Investigating the Impact of DAF-2 Knockdown on Lifespan Extension and Muscle Function in *C. elegans*: A Study on Longevity and Muscle Preservation

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Abstract

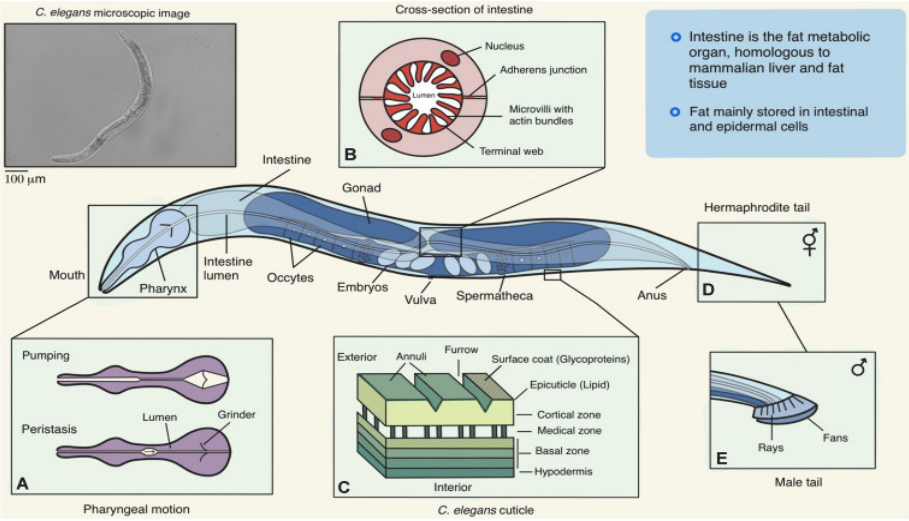
Aging is characterized by progressive declines in cellular function, leading to reduction in mobility, muscle degeneration, and shortened lifespan. In *Caenorhabditis elegans*, the insulin/IGF-1 signaling pathway plays a critical role in regulating aging processes, with DAF-2 functioning as a key receptor. This study investigated the impact of DAF-2 knockdown via RNAi on lifespan, locomotion, and muscle degeneration in *C. elegans*. Key achievements included the successful growth and maintenance of synchronized worm colonies and the optimization of FUdR concentrations to ensure non-lethal conditions. Although time constraints limited the scope of experimental results, these efforts provide a foundation for future studies to enhance replicates, complete planned assays, and validate RNAi efficacy. These findings contribute to understanding the role of DAF-2 signaling in aging and muscle integrity, providing a foundation for further exploration of age-related biological mechanisms.

Introduction

Caenorhabditis elegans as a Model Organism

Caenorhabditis elegans (*C. elegans*) is a nematode widely used in biological research due to its unique combination of simplicity and relevance to higher organisms. First proposed as a model organism by Sydney Brenner in 1963, *C. elegans (Figure 1)* has since become an important piece of genetic and molecular biology research (Goldstein, 2016). Its advantages include a short lifespan of 2–3 weeks, a rapid reproductive cycle, and a fully mapped genome with approximately 60–80% of its genes homologous to human genes (Kim et. al, 2018). Additionally, the organism's transparent body allows for direct visualization of biological processes, such as neuronal activity,

muscle function, and cellular aging, using advanced imaging techniques. These, position *C. elegans* as a cost-effective, high-throughput ideal model for studying aging, development, and for understanding the molecular mechanisms underlying human diseases.



Source:https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular biology/caenorhabditis-elegans

Figure 1. Overview of *C. elegans* anatomy, highlighting its well-studied structure and simple physiology. These characteristics, along with its short lifespan, make *C. elegans* an excellent model for investigating aging mechanisms and pathways related to longevity

Longevity and Muscle Degeneration

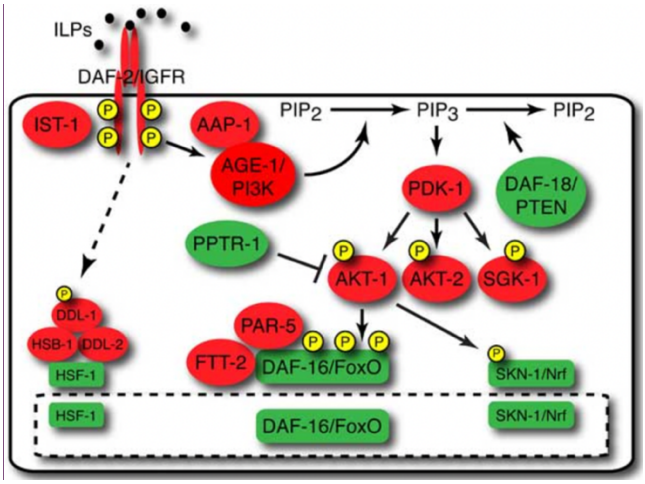
Aging is a fundamental biological process that leads to the progressive decline of various physiological systems, significantly impacting an organism's health and overall quality of life (WHO, 2024). Among the many consequences of aging, sarcopenia—characterized by the gradual degeneration of muscle tissue—stands out as a critical factor that reduces mobility, increases the risk of falls, and contributes to overall frailty in older individuals (Walston, 2012). Addressing muscle degeneration in the context of natural aging is essential to developing therapeutic strategies that promote healthy aging and maintain physical function throughout the lifespan. To better

understand these processes, many researchers have turned to the insulin/IGF-1 signaling pathway, which influences metabolism, stress resistance, and proteostasis (Kaletsky & Murphy, 2010).

