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ORIGINAL ARTICLE

Effect of Dietary Restriction on the Healthspan of *Caenorhabditis elegans*

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ABSTRAK

Pembatasan diet (DR) diketahui dapat meningkatkan jangka hayat Caenorhabditis elegans, namun kesannya terhadap tempoh kesihatan kurang jelas. Kajian ini menyiasat kesan DR terhadap parameter kesihatan. Cacing N2 (n=40) dikulturkan pada plat 'Nematode Growth Medium' dengan E. coli, di mana DR diinduksi dengan mencairkan kepekatan bakteria sebanyak 100 kali. Jangka hayat direkodkan setiap hari, manakala pengepaman faring dan pergerakan diukur pada hari ke-5, 9, 13, 17 dan 21. Jangka hayat maksimum untuk kumpulan DR ialah 28 hari, berbanding 25 hari untuk kumpulan kawalan. Penurunan pergerakan dari hari ke-5 hingga hari ke-21 adalah lebih perlahan dalam kumpulan DR (54.7%) berbanding kumpulan kawalan (62.9%). Selain itu, tahap pergerakan kumpulan DR kekal lebih tinggi secara konsisten berbanding kumpulan kawalan sepanjang tempoh kajian (p<0.05). Pengepaman faring menurun sebanyak 62.3% dalam kumpulan DR berbanding 89.2% dalam kumpulan kawalan, dengan kumpulan DR menunjukkan tahap pengepaman faring yang lebih tinggi di sepanjang kajian (p<0.05). Hasil ini mencadangkan bahawa DR mungkin memperbaiki jangka kesihatan dengan memelihara mobiliti semasa penuaan. Kajian lanjut dengan saiz sampel yang lebih besar disarankan untuk mengesahkan kesan DR terhadap jangka hayat.
Kata kunci: *Kesihatan; pemakanan; penuaan*

ABSTRACT

Dietary restriction (DR) is known to increase lifespan in *Caenorhabditis elegans*, but its impact on healthspan is less clear. This study investigated the effects of DR on health parameters. N2 strain nematodes (n=40) were cultured on Nematode Growth Medium (NGM) plates with *E. coli*, where DR was induced by diluting the bacterial concentration 100-fold. Lifespan was recorded

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daily, and pharyngeal pumping and locomotion were measured on days 5, 9, 13, 17 and 21. Maximum lifespan for the DR group was 28 days, compared to 25 days for the control. The decline in locomotion from day 5 to day 21 was slower in the DR group (54.7%) compared to the control (62.9%). Additionally, locomotion levels in the DR group remained consistently higher than the control throughout the study period ($p<0.05$). Pharyngeal pumping decreased by 62.3% in the DR group compared to 89.2% in the control group, with the DR group showing significantly higher pharyngeal pumping levels throughout the study ($p<0.05$). These results suggest that DR may improve functional healthspan by preserving mobility during ageing. Further studies with larger sample sizes are recommended to confirm DR's impact on lifespan.
Keywords: Ageing; diet; health

INTRODUCTION

Dietary restriction (DR) is the practice of reducing specific or overall nutrient consumption without inducing malnutrition. This comprehensive approach encompasses caloric restriction (CR), which involves decreasing total food intake, as well as investigations into limiting significant dietary elements such as protein, fat or carbohydrates, and altering the timing of food consumption, as seen in intermittent fasting, within mammalian subjects (Robertson & Mitchell 2013). Experimental evidence consistently demonstrates that DR effectively mitigates the age-related physiological alterations in mammals (Tonini et al. 2021a). DR effectively combats obesity, reduces the impact of free radicals, and enhances the availability of antioxidants, contributing to extended lifespans. It significantly reduces toxicity and lethality from various chemicals and drugs by promoting tissue repair and restoring organ function (Hahn et al. 2019). From a human health perspective, the manifold advantages of DR are noteworthy (Vidoni et al. 2021).

