lipid metabolism and a promoter of general health.

After seeing the results from the Oil Red O staining, the question arises, what are the potential effects of nicotinic acid (NA) on lipid metabolism and accumulation in C. elegans? How does nicotinic acid (NA) supplementation influence lipid metabolism and accumulation in C. elegans, and what implications does this have for the regulation of metabolic health and longevity? To answer these questions, I performed NA supplementation on the same six strains–RB1042 (*parp-1*), VC1171 (*parp-2*), HV727 (*pnc-1*), ZD101 (*tir-1*), and N2 (WT), as well as the HV826 (*pnc-1; parp-1*) –under the same environment.

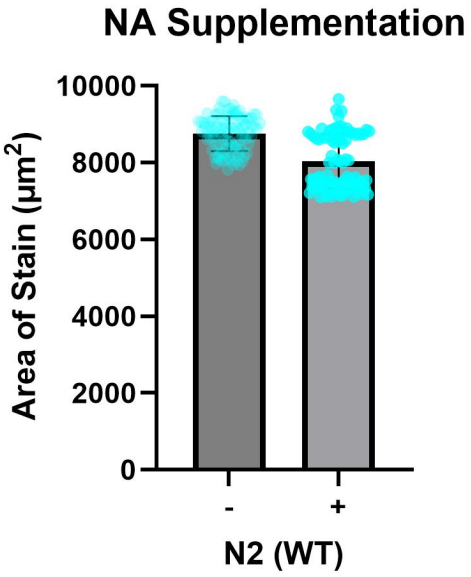

Figure 6. NA Supplementation of N2 (WT). This figure shows a comparison of NA supplementation done on N2 (WT) (n=89). N2 (WT) (-) (n=89), indicates no NA supplementation done and is compared to N2 (WT) (+) (n=87) where there was NA supplementation done.

In Figure 6, we can see how there is not much change before and after NA supplementation in wild-type worms. This data suggests that with NA supplementation in N2 animals, we see a trend towards a decrease in lipid accumulation, but it is not significant.

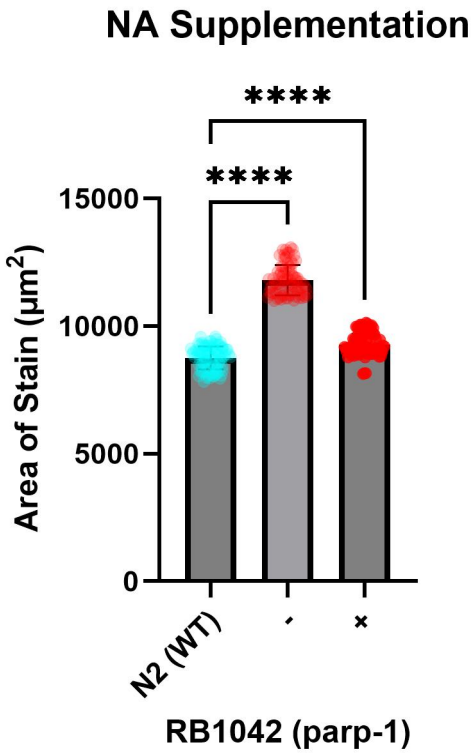

Figure 7. NA Supplementation of RB1042 (*parp-1*). Comparison of NA supplementation done on RB1042 (*parp-1*) with N2 (WT) (n=89). RB1042 (*parp-1*) (-) (n= 80), indicates no NA supplementation done and is compared to RB1042 (*parp-1*) (+) (n=87) where there was NA supplementation done. * = $p<.05$ using a t-test; error bars are standard deviation.

In Figure 7, we can clearly see a decrease lipid accumulation when NA supplementation was done on RB1042 (*parp-1*). When comparing N2 (WT) to RB1042 (*parp-1*) (+), we can see how lipid accumulation of RB1042 (*parp-1*) (+) decreased. While comparing N2 (WT) to RB1042 (*parp-1*) (-), we can see how there is a significant increase in the mutant strain. Moreover, after adding NA supplantation, lipid accumulation significantly decreased compared to when it was not added to RB1042 (*parp-1*).