The Insulin/IGF-1 Signaling Pathway and DAF-16 Activation

The insulin/IGF-1 signaling pathway (*Figure 2*) is well-characterized in *C. elegans*. DAF-2, the insulin/IGF-1 receptor, modulates the activity of DAF-16, a FOXO transcription factor that orchestrates stress resistance, metabolism, and longevity. Under normal conditions, active DAF-2 signaling triggers the activation of PI3K, which converts PIP2 to PIP3, a key lipid second messenger. PIP3 recruits and activates AKT, which subsequently phosphorylates DAF-16, preventing its translocation to the nucleus. This process inhibits DAF-16 from initiating the transcription of genes associated with stress resistance, proteostasis, and lifespan extension. (Murphy and Hu, 2013)

However, when DAF-2 signaling is reduced, PI3K activity is suppressed, leading to a decrease in PIP3 levels and the inactivation of AKT. This allows DAF-16 to become active and translocate to the nucleus, where it upregulates genes involved in stress response, proteostasis, and longevity. This activation enhances the cellular capacity to prevent toxic protein aggregation and repair muscle tissue, offering a potential strategy to mitigate muscle degeneration and preserve cellular health. (Murphy and Hu, 2013)

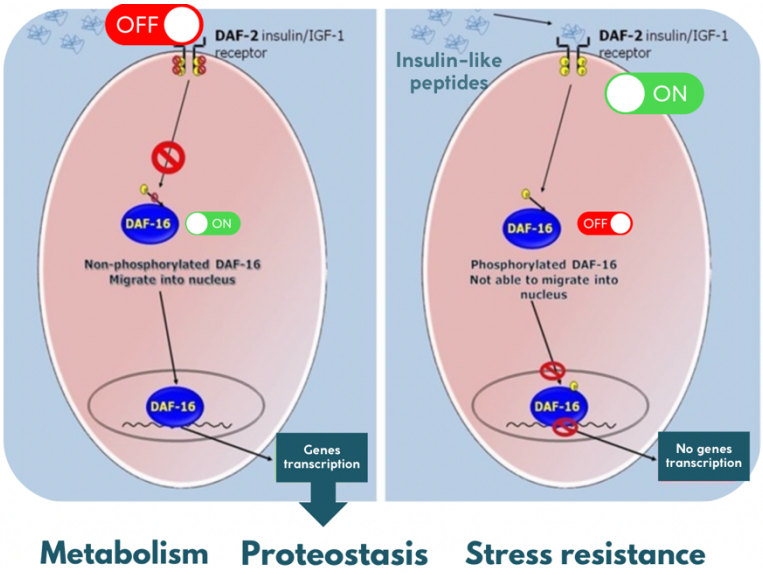


Source: http://www.wormbook.org/chapters/www_insulingrowthsignal/insulingrowthsignal.html

Figure 2. Schematic representation of the insulin/IGF-1 signaling (IIS) pathway in *C. elegans*, illustrating the interactions between key components such as DAF-2, PI3K, AKT, and the downstream activation of DAF-16/FoxO.

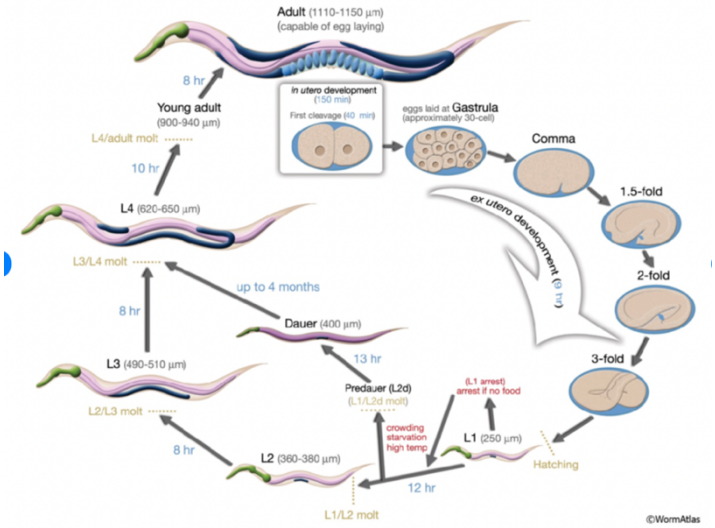
Previous Research on DAF-2 Knockdown in *C. elegans*

Studies on DAF-2 knockdown (*Figure 3*) have demonstrated remarkable effects on lifespan and muscle health. For instance, Kenyon et al. (1993), Kaletsky and Murphy (2010) showed that reducing DAF-2 activity doubles the lifespan of *C. elegans*. Similarly, Zhang et al. (2022) took a more focused approach by showing that the intestine-specific elimination of DAF-2 in *C. elegans* nearly doubled the organism's lifespan as well. In addition, Venz et al. (2021) emphasized RNA interference (RNAi) as a successful method for reducing DAF-2 expression in adult worms, leading to increased lifespan without triggering developmental side effects like Dauer formation (*Figure 4*). Dauer formation occurs as an alternative developmental stage that *C. elegans* can enter under unfavorable conditions (such as overcrowding or lack of food), allowing it to survive in a state of arrested development indicating that selective gene targeting could prolong lifespan without jeopardizing overall health.



