Sex Differences in the Response to Genetic and Chemical Pro-Longevity Interventions in *Caenorhabditis elegans*

by

Rose Al-Saadi

A dissertation accepted and approved in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

in Biology

Dissertation Committee:

Cristopher Niell, Chair

Patrick Phillips, Advisor

Matthew Barber, Core Member

Peter Ralph, Core Member

Michael Wehr, Institutional Representative

University of Oregon

Summer 2025

© 2025 Rose Al-Saadi

This work is openly licensed via CC BY 4.0.

DISSERTATION ABSTRACT

Rose Al-Saadi

Doctor of Philosophy in Biology

Title: Sex Differences in the Response to Genetic and Chemical Pro-Longevity Interventions in *Caenorhabditis elegans*

Women often live longer than men yet suffer worse health outcomes in old age. This sexual dimorphism in lifespan and aging trajectories is robust and has been frequently observed and comprehensively documented. Sex differences extend beyond natural aging and are additionally seen in the response to pro-longevity interventions. Despite that, our understanding of the mechanisms underlying such differences remains limited. In this dissertation, I utilize the model organism *Caenorhabditis elegans* to explore sex differences in aging. I leverage a healthspan measure that is unique to male *C. elegans*: mating. Males display a complex mating behavior that strongly declines with age due to physiological deterioration, making it an ideal screening phenotype to investigate the efficacy of pro-longevity interventions. Using male reproductive success as a complex healthspan measure, I investigate the effects of DAF-2/ IGF-1 insulin receptor degradation on male longevity and whether the effects of this intervention are sexually dimorphic. Additionally, I examine the effects of several pro-longevity chemical compounds on male lifespan and contrast them to previously published hermaphrodite data to determine whether their effects on longevity are sexually dimorphic or sex shared. Finally, I characterize the transcriptomic signatures of two chemical pro-longevity interventions, metformin and Thioflavin T, on aging *C. elegans* and determine whether the effects at the transcriptional level are sexually dimorphic or sex shared. This work provides a new experimental paradigm for the study of sex differences in aging using the model organism *C. elegans* and offers new mechanistic targets for future research. Understanding the mechanistic basis of sex differences in aging will aid in the development of interventions that can benefit individuals of any sex.

This dissertation includes previously unpublished coauthored material.

ACKNOWLEDGMENTS

I wish to express my deep and sincere gratitude to my advisor, Dr. Patrick Phillips. He has demonstrated to me that being a kind mentor and a rigorous scientist are not mutually exclusive. He has advocated for me even before I arrived at UO and has supported me every step of the way. He has always encouraged me to trust my scientific instincts and allowed me the freedom to explore my interests in his lab, which has been a truly invaluable experience. I would also like to thank my first academic advisor, Dr. Sarah Hall, who introduced me to *C. elegans* research and encouraged me to pursue a PhD and a career in science; without her guidance and mentorship, I would not be where I am today. I would like to also thank my current and previous committee members, Cris Niell, Matt Barber, Peter Ralph, Mike Wehr, and Nadia Singh for their support, mentorship, and insightful feedback on my projects throughout my PhD.

I am deeply grateful to the Phillips lab members, both current and past, who have fostered a positive and supportive work environment that made being in the lab both intellectually stimulating and incredibly fun. I want to especially thank Zach Stevenson and Christine Sedore for being the best coffee run crew, and for being amazing friends inside and outside the lab. Their enthusiasm for science is both unparalleled and infectious and they both continue to inspire me to be a better scientist. I want to thank Kristin Robinson and Aubrey Mayer for being great office mates and for allowing me to vent about failed experiments and worms that refuse to die, Megan Moerdyk-Schauwecker for ensuring I always had friends to share a Thanksgiving meal with, Hannah Lewack for being the best undergraduate mentee I could have asked for, and Anna Coleman-Hulbert and Erik Johnson for adding quirky and fun vibes to the lab and making our Tea Times the best.

My time as a PhD student has been enriched by a number of student groups including the Graduate Evolutionary Biology and Ecology Students (GrEBES), the Community for Minorities in STEM (CMiS), and Women in Graduate Science (WGS). These groups have offered me a sense of belonging, which was especially needed during the early days of the pandemic, and allowed me to integrate myself into a scientific community of my peers. I am also grateful for the financial support that I have received from all three groups that contributed to attending conferences and recognized my contributions in leadership roles.

I want to thank my funding sources who have made this work possible. This work was funded in part by the Genetics Training Program at the University of Oregon, which is funded by the National Institutes of Health (T32GM149387). Additional funding was provided by National Institutes of Health awards to Patrick C. Phillips (U01AG045829, U24AG056052, R01AG088629).

I would like to thank all the friends who have made my time in Eugene a happy and exciting time. I want to thank my cohort mates LyAndra Lujan and Masha Korchagina for their friendship and support, Elizabeth Vargas, Jordan Munroe, Kona Shaw, Ethan Shaw, Emily Dennis, and Max Horrocks for teaching me how to work hard and play hard, Leanne Kelley and Alex Nichitean, my worm sisters, and Mayara Ribeiro and Niko Wagner, for their support from afar; reminiscing about Café Disco and Chipotle Wednesdays will always bring me joy.

I want to thank my family for all the love and support that they have provided through the years. I am forever grateful for their courage to immigrate to the United States from Iraq and the sacrifices they made that have allowed me to pursue many opportunities I couldn't have otherwise.

I want to acknowledge the important contributions of my cats Sesame, Peppercorn, and Nimbus to this work through their moral support and unwavering love and purrs.

I feel incredibly lucky to have shared my grad school journey with my best friend and partner, Zac Bush, who has enriched my life with wonderful people, delicious food, running, and great knowledge of meiotic recombination and obscure internet memes. Thank you for being my main support system over the last three years of my PhD, for sharing and amplifying my enthusiasm for science, for inspiring me to become a better and more compassionate person, and for all the love and joy you bring into my life.

DEDICATION

This dissertation is dedicated to my mother Wajida. Her passion for knowledge and her drive to pursue education despite all obstacles has inspired me throughout my life and planted in me a love for science and education.

TABLE OF CONTENTS

Chapter Page

CHAPTER 1: INTRODUCTION 12

Aging Theories and Hallmarks 12

Sex Differences in Aging and Age-Associated Diseases 13

C. elegans as an Aging Model 15

Pro-longevity Interventions in *C. elegans* 16

Challenges and Benefits of *C. elegans* Males as an Aging Model 18

Dissertation Outline 19

Bridge to Chapter 2 20

CHAPTER 2: KNOCKING DOWN THE DAF-2 INSULIN-LIKE RECEPTOR IN MALE *C. ELEGANS* DRAMATICALLY INCREASES REPRODUCTIVE HEALTH AND LONGEVITY IN A SEX AND TISSUE-SPECIFIC MANNER 21

Abstract 21

Main 22

Discussion 28

Methods 31

Supplemental Figures 34

Supplemental Tables 37

Bridge to Chapter 3 45

CHAPTER 3: PRO-LONGEVITY COMPOUNDS EXTEND *CAENORHABDITIS ELEGANS* MALE LIFESPAN AND REPRODUCTIVE HEALTHSPAN 46
Abstract 46
Introduction 46
Materials and Methods 49
Results 51
Discussion 55
Supplemental Tables 61
Bridge to Chapter 4 70
CHAPTER 4: PRO-LONGEVITY INTERVENTIONS GENERATE SEXUALLY DIMORPHIC TRANSCRIPTOMIC SIGNATURES IN AGING *CAENORHABDITIS ELEGANS* 71
Introduction 71
Materials and Methods 73
Results 75
Discussion 87
Supplemental Figures 91
Supplemental Tables 93
CHAPTER 5: CONCLUSION 94
REFERENCES CITED 96

LIST OF FIGURES

Figure Page

Fig. 2.1 Ubiquitous and tissue-specific DAF-2 degradation extends male lifespan.... 25

Fig. 2.2 Ubiquitous DAF-2 degradation preserves late-life male reproductive success.......... 27

Fig. S2.1 Neuron- and Hypodermis-specific DAF-2 degradation does not extend male lifespan.......... 34

Fig. S2.2 Tissue-specific DAF-2 degradation does not preserve late-life male reproductive success.......... 35

Fig. S2.3 Turning success and LOV efficiency are not improved upon DAF-2 ubiquitous degradation.......... 36

Fig. 3.1 Pro-longevity compounds extend male lifespan.......... 53

Fig. 3.2 Sex differences in the percent change in median lifespan for different compounds compared to the control.......... 54

Fig. 3.3 Sulforaphane and metformin preserve late-life male reproductive success ... 56

Fig. 4.1 FUdR interacts with pro-longevity compounds in a sex-dependent manner.......... 77

Fig. 4.2 Hermaphrodites and males have distinct transcriptomic profiles associated with aging.......... 78

Fig. 4.3 Metformin elicits sexually dimorphic transcriptional changes.......... 80

Fig. 4.4 ThioT elicits sexually dimorphic and sex-shared transcriptional changes..... 82

Fig. 4.5 Metformin and ThioT interact with age to modulate gene expression changes.......... 84

Fig. 4.6 Sex interacts with compound treatments to modulate age-associated gene expression changes... 86
Fig. S4.1 Principal component analysis of RNA-seq replicates 91
Supplemental Fig. S4.2 Top genes whose expression change by compound treatment is sex biased 92

LIST OF TABLES

Table Page

Table S2.1 Lifespan assay summary statistics 37

Table S2.2 Lifespan assay CPH model output........ 38

Table S2.3 Mating assay summary statistics 39

Table S2.4 Mating assay GLM model output 41

Table S2.5 Behavior assay summary statistics 43

Table S2.6 Behavior assay GLM output........ 44

Table S2.7 Strains used in this study 45

Table S3.1 Lifespan assay summary statistics 61

Table S3.2 Lifespan assay CPH model output........ 62

Table S3.3 Change in median lifespan by sex summary statistics 63

Table S3.4 Median lifespan by sex CPH model output 64

Table S3.5 Mating assay summary statistics 66

Table S3.6 Mating assay GLMM model output 68

Table S4.1 Compound by FUdR by sex CPH model output 93

CHAPTER1: INTRODUCTION

Aging theories and hallmarks

Aging is defined as the progressive deterioration of physiological and cellular functions over time. The study of aging as a biological process is complicated by its multifactorial nature, and by the variable contribution of these factors depending on the genetic background and the environment of an individual (Gems 2022). Several theories and mechanisms for aging have been proposed, with some rooted in evolutionary concepts while others focused on mechanistic causes. One of the most prominent evolutionary explanations of aging is George Williams' antagonistic pleiotropy, which states that some genes have contrasting or "antagonistic" effects on fitness, beneficial in early life and detrimental in late life (Williams 1957). Given that selection is weaker following reproductive senescence, the detrimental effects of such alleles in late life are "shadowed", leading to an overall positive selection on loci promoting beneficial "wild type" processes in early life and contribute to aging in late life (Williams 1957).

Theories that have since emerged to explain antagonistic pleiotropy from a mechanistic standpoint can be divided into senescent theories and the programmatic aging theory (Gems 2022). The disposable soma theory is a senescent theory that was first proposed by T. B. L. Kirkwood (1977), which holds that given limited resources, natural selection will prioritize resource investment in the germline and reproduction, depleting resources from the soma and leading to an accumulation of damage without enough resources for maintenance mechanisms to reverse it. The programmatic theory, which was proposed independently by Mikhail Blagosklonny (2006) and João Pedro de Magalhães and Church (2005), states that although aging itself is not a programmed process, early programs that evolved to regulate growth and development can have run-on functions (hyperfunction) in late life that lead to aging and age-associated decline. Since their proposal, these theories have received varying degrees of empirical support (Mc Auley 2025). Interestingly, these evolutionary theories of aging do not necessarily predict that there will be conserved genetic pathways that directly influence the process of aging itself, yet the discovery of such pathways has completely revolutionized the field over the last 30 years.

In addition to proposing evolutionary and mechanistic theories, the field has benefitted from describing a set of aging hallmarks that are defined by (1) their age-associated onset or progression, and (2) the ability to accelerate or decelerate aging by modifying them (López-Otín et al. 2013; López-Otín et al. 2023). These hallmarks are divided into primary (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy), antagonistic (deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence), and integrative (stem cell exhaustion, altered intercellular communication, chronic inflammation, dysbiosis) (López-Otín et al. 2023). Moreover, the trans-NIH Geroscience Interest Group described aging pillars (adaptation to stress, epigenetics, inflammation, macromolecular damage, metabolism, stem cells and regeneration, and proteostasis) and emphasized their interconnectedness and the importance of understanding how these interactions regulate aging and age-associated disease (Kennedy et al. 2014). A key goal of the aging field is to uncover interventions that can modulate these hallmarks and thus modulate aging progression and trajectories.

Sex differences in aging and age-associated diseases

A remarkable phenomenon in the aging field and one of its most robust features is that women live longer than men (Austad 2011). This phenomenon is observed across the world and throughout history (Rochelle et al. 2015). In fact, sex-based differences in life expectancy have been documented in datasets dating back to 1751 from Sweden. These differences persisted despite the global average life expectancy increasing by more than two-fold, from 28.5 years in 1751 to 73.2 in 2020 (Dattani et al. 2023). Additionally, sex-based discrepancies are present even in extreme mortality conditions such as famine, slavery, and pandemics (Rochelle et al. 2015; Zarulli et al. 2018; Yan et al. 2024). While these differences may be in part due to the complex social structures and environmental factors in human populations, similar sex-based differences in longevity are observed in social groups where men and women share similar lifestyles (Luy 2003; Lindahl-Jacobsen et al. 2013). Similarly, sex differences in longevity are seen across many other wild mammalian species and commonly used model organisms (Austad and Fischer 2016; Lemaître et al. 2020).

Aging is often measured by lifespan (the chronological time between birth and death) and healthspan (the portion of lifespan that is disease-free). Both measurements show sex differences, albeit paradoxically; while women live longer than men, their healthspan is shorter (Oksuzyan et al. 2008; Gordon et al. 2017). Women are more likely than men to experience Alzheimer's disease and other dementias (Rajan et al. 2021), chronic conditions (Bello-Lujan et al. 2022), bone mineral density loss (Daly et al. 2013), inflammation (Milan-Mattos et al. 2019), and higher rates and levels of frailty as they age (Gordon et al. 2017). On the other hand, men have a higher incidence of ischemic heart disease, trachea, bronchus, and lung cancers, and colon and rectum cancers (Mauvais-Jarvis et al. 2020; Hägg and Jylhävä 2021).

Why do sex differences in aging exist? Sex-chromosomal and sex-hormonal mechanisms have been proposed as a proximate explanation for the longevity-gap between sexes (Hägg and Jylhävä 2021). Some of the sex-chromosomal explanations are focused on the X chromosome, largely related to X-chromosome inactivation (Marais et al. 2018). In humans, the homogametic sex has two X chromosomes with one undergoing random inactivation, and the heterogametic sex has one X and one Y chromosome. In the presence of X-linked deleterious mutations, the heterogametic sex has an "unguarded" X chromosome and is more vulnerable to expressing such mutations. Although this is supported by the higher incidence of X-linked diseases such as Duchenne muscular dystrophy and hemophilia in individuals with XY chromosomes, the effect size of this mechanism is not enough to explain the magnitude of lifespan differences (Connallon et al. 2022). Additionally, while X-chromosome inactivation is random, a skewed inactivation is associated with cardiovascular disease risk and cancer incidence, and a balanced inactivation is associated with healthy aging and longevity (Gentilini et al. 2012; Roberts et al. 2022).