Health span refers to the time starting at birth and extending until an individual's health is no longer optimal, marked by the onset of diseases or ageing-related disabilities. This concept, similar to lifespan, allows for the quantitative assessment of how genetics, environment or interventions influence health span. It also facilitates the comparison of these factors' effects on health span relative to lifespan. Clinically, interventions that extend health span would be of considerable value. Conversely, it is theoretically possible that some interventions could extend lifespan without a proportionate or greater extension of health span, a scenario that we would ideally want to avoid in human populations (Kaeberlein 2018). Ageing research has made significant strides in uncovering signaling pathways and conserved genes linked to lifespan extension. However, uncertainty persists about whether these interventions truly prolong the healthy phase of life or simply extend a period marked by frailty and increased age-related diseases. Beyond lifespan, preserving health span is increasingly recognised as the ultimate goal, as exceeding health span often leads to chronic illness and declining quality of life (Peterson 2017). Recent findings have highlighted that while genetic and dietary interventions, such as CR, often extend lifespan in model organisms, the translation of these benefits into increased health span in humans remains inconsistent (Rollins et al. 2023).

Emerging tools like biological ageing clocks and gut microbiome profiling are now being explored to address this gap, aiming to provide a more precise understanding of health span markers and their relationship with lifespan (Smith et al. 2023). Studies also reveal that lifestyle factors, including diet and exercise, play more significant roles than genetics in determining health outcomes, underscoring the importance of interventions that focus on improving health span rather than solely extending life (Tonini et al. 2021b). Preserving health span is increasingly recognised as the ultimate goal, as exceeding health span often leads to chronic illness and declining quality of life. This shift in focus represents a critical evolution in ageing research, highlighting the need to prioritise strategies that enhance both longevity and the quality of life during extended years (JAMA Network 2023).

Caenorhabditis elegans (*C. elegans)* is a leading model organism used to investigate the intrinsic relationship between lifespan and health span (Bansal et al. 2015). *C. elegans* is a nematode worm with a simpler anatomy than humans but shares many molecular similarities, making it an excellent model organism. It is easy to maintain during its short 2-week lifespan and its transparency allows for detailed observation of cell fates using basic or advanced microscopy techniques. Its distinct life phases provide valuable insights into ageing mechanisms, both physiologically and genetically (Mullan & Marsh 2019). *C. elegans* ease of handling, minimal nutritional and growth requirements and rapid self-fertilisation, which yields numerous offspring in a short time. It was the first multicellular organism to have its genome sequenced, marking a significant milestone in genomics (Meneely et al. 2019). *C. elegans* is able to mimic human diseases and has a rapid life cycle. It shares notable physiological parallels with humans, such as in the digestive, nervous and reproductive systems. Additionally, *C. elegans* demonstrates the capacity to recognise and respond to various stimuli, indicating a level of complex behaviour (Yokoyama 2020). The question remains whether the gap between lifespan and health span should be a concern when applying ageing research. The extended phase of frailty observed in long-lived *C. elegans* mutants could have severe economic and social repercussions if mirrored in humans (Rollins et al. 2017).

MATERIALS AND METHODS

General Methods and Strains

C. elegans nematodes were cultured on Nematode Growth Medium (NGM) plates, prepared in the laboratory using agar, sodium chloride, peptone and cholesterol (Sigma-Aldrich, USA). The NGM plates were seeded with the *E. coli* OP50 strain to serve as a food source. The *C. elegans* strain used in this study was the N2 strain, which was obtained from the Medical Molecular Biology Institute (UMBI) at Universiti Kebangsaan Malaysia (UKM) (Siti Bazilah et al. 2022). DR was achieved by diluting the concentration of *E. coli* 100 times, from $1x10^{11}$ cfu/ml to $1x10^{9}$ cfu/ml based on a previously established method (Loo et al. 2024).

Lifespan Analysis

The lifespan analysis of *C. elegans* was carried out based on previous studies (Bansal et al. 2015). The analysis was conducted using two distinct groups which were control and DR groups. A synchronised population of 40 first-stage larvae (L1) of the N2 strain was initially transferred onto new plates containing either the control *E. coli* concentration or the DR amount. The plates were replaced every two days. Each plate was supplemented with 5-fluorodeoxyuridine and antibiotics (kanamycin and carbenicillin). The number of living worms was documented daily under a light microscope. A platinum wire was used to gently tap the dead and inactive worms; a lack of response was recorded as dead to differentiate between dead and inactive worms. Health span parameters were measured on days 5, 9, 13, 17 and 21.