Source: https://europepmc.org/article/med/32110282

Figure 3. Simplified DAF-2/Insulin/IGF-1 pathway. Knocking down DAF-2 (OFF) allows DAF-16 to enter the nucleus, enhancing metabolism, proteostasis, and stress resistance. When DAF-2 is active (ON), DAF-16 is phosphorylated and unable to promote these effects.



Source:https://www.researchgate.net/figure/Life-cycle-of-C-elegans-at-22-C-The-life-cycle-of-C-elegans-consists-of-embryogenesis_fig3_335926740 ***Figure 4.*** Life cycle of *C. elegans* at 22°C, highlighting its developmental stages from embryogenesis through to adulthood. The diagram illustrates the stages of larval development (L1 to L4), the dauer stage as a survival mechanism, and the transition to adulthood.

Despite these insights into the mechanisms of longevity, the specific impact of DAF-2 knockdown on muscle health remains underexplored highlighting a gap in knowledge about the balance between lifespan extension and muscle preservation. For instance, Oh and Kim (2013) investigated DAF-2 knockdown in a muscular dystrophy model (dystrophin protein deficient models) and also found that reduced insulin signaling prevented muscle cell death and delayed degeneration (Oh and Kim, 2013), highlighting the critical role of DAF-2 in aging and proteostasis; however, while significant progress has been made in understanding the role of DAF-2 in dystrophin-deficient models, limited research has explored the independent effects of DAF-2 knockdown on muscle degeneration and lifespan in natural aging organisms (wild-type *C. elegans)* leaving a gap in understanding how DAF-2-reduction operates beyond specific disease models.

Research Question and Hypothesis

Building on the existing literature and addressing critical research gaps, this study focuses on the following question: *"How does DAF-2 knockdown via RNAi influence both lifespan extension and the progression of muscle degeneration in* C. elegans *over time?"* This question seeks to deepen our understanding of the well-established role of the DAF-2/DAF-16 insulin signaling pathway in promoting longevity, while also emphasizing the need to explore how this genetic manipulation impacts muscle health. Previous research has shown that certain small molecules, such as metformin, rapamycin, and sulforaphane, can influence the IIS pathway by either activating DAF-16 or reducing DAF-2 signaling, resulting in enhanced stress resistance and improved mitochondrial function (Qi et al., 2021). However, the effects of these interventions on muscle integrity remain unclear. By assessing muscle health through crawling speed tests and using staining techniques to analyze muscle fiber integrity, it is hypothesized DAF-2 knockdown

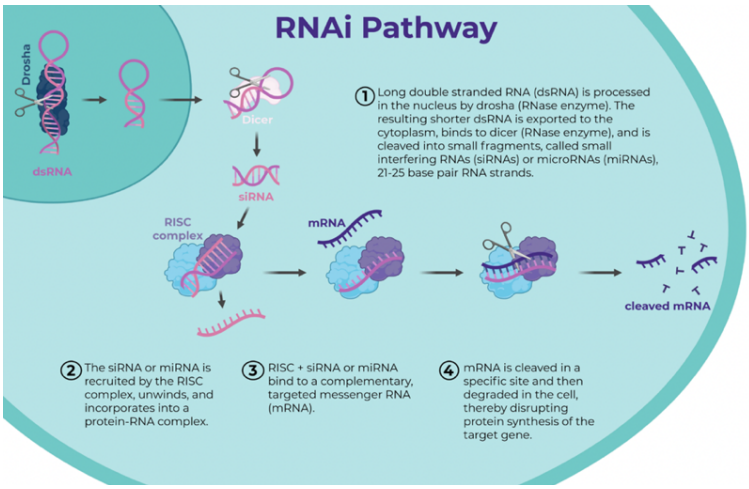
in *C. elegans* could directly contribute to muscle preservation, reducing muscle degeneration in addition to extending the organism's lifespan. The findings will provide a comprehensive view of the broader implications of modulating the IIS pathway in aging, shedding light on a less-explored aspect of how lifespan extension might affect physical function and overall health.

Materials and Methods

The experimental design of this study is set up to examine the influence of DAF-2 knockdown on lifespan and muscle function in *C. elegans*. It uses a comprehensive approach that integrates genetic, physiological, and structural analyses to better understand how IIS pathway modulation affects aging and muscle preservation.