Other sex-chromosomal explanations are centered around the Y chromosome including Y chromosome toxicity or loss (Marais et al. 2018). The toxic Y hypothesis postulates that the high density of transposable elements on the Y chromosome are activated during aging due to the age-related loss of heterochromatin, contributing to the shorter lifespan of males (Muyle et al. 2021). Although some studies in *Drosophila* provided support for this hypothesis by detecting a correlation between the number of Y chromosomes and longevity, a recent study refuted it, showing instead that lifespan is correlated with phenotypic sex rather than the number of Y chromosomes (Brown et al. 2020; Delanoue et al. 2023). The mosaic loss of Y chromosome,

which is considered the most common somatic mutation in individuals with XY chromosomes, is correlated with cancer and several neurodegenerative and cardiovascular diseases (Bruhn-Olszewska et al. 2025).

Sex-hormonal mechanisms lend themselves as an additional explanation for the large disparity in lifespan. While both males and females experience age-related decline in sex-hormone levels, only females experience a sharp decline in hormones during menopause. Natural menopause has been associated with a greater rate of neuromuscular and cognitive decline (Weber et al. 2012; Piasecki et al. 2024). Similarly, surgical menopause, or oophorectomy, has been associated with decline in cognitive function and an increase in the risk of Alzheimer's (Rocca et al. 2007; Bove et al. 2014; Gurvich et al. 2018). Longer durations of estrogen exposure are associated with longevity while shorter periods are associated with increased risk of cardiovascular diseases (Shadyab et al. 2017; Hägg and Jylhävä 2021; Mishra et al. 2021). All together, these differences have been hypothesized to be a major contributor to the mortality-morbidity paradox.

C. elegans **as an aging model**

A French librarian with a personal interest in biology, Émile Maupas, was the first to describe *Caenorhabditis elegans* (originally named *Rhabditis elegans*) in 1899, naming it for its rod-like shape (*rhabditis*) and elegant sinusoidal movement (*elegans*) (Maupas 1899). Although early work on *C. elegans* by Nigon and Dougherty described its development and reproductive systems (Nigon 1949; Dougherty et al. 1959), the establishment of *C. elegans* as a tractable genetic model system is credited to Sydney Brenner who started working on this nematode in 1967 (Brenner 2009). Brenner recognized the power of *C. elegans* as a model system: they are small and easily maintained, their generation time is short (about 3 days), and their brood size is large (300-350 progeny) which creates a large nearly isogenic population from a single hermaphrodite that is ideal for addressing questions about genetics, development, and behavior (Brenner 1974). Eight years later, Brenner published the first paper outlining *C. elegans* genetic methods and identified 300 mutations in about 100 genes that mostly affect behavior and morphology (Brenner 1974). The establishment of this model system catalyzed many groundbreaking discoveries and accomplishments including describing the complete cell lineage of an animal and the discovery of apoptosis (Kimble and Hirsh 1979; Hedgecock et al. 1983;

Sulston et al. 1983; Ellis and Horvitz 1986), the discovery of RNAi, microRNAs and their role in gene regulation (Lee et al. 1993; Wightman et al. 1993; Fire et al. 1998), and the establishment of green fluorescent proteins as gene expression markers (Chalfie et al. 1994). *C. elegans* was also the first animal to have its genome sequenced (The C. elegans Sequencing Consortium 1998). This small nematode facilitated one of the greatest discoveries in the aging field; that lifespan can be regulated genetically.

In 1983, Michael Klass proposed a method to screen for longevity in *C. elegans* and conducted the first screen for long-lived mutants in *C. elegans,* identifying eight such mutants (Klass 1983). Several of those mutants, however, displayed reduced food intake and therefore their long lifespan was attributed to caloric restriction. Tom Johnson's work later uncoupled the reduced food intake and the prolonged lifespan phenotypes in one of those mutants, *age-1* (Friedman and Johnson 1988). Although *age-1* mutants experienced reduced fertility which was hypothesized to underlie the increased longevity (in line with the disposable soma theory), they also had a reduced mortality rate (the probability of death at a given time); this provided the first evidence that mortality rate is genetically regulated (Johnson 1990). This work fascinated Cynthia Kenyon and fueled her curiosity about the genetic basis of aging, inspiring her to plan her own screen for long-lived mutants. However, before even conducting such screen, a lucky decision to use *daf-2* as the screening strain due to its dauer-constitutive phenotype (causing larval arrest) revealed that *daf-2* itself was a long-lived mutant, becoming the longest-lived mutant at the time (Kenyon et al. 1993; Kenyon 2011). The gene *daf-2* encodes for an insulin/IGF-1 receptor homolog that is conserved in mammals, a finding that revolutionized the aging field because it demonstrated that aging can be regulated genetically via evolutionarily conserved pathways (Kimura et al. 1997; Kenyon 2011).

Pro-longevity interventions in *C. elegans*

Since the discovery of *daf-2* as a long-lived mutant, *C. elegans* became a popular aging model, with studies leveraging its short lifespan and amenability to genetic manipulations to investigate a variety of interventions including genetic, pharmacological, and lifestyle interventions. The three major pathways that have been implicated in *C. elegans* lifespan regulation are the insulin/IGF-1 signaling (IIS), the AMP-activated kinase (AMPK), and the mechanistic target of

rapamycin (mTOR) pathways (Kishimoto et al. 2018). In addition to *daf-2*, two other genes in the IIS pathway play a significant role in its aging regulation aging via nutrient sensing: the PI3K homolog *age-1* and the FoxO transcription factor homolog *daf-16* (Morris et al. 1996; Lin et al. 1997; Ogg et al. 1997; Paradis and Ruvkun 1998; Uno and Nishida 2016). Under favorable conditions and food abundance, insulin-like peptides bind the DAF-2 receptor which modulates other proteins downstream that ultimately inhibit DAF-16 from translocating into the nucleus. Under unfavorable conditions, however, such as caloric restriction or DAF-2 defects via mutations or knockdowns, DAF-16 is phosphorylated and translocates into the nucleus, activating longevity and stress response pathways that promote longer lifespan (Kishimoto et al. 2018). Similarly, mTOR inhibition by low nutrient conditions of mutations that decrease its activity promote longevity in an AMPK- and DAF-16-dependent manner (Vellai et al. 2003; Jia et al. 2004; Uno and Nishida 2016). The effects of mTOR are mediated by the FoxA transcription homolog PHA-4 via its role in regulating autophagy (Sheaffer et al. 2008). Although there is some level of crosstalk between these signaling pathways, the effect of mTOR on lifespan is independent of DAF-16, and combinatorial attenuation of both pathways increases lifespan in an additive manner (Chen et al. 2013).

While genetic interventions offer invaluable mechanistic insights into longevity, they do not satisfy the need for interventions that are more viable for use in human health and longevity. Therefore, an additional important avenue of aging research is the development and investigation of chemical and pharmacological interventions. *C. elegans* represent an excellent screening platform to identify and characterize such interventions due to the properties mentioned previously and in chapter 3. This provided the basis for the formation of the *Caenorhabditis* Intervention Testing Program, a National Institute of Aging-funded program to screen for compound interventions in a reproducible and rigorous manner (Driscoll et al. 2025). CITP has screened over 75 compounds and identified 12 compounds that reliably extend hermaphrodite lifespan (Lucanic et al. 2017; Onken et al. 2022; Banse et al. 2024; Banse et al. 2024; Sedore et al. 2025). Due to this wealth of knowledge and established protocols and tools for *C. elegans* longevity, it represents an ideal model organism for the investigation of aging questions and mechanisms.

Challenges and benefits of *C. elegans* males as an aging model

C. elegans is an androdiecious species with two sexes: hermaphrodites which are genetically females that evolved the ability to produce sperm and self-fertilize, and males which mate with hermaphrodites to produce cross progeny. The two sexes show behavioral, neuronal, and physiological differences in addition to differences in aging and lifespan (Barr et al. 2018; Hotzi et al. 2018; Reilly et al. 2021; Weng and Murphy 2024; Purice et al. 2025). However, the studies that have investigated hermaphrodite development and aging far outnumber those that have investigated males. One of the reasons for that is males are rare in the common lab strain N2 Bristol, composing only 0.01% of the population. Moreover, males have a high drive to search for mates, leading them to escape the agar media they are grown on and desiccating on the plastic walls of petri dishes. Another feature of males is their toxic effect on other hermaphrodites and males (more in chapter 3 discussion).

Despite the challenges that males present, they offer a unique behavior that can be utilized as a comprehensive health metric: mating. Male mating behavior is considered one of the most complex behaviors in *C. elegans* nematodes that involves multiple steps, integrates a variety of inputs including chemical and mechanosensory, and requires proper function of neurons, muscle, and the germline (Barr and Garcia 2006). Most importantly, male mating declines with age due to an increase in turning defects that is correlated with neuronal deficits (Guo et al. 2012; Chatterjee et al. 2013).

Another unique benefit of using males is the lack of requirement of 5-Fluoro-2'-deoxyuridine (FUdR) supplementation. FUdR is commonly used in hermaphrodite aging studies, despite evidence showing its effect on hermaphrodite lifespan both directly and indirectly (more in chapter 3 introduction, (Mitchell et al. 1979; Gandhi et al. 1980; Anderson et al. 2016; Wang et al. 2019; McIntyre et al. 2021). Because males cannot produce self-progeny, the need for FUdR use is eliminated, allowing for the effects of interventions on lifespan to be interpreted directly without the confounding effect of FUdR.

Dissertation outline

Sexual dimorphism in aging trajectories has long been noted, yet the mechanisms underlying such differences remain elusive. In the face of an aging population, it is becoming increasingly important to expand our knowledge of the basis of such difference which would aid in the development of pro-longevity interventions that can benefit both men and women alike. This dissertation aims to explore sex differences in aging using *C. elegans* as the model organism due to its short lifespan, amenability to both genetic and chemical interventions, and the abundance of molecular and genetic resources available in it.

In chapter 2, I explore sex differences in the response to genetic pro-longevity interventions. In addition to myself, Hannah B. Lewack and Patrick C. Phillips were key contributors to this work, with Hannah B. Lewack as a co-lead author. We explore how IIS attenuation using an auxin-inducible degron system affect survival and reproductive healthspan in male *C. elegans*. We uncover an extraordinary longevity phenotype and investigate the specific tissue types that contribute to it. We additionally develop male mating success as a novel and complex healthspan measure and describe a novel phenotype that inhibits mating in older males and is repressed by IIS disruption.

In chapter 3, I explore sex differences in the response to chemical pro-longevity interventions at the physiological level. In addition to myself, Patrick Phillips was a key contributor to this work, which is currently under review. I describe a new compound-screening paradigm using male *C. elegans* survival and reproductive success as a complex healthspan metric. I screened several compounds that have been shown to promote hermaphrodite lifespan and found that their effect size on lifespan is sex specific. The chapter provides further support to idea that lifespan and healthspan are two separate aging metrics that can be uncoupled.

In chapter 4, I explore sex differences in the response to chemical pro-longevity interventions at the transcriptional level. In addition to myself, Christine A. Sedore, Erik Johnson, Erik Segerdell, and Patrick Phillips were key contributors to this work. We characterize the effects of two compounds, metformin and Thioflavin T, on the transcriptomic landscape of *C. elegans* hermaphrodites and males. Additionally, we explore how biological sex modulates these effects.

We uncover some sex-biased and sex-shared mechanisms by which these compounds extend lifespan.

Taken together, these chapters describe sex differences at the physiological and transcriptional level of a classic aging model in the response to pro-longevity interventions and propose a new experimental paradigm that leverages the unique benefits of *C. elegans* males as a screening tool for chemical and pharmacological interventions.

Bridge to chapter 2

In chapter 1, I provide an introduction into the aging field, reviewing historical evolutionary theories and current knowledge of the molecular pathways that contribute to aging. I highlight the prevalence and complexity of sex differences in aging trajectories and the growing demand for understanding the mechanisms that underlie differences in the response to pro-longevity interventions. In chapter 2, I explore sex differences in the response to genetic interventions. Specifically, I explore how targeting the IIS pathway in *C. elegans* males in a ubiquitous and tissue-specific manner affect lifespan and reproductive healthspan.

CHAPTER 2: KNOCKING DOWN THE DAF-2 INSULIN-LIKE RECEPTOR IN MALE *C. ELEGANS* DRAMATICALLY INCREASES REPRODUCTIVE HEALTH AND LONGEVITY IN A SEX AND TISSUE-SPECIFIC MANNER

Rose S. Al-Saadi, Hannah B. Lewack, Patrick C. Phillips

Author Contributions

RSA and PCP conceptualized the project. RSA conducted the lifespan assays, the mating assays for ages 12–21 days, conducted the statistical analysis and visualization, and co-wrote the original manuscript. HBL conducted the mating success and mating behavior assays and co-wrote the original manuscript. PCP supervised the project, reviewed and edited the manuscript, and acquired funding.

Abstract

Aging is a universal phenomenon experienced by nearly all multicellular organisms, with several factors including sex impacting its manifestation (Austad and Fischer 2016). In the nematode model *Caenorhabditis elegans*, most aging studies have been conducted with hermaphrodites, and little is known about male-specific responses to pro-longevity mutations. The auxin-inducible degron system has been used to degrade DAF-2/IGF-1 in hermaphrodites and it was found that both ubiquitous and tissue-specific degradation extended lifespan (Venz et al. 2021; Roy et al. 2022; Zhang et al. 2022). However, the role of sex in modulating this lifespan extension remains unknown. Here we show that ubiquitous degradation of DAF-2 in male *C. elegans* increases median lifespan by 446%, the longest lifespan extension by a single intervention to date. Degrading DAF-2 in the male germline decreased lifespan, which is the opposite of its effect on hermaphrodites (Zhang et al. 2022). We identified a novel age-associated male tail paralysis phenotype that prohibits aged males from mating. A significant reduction in this phenotype is observed with ubiquitous DAF-2 degradation and is associated with an increase in reproductive healthspan at old age by 39.6%. This work highlights the importance of studying sex differences in aging and builds upon a paradigm for examining pro-longevity genetic interventions and their effects on both lifespan and healthspan.

Main

Sex underlies incredibly robust differences in aging and longevity between males and females (Austad 2011). Such differences are seen in human populations and many other wild mammalian species (Rochelle et al. 2015; Lemaître et al. 2020). While complex social structures and other environmental factors undoubtedly contribute to this phenomenon, sex-based differences in longevity are nevertheless observed in social groups where both sexes share similar lifestyles (Luy 2003; Lindahl-Jacobsen et al. 2013). Precisely how sex regulates aging and longevity, especially at a molecular level, is still largely understudied. This is in part due to the complex nature of aging, and in part due to limited emphasis in research programs on how sex modulates different biological processes. Developing better tools and interventions that are effective at enhancing late life health and longevity for both males and females depends critically on increased understanding of the biological basis of sex-specific differences in aging responses.