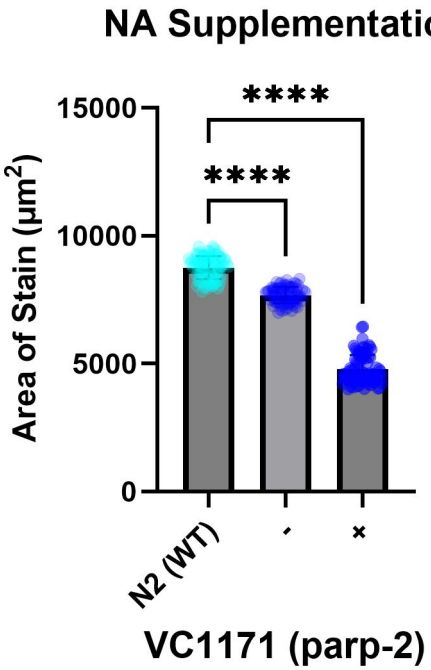

Figure 8. NA Supplementation of VC1171 (*parp-2*). Comparison of NA supplementation done on VC1171 (*parp-2*) with N2 (WT) (n=89). VC1171 (*parp-2*) (-) (n=82) indicates no NA supplementation done and is compared to VC1171 (*parp-2*) (+) (n= 87) where there was NA supplementation done. * = $p<.05$ using a t-test; error bars are standard deviation.

In Figure 8, we can detect a decrease in lipid accumulation when NA supplementation was supplied to VC1171 (*parp-2*). When comparing N2 (WT) to VC1171 (*parp-2*) (+), we can see how lipid accumulation of VC1171 (*parp-2*) (+) decreased. Moreover, after adding NA supplantation, lipid accumulation significantly decreased compared to when it was not added to VC1171 (*parp-2*). This further supports the role of parp-2 in regulating fat accumulation and lipid homeostasis.

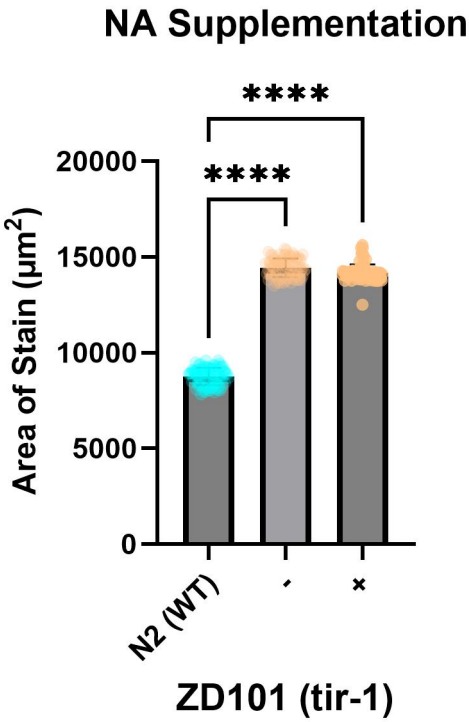

Figure 9. NA Supplementation of ZD101 (tir-1). Comparison of NA supplementation done on ZD101 (*tir-1*) with N2 (WT) (n=89). ZD101 (*tir-1*) (-) (n=80) indicates no NA supplementation done and is compared to ZD101 (*tir-1*) (+) (n=90) where there was NA supplementation done. * = p<.05 using a t-test; error bars are standard deviation.

In Figure 9, the data suggests no change in lipid accumulation when NA supplementation was done on ZD101 (*tir-1*). When comparing N2(WT) to ZD101 (*tir-1*) (+), we can see how lipid accumulation of ZD101 (*tir-1*) (+) is significantly greater than N2 (WT). Moreover, after adding NA supplantation, lipid accumulation had no major affect compared to when it was not added to ZD101 (*tir-1*) (-).

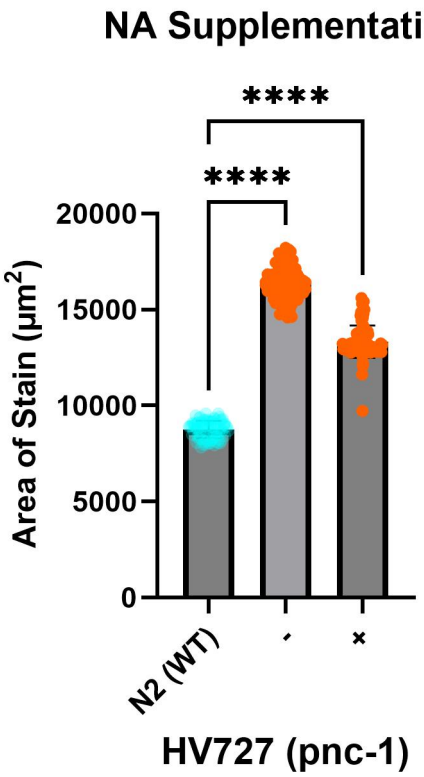

Figure 10. NA Supplementation of HV727 (*pnc-1*). Comparison of NA supplementation done on HV727 (*pnc-1*) with N2 (WT) (n=89). HV727 (*pnc-1*) (-) (n=81) indicates no NA supplementation done and is compared to HV727 (*pnc-1*) (+) (n=87) where there was NA supplementation done. * = p<.05 using a t-test; error bars are standard deviation.