RNA Interference (RNAi) for DAF-2 Knockdown

RNA interference (RNAi) (*Figure 5*) is a biological process through which gene expression is silenced by degrading specific messenger RNA (mRNA) molecules (Conte et al., 2015). This technique is widely used in *C. elegans* research to study gene function by selectively "knocking down" target genes (Conte et al., 2015). RNAi involves introducing double-stranded RNA (dsRNA) corresponding to the target gene into the organism. This dsRNA is recognized by the RNA-induced silencing complex (RISC), which degrades the complementary mRNA, thereby reducing or eliminating the production of the associated protein (Conte et al., 2015). Previous studies, such as those by Oh and Kim (2013), demonstrated the ability of RNAi-mediated DAF-2 knockdown to extend lifespan and improve muscle function in *C. elegans*. Based on these findings, RNAi was determined to be the most suitable method for this study.



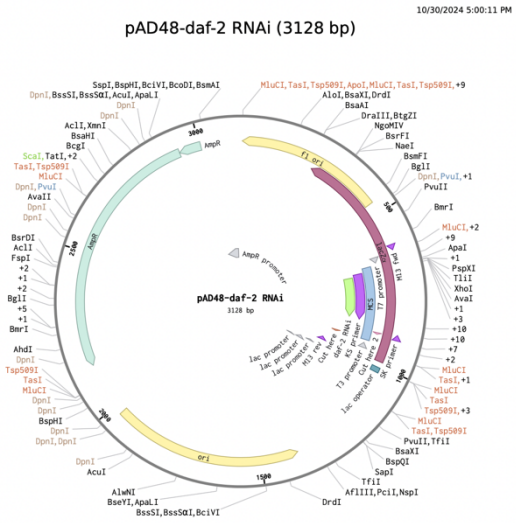
Source: https://www.umassmed.edu/rti/biology/rna/how-rnai-works/

` ***Figure 5.*** Overview of the RNA interference (RNAi) pathway to target and degrade specific messenger RNA (mRNA), thereby inhibiting gene expression.

The Role of IPTG in RNAi Method

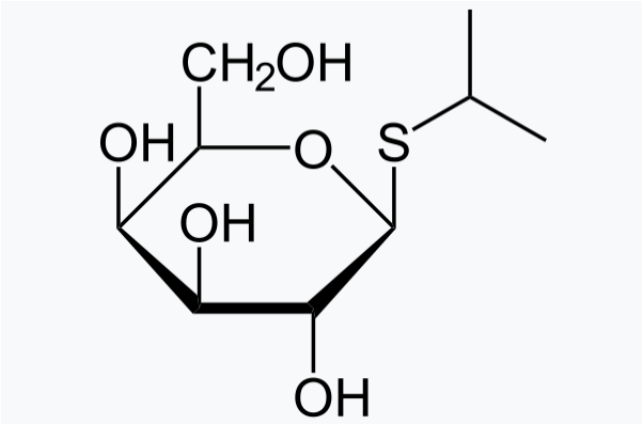
When RNAi is delivered through feeding, a common method involves genetically modified *E. coli* strains with pAD48-daf-2 RNAi plasmid, which produce dsRNA. These bacteria are engineered to contain these plasmids (*Figure 6*) with the gene of interest under the control of a T7 RNA polymerase promoter (Adler & Alvarado, 2018). To induce the production of dsRNA in the bacteria, IPTG (Isopropyl β-D-1-thiogalactopyranoside) (*Figure 7*) is used. IPTG acts as an inducer by mimicking allolactose, a natural metabolite of lactose metabolism (*Figure 8*). It binds to and inhibits the LacI repressor, which otherwise blocks the expression of the T7 RNA polymerase in the bacteria. By adding IPTG to the bacterial culture or worm growth medium, the T7 RNA polymerase is expressed, initiating the transcription of the target gene into dsRNA. When

the worms consume these transformed bacteria, the dsRNA is taken up by their cells and processed, triggering the RNAi response. (Kroemer)



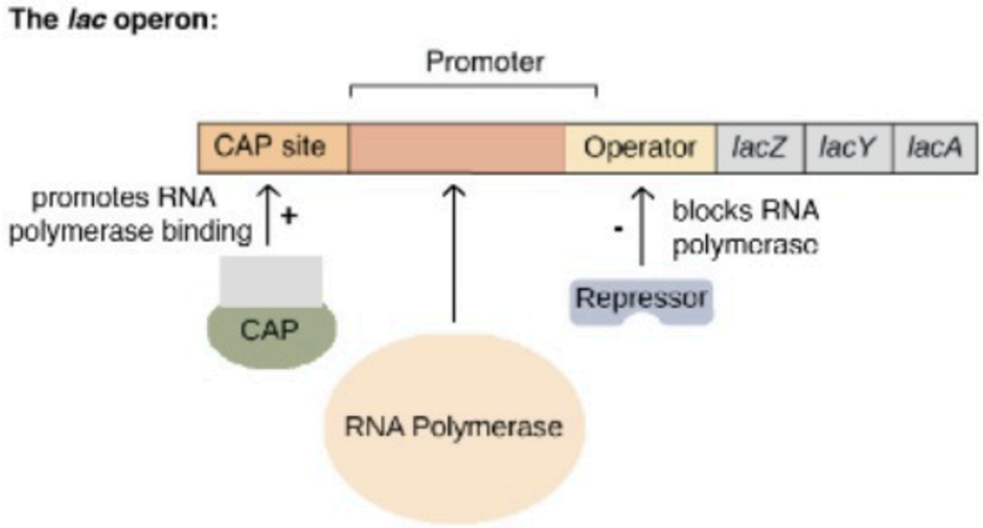
Source: https://www.addgene.org/34834

Figure 6. pAD48-daf-2 RNAi Plasmid Map for DAF-2 Knockdown in *C. elegans*. This 3128 bp plasmid targets the DAF-2 gene for RNAi, with T7 and T3 promoters driving gene silencing