While the influence of sex on the natural aging trajectories is clear, over the last two decades it has become evident that the sexes respond differently to pro-longevity interventions including genetic and pharmacological interventions (Knufinke et al. 2023; Bartke et al. 2024). While we know these differences in response to longevity interventions exist, the molecular mechanisms underlying these differences remain to be fully elucidated. Here, we use *Caenorhabditis elegans* as a genetic model to investigate how tissue-specific degradation of the classic DAF-2 insulin-like receptor enhances health and lifespan in a sex-dependent manner.

The insulin/ insulin-like growth factor 1 (IGF-1) signaling pathway (IIS) is one of the best described genetic pathways that modulate aging. Knocking out components of this pathway confers significant longevity extensions in worms, flies, mice, and other animals (Kenyon 2010). Sex differences in the IIS pathway have also been well documented in humans and other species (Tramunt et al. 2020). For example, women develop lower sensitivity to insulin than men as they age and generally have lower incidences of metabolic diseases (Ciarambino et al. 2023). Reduced IIS pathway activity in mice leads to a larger lifespan extension and better health outcomes in females than in males (Garratt 2020). Despite these differences, the molecular basis of sex-specific responses to insulin signaling remains relatively understudied. Investigating these

differences ensures that sex differences are included in the design and implementation of therapeutics aimed at enhancing health late in life.

Within *C. elegans, daf-2* encodes for an insulin-like receptor that, when mutated, can double individual lifespan (Kenyon et al. 1993; Kimura et al. 1997; Kenyon 2010). This lifespan-extension phenotype is dependent on the downstream FoxO transcription factor DAF-16 (Kenyon et al. 1993). Since the discovery of this phenotype, the molecular components and the signaling cascade regulated by DAF-2 have been extensively studied (Kenyon 2011). Recently, the auxin-inducible degron (AID) system has been used to knock down DAF-2 in a spatially and temporally controlled manner, demonstrating that DAF-2 regulates hermaphrodite lifespan primarily through the intestine, without negatively impacting development and reproduction (Venz et al. 2021; Roy et al. 2022; Zhang et al. 2022).

Here, we examined the sex-specific differences in longevity and healthspan in *C. elegans* by using the AID system to contrast the tissue-specific effects of DAF-2 degradation in males and hermaphrodites. Male mating represents an ideal measure of reproductive healthspan because it is a neurologically complex behavior that uses the majority of the male's 93 sex-specific neurons to achieve (Sulston et al. 1980; Barr et al. 2018; Molina-García et al. 2020). It also declines rapidly with age (Barr and Garcia 2006; Chatterjee et al. 2013) due to behavioral and neuronal deficits (Guo et al. 2012; Chatterjee et al. 2013) rather than a decrease in mating drive or sperm quality. We find that ubiquitous downregulation of insulin-like signaling leads to a much larger increase in lifespan in males compared to hermaphrodites and greatly enhances male sexual function late in life. In contrast, downregulating insulin-like signaling in the male germline decreases lifespan (Zhang et al. 2022). This study demonstrates the importance of studying sex differences in longevity and adds to a platform for investigating these effects in one of the most important model systems for the study of the biology of aging.

DAF-2 degradation drastically extends male lifespan

The two major components of the AID system are: 1. a short sequence called a degron tag that is fused to the protein of interest, and 2. the plant F-box protein Transport Inhibitor Response 1 (TIR1) (Nishimura et al. 2009; Zhang et al. 2015). TIR1, along with other endogenous proteins,

forms an E3 ubiquitin ligase complex that, when auxin is added, will target the protein of interest for degradation. However, auxin treatment alone has been previously shown to increase hermaphrodite lifespan (Loose and Ghazi 2021). To account for the effects of TIR1 and auxin treatment on male lifespan independent of protein degradation, we measured survival using the CA1200 strain that contains ubiquitously expressed TIR1 but no degron tag. We found that although there was a significant difference in survival between the ethanol control and auxin ($p = 0.0008$), there was no difference in the median lifespan for the two treatments (Supplemental Fig. S2.1a).

Three different groups have previously shown that ubiquitous DAF-2 degradation increased hermaphrodite lifespan significantly (Venz et al. 2021; Roy et al. 2022; Zhang et al. 2022). However, the effects of DAF-2 degradation on male lifespan remains unexplored. Using previously published AID strains, we asked whether the effect of ubiquitous DAF-2 degradation on lifespan is sexually dimorphic. We degraded DAF-2 in both males and hermaphrodites and measured their lifespan. We note that the whole-body DAF-2 AID strain used here produced occasional spontaneous dauers, indicative of leaky DAF-2 degradation without auxin exposure. Therefore, we used the TIR1-only strain on auxin as our negative control as reported previously (Zhang et al. 2022). We placed 40 animals per plate (in triplicates), with hermaphrodites and males housed separately, and measured their lifespan, finding that DAF-2 degradation, sex, and the interaction between the two factors have a significant effect on survival. Ubiquitous DAF-2 degradation in males led to a significant increase in survival ($p < 0.0001$) accompanied by a dramatic increase in median lifespan (+446%, Fig. 2.1a), with a maximum lifespan of 127 days compared to 38 days in controls. This is much greater than the increase in hermaphrodite lifespan reported here (+109%, Fig. 2.1a) and previously (+70–135% in Venz et al. 2021, +167% in Zhang et al. 2022, and +88–117% in Roy et al. 2022). Sex clearly plays a key role in regulating the response to IIS signaling disruption, consistent with previous findings in the canonical *daf-2* mutant (Hotzi et al. 2018).

Male-specific pheromones in *C. elegans* have been shown to decrease both male and hermaphrodite lifespan, resulting in "male-induced demise" (Maures et al. 2014; Shi and Murphy 2014; Shi et al. 2017). To eliminate the effect of male pheromones on lifespan, we housed males

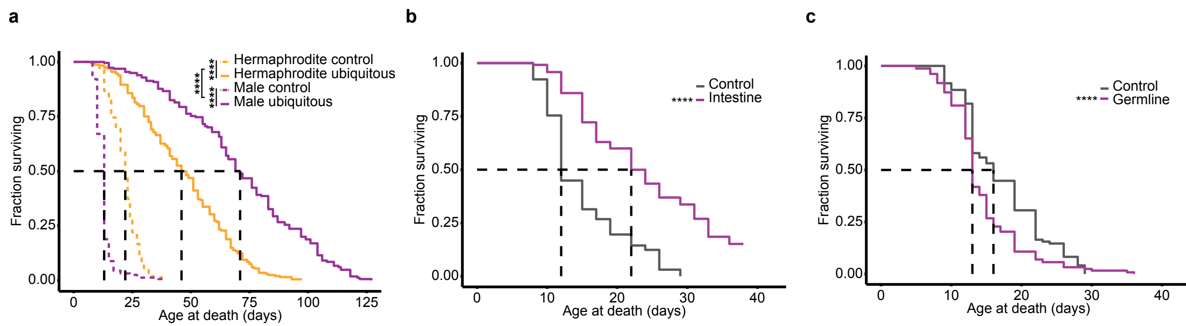


Fig. 2.1 Ubiquitous and tissue-specific DAF-2 degradation extends male lifespan. (a) Kaplan-Meier curves showing survival of *C. elegans* hermaphrodites and males following ubiquitous DAF-2 degradation. Yellow lines denote hermaphrodites and purple lines denote males. Dashed lines denote the negative controls treated with ethanol and solid lines denote DAF-degradation with 1mM auxin treatment. Each line represents at least two biological replicates with total n = 183-235. (b, c) Kaplan-Meier curves showing survival of *C. elegans* males following DAF-2 degradation in the (b) intestine and (c) germline. Gray lines denote the negative controls treated with ethanol and purple lines denote DAF-degradation with 1mM auxin treatment. The intestine lifespan curve represents one biological replicate with n = 80-109 and the germline represents two biological replicates with n = 137 – 202. The black dashed lines denote the age at which 50% of the population has died. The asterisks denote *p*-values from a Cox Proportional Hazards model where ****p<.0001. For additional information and the output of the CPH model, see Supplemental Tables S2.1 and S2.2

on individual plates then measured the effects of DAF-2 degradation on their lifespan. We found that when males are housed individually, ubiquitous DAF-2 degradation resulted in a significant increase in lifespan (+230%, Supplemental Fig. S2.1b). We hypothesized that individually housed males would display increased lifespan. Indeed, control males lived longer when housed individually. However, DAF-2 AID males did not display an added benefit of being housed individually. This indicates the DAF-2 degradation confers the maximum benefit to lifespan extension in males, and eliminating male pheromones provides no added benefits. This increase in the baseline lifespan also explains the decreased effect size of this intervention on single males compared grouped males.

Next, we wanted to identify which tissues contribute to this dramatic lifespan extension. Using strains with tissue-specific promoters driving DAF-2 degradation, we targeted DAF-2 in tissues known to regulate hermaphrodite lifespan (intestine, germline, neurons, hypodermis) and measured male lifespan (Venz et al. 2021; Roy et al. 2022; Zhang et al. 2022). We found that intestinal degradation of DAF-2 in males extended median lifespan by 83% ($p < 10^{-14}$, Fig. 2.1b). Germline degradation of DAF-2, however, decreased median lifespan by 19% (p =0.00001, Fig.

2.1c). This negative effect of germline-specific degradation on lifespan is the opposite of what was observed previously in hermaphrodites (Zhang et al. 2022). Degradation of DAF-2 in the neurons and hypodermis did not alter survival (Supplemental Fig. S2.1c, d). Overall, we find that sex plays a significant role in modulating the magnitude of response to DAF-2 degradation in *C. elegans,* with males displaying a much greater increase in lifespan in response to this intervention compared to hermaphrodites.

Ubiquitous DAF-2 degradation prolongs reproductive healthspan in old males

Lifespan and healthspan can often be decoupled, and it has become increasingly important to test the effects of pro-longevity interventions on both metrics (Bansal et al. 2015; Hahm et al. 2015; Banse et al. 2024). Therefore, we tested whether the extension in male lifespan produced by DAF-2 degradation was accompanied by an extension in reproductive healthspan. We used reproductive success as our healthspan metric because it is a complex behavior that requires fidelity of multiple tissues and systems (muscles, neurons, reproductive organs) (Barr and Garcia 2006), and declines with age due to physiological changes and not due to a decrease in motivation (Guo et al. 2012; Chatterjee et al. 2013). We tested the mating success of young (day 1 of adulthood), middle-aged (day 7), and old (day 9) adults. Because hermaphrodites produce sperm and can self-fertilize, we used *fog-2* pseudo-females, which are essentially hermaphrodites that are unable to produce sperm (Schedl and Kimble 1988), as mating partners for the males to ensure that only cross progeny were counted. To assess mating success, one male and two virgin *fog-2* pseudo-females were allowed to mate for 24 hours, then mating success was measured. Using a logistic regression model, we asked whether age, DAF-2 degradation, and the interaction between the two factors have an effect on mating success. When DAF-2 is degraded ubiquitously, mating success at both days 7 and 9 was maintained while the no-auxin controls displayed the anticipated decline in mating success. Because the males were still capable of mating at day 9, we additionally tested days 12, 15, 18, and 21 and found that ubiquitous DAF-2 degradation decreased the overall rate of reproductive decline by 40% ($p = 4 \times 10^{-5}$) compared to the no-auxin control (Fig. 2.2a). Ubiquitous DAF-2 AID males mate successfully later in life, with successful mating events occurring until day 18 of adulthood, a substantial extension compared to the no-auxin controls that stop mating successfully at day 9 of adulthood (Fig. 2.2a). Despite extending lifespan, intestinal DAF-2 degradation was not sufficient to preserve

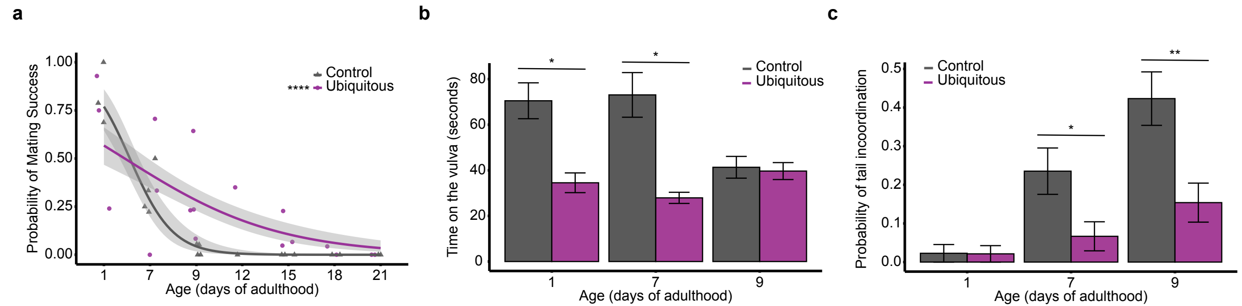


Fig. 2.2 Ubiquitous DAF-2 degradation preserves late-life male reproductive success. (a) Logistic regression lines showing mating success for *C. elegans* males following ubiquitous DAF-2 degradation. Gray lines denote untreated controls, and purple lines denote DAF-2 degradation with 1mM auxin treatment. Shapes represent biological replicates, with 4–58 technical replicates in each. The gray shading around the regression lines represents SEM. A generalized linear mixed model with a binomial distribution was used to assess the effect of treatment and age (and the interaction) on mating success. (b, c) Bar graphs showing (b) the time on the vulva and (c) the probability of tail incoordination in *C. elegans* males following ubiquitous DAF-2 degradation. Gray bars denote untreated controls, and purple bars denote DAF-2 degradation with 1 mM auxin. Each bar represents at least two biological replicates, with a total n = 48–52. Error bars represent SEM. A generalized linear mixed model with a binomial distribution for tail incoordination and a gaussian distribution for the time on the vulva was used to assess the effect of treatment and age (and the interaction) on behavior. The asterisks denote *p*-values the generalized linear models followed by planned comparisons where *p<.05, **p<.01, ****p<.0001. For additional information and the output of the linear models, see Supplemental Tables S2.3–S2.6

reproductive success in late life (Supplemental Fig. S2.2a). In fact, degradation of DAF-2 in any single tissue type did not preserve reproductive success in late life (Supplemental Fig. S2.2b-d). Overall, we found that ubiquitous degradation of DAF-2 is the only intervention that prolongs reproductive healthspan in males.

