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New Haystacks Reveal New Needles: Using *Caenorhabditis elegans* to Identify Novel Targets for Ameliorating Body Composition Changes during Human Aging

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Abstract

Dramatic changes in body composition accompany aging in humans, particularly with respect to adiposity and the musculature. People accumulate fat as they age and lose muscle mass and strength. *Caenorhabditis elegans* nematodes are small, hermaphroditic soil nematodes that offer a flexible model for studying genetic pathways regulating body composition in humans. While there are significant physiological differences between worms and people, many of the genetic pathways relevant to human lipid and muscle homeostasis are present in worms. Initial studies indicate that adiposity increases in *C. elegans* during aging, as occurs in humans. Furthermore, substantial evidence demonstrates age-related loss of muscle mass in worms. Possible mechanisms for these changes in *C. elegans* are presented. Recent studies have highlighted neuroendocrine and environmental signals regulating *C. elegans* fat metabolism. Potential dysfunction of these pathways during aging could affect overall fat accumulation. By contrast, muscle decline in aging worms results from accumulated damage and ‘wear-and-tear’ over life span. However, neuroendocrine pathways also regulate muscle mass in response to food availability. Such pathways might provide useful therapeutic approaches for combating muscle loss during aging. From this chapter, readers will develop a deeper understanding of the ways that *C. elegans* can be used for mechanistic gerontological Studies.

Introduction to *Caenorhabditis elegans*

C. elegans as a Model for Gerontological Studies

C. elegans nematodes are small soil nematodes that have been adopted by researchers as a simple experimental model for investigating physiological and developmental processes in a whole organism [1]. There are several characteristics that make *C. elegans* a powerful experimental approach for gerontology. One, the body is transparent and the tissues can be easily visualized through a microscope in live animals. This allows visual inspection for aging-related anatomical changes. Two, worms are small, have a short life cycle and can be maintained economically on agar-based medium in Petri dishes, without any specialized equipment. These features facilitate age-synchronization of large populations for longitudinal or cross-sectional studies, providing statistical robustness. Three, *C. elegans* are

amenable to a variety of experimental manipulations facilitating mechanistic studies. Investigations commonly involve genetic analysis, through forward and reverse genetic approaches, which are complemented by cellular and behavioral studies.

Genetic studies have been tremendously successful for identifying new pathways regulating *C. elegans* longevity [2]. In many cases, genes controlling longevity also impact tissue aging, such that mutants maintain high functionality late into life [3]. Genes with this phenotype activate stress-resistance programs, for example the insulin/IGF-I pathway which regulates expression of heat-shock proteins and antioxidant enzymes [4–6]. Similarly, the antioxidants regulated by the SKN-1/Nrf family transcription factor also promote longevity and stress resistance [7]. This link between stress resistance and longevity suggests that tissue decline during *C. elegans* aging results at least in part from tissue damage, potentially by oxidative insult and proteotoxicity.

Regulation of Adiposity in *C. elegans*

Evidence for Fat Accumulation during Aging in *C. elegans*—Worms lack specialized lipid storage cells, but accumulate fat in the intestine and hypodermis [9]. The *C. elegans* intestine serves digestive, detoxification and metabolic storage functions, thus combining the functions of the human intestine, liver and adipocyte [8]. Under standard growth conditions, wild-type adults contain moderate amounts of fat in the intestine and hypodermis that can be visualized by Sudan black or oil red O staining [9, 10]. These depots constitute the major metabolic fat stores and are depleted during starvation [10].

Only one study has closely examined adiposity during aging in *C. elegans* [11]. Using two different fat-specific stains, Nile Red and Sudan Black, fat levels were found to increase over life span in wild-type animals. Most of this increase occurred between adult days 5 and 10, which is the period following reproductive senescence when animals also exhibit slowing locomotory behaviors [12, 13]. Thus, increased fat accumulation corresponds with sedentary behavior and possibly reflects a metabolic shift related to cessation of egg laying and locomotion. However, mechanisms contributing to age-related adiposity have not been elucidated. Therefore, the following sections discuss the current understanding of *C. elegans* fat accumulation and metabolic regulation. Shifts in these pathways during aging could be factors contributing to increased adiposity in old worms.

Function and Regulation of Fat Storage in *C. elegans*—Fat serves as a source for metabolic energy for worms under several conditions. First, there is a requirement for basal fat metabolism during growth and development. Mutant worms unable to synthesize certain fats fail to develop normally or die prematurely, suggesting that fat performs essential functions for development and survival [14, 15]. In adults, intestinal fat is mobilized to oocytes where it provides nutrients to developing embryos [16, 17]. Finally, in adults and diapaused dauer larvae, stored fat serves as an energy source in the absence of environmental food sources [18]. Using fat-specific dyes, fat depletion can be observed as animals endure starvation conditions [14].