Locomotion Assay

Locomotion analysis was conducted on NGM plates (Bansal et al. 2015). Five worms were randomly selected from each plate and observed under a light microscope (Nikon, Japan) equipped with a camera and computer software for visualising and recording their movements. The polyline tool in the software was used to track the distance traveled by the worms in real-time over a one-minute period, with the recorded distances measured in micrometers.

Food Seeking Behaviour (Pharyngeal Pumping)

Five worms were randomly selected from each plate and observed under a light microscope equipped with a camera and software for visualisation and recording. The pharyngeal pumping behaviour, reflecting food-seeking activity, was manually counted for one minute while the worms were on seeded NGM plates. Measurements were taken on days 5, 9, 13, 17 and 21. The recordings were captured using VLC software (version 2.1.3).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, IBM Corp, Armonk, New York) software. Lifespan was compared between the control and DR groups using an independent t-test. Analysis of variance (ANOVA) was conducted for each health marker to estimate the effects of treatment, age and their interaction.

RESULTS AND DISCUSSION

Lifespan of *C. elegans*

Lifespan is influenced by a combination of genetic and environmental factors, including diet and metabolism (Lee et al. 2006). This study explored an effective approach to examine how DR affects longevity in *C. elegans*. We utilised dietary deprivation, achieved by limiting the food source (*E. coli*), to extend the lifespan of *C. elegans*.

In our experiment, we compared two groups: a control group (N=39) and a DR group (N=20). The mean lifespan for the DR group was 18.85 days, slightly longer than the control group's mean lifespan of 18.13 days (Figure 1). Previous studies suggested that DR can provide modest increases in lifespan (Smith et al. 2008). However, we found that the differences between the lifespan of DR group was not statistically significant ($p>0.05$) compared to the control. The lack of a statistically significant difference in the lifespan could be attributed to several factors including suboptimal DR regimen. DR is highly sensitive to the degree of food limitation, and both excessive and insufficient restriction can negate lifespan benefits. Greer and Brunet (2009) demonstrated that DR's impact on *C. elegans* lifespan depends on precise modulation of dietary intake, suggesting that deviations could nullify its effects. Variations in environmental factors such as temperature, bacterial food quality or contamination could also influence the outcomes. For example, the composition and freshness of *E. coli OP50*,

FIGURE 1: Lifespan assay of *C. elegans* in control and DR groups

commonly used as food for *C. elegans*, has been found to affect metabolic and longevity outcomes (Gomez-Amaro et al. 2015). In addition, the small sample size used in this study could reduce the statistical power to detect differences. Therefore, a larger cohort is necessary to observe subtle effects in the future.

Although the DR group had slightly higher mean and maximum lifespan values compared to the control group, the difference was not statistically significant ($p>0.05$). This suggests that the results may be due to technical limitations, such as worms moving away or missing from the media. Based on previous research, transient DR has been shown to confer long-term benefits, including increased stress resistance and longevity (Smith et al. 2008). Additionally, it is noted that DR correlates with reduced food sensing, which leads to decreased food consumption

Previous studies have highlighted that DR regimens impact the lifespan of *C. elegans*. For instance, AMP-activated protein kinase (AMPK) and the Forkhead transcription factor FoxO (daf-16) play crucial roles in promoting longevity under certain DR regimens (Greer & Brunet 2009). AMPK is activated in response to low energy levels, helping to maintain cellular energy balance, while FoxO regulates genes involved in stress resistance and metabolism. Together, these proteins are essential for lifespan extension, as AMPK activation enhances FoxO activity, leading to improved stress resilience, enhanced metabolic processes and ultimately increased longevity.

Furthermore, previous study demonstrated that DR affects the insulin/insulin-like growth factor-1 (IGF-1) signaling pathway in *C. elegans* (Dall & Færgeman 2019). Reduced food intake lowers insulin-like peptide levels which decrease IGF-1 signaling. IGF-1 signaling is closely tied to the PI3K/Akt/mTOR pathway, a regulator of cell growth and metabolism. Lower IGF-1 levels reduce mTOR activity, which in turn decreases cell proliferation and metabolic rates, slowing the ageing process. This reduced metabolic activity helps conserve energy and reduces oxidative damage from reactive oxygen species (ROS), which are key contributors to ageing.