DAF-2 degradation lowers the incidence of male tail incoordination

For a mating event to be successful, a male must complete a series of complex behaviors. Males must locate a hermaphrodite, scan the body using its tail via backward movement, turn once it reaches the head or the tail of the hermaphrodite, locate the vulva, insert the spicule, and transfer sperm (Barr and Garcia 2006). This sequence of behaviors, which is considered the most complex in *C. elegans*, declines rapidly with age (Chatterjee et al. 2013). To investigate which changes at the behavioral level contribute to sustained mating success, we degraded DAF-2 ubiquitously in young (day 1 of adulthood), middle-aged (day 7), and old (day 9) males and

allowed them to mate with immobilized hermaphrodites then quantified different mating behaviors: response rate (whether or not a male made contact with a hermaphrodite), turning ability, location of vulva (LOV) efficiency (vulva location success "1" or failure "0" divided by the number of passes), and the amount of time on the vulva. We note that the sample size for turning success, LOV, and time on the vulva at day 9 is limited due to many assayed males not making contact with hermaphrodites and therefore not reaching a point at which those behaviors are relevant. DAF-2 degradation in males did not have an effect on either turning ability or LOV efficiency (Supplemental Fig. S.2.3a, b). The amount of time on the vulva, however, was significantly lower at both one and seven days of adulthood when DAF-2 was degraded (Fig. 2.2b). This potentially explains the lower mating output of the ubiquitous DAF-2 AID in the early ages (Fig. 2.2a, day 1). We also noted a novel male aging phenotype that we termed "tail incoordination" (Fig. 2.2c). While young males are able to perform swift sinusoidal movements, older males seem to have a stiff tail that remains immobile while the rest of their body continues in the sinusoidal motion. Males with this phenotype were rarely able to begin the mating sequence. We found that DAF-2 degradation significantly reduces the incidence of tail incoordination in older males at both days 7 (-72%, $p = 0.03$) and 9 of adulthood (-64%, $p = 0.003$, Fig. 2.2d). Overall, our results support the hypothesis that the decrease in tail incoordination incidence following DAF-2 degradation likely underlies the sustained reproductive healthspan in males.

Discussion

Sex differences in aging and the response to pro-longevity interventions have been documented widely (Austad 2011; Austad and Bartke 2016). Yet, the molecular mechanisms that underlie these differences remain to be fully elucidated. Here, we show that tissue-specific and ubiquitous knockdown of the insulin-like signaling pathway confer sexually dimorphic changes in longevity. We targeted the protein DAF-2 for degradation in the whole body of *C. elegans* males and hermaphrodites and found that the lifespan extension in males significantly exceeded that in hermaphrodites, leading to one of the largest reported lifespan extensions in *C. elegans* via a single intervention. Three different studies have previously shown that DAF-2 degradation in the intestine and neurons extends median lifespan in hermaphrodites (Venz et al. 2021; Roy et al. 2022; Zhang et al. 2022). Here, we show that DAF-2 degradation in male intestine also prolongs

median lifespan dramatically, while neuronal degradation had no effect on lifespan. This difference in the effect of neuronal degradation is likely due to the sexually dimorphic nature of *C. elegans* neurons. The two sexes have several sex-specific neurons (8 in hermaphrodites and 93 in males), and some of the 294 shared neurons are sexually dimorphic (Kim and Kim 2022). Additionally, it has been shown recently that neuronal aging in *C. elegans* results in sexually dimorphic transcriptional changes (Weng and Murphy 2024). Future work could explore whether the differences in the neuronal effect of DAF-2 degradation is via sex-specific neurons or sexually dimorphic shared neurons.

Zhang et al. (2022) also found that DAF-2 degradation in the hypodermis and germline lead to median lifespan extension in hermaphrodites, while Venz et al. (2021) observed no effects of either of these tissues and Roy et al. (2022) observed no effects of germline degradation on lifespan. Here, we show that hypodermis degradation had no effects on median lifespan in males while degradation in the germline led to a decrease in their median lifespan. Because the AID system produces a strong knockdown but not a fully null mutation, it is possible that the discrepancies between these studies is due to variable levels of DAF-2 knockdown in the hypodermis and germline.

Males are known to produce pheromones that are toxic to other males (Shi et al. 2017; Ludewig et al. 2019), therefore, we chose to culture them in groups as well as individually. Although it has been shown that *daf-2* and other classical long-lived mutant hermaphrodites are especially susceptible to male-induced demise (Booth et al. 2022), our results show that DAF-2 AID males are still extraordinarily long-lived. When DAF-2 is ubiquitously degraded, both single and grouped males live significantly longer, with comparable median lifespans of 76 and 71 days, respectively. On the other hand, the DAF-2 AID strain without auxin exposure and DAF-2 degradation suffered from male-induced demise, as evident by the difference of median lifespans of single and grouped males (23 and 15 days, respectively). This alludes to a mechanism in DAF-2 AID males that is protective against this male pheromone-induced demise. Future studies could explore the effects of DAF-2 degradation in the context of male pheromones and whether this degradation is protective of male-induced demise, thus contributing to the dramatic lifespan extension.

Incorporating both lifespan and healthspan measures into aging studies is important especially in the context of our aging population; people are living longer but suffering more diseases and disabilities in old age (Olshansky 2018). Here, we provide additional evidence for the uncoupling of lifespan and healthspan. Despite the lifespan extension conferred by intestinal DAF-2 degradation in males, a similar effect on reproductive healthspan was not observed. However, previous work had shown that in the canonical *daf-2* mutant, several health metrics such as pharyngeal pumping (Huang et al. 2004), mobility (Mulcahy et al. 2012; Hahm et al. 2015), learning (Weng et al. 2024), and resistance to microbial pathogens (Murphy et al. 2003) are improved compared to wild type animals. The discrepancy is attributed to the complexity of the health metric we used in our study. In order to maintain reproductive success into old age, several systems and tissues need to maintain their function. Therefore, DAF-2 likely needs to be degraded in multiple tissues to produce a meaningful effect on reproductive success, which is what we observed in the ubiquitous DAF-2 AID strain.

In Roy et al. (2022) the dauer phenotype of *daf-2* mutants could not be recapitulated by degrading DAF-2 in single tissues. Additionally, the phenotype of reduced motility in early life and increased motility in late life was under combinatorial regulation of both neurons and muscles (Roy et al. 2022). Consistent with these results, we show here that male reproductive success in late life could not be promoted by single-tissue degradation but requires DAF-2 to be degraded in multiple tissues, accomplished here only via ubiquitous degradation. Future work could explore the effects of combined degradation in neurons and muscles, tissues known to play a role in motility and mating, on reproductive success in late life.

It has been shown previously that older males display deficits in turning behavior that is abolished in *daf-2* mutants, accompanied with improvements in the responsiveness to hermaphrodites, LOV efficiency, and turning ability (Chatterjee et al. 2013). We anticipated observing similar changes in the ubiquitous DAF-2 AID. However, we did not see differences in the response rate of LOV efficiency and saw a reduction in the time on the vulva. Instead, we discovered a novel tail immobility phenotype in aging males is the likely cause of sexual dysfunction, one that is ameliorated by DAF-2 degradation in the whole body. Future work could explore the basis of this phenotype and its relation to muscle health and function which had been

shown previously to decline with age, leading to motor deficits that are reduced in *daf-2* mutants (Glenn et al. 2004; Roy et al. 2022)

Our work shows that genetic interventions targeting the insulin-like signaling pathway extend not only the lifespan of the animal, but also the reproductive healthspan. While we focus on reproductive healthspan here, future studies could explore other healthspan metrics such as oxidative stress resistance, motility, pharyngeal pumping, and lipofuscin accumulation (Bansal et al. 2015). Our study expands on a framework for measuring healthspan in males as well as describing a novel tail paralysis phenotype. Taken together, these results suggest that males represent a useful tool for future aging research as they may be more susceptible to certain interventions and provide a complex behavior that can be used to assess the efficacy of interventions on reproductive healthspan.

Methods

C. elegans strains and maintenance

The strains used in this study were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The list of strains used in this study is provided in Supplemental Table S.2.7, many of which were sourced from Zhang et al. (2022). Strains were maintained at 20°C on 60 mm plates filled with standard nematode growth medium (NGM) seeded with 10 mm of *Escherichia coli* OP50, unless specified otherwise. Worms were transferred every Monday and Friday to fresh plates. Plant auxin at a final concentration of 1mM for knockdown experiments was prepared by adding 400 mM auxin stock (0.350g indole-3-acetic acid added to 5mL of 190-proof ethanol) to cooled agar in a 2.5mL to 1 L ratio and poured onto plates once mixed. Plates were then covered with foil/towels and stored in light-proof boxes at 4°C after being seeded with OP50. To decrease the likelihood of spontaneous dauer formation, the ubiquitous DAF-12 AID strain was kept in a separate box from other strains, and only a few worms during maintenance were transferred to prevent overcrowding. To maintain male stocks, 8–10 males and 4–5 hermaphrodites were transferred to new plates 1–2 times a week. Plates older than 1–2 weeks were discarded frequently.

Lifespan assay

The lifespan protocol used here is based on the previously published CITP lifespan protocol (Lucanic et al. 2017). A day prior to the start of the experiment, L4 males are staged visually using the male tail morphology and picked to 60 mm NGM plates seeded with *E. coli* OP50 at a concentration of 50 males per plate. Approximately 24 hours later, the adult males are transferred to experimental plates (60 mm NGM plates seeded with *E. coli* OP50, 40 males per plate, 3 plates per condition). Worms were scored three times a week and transferred to new plates weekly until the worms were all dead. Worms were scored as either alive, dead, or lost (if burrowed, missing, or walled). Mobile worms were scored as alive. Immobile worms were assayed by touching their tail or head with a platinum worm pick. If they do not respond to touch, they are scored as dead. Dead worms are removed from the plate, and live worms are counted.

Mating assay

For each replicate, a single male was picked at the L4 larval stage and placed on its respective treatment plate: a small 35 mm NGM plate or a 35 mm auxin plate seeded with 10 mm of *E. coli* OP50. Each trial consisted of 16 replicates per treatment. We tested the effects of DAF-2 degradation on young, middle-age, and old males, looking at days 1, 7, and 9 of adulthood, respectively. Once the males reached their target age, they were moved to a mating plate with two 1-day old adult feminized *fog-2* hermaphrodites. These hermaphrodites do not produce sperm, therefore any progeny observed must be a product of mating with a male (Schedl and Kimble 1988). Worms were allowed to mate for 24 hours at 20°C before being assayed. Mating success was scored as 1 for successful mating (progeny present), and 0 for unsuccessful mating (progeny absent). Plates with contamination or missing/dead males were censored.

Behavior assay

For each strain, 30 males were picked at their L4 stage and put on either a 100 mm NGM plate or a 100 mm auxin plate. They were then aged to their respective days of adulthood (one, seven, and nine). The day prior to the behavior assay, 300, L4 immobilized *unc-31* hermaphrodites were picked at their L4 stage and aged to day one of adulthood on a 150 mm NGM plate seeded with

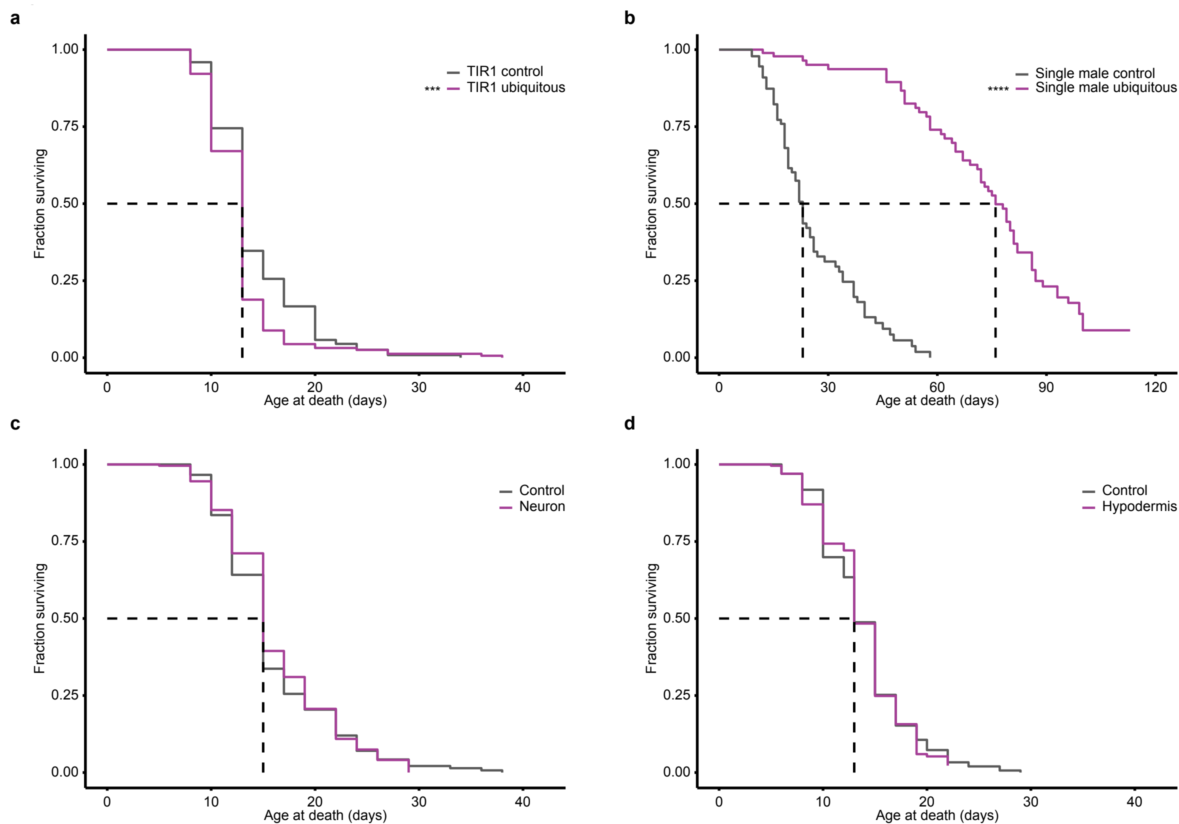
E. coli OP50. Behavior assays were based on methods described in Chatterjee et al. (2013). Five *unc-31* hermaphrodites were allowed to equilibrate on a 60 mm NGM plate seeded with 10 mm *E. coli* OP50 for 10 minutes. One aged male was placed on the plate and its behavior recorded for four minutes using the Dinocam software. If the worm did not respond to the hermaphrodites after four minutes, it was excluded from mating measures, if it began mating in the final minute, recording time was extended to six minutes to include the entirety of the behavior. Mating was evaluated using six measurements: contact with vulva rate (the amount of trials where males made and held contact with the vulva over total trials), Number of Passes (the amount of passes a worm made over the vulva with its tail before successfully mating), LOV efficiency (Successful vulva location (1) or unsuccessful (0) divided by the number of passes over the vulva), the amount of time on the vulva (the amount of time the male kept contact with the vulva), turning success (the percentage of worms that can successfully complete turning behaviors), and tail paralysis (the amount of worms that had upper bodies that were mobile but whose tails were immobile, over the total amount of trials).