Source: https://gendepot.com/products/iptg-isopropyl-beta-d-thiogalactopyranoside

Figure 7. Structure of IPTG (Isopropyl β-D-1-thiogalactopyranoside) a synthetic lactose analog used to induce gene expression by inactivating the lac repressor



Source: Capilano University, Eugene Chu Biol-300

Figure 8. The lac operon system illustrating the regulatory components involved in RNA polymerase activity.

Experimental Setup

Overview

The experimental workflow shown in *Figure 9* outlines the process for assessing the impact of DAF-2 knockdown on *C. elegans* lifespan, locomotion, and muscle integrity. The procedure consisted of three main phases: population maintenance, synchronization to align developmental stages, and experimental setup. Worms were divided into control and experimental groups, treated with FUdR to prevent reproduction, and monitored over defined time points for lifespan, mobility, and muscle structure assessments. This structured approach ensured consistency and reliability throughout the study.

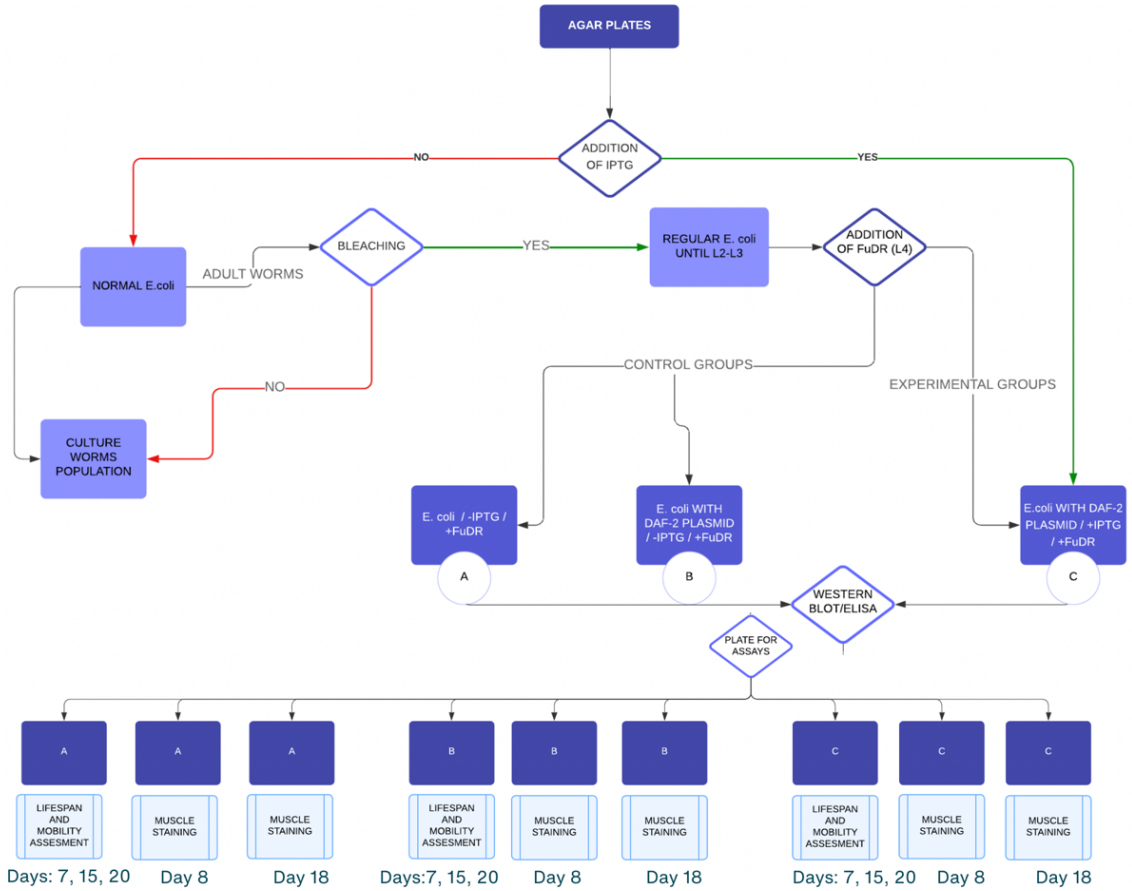


Figure 9. Overview of the study design illustrating the workflow for DAF-2 knockdown in *C. elegans*.