In Figure 10, we can clearly see a decrease lipid accumulation when NA supplementation was done on HV727 (*pnc-1*). When comparing N2 (WT) to HV727 (*pnc-1*) (+) and (-), we can see how lipid accumulation of the mutant strain is significantly greater than N2 (WT). Moreover, the data suggest, after adding NA supplantation, lipid accumulation had a significant decrease, when compared to when it was not added to HV727 (*pnc-1*)(-). NA's ability to restore fat accumulation in *pnc-1* mutants supports the role of NAD^+ biosynthesis fueling this process.

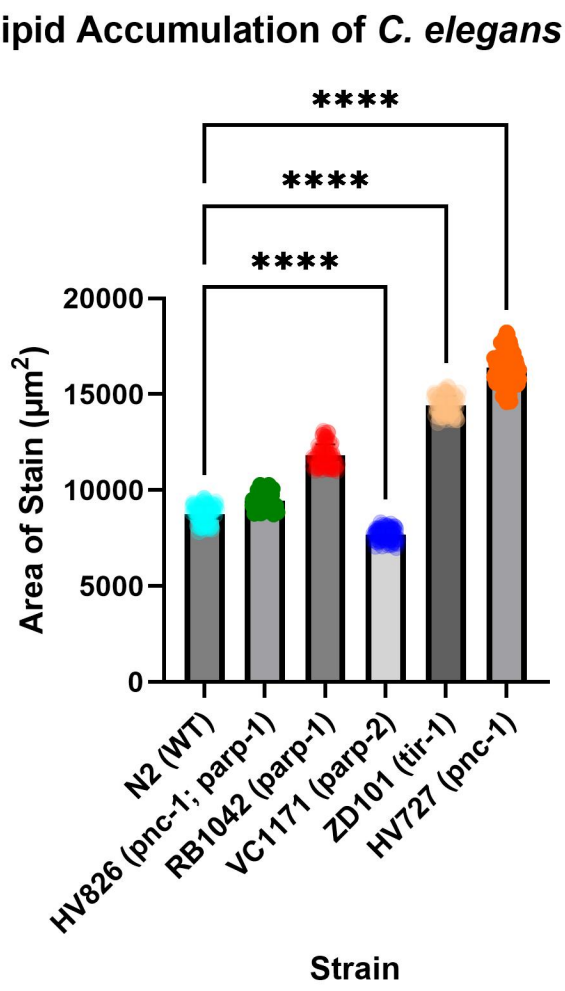

Figure 11. Lipid Accumulation of *C.elegans* Strains at 62 hrs. with NA supplementation.

Comparison of NA supplementation done on the mutant strains, RB1042 (*parp-1*) (n=87), VC1171 (*parp-2*) (n=87), HV727 (*pnc-1*) (n=87), ZD101 (*tir-1*) (n=90), and N2 (WT) (n= 89), as well as the HV826 (*pnc-1; parp-1*). * = p<.05 using a t-test; error bars are standard deviation.

In Figure 11, this data suggests VC1171 (*parp-2*) has the lowest lipid accumulation compared to the rest of the strains. Whereas HV727 (*pnc-1*) has the greatest area of lipid accumulation when compared

to the rest of the strains. There is significance seen with N2 (WT) and VC1171 (*parp-2*), ZD101(*tir-1*), and HV727 (*pnc-1*). Taken together, these data support the role that NAD^+ biosynthesis plays in fat accumulation and suggests PARPs are the NAD^+ consumers contributing to lipid biology and homeostasis.

Chapter 4 Discussion

NAD-Induced Lipid Homeostasis: Implications for NAD^+ and Aging

Findings suggest that nicotinic acid (NA) supplementation in C. elegans influences lipid metabolism by promoting fatty acid oxidation, reducing lipid accumulation, and modulating the expression of genes related to lipid metabolism. This supplementation extends lifespan and health span in C. elegans. These results imply that NA supplementation could serve as a regulator of lipid metabolism and a promoter of general health in C. elegans and potentially other animals.