Despite differences in the sites of fat storage between mammals and invertebrate nematodes, several of the genetic factors that regulate mammalian adiposity have also turned up as

significant regulators of *C. elegans* fat metabolism [19]. In mammals, fat storage is regulated by transcription factors that specify adipocyte cell fate and then induce the expression of adipocyte-specific fat storage enzymes. Mammalian adipocyte differentiation and adipogenesis are regulated by the C/EBP transcription factor. A C/EBP homolog in worms, LPD-2, regulates the expression of lipogenic genes [14]. A second transcription factor, SREBP, is critical in mammals for lipid homeostasis. Similarly, a *C. elegans* SREBP homolog, LPD-1/SBP-1, also regulates lipogenic gene expression [14]. Expression of *fat-5*, *fat-6* and *fat-7*, which encode fatty acid desaturases, is also under *lpd-1* control. Mutants lacking *lpd-1*/SREBP function show an absence of intestinal fat and growth defects, suggesting that fatty acids are necessary for normal development [14].

Nuclear hormone receptors regulate fatty acid metabolism in both worms and people. A central regulator of mammalian lipid metabolism is HNF4, a member of the steroid nuclear receptor transcription factor family. Similarly, fatty acid metabolism in *C. elegans* is regulated by two HNF4-like receptors, encoded by the *nhr-49* and *nhr-80* genes [20, 21, 15]. Both NHR-49 and NHR-80 regulate the expression of delta-9 desaturases (stearoyl-CoA desaturases). The *C. elegans* genome contains three delta-9 desaturase genes, *fat-5*, *fat-6* and *fat-7*. Deletion of all three delta-9 desaturases is lethal, demonstrating the necessity of the lipid products for survival [15]. While these three desaturase enzymes function redundantly, *fat-6* appears to encode the major delta-9 desaturase active under standard growth conditions [15]. Under certain conditions, such as when *fat-6* activity is limited, *fat-7* expression is highly induced and this induction involves *nhr-80* [15]. In contrast, *nhr-49* activity is necessary for *fat-7* expression to be repressed under starvation conditions [21]. While both NHR-49 and NHR-80 regulate delta-9 desaturase expression, NHR-49 has additional roles regulating the expression of genes involved in beta-oxidation and other starvation-regulated genes [20, 21]. An important cofactor for both NHR-49 and LPD-1/SBP-1 is the mediator protein, MDT-15 [22]. In the absence of *mdt-15* activity, *nhr-49*-mediated induction of FA desaturases during starvation is compromised. Consistent with this, levels of unsaturated fatty acids are elevated in *mdt-15* mutants. Mutations in either *nhr-49* or *mdt-15* significantly shortened adult life span, demonstrating that fatty acid metabolism is an essential aspect of *C. elegans* physiology [20, 22]. Further evidence that fats play important roles in *C. elegans* physiology is the finding that elongase mutants develop into sickly adults with low fertility, or arrest development completely in the first larval stage [23].

Neuroendocrine and Environmental Factors Promote Mobilization of Fat Reserves—Fat metabolism requires the action of lipases, esterases, enzymes of beta-oxidation and the mitochondria electron transport chain. When environmental nutrients are depleted, fat reserves in the intestine and hypodermis provide metabolic substrates for growth and survival. Neuroendocrine pathways regulate fat utilization under these conditions. In adults, sensory neurons detecting food secrete a TGF- β ligand, DAF-7, into the body [24]. Low levels of food in the environment correspond to reduced *daf-7* expression. Low *daf-7* expression stimulates fat accumulation in the intestine and hypodermis [25]. Environmental food signals also increase serotonin levels, which, in turn, inhibits fat accumulation and serotonin-deficient mutants accumulate high levels of fat [26]. Serotonin signaling also regulates the rate of fat metabolism in peripheral tissues [27]. It is

unlikely that a simple decline in serotonin signaling is the cause for fat accumulation in older worms. In addition to regulating fat accumulation, serotonin also regulates feeding and locomotory behaviors, several of which are altered during aging [28, 29]. Mutations disrupting serotonin receptors can suppress these age-related changes, suggesting that serotonergic neurotransmission is elevated, rather than depressed, during normal aging. Thus, the mechanisms driving fat accumulation during worm aging may occur downstream of serotonergic input.

Increased utilization of fat reserves can have a prolongevity effect in *C. elegans*. Both fat mobilization and longevity are enhanced by disruption of *ctbp-1*, which encodes a homolog of the mammalian NAD(H)-dependent transcriptional corepressor, C-terminal binding protein [30]. In *ctbp-1* mutants, triacylglycerol levels are reduced due to the induction of *lips-7*, encoding a TAG lipase. Interestingly, *ctbp-1* mutants are long-lived due, in part, to the induction of *lips-7*.