In general, DR has a notable impact on

longevity, primarily through modulation of the insulin/IGF-1 signaling pathway, nutrient-sensing pathways and stress responses. Although the observed lifespan extension in *C. elegans* was modest, these findings underscore the potential of DR in influencing the ageing process. The observed results could be limited by technical factors as mentioned previously. Improvising these limitations in the future may provide better outcome which may be valuable for broader applications, potentially extending to higher organisms, including humans.

Locomotion Assay

Our study demonstrated that DR significantly influenced the locomotion of *C. elegans*, as evidenced by the higher mean values of distance covered by the DR group compared to the control group (Figure 2). These higher mean values were seen on all data collection days; day 5, day 9, day 13, day 17 and day 21. Despite the steady decline of mean for both groups as they aged, the DR group consistently maintained a higher mean until the end. These findings align with those of previous studies that showed worms which were provided with DRs had a higher movement capacity (Loo et al. 2023).

The movement capacity of *C. elegans* is used as an indicator of its neuromuscular health (Siti Bazilah et al. 2022). The decreasing trend of locomotion values for both the DR and control group can be ascribed to the mitochondrial dysfunction and sarcopenia-related myofibril disorganisation that occurs as the worms age (Loo et al. 2023). The observed higher locomotion in DR may be due to a slower muscular degradation rate (Loo et al. 2023). In addition, DR may improve mitochondrial function and energy efficiency through the activation of AMPK and sirtuins (Mair et al. 2009). These pathways increase ATP production, thereby supplying more energy for muscle contraction and movement. Alternatively, the increased activity could reflect an adaptive behavioural response to food scarcity, where enhanced locomotion increases the likelihood of encountering food sources, suggesting an evolutionary survival

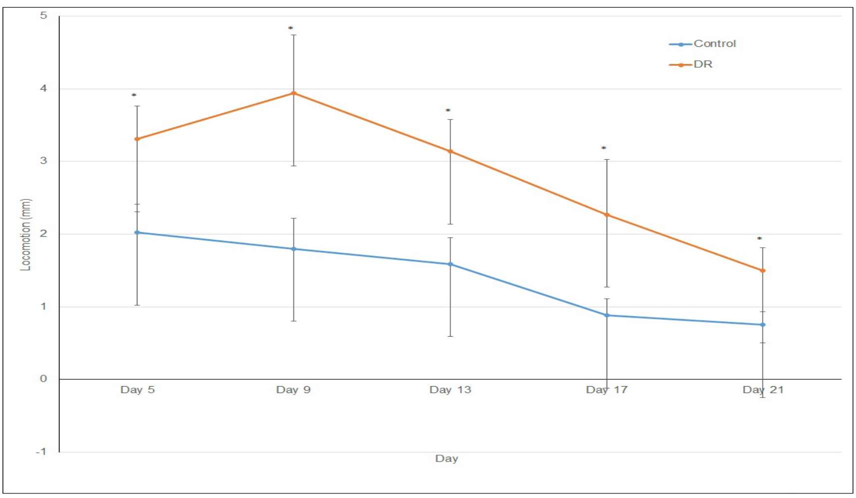

FIGURE 2: Locomotion assay of *C. elegans* for DR and control groups. Nematodes on DR had higher locomotion than the control group across different days (* $p<0.05$)

mechanism.

Interestingly, the increased locomotor activity observed in DR worms may represent an adaptive behavioural response (Shtonda & Avery 2006). Under food-scarce conditions, increased movement might enhance the likelihood of locating food sources, reflecting an evolutionary survival mechanism in *C. elegans*.

The effect of DR on locomotion can be translated into humans as CR effect on physical activity. It was observed in one study that CR increases energy efficiency as well as boosts physical performance (Capó et al. 2020). Despite these promising insights, it remains unclear whether the observed effects on locomotion are directly due to improved muscle function or altered neural signaling. Additionally, DR-induced changes in other physiological systems, such as metabolic rate or stress resistance, could indirectly influence locomotor activity. Further research is needed to disentangle these effects and identify the molecular pathways involved.

Our results indicate that DR positively affects the locomotor activity of *C. elegans*, shedding light on the complex interplay between diet, metabolism and behaviour. This work provides a foundation for future studies to understand how dietary interventions may mitigate age-related declines in physical activity.