Preparation of Agar Plates and Induction Media

Agar plates were prepared using Nutrient Growth Media (NGM) to create RNAi-inducing and non-inducing conditions by supplementing the media with or without 1 mM IPTG. To prepare NGM plates, 3 g of Beef Extract, 15 g of agar, and 5 g of peptone were dissolved in 1 L of distilled water, sterilized via autoclaving, and allowed to cool. The medium (30 ml per plate) was poured into sterile Petri dishes and left to solidify at room temperature. Excess moisture was removed by drying the plates for 24 hours at room temperature. NGM agar plates were stored at 4°C and brought to room temperature before use.

The IPTG solution was prepared by dissolving 0.23831 g of IPTG in distilled water and adjusting the volume to 1 L to achieve a 1 mM concentration. Plates containing IPTG were prepared 4 days prior to the synchronization step to allow the solution to be fully absorbed and dried (Murphy & Hu, 2013).

Culturing and Seeding transformed and regular E. coli OP50 *for* C. elegans

For the *E. coli* OP50 stock culture, nutrient broth was prepared by dissolving 3 g of beef extract and 5 g of peptone in 1 L of distilled water. The medium was poured into multiple tubes and autoclaved to ensure sterility. When needed, tubes were inoculated with *E. coli* OP50 and incubated overnight at 37°C to establish a robust bacterial stock. Approximately 0.5 ml of OP50 *E. coli* from a stock solution was spread onto nutrient agar plates for regular OP50.

The stock solution for transformed *E. coli* was ordered from the laboratory and contained the RNAi plasmid as referenced in Figure 6. For *E. coli* containing the RNAi plasmid, IPTG was added to the plates 4 days prior, as outlined in Step 1, at a final concentration of 1 mM to induce dsRNA production for RNAi experiments. Regular OP50 *E. coli*, which does not require IPTG, was used as a control food source for *C. elegans*. The plates were incubated at 37°C for 24 hours to allow the formation of bacterial growth.

C. elegans *seeding into food source*

To begin the *C. elegans* culture, a sterile scalpel was used to excise small agar sections, approximately 1 cm x 1 cm, containing worms from the stock plate included in the *C. elegans* Culture Kit (Carolina Biological Supply, Catalog No. 173525). The kit supplied all necessary

materials, ensuring consistency and preventing contamination during worm maintenance. These agar segments, carrying worms, were gently placed onto pre-prepared plates seeded with *E. coli* OP50 as the food source. The plates were kept at 20°C, the optimal temperature for worm growth and reproduction. This procedure facilitated smooth transfer and allowed the worms to adapt and expand their population effectively while maintaining a contamination-free environment. This setup provided a stable foundation for further experimental work.

Synchronization of Worms Using the Bleaching Method

To synchronize *C. elegans* at the same developmental stage, a bleaching protocol was performed (Stiernagle, 2006). Adult worms were washed off NGM agar plates using M9 buffer, which, was prepared by dissolving 64 g of sodium phosphate dibasic heptahydrate, 15 g of potassium phosphate monobasic, 2.5 g of sodium chloride, and 5 g of ammonium chloride in distilled water to a final volume of 1 liter. M9 solution containing the worms was transferred to labeled microcentrifuge tubes for further bleaching and washing. That said, M9 supernatant was removed and 20% commercial bleach was added to lyse the adult worms and release the eggs. The mixture was vortexed for 1 minute and centrifuged at 1,000 rpm to pellet the eggs. The supernatant containing worm debris and excess bleach was carefully removed, and the eggs were washed three more times with sterile M9 buffer. For each wash, the pellet was resuspended in M9 buffer and centrifuged at 1,000 rpm for 1–2 minutes. After the final wash, the eggs were resuspended in M9 buffer, leaving the microcentrifuge tube half full, and placed on an orbital shaker at 20°C for 24 hours to allow the eggs to hatch into synchronized larvae (Stiernagle, 2006).

On the first day of their development cycle (24 hours after being placed on the shaker), the hatched larvae were transferred onto plates seeded with standard *E. coli* OP50 and allowed to grow until the third day.

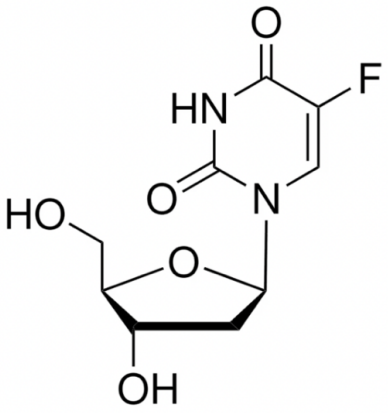
Experimental Groups

On Day 3, the synchronized worms were divided into three experimental groups and transferred to the respective plates to compare the effects of RNAi induction. The first group (Control 1) was fed with regular *E. coli* OP50, which contained no plasmid or IPTG, serving as a baseline control. The second group (Control 2) was fed *E. coli* containing the RNAi plasmid but without IPTG, ensuring no RNAi induction occurred. The third group (Experimental Group) was fed *E. coli* containing the RNAi plasmid, with IPTG added to induce dsRNA expression for RNAi. These experimental conditions were designed to distinguish between the baseline condition, the presence of the RNAi plasmid without induction, and the full RNAi induction to assess the specific effects of the RNAi process on the experimental outcomes.