Experimental data demonstrates that NA supplementation decreases lipid accumulation in mutant strains, particularly in *parp-1* and *parp-2* mutants. These mutants showed significant reductions in lipid accumulation after NA supplementation compared to controls. In contrast, supplementation had no significant effect on tir-1 mutants and had varying impacts on pnc-1 mutants. Notably, VC1171 (*parp-2*) exhibited the lowest lipid accumulation among the strains studied, while HV727 (*pnc-1*) showed the highest.

Overall, the findings support the role of NAD+ biosynthesis in fat accumulation and suggest that PARPs are significant NAD+ consumers involved in lipid biology and homeostasis. These results highlight the potential of NA supplementation as a regulator of lipid metabolism and promoter of metabolic health and longevity.

By extending lifespan and health span in C. elegans, NA supplementation suggests a potential for enhancing overall health and longevity in other organisms, including humans. NA supplementation encourages fatty acid oxidation and decreases lipid buildup, potentially mitigating lipid-related diseases such as obesity and dyslipidemia. NA influences gene expression related to lipid metabolism, suggesting a mechanism for its effects on cellular physiology and metabolic health. The observed effects of NA supplementation on lipid metabolism and longevity in C. elegans may pave the way for future research into NA-based therapies for metabolic disorders and age-related diseases in humans.

A vital component of several biological processes, such as energy metabolism, DNA repair, epigenetic control, and cellular signaling, is nicotinamide adenine dinucleotide. Its participation in several processes linked to aging, cancer, neurological illnesses, and metabolic problems makes it a promising target for medication. As people age, their levels of NAD^+ decrease, which impairs cellular function and makes them more vulnerable to age-related illnesses. Preclinical research has indicated that increasing NAD^+ levels by supplementing or activating NAD^+ biosynthesis pathways may be able to slow down age-related decline and lengthen life (Claudia et al. 2017).

In metabolic processes like glycolysis, the tricarboxylic acid cycle (TCA cycle), and oxidative phosphorylation, NAD^+ is an essential cofactor. Obesity, diabetes, and fatty liver disease are metabolic diseases associated with dysregulation of NAD^+ metabolism (Claudia *et al*.

2017*).* One possible treatment approach for these disorders is to target NAD^+ metabolism (Claudia *et al.* 2017).

To keep neurons healthy and functioning properly, NAD^+ is essential. Neurodegenerative illnesses like Alzheimer's, Parkinson's, and Huntington's disease are linked to declining NAD^+ levels (Imai S, 2014). In preclinical models, approaches targeted at increasing NAD^+ levels or stimulating NAD^+ -dependent enzymes exhibit promise as possible neurodegenerative disease therapies (Imai S, 2014). Numerous facets of cancer biology, such as DNA repair, cell survival, and tumor development, are influenced by NAD^+ metabolism (Yong *et al* .2023). A promising approach to cancer treatment is to target NAD^+ metabolism, either by stopping cancer cells from synthesizing NAD^+ or by taking advantage of weaknesses in NAD^+ -dependent mechanisms (Yong *et al* .2023). The pivotal function of NAD^+ in numerous cellular processes and its association with a range of ailments make it a potentially effective therapeutic target. One possible treatment approach for aging, metabolic diseases, cancer, and neurological illnesses is to modify NAD^+ metabolism.

Nicotinic acid (NA) supplementation in *C. elegans* demonstrates promising implications for regulating lipid metabolism, promoting longevity, and improving overall health. Further research into the mechanisms underlying these effects could lead to novel therapeutic strategies for combating metabolic diseases and extending lifespan in humans.

Future Directions

These findings open the door to understanding PARPs in lipid homeostasis. The findings data in parp-1 shows what is to be expected. However, we can clearly see that in *parp-2* lipid accumulation decreased. As well as a new player, tir-1, and its role in lipid homeostasis is brought forth. In the first trial we discovered significance when comparing each strain's area to VC1171, there is significance seen between RB1042, ZD101, and HV727. It was discovered here how *parp-2* can play a crucial role in the NAD^+ biosynthesis pathway. As well as new information on tir-1's role. To validate the information found in trial 1, a second trial was done. In the second trial, we discovered how there is significance seen from N2 between VC1171, ZD101, and HV727. The greatest significance is seen between N2 and HV727.