Food-Seeking Behaviour

The rate of pumping determines both the amount of food consumed and the rate of growth. The motor neuron (MC) that initiates pharyngeal muscle contraction directly determines the pumping rate (Avery & You 2012). Therefore, *C. elegans*' food-seeking behaviour is dependent on the action of the pharynx, a neuromuscular organ with typical pumping motions (Sherman & Harel 2024).

The worms with DR showed a significantly higher pumping rate compared to the control (Figure 3). The impact of the animal's nutritional status and prior feeding history, both of which influence feeding and satiety in mammals, on feeding has never been explored. Therefore, previous research shows that under routine

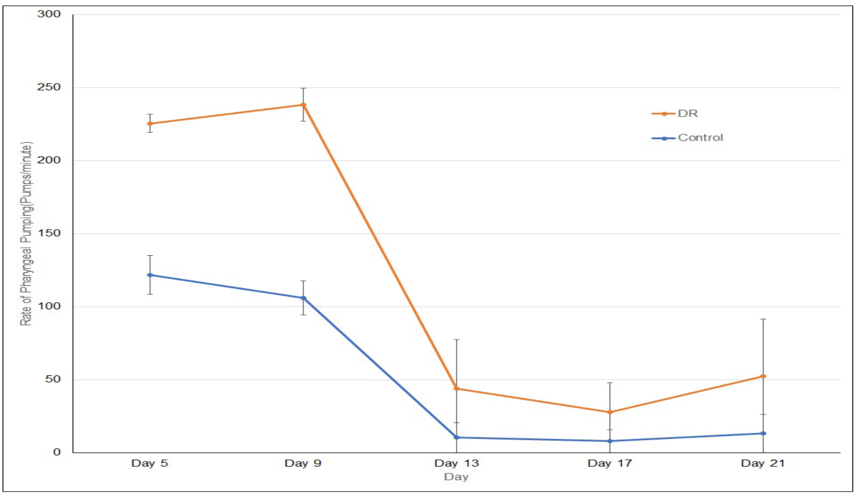

FIGURE 3: Rate of Pharyngeal Pumping for control and DR group in one minute. There a significant difference in pharyngeal pumping between control and DR groups across different days (*p<0.05).

laboratory conditions, worms feed and move constantly. However, under certain conditions, worms stop feeding and moving and become quiescent. Thus, the previous study discovered that in response to food quality, food intake and rate of food absorption in the intestine, led to the discovery of another feeding behaviour known as satiety quiescence (You et al. 2008).

In *C. elegans*, satiety quiescence resembles behavioural aspects of satiety and postprandial sleep in mammals such as in humans where they act when they feel full and sleepy after eating (Gallagher et al. 2013). This suggests why worms with control have lower numbers of pharyngeal pumping than the DR group. Another study discovered that hunger causes the release of a protein called acyl-CoA-binding protein (ACBP) from cells, increasing its levels in the blood. Extra ACBP increases appetite and promotes weight gain, whereas blocking ACBP decreases hunger. This suggests that ACBP plays an important role in increasing appetite and fat storage. Therefore, in *C. elegans* ACBP increased bacterial feeding and pharyngeal pumping, indicating that ACBP is an ancient hunger signal that encourages eating across species (Charmpilas et al. 2020). Consequently, this suggests that the DR group may exhibit increased ACBP due to reduced concentration of the food.

As *C. elegans* worms get older, their pharyngeal pumping declines and becomes very weak by day 12. Our results showed a rapid decline in both worms with control and DR at day 13. Our data indicated that age had a significant effect on pharyngeal pumping ($p<0.05$). Constant pumping of the pharynx causes their muscles to work harder, which can lead to faster muscle damage during ageing. As a result, eating less helps protecting their muscles and delaying ageing (Loo et al. 2023). Even though both control and DR groups showed rapid decline particularly at day 13, worms with DR still had a higher rate of pharyngeal pumping than the control group. Worms with DR may have a higher pharyngeal pumping rate due to their active search for food, resulting in slower degradation of muscle function (Charmpilas et al. 2020).