Addition of FudR

FUdR (5-fluorodeoxyuridine) (*Figure 10*) was added to the plates on Day 6 to sterilize the worms by inhibiting DNA replication in germ cells. FUdR, a thymidine analog, interferes with DNA synthesis, specifically targeting highly mitotic cells while sparing somatic cells (Sutphin & Kaeberlein, 2009). Initially, a concentration of 25 μM was used; however, this dose proved lethal to the worms in our experiment.

To resolve this issue, we conducted troubleshooting by testing varying concentrations of FUdR (25 µM, 20 µM, 15 µM, and 10 µM) to identify a dose that was non-lethal yet effective for sterilization. These concentrations were prepared in 1 L solutions using the following quantities: 0.00615 g for 25 µM, 0.00492 g for 20 µM, 0.00369 g for 15 µM, and 0.00246 g for 10 µM.



Source:https://www.sigmaaldrich.com/CA/en/substance/5fluoro2deoxyuridine2461950919

Figure 10. Structure of FUdR (5-Fluoro-2'-deoxyuridine)

Planned Assays and Measurements

Several assays were planned to assess lifespan, locomotion, muscle degeneration, and protein quantification in *C. elegans*. Protein quantification was planned to assess the levels of DAF-2 protein to validate RNAi delivery method. For the lifespan assay, survival would have been monitored daily by counting live worms on dedicated plates until all worms perished. Kaplan-Meier survival analysis was intended to analyze the lifespan data (DataTab, 2024).

Following the study design (*Figure 9*) Locomotion was to be assessed using the Time-Off-Pick assay on Days 7, 15, and 20, where worms would be gently touched with a platinum pick or

eyebrow hair, and their movement response time recorded to quantify locomotion speed (Walker et al., 2022). Muscle degeneration was planned to be evaluated using eosin staining, a method that highlights muscle structure and integrity (Fischer et al., 2008). Separate plates were to be prepared for staining at two key time points: Day 8, to assess muscle structure in young worms, and Day 18, to evaluate age-related degeneration. The staining protocol involved transferring worms to slides, fixing them with paraformaldehyde, and applying eosin stain (Fischer et al., 2008). Since this process is lethal to the worms, separate plates would have been used for each time point.

Results

Current Progress and Observations

The study has successfully developed a comprehensive and detailed experimental design aimed at investigating the effects of DAF-2 knockdown on *Caenorhabditis elegans* lifespan and muscle degeneration. Despite the carefully outlined methodology, the limited time frame and logistical constraints have so far allowed for the collection of only one type of measurement: the survival assay which is still ongoing. Other planned assays, including those for DAF-2 quantification, locomotion and muscle degeneration, have yet to be conducted.

Key achievements during this experimental phase include the successful cultivation and maintenance of a synchronized *C. elegans* colony and the optimization of FUdR concentration, critical for ensuring accurate lifespan measurements. Through the series of trials (refer to methods section), the 20 μM concentration of FUdR, was identified as the most optimal dose. This concentration was non-lethal to the worms, providing a suitable environment for subsequent experimental procedures.

While the collected data is insufficient to reject or fail to reject the hypothesis at this stage, the study's foundational work ensures the feasibility of future trials to expand the dataset and explore the proposed research question in greater depth.

Expected Results

While results are not yet available, the expected outcomes (*Figure 11*) are based on prior research and the hypothesized impact of DAF-2 knockdown in *C. elegans*.

Speed Over Time (Time-Off-Pick Assay)

Time Point	Control (Speed)	DAF-2 Knockdown (Speed)
Early Stage	+++ (High)	++++ (Very High)
Mid Stage	++ (Moderate)	+++ (High)
Late Stage	+ (Low)	++ (Moderate)

Muscle Degeneration (Eosin Staining)

Time Point	Control (Degeneration)	DAF-2 Knockdown (Degeneration)
Early Stage	Low	Very Low
Mid Stage	Moderate	Low
Late Stage	High	Moderate

Kaplan-Meier Survival Trends

Time Point	Control (Survival)	DAF-2 Knockdown (Survival)
Early Stage	+++ (High)	++++ (Very High)
Mid Stage	++ (Moderate)	+++ (High)
Late Stage	+ (Low)	++ (Moderate)

Figure 11. Expected results for DAF-2 knockdown in *C. elegans*, showing improved speed and survival with reduced muscle degeneration across life stages compared to controls.

Lifespan Extension

The knockdown of DAF-2 is expected to result in a significant extension of *Caenorhabditis elegans* lifespan. Previous studies, such as Oh and Kim (2013), have shown that reducing insulin/IGF-1 signaling through DAF-2 knockdown can double the lifespan of worms. In this study, the experimental group is hypothesized to live 40–50 days, compared to the normal lifespan of 15–20 days observed in control groups. This extension is hypothesized to occur due to reduced signaling that prioritizes maintenance and survival pathways over reproduction and growth.