When HV727 (*pnc-1*) was supplemented with NA, there was a significant decrease seen in lipid accumulation. However, when compared to N2 (WT), we can see how regardless of NA supplementation, lipid accumulation in N2 (WT), was consistently lower than HV727 (*pnc-1*). Lipid accumulation for RB1042 (*parp-1*) and VC1171 (*parp-2*), were seen to have a significant decrease when supplemented with NA. This supports how RB1042 (*parp-1*) and VC1171 (*parp-2*), mutant strains, play a role as NAD^+ consumers. However, the data suggests ZD101(*tir-1*), does not play a role in a NAD^+ consumer. ZD101(*tir-1*) shows little to no change when supplanted with NA. These findings support how NA rescues lipid accumulation in PARPs mutants.

The research findings demonstrate that nicotinic acid (NA) supplementation has significant effects on lipid metabolism and accumulation in C. elegans. This study reveals that NA administration controls the expression of genes related to lipid metabolism, favoring lipid energy consumption. This supplementation also extends the lifespan and health span of C. elegans, indicating broader implications for metabolic health and longevity.

The experiments show that NA supplementation decreases lipid accumulation in mutant strains, particularly in RB1042 (*parp-1*) and VC1171 (*parp-2*) mutants. These mutants exhibit significant reductions in lipid accumulation after NA supplementation compared to untreated conditions. However, NA supplementation has less pronounced effects on strains such as ZD101 (*tir-1*), where lipid accumulation remains largely unchanged. Even more, this data opens the door to investigates how Nicotinamide (NAM) affects mutant strains. The data suggests that Nicotinamide (NAM) would block consumers from performing correctly. This data would ultimately prove that NAM has a role of inhibition in NAD^+ consumers.

Overall, the data suggest that NA supplementation influences lipid metabolism by reducing fat accumulation in certain mutant strains of C. elegans. The findings underscore the potential of NA as a regulator of lipid metabolism and a promoter of general health, with implications for metabolic health and longevity. Additionally, the study highlights the role of NAD+ biosynthesis in fat accumulation and suggests that PARPs may be key NAD+ consumers contributing to lipid biology and homeostasis.

Chapter 5 Methods and Materials

C.elegans Maintenance

In OP50, *E.coli* was inoculated colony into 50 ml LB broth in a 200 ml flask and incubated with gentle agitation at 37°C overnight. Approximately 200 µl of OP50 liquid culture was applied to a 60 mm NGM plate. Seeded plates were incubated at room temperature for 3-7 days before use. *C. elegans* was maintained under standard conditions at 20˚C using E. coli strain OP50 as food source and grew worms until L4 stage. This was repeated every 3 days for maintenance of *C.elegans* cultures.

Oil-Red O Staining

Oil-Red O staining was performed based on "*Quantification of Lipid Abundance and Evaluation of Lipid Distribution in Caenorhabditis Elegans by Nile Red and Oil Red O Staining: Protocol*". Synchronized young adult, L4 at 64 hrs., *C. elegans* that were collected in 1-2 plates and rinsed with PBST. Worms were pelted by centrifuging. After removing most supernatants, 100 µL of PBST was left and added 600 µL of ORO working solution to the worm pellet and the mixture was incubated at room temperature for 2 hours with gentle rocking. Prepared ORO working solution by diluting ORO stock solution in water to 60% isopropanol and filtered through sterile syringe before usage. The Oil-Red O staining working solution, which was originally 100% isopropanol, was then diluted in water to 60% isopropanol and left mixing in the dark overnight before they were stained. Stained worms were washed with PBST and mounted on

agarose slides for imaging. Pictures were captured using ECHO microscope and processed with a MATLAB program.

Microscopy with ECHO

Imaging of stained worms was taken by ECHO microscope using 50% bright field under 10X lens.

NA Supplementation

Following the McReynold's lab approach, 1M stock solution of NA and NAM was created and pH was adjusted of the NA solution to pH 5.4 using 10 N NaOH for solubility. Both solutions were filtered and sterilized after making and adjusting the pH as necessary. An OP50 lawn was established on NGM plates in the same normal conditions as the previous experiment. 25mM of NA was added and filter-sterilized, to NGM plates and with OP50 lawns. The plates were incubated plates at room temperature for 2-3 days to allow chemicals to diffuse. Transferred gravid/L4 hermaphrodites to supplemented plates and followed the same Oil Red O staining protocol as mentioned above.

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