Defects in mitochondrial function caused by prohibitin mutations differentially affect longevity of wild-type and long-lived mutants. Prohibitins are conserved components of mitochondrial membranes that are required for mitochondrial function, which is also involved in fatty acid metabolism. *C. elegans* prohibitin mutants are unable to metabolise fat, and have substantial decreases in stored fats [11]. This fat storage defect was enhanced in long-lived *daf-2*/insulin-receptor mutants. Although prohibitin mutations shortened life span in a wild-type animals, they were found to further extend life span of long-lived insulin receptor mutants. These findings suggest a complex interaction between prohibitin-dependent aspects of mitochondrial function and fat metabolism in *C. elegans*.

AMPK, a multisubunit kinase regulated by cellular energy levels, coordinates the breakdown of intestinal and hypodermal fat with energy requirements of the periphery. Dauer larvae lacking adequate AMPK activity in hypodermal cells prematurely exhaust their lipid stores and die [18]. The excretory cell constitutes the major metabolic sink required for dauer survival, and AMPK mutant dauers die from osmotic stress due to inadequate excretory cell function. This phenotype reflects inappropriately high activity of the AMPK target, ATLG-1, an adipose triglyceride lipase, in the AMPK mutant dauers. Since AMPK disruption increases fat utilization and depletes fat reserves, it is possible that fat accumulation during aging reflects elevated AMPK activity.

Intestine as a Central Regulator of *C. elegans* Longevity—Changes in intestinal composition during aging may reflect alterations in the function of aging-regulated transcription factors. This idea is supported by findings from microarray analyses of transcription during aging and in long-lived mutants which found that a substantial number of age-regulated transcripts are targets of the ELT-3/GATA transcription factor, which is expressed in the worm intestine and hypodermis [31]. Indeed, the group of age-regulated transcripts identified by microarray analysis was enriched for intestinal genes. It is possible that changes in body composition in humans might also reflect tissue-specific alterations in gene expression due to age-regulated transcription factors.

The intestine plays a central role in determining *C. elegans* life span. This is due to the fact that the FOXO transcription factor, DAF-16, appears to function primarily from the intestine to promote longevity [32]. DAF-16 directs the expression of pro-longevity genes with a variety of functions, such as antioxidant defense, xenobiotic detoxification and fatty acid metabolism [33, 34]. DAF-16 was first identified as the major target of DAF-2, an insulin/IGF-I receptor like protein that regulates *C. elegans* development, reproduction and longevity [35, 36]. One possibility is that DAF-16 target genes protect the intestine from aging-related decline, allowing the intestine to carry on essential functions for longer time. In addition, there may be DAF-16-regulated changes in intestinal function that have pro-longevity effects. One example is a shift to fat-based metabolism. The *C. elegans* insulin pathway regulates the expression of a large number of transcripts, either directly or indirectly through *daf-16* [34]. Among these insulin-pathway target genes is a lipase, corresponding to the *K04A8.5* open reading frame [37]. Inactivation of *K04A8.5* function partially suppressed the longevity of insulin receptor mutant adults, suggesting that utilization of fat stores via *K04A8.5* lipase activity was partially responsible for the extreme longevity of adults with defective insulin signaling.

Muscle Aging and Sarcopenia in *C. elegans*

Sarcopenia in Feeding and Locomotory Muscles

In addition to increased adiposity, loss of muscle mass is a major body composition change of human aging. This loss of muscle mass is named sarcopenia and the causes remain mysterious, although they are likely to be complex in the human population. Clinical and laboratory studies of sarcopenia have implicated a variety of factors, including disruptions in neuromuscular connectivity, immune dysfunction, and mitochondrial dysfunction [38, 39]. Changes in musculature similar to human sarcopenia have been noted in *C. elegans* locomotory and feeding muscles [13, 40–42]. Identifying the factors driving muscle sarcopenia during worm aging would identify candidate pathways involved in human sarcopenia and potentially lead to the development of new therapeutic approaches for its treatment.

The locomotory muscles are situated longitudinally along the body axis in four groups and function as pairs to promote sinusoidal movement along the substrate. Sarcomeres in the *C. elegans* locomotory muscles degenerate as animals age, and this damage can be visualized by GFP-labeled sarcomeric proteins, phalloidin-stained sarcomeres or ultrastructurally by electron microscopy [13,41]. Prior to the appearance of sarcomeric damage, however, animals exhibit locomotory deficiencies that reflect weakness in the muscle sarcomeres [12, 13, 41]. At this stage of aging, when the muscles appear generally intact but locomotion has slowed, muscles become more susceptible to damage [13]. In long-lived insulin pathway mutants, both muscle damage and locomotory declines are delayed [12, 41]. This correlates with the induction of stress resistance enzymes that could protect muscles from damage over life span [5, 34,43].