CONCLUSION

Our study explored the impact of DR on the lifespan, locomotion and pharyngeal pumping behaviour of *C. elegans*, aiming to understand the relationship between lifespan extension and health span maintenance. Although the lifespan extension observed in the DR group was modest and not statistically significant, the trends suggest that dietary interventions could potentially influence longevity, particularly with the use of a larger sample size and a tightly controlled DR regimen in future studies.

The DR group exhibited better locomotor activity than the control group throughout the observation period, despite both groups showing a natural decline with age. This supports the notion that DR may preserve neuromuscular function by enhancing mitochondrial efficiency and energy production or through adaptive responses to food scarcity. Similarly, increased pharyngeal pumping rates in DR worms indicate heightened food-seeking behaviour, likely reflecting an evolutionary survival strategy under nutrient-limited conditions. These behavioural adaptations, while is beneficial in *C. elegans*, could potentially translate to improvements in physical activity and muscle function in higher organisms, including humans.

Our findings contribute to the understanding of how DR influences both lifespan and healthspan, emphasising the need for future studies to dissect the molecular underpinnings of these effects and address the limitations noted in our study. Refining experimental techniques to minimise technical factors, such as worm loss and plate conditions, will help achieving more conclusive results. Expanding this research to explore the potential translatability of DR's effects on health maintenance in higher organisms could provide valuable insights for ageing interventions aim at enhancing quality of life without merely extending frailty.

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REFERENCES

Avery, L., You, Y.J. *C. elegans* feeding. 2012. WormBook **21**: 1-23.

Bansal, A., Zhu, L.J., Yen, K., Tissenbaum, H.A. 2015. Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proc Nat Acad Sci* **112**(3): 277-86.

Capó, X., Martorell, M., Ferrer, M.D., Sureda, A., Pons, V., Domingo, J.C., Drobnic, F., Martínez-Rodríguez, A., Leyva-Vela, B., Sarabia, J.M., Herranz-López, M., Roche, E., Tur, J.A., Pons, A. 2020. Calorie restriction improves physical performance and modulates the antioxidant and inflammatory responses to acute exercise. *Nutrients* **12**(4): 930.

Charmpilas, N., Ruckenstuhl, C., Sica, V., Büttner, S., Habernig, L., Dichtinger, S., Madeo, F., Tavernarakis, N., Bravo-San Pedro, J.M., Kroemer, G. 2020. Acyl-CoA-binding protein (ACBP): A phylogenetically conserved appetite stimulator. *Cell Death Dis* **11**(1): 7.

Dall, K.B., Færgeman N.J. 2019. Metabolic regulation of lifespan from a *C. elegans* perspective. *Genes Nutr* **14**: 25.

Gallagher, T., Kim, J., Oldenbroek, M., Kerr, R., You, Y.J. 2013. ASI regulates satiety quiescence in *C. elegans*. ASI Regulates Satiety Quiescence in *C. elegans*. *J Neurosci* **33**(23): 9716-24 .

Gomez-Amaro, R.L., Valentine, E.R., Driscoll, M. 2015. Absence of dietary restriction-induced longevity in *C. elegans* that eat liquid bacterial suspension. *Aging Cell* **14**(1): 20-8.

Greer, E.L., Brunet, A. 2009. Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. *Aging Cell* **8**(2): 113-27.

Hahn, O., Drews, L. F., Nguyen, A., Tatsuta, T., Gkioni, L., Hendrich, O., Zhang, Q., Langer, T., Pletcher, S., Wakelam, M.J.O., Beyer, A., Grönke, S., Partridge, L. 2019. A nutritional memory effect counteracts the benefits of dietary restriction in old mice. *Nat Metab* **1**(11): 1059-73.

Kaeberlein, M. 2018. How healthy is the healthspan concept? *Geroscience* **40** (4): 361-4.

Lee, G.D., Wilson, M.A., Zhu, M., Wolkow, C.A., De Cabo, R., Ingram, D.K., Zou, S. 2006. Dietary deprivation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* **5**(6): 515-24.

Loo, J., Gunasekaran, G., Tan, J.K., Goon, J.A. 2024. Elucidating the effective age for dietary restriction and the key metabolites involved. *Exp Gerontol* **197**: 112601.