Muscle Degeneration and Mobility

Worms with DAF-2 knockdown are expected to maintain higher locomotion speeds compared to controls across their lifespan. Locomotion is anticipated to decline more gradually in the experimental group, with significantly better performance during mid-adulthood (Day 15) and late-adulthood (Day 20). This preservation of mobility aligns with previous findings that reduced insulin/IGF-1 signaling supports muscle function by mitigating age-related decline in movement speed. (Oh & Kim, 2013)

Structure

Muscle structural integrity, assessed through eosin staining, is expected to be better preserved in worms with DAF-2 knockdown. Experimental worms are hypothesized to exhibit lower levels of muscle deterioration at Day 8 (young adults) and Day 18 (older adults) compared to control groups. Structural analysis is anticipated to reveal fewer instances of fragmented or degraded muscle fibers in the experimental group, indicating delayed onset and reduced severity

of muscle degeneration. These results are consistent with prior research suggesting that reduced insulin/IGF-1 signaling protects muscle integrity over time. (Oh & Kim, 2013)

Discussion

Despite the absence of comprehensive results, this study has laid the groundwork for further investigation into the effects of DAF-2 knockdown on lifespan and muscle degeneration. The detailed study design and troubleshooting efforts ensure a robust experimental setup for future trials.

Limitations

This study faced several limitations that impacted its progress of the project. One key limitation was the optimization of IPTG for RNAi induction. Although IPTG was successfully used to induce RNAi, only a single concentration was tested throughout the experiment. Similar to the approach taken with FUdR, a systematic evaluation of varying IPTG concentrations would have provided insights into the optimal dose for consistent DAF-2 knockdown. Without such optimization, the effectiveness of RNAi induction may have varied, potentially affecting the results. This can be also be complemented with the lack of access to advanced molecular techniques, such as Western blotting as used by Koyuncu et al. (2021), or Northern blotting, which are essential for confirming RNAi efficiency and quantifying DAF-2 protein levels. These methods require a larger sample size (approximately 1,000–2,000 worms) and more time than was available for this study (Koyuncu et al., 2021). Similarly, qPCR validation was not feasible due to equipment constraints, limiting the ability to verify gene silencing at the transcript level.

Muscle degeneration analysis, initially planned using GFP imaging to visualize structural changes in muscle fibers, was another area where limitations were encountered. Although GFP imaging offers real-time insights into muscle health, the unavailability of advanced microscopy equipment and the need for further optimization prevented its implementation within the study timeline. Additionally, while the study included two controls—worms fed regular *E. coli* without a plasmid (to establish baseline conditions) and worms fed RNAi-inducing *E. coli* without IPTG (to assess plasmid effects without RNAi induction)—an empty vector control would have been an even better baseline. This control could help isolate any potential effects of the plasmid itself, independent of the RNAi process. By eliminating variables introduced by the plasmid backbone, an empty vector control would provide a more robust comparison and strengthen the study's conclusions. These limitations highlight areas for improvement in future iterations of the experiment, emphasizing the need for optimized protocols, additional controls, and access to advanced molecular tools.

Conclusion

Future Research Directions

Future research will focus on addressing the current limitations by enhancing experimental rigor and expanding the dataset. Additional trials will be conducted to improve the reproducibility and reliability of the findings, ensuring that results are statistically robust and representative. These trials will include increased replicates for the lifespan and locomotion assays, as well as the completion of the muscle degeneration analysis. Furthermore, the ELISA method will be performed to validate the knockdown of DAF-2 at the protein level, providing critical molecular evidence to strengthen the connection between observed phenotypes and reduced insulin/IGF-1

signaling. Additionally, future studies could explore the translational relevance of these findings by investigating how specific dietary interventions, such as low glycemic index or caloric restriction diets, mimic reduced insulin/IGF-1 signaling in humans. This step would help identify dietary strategies that could potentially delay aging and improve muscle health, bridging the gap between model organism research and human health applications.

Broader Implications and Clinical Relevance

The findings of this study hold significant implications for both aging research and broader societal health. By elucidating the role of insulin/IGF-1 signaling in lifespan and muscle degeneration, this research contributes to the growing body of knowledge on age-related diseases, such as sarcopenia and muscular dystrophy. Potential therapeutic strategies targeting this pathway could delay muscle atrophy and extend healthspan, improving the quality of life for aging populations.

Moreover, this study highlights the broader importance of metabolic regulation and its impact on cellular health. The role of glucose and insulin in the insulin/IGF-1 signaling pathway demonstrates a critical trade-off between reproduction and survival. Active insulin signaling, associated with high glucose availability, prioritizes growth and reproduction but accelerates aging by reducing repair and stress resistance. Conversely, reduced insulin signaling, triggered by low glucose levels, shifts focus toward energy conservation, repair processes, and stress resistance, promoting longevity. Insights from *C. elegans* research provide a deeper understanding of how modern dietary patterns and metabolic regulation influence aging processes. These findings could inform public health strategies aimed at improving metabolic health and longevity, empowering individuals to make lifestyle choices that support overall well-being and healthy aging.

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