Statistical analysis

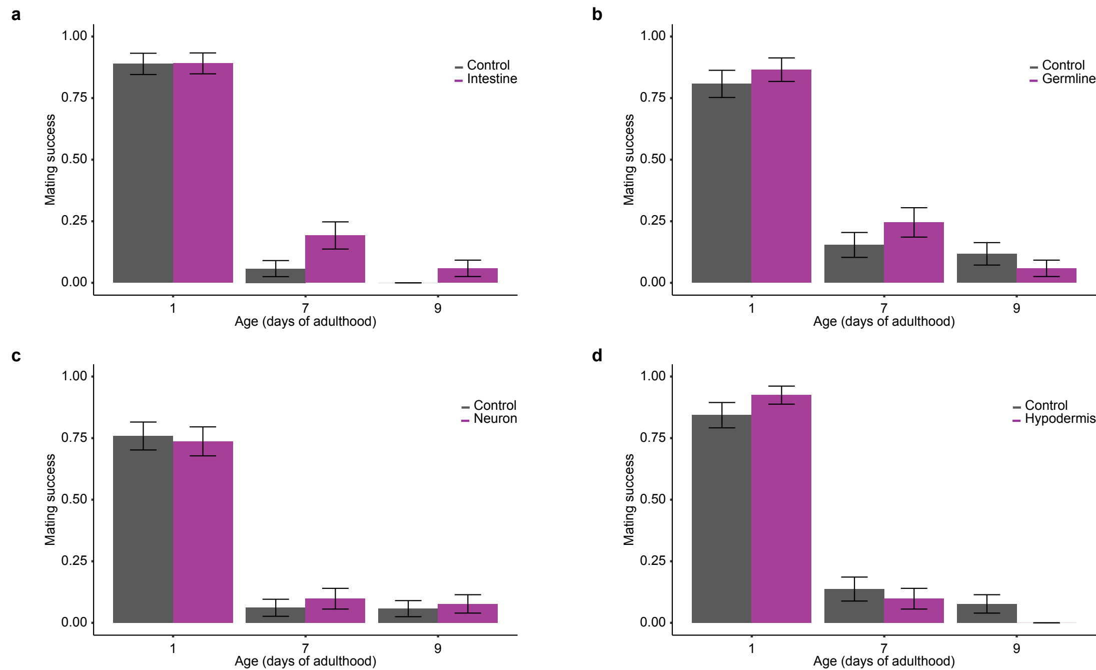
All the statistical analyses reported here were conducted in RStudio version 4.5.1 (R Core Team, 2024). A mixed effects Cox-Proportional Hazards (CPH) model was used for each strain (single tissue degradation) to analyze survival data, where treatment (control or auxin) was used as the fixed effect and technical (Rep) and biological replicates (StartDate) were used as the random effects. To test for the effect of sex, treatment, and the interaction between the two on survival in the ubiquitous DAF-2 AID strain, a CPH model was fit where, sex, treatment, and their interaction were the fixed effects, and technical (Rep) and biological replicates (StartDate) were the random effects. The coxme package version 2.2-22 (Therneau 2024) was used to fit the CPH model and the survival package version 3.8-3 (Therneau and Grambsch 2000; Therneau 2024) was used to construct the Kaplan-Meier survival curves. To analyze mating success, we fit a generalized linear model with a binomial distribution followed by paired comparisons to test for the effects of age, treatment, and their interaction on mating success within each strain. To analyze the effect of treatment behavior, we fit a generalized linear model with a binomial distribution followed by planned comparisons to test the effects of age, treatment, and their interaction on turning success, tail incoordination, and LOV efficiency and a gaussian

distribution for the time on the vulva. The lme package version 1.1-37 (Bates et al. 2015) was used to fit the linear model and the emmeans package version 1.11.2 (Lenth 2025) was used for planned comparisons.

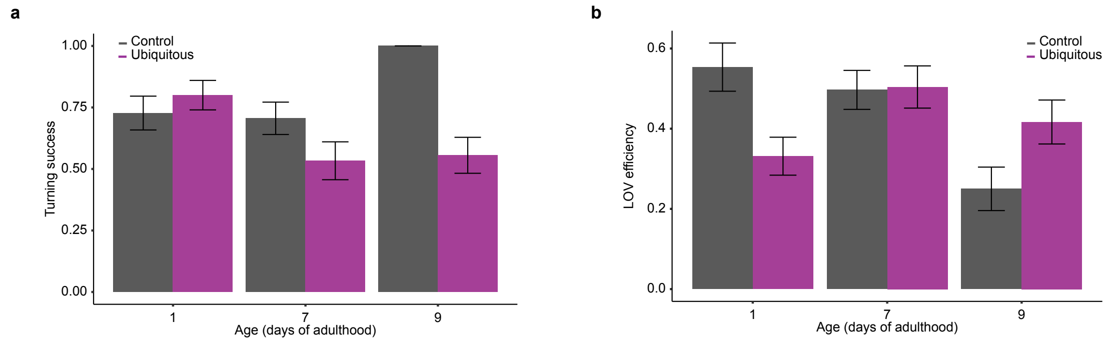
Supplemental Figures



Supplemental Fig. S2.1 Neuron- and Hypodermis-specific DAF-2 degradation does not extend male lifespan. (a) Kaplan-Meier curves showing survival of CA1200 TIR1-only *C. elegans* males. Gray lines denote the negative controls treated with ethanol and purple lines denote 1mM auxin treatment. Each line represents at least two biological replicates with total n = 179–184. (b) Kaplan-Meier curves showing survival of *C. elegans* males aged on individual plates. Gray lines denote the negative controls treated with ethanol and purple lines denote ubiquitous DAF-degradation with 1mM auxin treatment. Each line represents three biological replicates with total n = 62–70. (c, d) Kaplan-Meier curves showing survival of *C. elegans* males following DAF-2 degradation in the (b) neurons and (c) hypodermis. Gray lines denote the negative controls treated with ethanol and purple lines denote DAF-degradation with 1mM auxin treatment. Each line represents at least two biological replicates with total n = 180-202. The black dashed lines denote the age at which 50% of the population has died. The asterisks denote *p*-values from a Cox Proportional Hazards model where ****p<.0001, ***p<.001. For additional information and the output of the CPH model, see Supplemental Tables S2.1 and S2.2



Supplemental Fig. S2.2 Tissue-specific DAF-2 degradation does not preserve late-life male reproductive success. (a) Bar graphs showing mating success for *C. elegans* males following DAF-2 degradation in the (b) intestine, (c) germline, (d) neurons, and (e) hypodermis. Gray bars denote untreated controls, and purple bars denote DAF-2 degradation with 1 mM auxin. Each bar represents at least two biological replicates, with total n =49–59. Error bars represent SEM. A generalized linear mixed model with a binomial distribution was used to assess the effect of treatment and age (and the interaction) on mating success. For additional information and the output of the linear model, see Supplemental Tables S2.3 and S2.4



Supplemental Fig. S2.3 Turning success and LOV efficiency are not improved upon DAF-2 ubiquitous degradation. Bar graphs showing (a) turning success and (b) LOV efficiency for *C. elegans* males following ubiquitous DAF-2 degradation. Gray bars denote untreated controls, and purple bars denote DAF-2 degradation with 1 mM auxin. Each bar represents at least two biological replicates, with total n =44–52. Error bars represent SEM. A generalized linear mixed model with a binomial distribution was used to assess the effect of treatment and age (and the interaction) on behavior. For additional information and the output of the linear model, see Supplemental Tables S2.5 and S2.6

Supplemental Tables

Supplemental Table S2.1 Lifespan assay summary statistics

Sex (housing)	Strain	Tissue knockdown	Condition	Number dead	Number censored	Median Time	Mean	Std Error	Lower 95%	Upper 95%	90th percentile
Male (group)	CA1200	NA	Ethanol	184	13	13	14.20	0.32	13	13	20
Male (group)	CA1200	NA	Auxin	179	20	13	12.87	0.31	13	13	15
Male (group)	MQD2356	Neuron	Ethanol	202	38	15	15.98	0.39	15	15	24
Male (group)	MQD2356	Neuron	Auxin	192	48	15	16.24	0.36	15	15	24
Male (group)	MQD2374	Intestine	Ethanol	109	10	12	15.08	0.55	12	15	26
Male (group)	MQD2374	Intestine	Auxin	80	41	22	24.03	0.91	22	26	NA
Male (group)	MQD2375	Germline	Ethanol	137	103	16	17.26	0.48	14	19	26
Male (group)	MQD2375	Germline	Auxin	202	38	13	14.47	0.37	13	13	22
Male (group)	MQD2378	Hypodermis	Ethanol	180	60	13	13.98	0.32	13	15	20
Male (group)	MQD2378	Hypodermis	Auxin	202	38	13	13.98	0.30	13	15	19
Male (group)	MQD2453	Whole body	Ethanol	200	36	15	16.97	1.09	15	17	22
Male (group)	MQD2453	Whole body	Auxin	183	61	71	71.40	1.94	66	78	106
Hermaphrodite (group)	MQD2453	Whole body	Ethanol	235	41	33	35.29	0.71	32	37	51
Hermaphrodite (group)	MQD2453	Whole body	Auxin	223	106	46	46.96	1.27	42	51	72
Male (single)	MQD2453	Whole body	Ethanol	70	25	23	26.23	1.43	21	26	45
Male (single)	MQD2453	Whole body	Auxin	62	31	76	74.49	2.68	72	82	100
Hermaphrodite (single)	MQD2453	Whole body	Ethanol	55	20	40	41.62	1.67	37	46	60
Hermaphrodite (single)	MQD2453	Whole body	Auxin	44	31	39	46.89	3.28	34	60	79

Supplemental Table S2.2 Lifespan assay CPH model output

Male (group)				
CA1200	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	0.36	0.11	3.37	0.0008
MQD2356_neurons	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	-0.09	0.10	-0.87	0.38
MQD2374_intestine	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	-1.20	0.16	-7.75	8.88E-15
MQD2375_germline	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	0.50	0.11	4.39	0.00001
MQD2378_hypodermis	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	0.02	0.10	0.18	0.86
MQD2453_wholebody	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	-4.00	0.29	-0.14	<2E-16
Hermaphrodite (group)				
MQD2453_wholebody	Cox Proportional Hazards			
Compound	Effect	Stderr	z-value	p-value
Auxin	-0.91	0.11	-8.67	<2E-16
Males and Hermaphrodites (single) interaction model				
MQD2453_wholebody	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Auxin	-0.58	0.22	-2.63	0.008
Sex	1.02	0.19	5.49	4.07E-08
Interaction	-2.30	0.29	-8.03	9.38E-16

Supplemental Table S2.3 Mating assay summary statistics

Strain	Tissue Knockdown	Age	Compound	Mean Mating Success	SD	Count	SEM
CA1200	NA	1	No auxin	0.75	0.44	51	0.06
CA1200	NA	1	Auxin	0.71	0.46	52	0.06
MQD2356	neuron	1	No auxin	0.76	0.43	58	0.06
MQD2356	neuron	1	Auxin	0.74	0.44	57	0.06
MQD2374	intestine	1	No auxin	0.89	0.32	54	0.04
MQD2374	intestine	1	Auxin	0.89	0.31	55	0.04
MQD2375	germline	1	No auxin	0.81	0.40	52	0.06
MQD2375	germline	1	Auxin	0.87	0.34	52	0.05
MQD2378	hypodermis	1	No auxin	0.84	0.37	51	0.05
MQD2378	hypodermis	1	Auxin	0.92	0.27	53	0.04
MQD2453	whole body	1	No auxin	0.76	0.43	51	0.06
MQD2453	whole body	1	Auxin	0.58	0.50	59	0.06
CA1200	NA	7	No auxin	0.04	0.19	52	0.03
CA1200	NA	7	Auxin	0.10	0.30	51	0.04
MQD2356	neuron	7	No auxin	0.06	0.24	49	0.03
MQD2356	neuron	7	Auxin	0.10	0.30	51	0.04
MQD2374	intestine	7	No auxin	0.06	0.24	52	0.03
MQD2374	intestine	7	Auxin	0.19	0.40	52	0.06
MQD2375	germline	7	No auxin	0.15	0.36	52	0.05
MQD2375	germline	7	Auxin	0.25	0.43	53	0.06
MQD2378	hypodermis	7	No auxin	0.14	0.35	51	0.05
MQD2378	hypodermis	7	Auxin	0.10	0.30	51	0.04
MQD2453	whole body	7	No auxin	0.29	0.46	52	0.06
MQD2453	whole body	7	Auxin	0.35	0.48	52	0.07
CA1200	NA	9	No auxin	0.04	0.19	56	0.03
CA1200	NA	9	Auxin	0.06	0.24	52	0.03
MQD2356	neuron	9	No auxin	0.06	0.24	52	0.03
MQD2356	neuron	9	Auxin	0.08	0.27	52	0.04
MQD2374	intestine	9	No auxin	0.00	0.00	51	0.00
MQD2374	intestine	9	Auxin	0.06	0.24	51	0.03
MQD2375	germline	9	No auxin	0.12	0.33	51	0.05
MQD2375	germline	9	Auxin	0.06	0.24	51	0.03
MQD2378	hypodermis	9	No auxin	0.08	0.27	52	0.04
MQD2378	hypodermis	9	Auxin	0.00	0.00	49	0.00
MQD2453	whole body	9	No auxin	0.04	0.21	46	0.03
MQD2453	whole body	9	Auxin	0.30	0.46	56	0.06

Strain	Tissue Knockdown	Age	Compound	Mean Mating Success	SD	Count	SEM
MQD2453	whole body	12	No auxin	0.00	0.00	17	0.00
MQD2453	whole body	12	Auxin	0.35	0.49	20	0.11
MQD2453	whole body	15	No auxin	0.00	0.00	23	0.00
MQD2453	whole body	15	Auxin	0.12	0.33	58	0.04
MQD2453	whole body	18	No auxin	0.00	0.00	4	0.00
MQD2453	whole body	18	Auxin	0.03	0.17	33	0.03
MQD2453	whole body	21	No auxin	0.00	0.00	11	0.00
MQD2453	whole body	21	Auxin	0.00	0.00	34	0.00

Supplemental Table S2.3, continued

Supplemental Table S.2.4 Mating assay GLM model output

CA1200	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	1.07	0.32	3.34	0.0008
Auxin	-0.17	0.44	-0.38	0.70
Age 7	-4.29	0.79	-5.44	5.45E-08
Age 9	-4.37	0.79	-5.54	3.02E-08
Auxin: Age 7	1.17	0.97	1.21	0.23
Auxin: Age 9	0.67	1.03	0.65	0.52
Germline	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	1.44	0.35	4.08	0.00005
Auxin	0.43	0.54	0.79	0.43
Age 7	-3.14	0.52	-6.03	1.69E-09
Age 9	-3.45	0.56	-6.17	6.85E-10
Auxin: Age 7	0.16	0.73	0.21	0.83
Auxin: Age 9	-1.18	0.91	-1.30	0.19
Hypodermis	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	1.68	0.39	4.37	0.00001
Auxin	0.82	0.65	1.27	0.20
Age 7	-3.52	0.56	-6.28	3.31E-10
Age 9	-4.17	0.65	-6.44	1.22E-10
Auxin: Age 7	-1.20	0.90	-1.34	0.18
Auxin: Age 9	-16.90	931.81	-0.02	0.99
Intestine	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	2.08	0.43	4.80	0.000001
Auxin	0.02	0.61	0.03	0.97
Age 7	-4.87	0.74	-6.62	3.51E-11
Age 9	-20.65	913.35	-0.02	0.98
Auxin: Age 7	1.34	0.92	1.45	0.15
Auxin: Age 9	15.77	913.35	0.02	0.99

Neuron	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	1.15	0.31	3.73	0.0001
Auxin	-0.12	0.43	-0.27	0.79
Age 7	-3.88	0.67	-5.78	7.39E-09
Age 9	-3.94	0.67	-5.89	3.99E-09
Auxin: Age 7	0.63	0.87	0.72	0.47
Auxin: Age 9	0.42	0.90	0.47	0.64
Whole body	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	1.18	0.33	3.57	0.0004
Auxin	-0.87	0.42	-2.063	0.04
Age 7	-2.08	0.45	-4.624	0.000004
Age 9	-4.27	0.79	-5.372	7.78E-08
Age 12	-19.74	1581.97	-0.012	0.99
Age 15	-19.74	1360.06	-0.015	0.99
Age 18	-19.74	3261.32	-0.006	0.99
Age 21	-19.74	1966.65	-0.01	0.99
Auxin: Age 7	1.14	0.60	1.905	0.057
Auxin: Age 9	3.13	0.89	3.534	0.0004
Auxin: Age 12	18.82	1581.97	0.012	0.99
Auxin: Age 15	17.45	1360.06	0.013	0.99
Auxin: Age 18	15.97	3261.32	0.005	1.00
Auxin: Age 21	0.87	2262.53	0	1.00