Sarcopenia has also been studied in the *C. elegans* pharynx, which is the neuromuscular feeding organ in the head. In contrast to the longitudinal muscles promoting locomotion, the pharynx muscles are radially organized and contract to crush the food, which consists of

bacteria and slime molds [44]. Damage to the pharynx muscles is more readily apparent than in the body muscles and has been visualized by Nomarski optics in live animals, but can also be seen by GFP-marked muscle components [3]. There is evidence to suggest that pharynx deterioration results directly from damage accumulated over a lifetime of contractions, as pharynx function was protected in animals that were prevented from fast contractions for several days [42].

Functional decline of the feeding (pharynx) and locomotion (body) muscles is correlated with life span [12]. Individual animals that maintained longer spans of high pharynx or body muscle function were more likely to have longer life span, and vice versa. In these studies, there was no correlation between self-fertile reproductive period and life span showing that progeny production in these hermaphrodites was not a measureable predictor of relative longevity. A computer learning-based analysis of pharynx muscle morphology across adult life span revealed that morphology changes during aging occurred in a stepwise, rather than progressive, fashion [45]. This revealed that structural aging may be modulated by cellular homeostatic mechanisms for maintaining cellular integrity.

Starvation Alters Muscle Composition in Ways that Mimic Aging

As for human muscles, the causes of sarcopenia in worm muscles remain mysterious. One possibility is a connection to the decline in feeding that occurs during normal aging [12]. Evidence of feeding decline is a progressive decrease in the rate of food intake by the pharynx muscles as well as atrophic changes in the intestine. Thus, a potential approach to studying sarcopenia in worms is through the starvation response in young adults. Such studies have shown that one response to starvation in pharynx muscles is an increase in muscarinic signaling [46]. Increased pharynx muscarinic signaling leads to activation of the MPK-1 kinase, which is a worm ortholog of the mammalian stress-inducible kinase ERK. This is believed to prime the pharynx muscles to quickly activate pumping upon future encounters with food. Overactive muscarinic activation during starvation can have detrimental consequences. For example, *gbp-2* mutants that are unable to contain muscarinic signaling due to the absence of an inhibitory G-protein subunit, have significantly shortened survival times under starvation conditions [46,47].

One output of starvation-induced muscarinic signaling in the pharynx is activation of the autophagy pathway. Autophagy is a process that enables cells to recycle components through lysosomal proteolysis to obtain nutrients [48]. There is evidence suggesting that autophagy is also activated in mouse models of sarcopenia [49]. The starvation-sensitive *gbp-2* mutants demonstrate overactive autophagy in the pharynx [50]. Autophagic overactivity is one cause of the early death in *gbp-2* mutants, as genetic manipulations that depress autophagy can partially rescue the survival of starved *gbp-2* animals. Induction of autophagy is not always detrimental, however. In fact, current evidence indicates that autophagy is activated during dietary restriction, which extends life span [51]. Thus, there appears to be a range in the level of autophagy activation in response to changes in food availability. A moderate level of autophagy within cells appears to be beneficial, possibly providing cells with a variety of metabolic precursors and clearing damaged cellular

components. However, high levels of autophagy can be detrimental, contributing to tissue destruction and death.

Another set of factors that may contribute to the decline in muscle mass during aging in worms and other species are micro-RNAs, which modulate gene expression in many different scenarios. Ibenez et al. [52] reported the identification of several micro-RNAs that are age-regulated. Computational prediction of their targets revealed that several age-regulated micro-RNAs in *C. elegans* may disproportionately affect targets expressed in muscles. This led to the hypothesis that muscle aging might reflect changes in target regulation by age-regulated micro-RNAs.

Concluding Remarks

Body composition changes are a major challenge for extending human health span and life span. Increased adiposity and decreased muscle mass can limit the independence of elders and can also predispose to disease. Due to the complex effects of aging on the body, it is difficult to isolate causes and modifying factors of these conditions in clinical studies. Rather, the use of model organisms, including rodents, has been the mainstay for studying these conditions. The utility of invertebrate model organisms, such as *C. elegans* nematodes, has been appreciated in this current era of biogerontology. This chapter highlighted recent studies in *C. elegans* focusing on processes analogous to human adiposity and sarcopenia. These findings may lead to the development of new hypotheses about etiology of these conditions in humans that can be tested using rodent models and clinical studies. In particular, future studies may find therapeutic strategies by targeting neuroendocrine pathways that converge on lipogenic targets. Furthermore, antisarcopenic therapies may be developed that modulate muscle muscarinic or autophagic targets related to those promoting muscle decline during starvation in worms.

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