Loo, J., Shah Bana, M.A.F., Tan, J.K., Goon, J.A. 2023. Effect of dietary restriction on health span in *Caenorhabditis elegans*: A systematic review. *Exp Gerontol* **182**: 112294

Mair, W., Panowski, S.H., Shaw, R.J., Dillin, A. 2009. Optimizing dietary restriction for genetic epistasis analysis and gene discovery in *C. elegans*. *PLoS ONE* **4**(2): 4535.

Meneely, P.M., Dahlberg, C.L., Rose, J.K. 2019. Working with worms: *Caenorhabditis elegans* as a model organism. *Curr Protoc Ess Lab Tech* **19**(1): 35.

Mullan, A., Marsh, A. 2019. Advantages of using *Caenorhabditis Elegans* as a Model Organism. [Oxford Insturments]. https://andor.oxinst.com/learning/view/article/advantages-of-using-caenorhabditis-elegans-as-a-model-organism [Accessed 24 September 2024]

Peterson, T. 2017. Healthspan is more important than lifespan, so why don't more people know about it? Harvey [A. Friedman Center for Aging]. https://publichealth.wustl.edu/heatlhspan-is-more-important-than-lifespan-so-why-dont-more-people-know-about-it/ [Accessed 30 May 2017]

Robertson, L.T., Mitchell, J.R. 2013. Benefits of short-term dietary restriction in mammals. *Exp Gerontol* **48**(10): 1043-8.

Rollins, J., Smith, T., Nguyen, L., Patel, R., Chen, D. 2023. Recent advancements in *C. elegans* aging research. *J Aging Res* **45**(3): 202-15.

Rollins, J.A., Howard, A.C., Dobbins, S.K., Washburn, E.H., Rogers, A.N. 2017. Assessing health span in *Caenorhabditis elegans*: Lessons from short-lived mutants. *J Gerontol A Biol Sci Med Sci* **72**(4): 473-80.

Sherman, D., Harel, D. 2024. Deciphering the underlying mechanisms of the pharyngeal pumping motions in *Caenorhabditis elegans*. *Proc Nat Acad Sci* **121**(7): 2302660121.

Shtonda, B.B., Avery, L. 2006. Dietary choice behavior in *Caenorhabditis elegans*. *J Exp Biol* **209**(1): 89-102.

Siti Bazilah, Z., Geetha G, Goon JA. 2022. Uncoupling healthspan and lifespan in long-lived Daf-2, Pmk-3 and Sgk-1 knockdown *Caenorhabditis elegans*. *Med Health* **17**(2): 198-215

Smith, E.D., Kaeberlein, T.L., Lydum, B.T., Sager, J., Welton, K.L., Kennedy, B.K., Kaeberlein, M. 2008. Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in *Caenorhabditis elegans*. *BMC Dev Biol* **8**: 49.

Smith, P.J., White, J.L., Gonzalez, R.T., Taylor, E.P., Andrews, M.E. 2023. Perspectives on the healthspan-lifespan relationship and clinical implications. *JAMA* **329**(15): 1205-10.

Tonini, C., Salazar, G., Varela, R., Henderson, M., Yu, S. 2021a. Dietary interventions and healthspan: Lessons from model organisms. *Ageing Res Rev* **65**: 101234.

Tonini, C., Segatto, M., Martino, F., Cigliano, L., Nazzaro, M., Barberio, L., Mandalà, M., Pallottini, V. 2021b. Effects of late-life caloric restriction on age-related alterations in the rat cortex and hippocampus. *Nutrients* **13**(1): 232.

Vidoni, C., Ferraresi, A., Esposito, A., Maheshwari, C., Dhanasekaran, D.N., Mollace, V., Isidoro, C. 2021. Calorie restriction for cancer prevention and therapy: Mechanisms, expectations, and efficacy. *J Cancer Prev* **26**(4): 224-36.

Yokoyama, M. 2020. *C. elegans as a Model Organism*. [News Medical Life Sciences]. https://www.news-medical.net/life-sciences/C-elegans-as-a-Model-Organism.aspx [Accessed 27 May 2020].

You, Y.J., Kim, J., Raizen, D.M., Avery, L. 2008. Insulin, cGMP, and TGF-β signals regulate food intake and quiescence in *C. elegans*: A model for satiety. *Cell Metab* **7**(3): 249-57.