Supplemental Table S.2.4, continued

Supplemental Table S.2.5 Behavior assay summary statistics

Variable	Strain	Tissue Knockdown	Age	Compound	Mean	SD	Count	SEM
LOV	MQD2453	Whole body	1	No auxin	27.92	16.37	44	2.44
LOV	MQD2453	Whole body	1	Auxin	41.33	34.43	47	4.77
LOV	MQD2453	Whole body	7	No auxin	39.67	26.79	51	3.72
LOV	MQD2453	Whole body	7	Auxin	0.80	0.41	45	0.06
LOV	MQD2453	Whole body	9	No auxin	0.68	0.36	52	0.05
LOV	MQD2453	Whole body	9	Auxin	0.64	0.41	52	0.06
Tail incoordination	MQD2453	Whole body	1	No auxin	0.27	0.39	44	0.06
Tail incoordination	MQD2453	Whole body	1	Auxin	0.25	0.40	47	0.06
Tail incoordination	MQD2453	Whole body	7	No auxin	0.29	0.38	51	0.05
Tail incoordination	MQD2453	Whole body	7	Auxin	0.27	0.39	45	0.05
Tail incoordination	MQD2453	Whole body	9	No auxin	0.00	0.00	52	0.00
Tail incoordination	MQD2453	Whole body	9	Auxin	0.00	0.00	52	0.00
Time on vulva	MQD2453	Whole body	1	No auxin	0.29	0.46	44	0.07
Time on vulva	MQD2453	Whole body	1	Auxin	0.29	0.46	47	0.06
Time on vulva	MQD2453	Whole body	7	No auxin	0.50	0.50	51	0.07
Time on vulva	MQD2453	Whole body	7	Auxin	0.44	0.50	45	0.07
Time on vulva	MQD2453	Whole body	9	No auxin	64.61	73.06	52	10.13
Time on vulva	MQD2453	Whole body	9	Auxin	59.81	56.06	52	7.77
Turning success	MQD2453	Whole body	1	No auxin	0.02	0.15	44	0.02
Turning success	MQD2453	Whole body	1	Auxin	60.00	93.10	47	13.44
Turning success	MQD2453	Whole body	7	No auxin	34.53	29.68	51	4.33
Turning success	MQD2453	Whole body	7	Auxin	35.17	33.62	45	4.66
Turning success	MQD2453	Whole body	9	No auxin	73.07	69.88	52	9.79
Turning success	MQD2453	Whole body	9	Auxin	17.00	4.47	52	0.62

Supplemental Table S.2.6 Behavior assay GLM output

Turning difficulty	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	-0.98	0.48	-2.05	0.04
Auxin	-0.41	0.74	-0.55	0.58
Age 7	0.11	0.72	0.15	0.88
Age 9	-15.59	1199.77	-0.01	0.99
Auxin: Age 7	1.15	1.05	1.10	0.27
Auxin: Age 9	16.75	1199.77	0.01	0.99
Tail incoordination	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	-3.76	1.01	-3.72	0.0002
Auxin	-0.07	1.43	-0.05	0.96
Age 7	2.58	1.06	2.43	0.015
Age 9	3.45	1.05	3.29	0.001
Auxin: Age 7	-1.39	1.58	-0.88	0.38
Auxin: Age 9	-1.33	1.51	-0.88	0.38
LOV	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	-0.66	0.29	-2.29	0.02
Auxin	-0.49	0.41	-1.21	0.23
Age 7	0.05	0.44	0.10	0.92
Age 9	-0.54	0.72	-0.75	0.45
Auxin: Age 7	0.63	0.63	1.00	0.32
Auxin: Age 9	1.09	0.93	1.18	0.24
Time on vulva	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	4.26	0.03	151.60	<2.00E-16
Auxin	-0.71	0.05	-14.30	<2.00E-16
Age 7	0.04	0.04	0.85	0.39
Age 9	-0.53	0.09	-5.68	1.39E-08
Auxin: Age 7	-0.25	0.08	-3.15	0.002
Auxin: Age 9	0.67	0.12	5.54	3.08E-08

Supplemental Table S.2.7 Strains used in this study

Strain Name	Genotype	Tissue knockdown
MQD2374	*ieSi61 II; daf-2(hq363[daf-2::degron::mNeonGreen]) unc-119(ed3) III.*	Intestine
MQD2378	*hqSi9 II; daf-2(hq363[daf-2::degron::mNeonGreen]) unc-119(ed3) III.*	Hypodermis
MQD2356	*hqSi8 II; daf-2(hq363[daf-2::degron::mNeonGreen]) unc-119(ed3) III.*	Neurons
MQD2453	*ieSi57 II; daf-2(hq363[daf-2::degron::mNeonGreen]) unc-119(ed3) III.*	Whole body
MQD2375	*daf-2(hq363[daf-2::degron::mNeonGreen]) unc-119(ed3) III; ieSi38 IV.*	Germline
JK574	*fog-2(q71) V.*	NA
CA1200	*ieSi57 II; unc-119(ed3) III.*	NA
CB169	*unc-31(e169) IV.*	NA

Bridge to chapter 3

In chapter 2, I explored the sex differences in the response to a genetic pro-longevity intervention, targeted DAF-2 degradation, on lifespan. Additionally, I explored whether the resulting beneficial effects on lifespan in males can be extended to reproductive healthspan or whether the two metrics are decoupled. In chapter 3, I extend this framework to test the effects of a second type of intervention, chemical interventions, and whether such interventions result in a sex-specific benefit on lifespan and healthspan.

CHAPTER 3: PRO-LONGEVITY COMPOUNDS EXTEND *CAENORHABDITIS ELEGANS* MALE LIFESPAN AND REPRODUCTIVE HEALTHSPAN

(In review at *GeroScience*)

Rose S. Al-Saadi, Patrick C. Phillips

Author contributions

RSA and PCP conceptualized the project. RSA conducted the lifespan and mating success assays, conducted the statistical analysis and visualization, and wrote the original manuscript. PCP supervised the project, reviewed and edited the manuscript, and acquired funding.

Abstract

Sex differences in aging are robust and ubiquitous. Demographic differences in aging generated by sex have long been recognized, but the underlying biological basis for these differences and the potential for sex-specific interventions remain understudied. To explore sex differences in the response to pro-longevity interventions, we utilized the *C. elegans* aging model and asked whether male lifespan and reproductive healthspan can be extended via compounds known to have pro-longevity effects in hermaphrodites. We tested seven different compounds at two concentrations each and found that lifespan was extended under all tested conditions. However, reproductive healthspan measured by mating success in late life improved under only two tested conditions, sulforaphane and metformin. These results demonstrate that lifespan and healthspan can be decoupled in *C. elegans* males and offer a new framework for screening pro-longevity compounds and for studying sex differences in aging in a classical aging model.

Introduction

Sex differences in aging are robust and ubiquitous (Austad and Fischer 2016). These differences are observed in both lifespan (the chronological time between birth and death) and healthspan (the proportion of lifespan that is disease-free). In humans, women live longer but tend to suffer worse health outcomes compared to men (Oksuzyan et al. 2008; Gordon et al. 2017). Several age-associated conditions such as chronic conditions (Bello-Lujan et al. 2022), bone mineral

density loss (Daly et al. 2013), inflammation (Milan-Mattos et al. 2019), and frailty (Gordon et al. 2017) exhibit sex differences in incidence and severity (Hägg and Jylhävä 2021). This sexual dimorphism extends to modulators of aging such as pro-longevity interventions, with many tested regimes (genetic, chemical, or environmental) showing sex differences in efficacy (Bartke et al. 2024). However, our knowledge of the molecular basis of these differences is limited due to a historic focus on male health and disease (Holdcroft 2007).

Caenorhabditis elegans have a short lifespan (2–3 weeks), have conserved genetic pathways that modulate aging (Zhang et al. 2020), have genetic diversity comparable to that of human populations (Andersen and Rockman 2022; Teterina et al. 2022), and have longevity that is amenable to both genetic and chemical interventions (Zhang et al. 2020), making them a pivotal model system for understanding the biology of aging. *C. elegans* is also sexually dimorphic, with self-fertile hermaphrodites and rare males (Anderson et al. 2010; Emmons 2014). While a few recent studies have included sex differences in *C. elegans* aging (McCulloch and Gems 2007; Liggett et al. 2015; Honjoh et al. 2017; Hotzi et al. 2018; Weng and Murphy 2024), the vast majority of aging research using *C. elegans* focuses solely upon hermaphrodites. This is largely due to the fact that males are rare in the commonly used lab strain N2 (0.1%), as well as being shown to have detrimental effects on hermaphrodite lifespan (Maures et al. 2014; Shi and Murphy 2014; Booth et al. 2022). Overall, our understanding of sex differences in aging processes in *C. elegans* remains extremely limited.

The *Caenorhabditis* Intervention Testing Program (CITP) is a National Institutes of Aging supported collaborative effort across three laboratories in the USA that leverages the benefits of *Caenorhabditis* nematodes as an aging model to test the effects of pharmacological interventions on the lifespan and healthspan of different species in the *Caenorhabditis* genus in a rigorous and robust fashion. The program has tested dozens of compounds and has found that many do indeed extend hermaphrodite lifespan (Driscoll et al. 2025). However, it remains unknown whether these compounds are similarly efficacious in males. This seems especially pertinent because the CITP's long-running sister program using mice, the ITP, has found that males tend to be more responsive to compound interventions than females (Strong et al. 2008; Harrison et al. 2014; Bartke et al. 2024).

Male *C. elegans* represent an exciting tool to test pharmacological interventions for two main reasons. First, males provide a complex health measure that integrates the health of multiple tissues and structures into one output: reproductive success. Male motivation for mating remains high throughout life and failure to sire progeny is caused by physical deterioration rather than a decrease in motivation per se (Guo et al. 2012; Chatterjee et al. 2013). This is in contrast to hermaphrodites, which appear to change reproductive patterns due a variety of environmental inputs, including the presence of males (Aprison and Ruvinsky 2016). Males need to locate a hermaphrodite via chemotaxis, scan their mate's body to locate the vulva via mechanosensory neurons, insert the spicule, and ejaculate, thereby transferring sperm to the hermaphrodite uterus (Barr and Garcia 2006). If any of these processes exhibit age-associated defects, reproductive success will decrease. The second reason males are an excellent tool for screening pharmacological compounds is that males do not produce self-progeny, eliminating the need to abrogate offspring production, for instance using the chemotherapy agent 5-Fluoro-2'-deoxyuridine (FUdR), which blocks cell division by inhibiting DNA synthesis (Mitchell et al. 1979; Gandhi et al. 1980). In hermaphrodite lifespan experiments, FUdR is commonly used to sterilize hermaphrodites and thereby eliminate the need for daily transfers during the first 5–6 days of adulthood (which is very labor and resource intensive). However, FUdR is known to affect hermaphrodites directly, as well as affecting the bacteria upon which they feed (Wang et al. 2019; McIntyre et al. 2021). Male lifespan experiments therefore offer the same ease as FUdR-supplemented hermaphrodites but without confounding effects of FUdR per se.

Here, we tested the potential pro-longevity effects of several CITP-validated compounds on male lifespan and healthspan, finding that all tested compounds increase male lifespan, even at lower concentrations than hermaphrodites. However, only two compounds reliably improved male healthspan, as measured via mating success in late life. The work here describes a new framework for pharmacological intervention screening using male *C. elegans* in addition to hermaphrodites to expand our understanding of sex differences in aging and to enable us to create interventions that are effective for both sexes.

Materials and methods

C. elegans strains and maintenance

All the strains used in this study were provided by the *Caenorhabditis* Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The strains used were CB1489 (*him-8(e1489)* IV) and JK574 (*fog-2(q71)* V). The strains were maintained on 60 mm plates of standard NGM media seeded with *E. coli* OP50-1. Worms were transferred to fresh plates 2–3 times weekly. Unless otherwise specified, all the stocks and assays were maintained at 20ºC.

Compound treatment

We used the following equation to determine the working stock concentration (X), where the treatment volume is 450 µL for water-soluble compounds and 25 µL for DMSO-soluble compounds and the plate volume is 10 mL for both types:

$$Treatment\ volume * X = final\ concentration * plate\ volume$$

For water-soluble compounds (gold sodium thiomalate, metformin, thioflavin T), 450 µL of the working stock was added to 60 mM NGM plates seeded with OP50-1 and allowed to dry at room temperature for approximately 3 days. The working stock concentrations for metformin were 0.78 M and 1.56 M for final concentrations of 35 mM and 70 mM, respectively. The working stock concentrations for gold sodium thiomalate were 1.11 mM and 2.22 mM for final concentrations of 50 µM and 100 µM, respectively. The working stock concentrations for thioflavin T were 0.56 mM and 1.11 mM for final concentrations of 25 µM and 50 µM, respectively. For DMSO-soluble compounds (all *trans* retinoic acid, propyl gallate, resveratrol, sulforaphane), 25 µL of the working stock mixed with 425 µL of water was added to 60 mM NGM plates seeded with OP50-1 and allowed to dry at room temperature for approximately 3 days. The working stock concentrations for all *trans* retinoic acid for were 30 mM and 60 mM for final concentrations of 75 µM and 150 µM, respectively. The working stock concentrations for propyl gallate were 40 mM and 80 mM for final concentrations of 100 µM and 200 µM, respectively. The working stock concentrations for resveratrol were 20 mM and 40 mM for final

concentrations of 50 µM and 100 µM, respectively. The working stock concentrations for sulforaphane were 20 mM and 40 mM for final concentrations of 50 µM and 100 µM, respectively.

Lifespan assays

A modified CITP lifespan protocol was used (Lucanic et al. 2017). Males at the L4 stage (identified by the distinct tail morphology in that stage) were selected from a mixed population plate and transferred to treated plates at a density of 40 worms per plate. Males were counted 3 times weekly, removing any dead worms, and transferred once weekly until all the worms were dead. Lost and walled worms and contaminated plates were censored from the final analyses.

CITP data comparison

We calculated the change in survival and percent change in median lifespan in hermaphrodites using data obtained from the CITP Data Portal (citpaging.org) for each compound (and unpublished data for gold sodium thiomalate) and compared the results to the survival and percent change in median lifespan in males when treated with the same compound and concentration. We filtered the hermaphrodite data to include the *C. elegans* N2 strain data from the Oregon location only to minimize variability since the male lifespan experiments were conducted in Oregon. We additionally filtered the compound concentrations to include only those tested in males. The change in median lifespan was calculated from individual treatment replicates and their corresponding control replicates by subtracting the median lifespan of the control replicate from the median lifespan of the treated replicate then divided by the median lifespan of the control.

Mating success assays

Males at the L4 stage (identified by the distinct tail morphology in that stage) were selected from a mixed population plate and transferred to treated plates at a density of 40 worms per plate 40. To generate seven-day old males, L4 males were moved to treated plates seven days prior to the assay. To generate five-day old males, L4 males were moved to treated plates five days prior to the assay. To generate one-day old males, L4 males were moved to treated plates one day prior

to the assay. To generate virgin *fog-2* pseudo-females, L4 pseudo-females were moved to separate plates away from males one day prior to the assay to prevent them from mating. On the day of the assay, one male and two pseudo-females are placed on a 35 mm plate seeded with 100 µL of OP50-1 and allowed to mate. Approximately 24 hours later, the plates are scored for the presence or absence of progeny. For each compound, at least two trials (biological replicates) were conducted with approximately 20 plates (technical replicates) per trial.

Statistical analysis

All the statistical analyses presented here were conducted using R version 4.4.1 (R Core Team, 2024). Lifespan data were analyzed using R scripts modified from the CITP's standardized analysis pipeline (Lucanic et al. 2017). To compare survival of each treatment group to the control group, a mixed effects Cox-Proportional Hazards model was used where the compound treatment was the fixed effect and both biological (Start Date) and technical (Rep) replicates were the random effects. To fit the mixed effects model, we used the coxme package version 2.2-20 (Therneau 2024). Kaplan-Meier curve plots were generated using the survival package version 3.6-4 (Therneau and Grambsch 2000; Therneau 2024). To test for the effects of sex on the response to compound treatment, a mixed effects Cox-Proportional Hazards model was used where the compound treatment, sex, and the interaction were the fixed effects and both biological (Start Date) and technical (Rep) replicates were the random effects. To compare the mating success of each treatment group to the control group, a generalized linear mixed model with a binomial distribution was used where the compound treatment and age (and the interaction) were the fixed effects and biological replicate (Trial) was the random effect. To fit the linear model, we use the lme4 package version 1.1-35.5 (Bates et al. 2015).

Results

Hermaphrodite pro-longevity chemical compounds also extend lifespan in *C. elegans* males

We first asked if male lifespan can be extended via compounds known to have pro-longevity effects in hermaphrodites. Since the commonly used wildtype N2 strain has a low incidence of males (0.1%), we used *him-8* mutants to enrich for males in our populations. We selected seven compounds that have been previously shown by CITP and others to extend hermaphrodite

lifespan. We tested the Vitamin A derivative all-*trans* retinoic acid (2024), the anti-rheumatic compound gold sodium thiomalate (personal communication with CITP), the type 2 diabetes medication metformin (Onken et al. 2022), the antioxidants propyl gallate and resveratrol (Benedetti et al. 2008; Lucanic et al. 2017), the cruciferous plant derivative sulforaphane (Qi et al. 2021; Sedore et al. 2025), and the amyloid dye thioflavin T (Alavez et al. 2011; Lucanic et al. 2017). For each compound, we selected the lowest effective concentration previously reported to extend N2 hermaphrodite lifespan and asked whether those concentrations extend male lifespan in a similar manner. We found that all tested compounds significantly extended male median lifespan (Fig. 3.1, darker survival curves), with thioflavin T and sulforaphane resulting in the largest extension in median lifespan (77% and 44%, respectively) and maximum lifespan (100% and 43%, respectively). Notable extension in median and maximum lifespan was observed with all-*trans* retinoic acid (19% and 10%), gold sodium thiomalate (23% and 44%), metformin (23% and 44%), propyl gallate (19% and 14%), and resveratrol (25% and 33%) treatments. Since males have been shown to be more sensitive than hermaphrodites to certain chemicals (Inoue and Nishida 2010; Shi et al. 2017), we next asked whether a lower concentration of each compound was sufficient to extend lifespan. We lowered the concentration of each tested compound by half and found that lifespan was significantly extended at these concentrations as well (Fig. 3.1, lighter survival curves). Surprisingly, we found that the magnitude of median lifespan increases for gold sodium thiomalate, metformin, and thioflavin T was very similar at these lower concentrations, suggesting that males are more susceptible to chemical treatments and could represent a new tool for testing compound interventions in a more efficient and cost-effective manner.

While all the tested compounds extended male lifespan significantly, we wondered if the effect sizes for the compound treatment between males and hermaphrodites were different. Using previously published data for hermaphrodites from the CITP, we asked whether males respond quantitatively differently than hermaphrodites to compound treatments. We found that the magnitude of survival increase elicited by four compounds (all-*trans* retinoic acid, sulforaphane, gold sodium thiomalate, thioflavin T) is dependent on sex. Hermaphrodites treated with all-*trans* retinoic acid, sulforaphane, and gold sodium thiomalate display a larger increase in median lifespan than males. Males, on the other hand, display a larger increase in median lifespan than

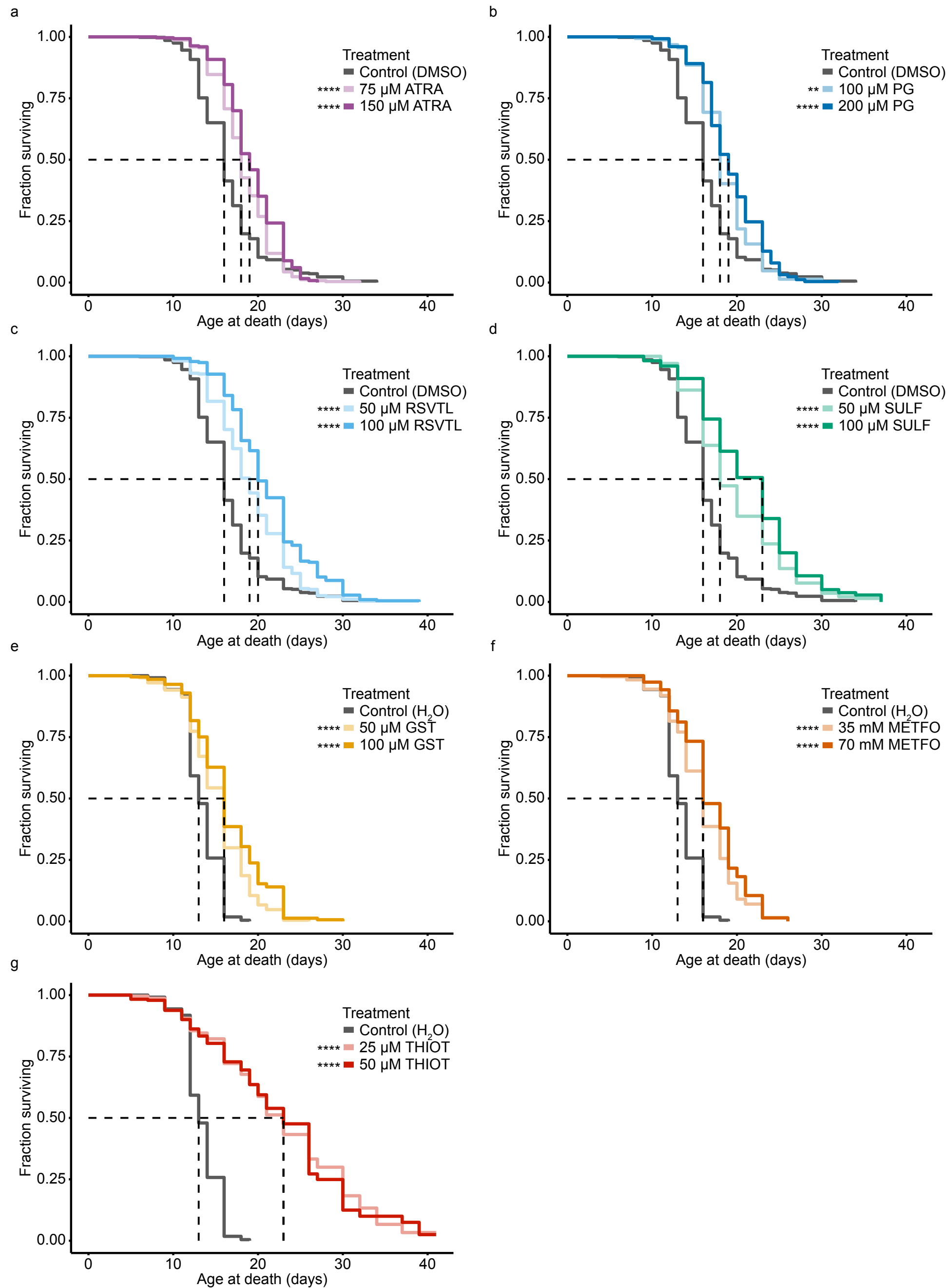


Fig. 3.1 Pro-longevity compounds extend male lifespan. Kaplan-Meier curves showing survival of *C. elegans* males treated with either DMSO-soluble (a-d) or water-soluble (e-g) compounds. Solid lines represent survival on the negative control (DMSO or water), all-*trans* retinoic acid (ATRA), propyl gallate (PG), resveratrol (RSVTL), sulforaphane (SULF), gold sodium thiomalate (GST), metformin (METFO), and thioflavin T (THIOT). Each line represents at least two biological replicates with total n = 113–496. The dashed lines denote the age at which 50% of the population has died. The asterisks denote *p*-values from a Cox Proportional Hazards model where **p<.01, ****p<.0001. For additional information and the output of the CPH model, see Supplemental Tables S3.1 and S3.2

hermaphrodites when treated with thioflavin T (Fig. 3.2). Although the median lifespan change induced by metformin treatment is greater in hermaphrodites than males, greater replicate variance at the individual sampling level for metformin treatment reduced the power, therefore the difference itself was not significant. Overall, we find evidence for differences in the efficacy of compound treatment based on sex in *C. elegans*.

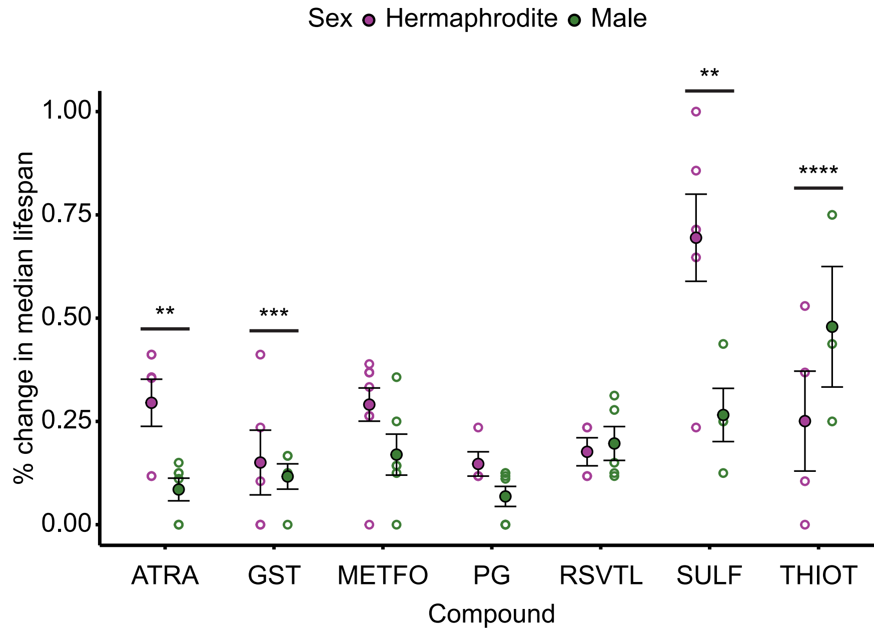


Fig. 3.2 Sex differences in the percent change in median lifespan for different compounds compared to the control. Dot plot showing the percent change in median lifespan for each compound compared to the control. Solid circles represent the mean and open circles represent individual replicates. The error bars represent SEM. Only the higher concentrations (shown in Fig. 3.1) are represented here as lower concentrations were not tested on hermaphrodites. A CPH model was used to assess the effects of compound, sex, and the interaction of the two factors on survival. The asterisks denote *p*-values from the CPH model where **p<.01, ***p<.001, ****p<.0001. For additional information and the output of the linear model, see Supplemental Tables S3.3 and S3.4

Reproductive success is preserved late in life under sulforaphane and metformin treatment

We next asked whether the increase in lifespan is accompanied by an improvement in health, specifically reproductive health. We selected reproductive success in late life as our health measure as it is a complex behavior that declines precipitously with age (Chatterjee et al. 2013). We treated males with the seven selected chemical compounds and measured their reproductive

success at three different ages: day 1 of adulthood when reproductive success is at its peak, day 5 of adulthood when reproductive success drastically declines, and day 7 of adulthood when reproductive success is severely diminished. Because hermaphrodites can produce self-progeny and do not depend on males for providing sperm, we used *fog-2* mutant hermaphrodites that fail to produce sperm, making them pseudo-females (Schedl and Kimble 1988). To measure reproductive success, a single male and two virgin *fog-2* pseudo-females were placed on a plate and allowed to mate for 24 hours. Plates were then screened for presence or absence of progeny, indicating successful mating or no/failed mating, respectively. We found that, despite the significant increase in lifespan with all treatments, only two of them improved reproductive success (Fig. 3.3). Sulforaphane increased mating success on day 7 by 201% percent and metformin increased mating success on day 5 by 215% percent (Fig. 3.3 d and f). Overall, we find that out of seven tested compounds that significantly increase male survival, only two improved male reproductive success in old age. This further validates the use of males to screen for pro-longevity interventions and the use of male mating success as a comprehensive healthspan metric.

Discussion

In this study, we leverage *C. elegans* males to investigate whether biological sex affects the response to a set of pro-longevity compounds validated by CITP. We found that treatment with all tested compounds in this study significantly extended male lifespan. Despite this, reproductive healthspan as measured by mating success was extended under only two tested compounds. To our knowledge, these findings represent the first effort to explore sex differences in response to pro-longevity compounds in the *C. elegans* model system.

Sex differences in biological processes have been historically neglected or ignored in both basic and clinical research (Holdcroft 2007). This oversight was in part due to the incorrect assumption that female traits are generally more variable due to hormonal fluctuations (Holdcroft 2007). Although this assumption was later debunked (Zajitschek et al. 2020), the bias for using males in research persisted for many years, significantly limiting our knowledge of sex differences in biological processes and inappropriately generalizing male-specific findings to both sexes (Zucker and Beery 2010; Beery and Zucker 2011). To address this knowledge gap, the National

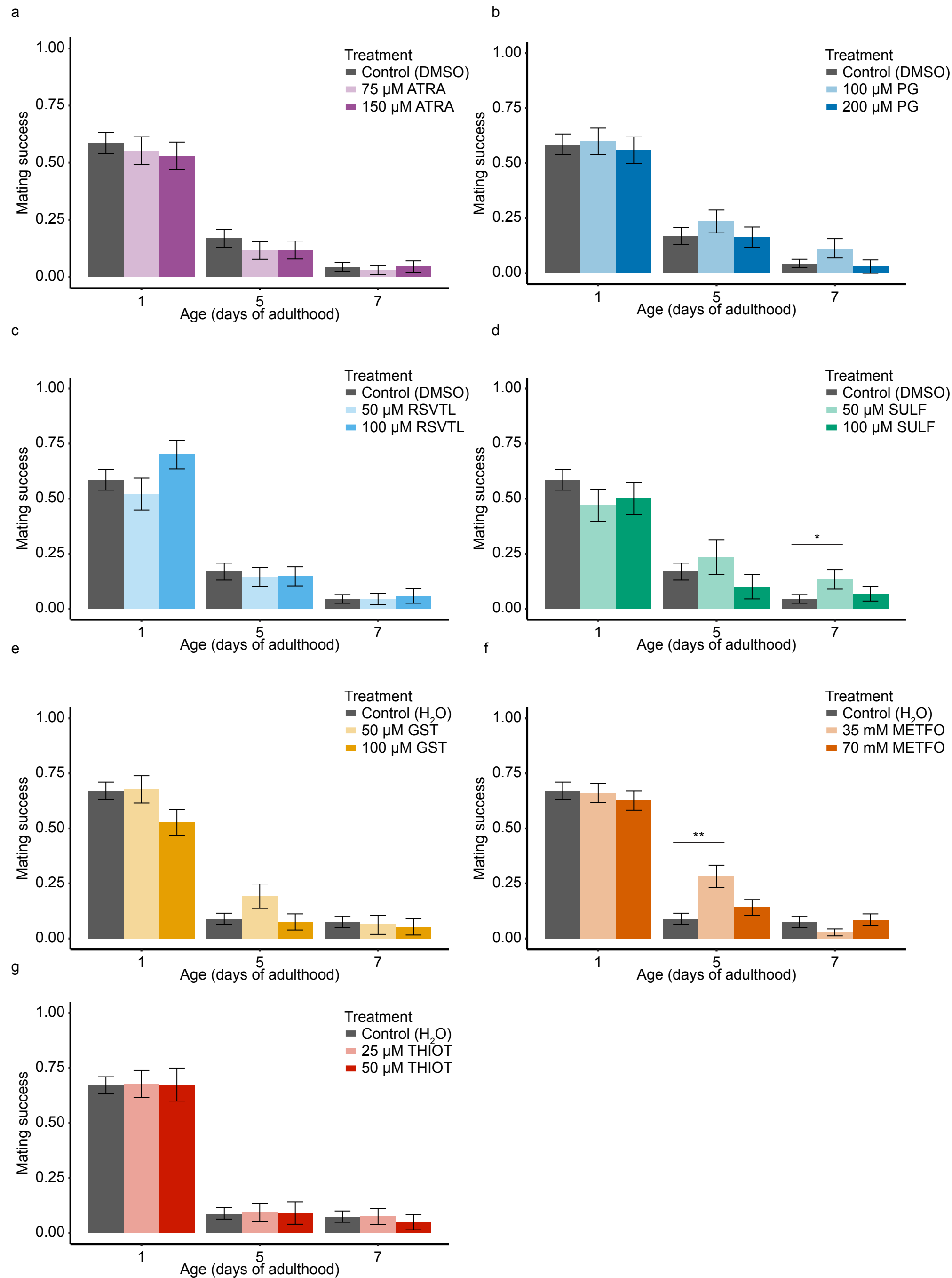


Fig. 3.3 Sulforaphane and metformin preserve late-life male reproductive success. Bar graphs showing mating success for *C. elegans* males treated with either DMSO-soluble (a-d) or water-soluble (e-g) compounds then mated with *fog-2* pseudo-females. Each bar represents at least two biological replicates, with total n = 30–113. Error bars represent SEM. A generalized linear mixed model with a binomial distribution was used to assess the effect of treatment and age (and the interaction) on mating success. The asterisks denote *p*-values for age by compound interaction from the linear model where *p<.05, **p<.01. For additional information and the output of the linear model, see Supplemental Tables S3.5 and S3.6

Institutes of Health mandated in 1993 that women be included in clinical studies (National Institutes of Health 1994) and in 2015 that sex as a biological variable be included in study design, analysis, and reporting (National Institutes of Health 2015). Sex plays an especially important role in aging; there are robust differences between the sexes in lifespan, healthspan, age-associated disease susceptibility, and the response to pro-longevity interventions (Hägg and Jylhävä 2021; Bartke et al. 2024). Despite institutional efforts and scientific findings, research on sex differences in aging biology has remained fairly limited.

In 2002, the Intervention Testing Program (ITP) was created to screen compounds (chemical, pharmaceutical, or plant extracts) that extend lifespan or delay onset of diseases in a genetically heterogeneous mouse model (Macchiarini et al. 2021). The program emphasized robust and reproducible experiments and has identified 13 compounds that significantly increase median lifespan in mice (Barardo et al. 2017; Macchiarini et al. 2021), many of which show sex differences in both the presence and magnitude of response. Of the ITP compounds that increase median lifespan, only five (rapamycin (Strong et al. 2020), meclizine (Harrison et al. 2024), glycine (Miller et al. 2019), captopril (Strong et al. 2022), acarbose (Harrison et al. 2014; Strong et al. 2016; Harrison et al. 2019)) increase lifespan in both sexes, with rapamycin producing a greater response in females and captopril producing a greater response in males (Barardo et al. 2017). Several compounds, however, work exclusively in one sex; five compounds (NDGA (Strong et al. 2008; Strong et al. 2016), Protandim (Strong et al. 2016), astaxanthin (Harrison et al. 2024), Aspirin (Strong et al. 2008), 17-alpha-estradiol (Harrison et al. 2014; Strong et al. 2016; Harrison et al. 2021) extend median lifespan in males only, one compound ((R/S)-1,3-butanediol (Strong et al. 2022) extends median lifespan in females only, and two compounds (canagliflozin (Miller et al. 2020; Miller et al. 2024), 16-alpha-hydroxyestradiol (Miller et al. 2024) extend male lifespan while reducing female lifespan (Barardo et al. 2017). Although the ITP successfully identified and validated the effects of many interventions, the need for a model for high-throughput screens emerged, leading to the formation of the CITP (Driscoll et al. 2025). Inspired by the model of the ITP, the CITP exploits the genetic diversity of *Caenorhabditis* in addition to the short generation time and relatively low-cost of maintenance of nematode strains to conduct rigorous and reproducible experiments to identify compounds that extend lifespan and/ or healthspan on *Caenorhabditis* hermaphrodites. To date, the CITP has tested over 75

compounds and identified 12 that extend the median lifespan by at least 20% (Driscoll et al. 2025). Of the 5 compounds tested by both the ITP and CITP, 3 significantly increased median lifespan in both species (Driscoll et al. 2025). While the ITP tests compounds using both male and female mice, the CITP follows the convention of *C. elegans* work, using only hermaphrodites across multiple species for lifespan experiments. This has left a need to investigate sex differences in the response to compounds in this classical aging model. Here, we provide additional evidence for sexual dimorphism in the response to pro-longevity interventions. Of the seven tested compounds, sex modulates the effect size of four of them.

In the *C. elegans* aging field, males have been excluded from experiments due to multiple factors. First, males are rare in the commonly used N2 lab strain, representing around 0.1% of the population (Anderson et al. 2010). Second, Lifespan experiments can be complicated by the adverse effects of males on hermaphrodite lifespan, induced by mating or exposure to male pheromones (Maures et al. 2014; Shi and Murphy 2014; Booth et al. 2022). Other males are also affected by male pheromones, leading to a density-dependent toxicity and lifespan decrease (Shi et al. 2017). Third, males are highly motivated to search for mates, occasionally leading them to leave their agar media, crawl onto the plastic walls of petri dishes and desiccate. This leads to a decrease in the sample size, as these males are marked as "lost" and censored from final analyses. To circumvent the first problem, the male-enriched *him-8* strain was used in this study. The HIM-8 protein binds the meiotic pairing center of the X chromosome, and its loss leads to an increase in X chromosome nondisjunction events and a higher incidence of males (Phillips et al. 2005). Although we note the negative effect of male-male interactions on lifespan, this was not severe enough to abolish the positive lifespan extension effects of the compounds tested in this study and actually highlights the efficacy of these interventions. Future work could investigate the effects of these compounds on males with mutations in *daf-22* that are deficient in pheromone production, thereby causing weaker male-induced demise (Maures et al. 2014; Shi et al. 2017). Finally, in our hands on average 10% of males were marked "lost" (excluding losses due to contamination) and censored from the lifespan experiments, which is not high enough to interfere with robust assessment of the treatments.

Despite these potential challenges, males provide a unique tool for measuring the effects of compounds on lifespan and healthspan. One of these benefits is eliminating the need for progeny

abrogation using FUdR thus eliminating its confounding effects on lifespan because males, unlike hermaphrodites, are not capable of producing self-progeny (Wang et al. 2019; McIntyre et al. 2021). Males additionally provide a unique measure for reproductive healthspan: mating success. Mating is a complex behavior that is controlled by multiple tissue types and motivated by a consistent drive. Indeed, males lose their ability to mate in old age due to neuromuscular deficits and not due to a decrease in drive or sperm quality (Guo et al. 2012; Chatterjee et al. 2013). Mating ability offers a complex yet easily-screenable phenotype (presence or absence of progeny) that could indicate a systemic healthspan extension. We show here that reproductive success in late life can be modulated by compound interventions, thus validating male mating success as a viable healthspan measure for future studies. Given the sensitivity of the system and the number of biological processes that modulate this phenotype, it could potentially be used as a highly sensitive screening tool for healthspan effects independent of lifespan extension.

The importance of describing both lifespan and healthspan while measuring the effects of pro-longevity interventions has been gaining increasing attention within the aging field (Bansal et al. 2015; Hahm et al. 2015; Rollins et al. 2017; 2024). Our findings further highlight the independent nature of lifespan and healthspan, as our study provides further evidence that lifespan extensions are not necessarily coupled with healthspan extensions. In fact, the compounds tested in this study mostly increased lifespan without improving the healthspan measure used here. Because our reproductive healthspan measure encompasses a number of systems, such as neurological and muscular functions, that are of central interest in human aging (Yuan and Larsson 2023; Livingston et al. 2024), this decoupling offers a potential opportunity. Indeed, it should be possible to screen for compounds that enhance late-life male reproductive function more directly, which could reveal novel pathways for future interventions. Future work could investigate the molecular mechanisms and behavioral changes underlying the improved reproductive success in late life induced by sulforaphane and metformin. Additionally, future work could investigate whether these compounds improve any other health metrics such as motility, oxidative stress resistance, and other standard healthspan measures (Bansal et al. 2015).

In conclusion, we describe here the first study to use male *C. elegans* to investigate the effects of pro-longevity compounds on lifespan and reproductive healthspan. We emphasize the benefits, which largely outweigh the challenges, of including males in future studies and analyses

especially when asking questions about aging. This will add to the fundamental, yet limited, knowledge of how sex influences biological processes in the classical *C. elegans* aging model. Ultimately, this will bring us one step closer to identifying interventions and regimes that benefit both sexes.

Acknowledgements

We thank the CITP members Christine Sedore and Anna Coleman-Hulbert at the University of Oregon for their support and guidance throughout this project, their advice on data analysis, and their help preparing compounds and media. Some data were downloaded from the CITP Data Portal (citpaging.org), which is supported by funding from NIH's NIA Division of Aging Biology (U24AG056052). Strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Funding

RSA was funded in part by the Genetics Training Program at the University of Oregon, funded by the National Institutes of Health (T32GM149387). Additional funding was provided by National Institute of Health awards to PCP (U01AG045829, U24AG056052, R01AG088629).

Competing interests

The authors declare no competing interests.

Supplemental Tables

Supplemental Table S3.1 Lifespan assay summary statistics

Compound	Number dead	Number censored	Median Time	Mean	Std Error	Lower 95%	Upper 95%	90th percentile
CTRL_DMSO	496	64	16	16.53	0.18	16	16	21
75 µM ATRA	277	45	18	18.27	0.19	18	18	23
150 µM ATRA	257	23	19	19.22	0.21	18	20	23
100 µM PG	151	9	18	18.54	0.27	18	18	23
200 µM PG	262	17	19	19.24	0.22	18	20	24
50 µM RSVTL	301	19	19	19.10	0.25	18	20	25
100 µM RSVTL	221	19	20	21.20	0.32	20	21	28
50 µM SULF	198	42	18	19.93	0.38	18	20	27
100 µM SULF	191	89	23	21.51	0.40	20	23	30
CTRL_H2O	316	44	13	13.42	0.11	13	14	16
50 µM GST	229	11	16	15.32	0.23	14	16	20
100 µM GST	186	13	16	16.46	0.30	16	16	23
35 mM METFO	213	27	16	16.03	0.25	16	16	20
70 mM METFO	202	39	16	17.01	0.25	16	18	23
25 µM THIOT	132	106	23	22.82	0.64	21	26	34
50 µM THIOT	113	127	23	22.81	0.76	21	26	32

Supplemental Table S3.2 Lifespan assay CPH model output

ATRA	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
75 µM	-0.46	0.09	-5.35	8.97E-08
150 µM	-0.57	0.09	-6.42	1.33E-10
PG	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
100 µM	-0.28	0.11	-2.70	0.007
200 µM	-0.64	0.09	-7.31	2.59E-13
RSVTL	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
50 µM	-0.51	0.08	-6.04	1.51E-09
100 µM	-0.82	0.09	-8.63	<2E-16
SULF	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
50 µM	-0.78	0.10	-7.67	1.75E-14
100 µM	-1.06	0.10	-10.54	<2E-16
GST	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
50 µM	-1.06	0.11	-10.03	<2E-16
100 µM	-1.29	0.11	-11.36	<2E-16
METFO	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
35 mM	-0.95	0.11	-8.89	<2E-16
70 mM	-1.23	0.11	-11.11	<2E-16
THIOT	Cox Proportional Hazards			
Concentration	Effect	Stderr	z-value	p-value
25 µM	-2.23	0.14	-15.82	<2E-16
50 µM	-2.26	0.14	-15.74	<2E-16

Supplemental Table S3.3 Change in median lifespan by sex summary statistics

Sex	Compound	Number of technical replicates	Mean % change in median lifespan compared to control	SEM
Hermaphrodite	ATRA	6	29%	6%
Hermaphrodite	PG	4	15%	3%
Hermaphrodite	RSVTL	4	18%	3%
Hermaphrodite	SULF	6	69%	11%
Hermaphrodite	GST	5	15%	8%
Hermaphrodite	METFO	9	29%	4%
Hermaphrodite	THIOT	4	25%	12%
Male	ATRA	6	9%	3%
Male	PG	7	7%	2%
Male	RSVTL	5	20%	4%
Male	SULF	4	27%	6%
Male	GST	5	12%	3%
Male	METFO	6	17%	5%
Male	THIOT	3	48%	15%

Supplemental Table S3.4 Median lifespan by sex CPH model output

ATRA	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-0.88	0.09	-10.16	<2.00E-16
Sex	0.37	0.18	2.12	0.03
Interaction	0.38	0.12	3.09	0.002
PG	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-0.52	0.09	-5.62	1.94E-08
Sex	0.36	0.18	2.04	0.04
Interaction	-0.06	0.13	-0.45	0.65
RSVTL	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-0.58	0.09	-6.30	2.94E-10
Sex	0.35	0.18	1.93	0.05
Interaction	-0.20	0.13	-1.54	0.12
SULF	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-1.51	0.09	-16.74	<2.00E-16
Sex	0.30	0.17	1.81	0.07
Interaction	0.41	0.13	3.03	0.002
GST	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-0.62	0.09	-6.69	2.28E-11
Sex	2.27	0.24	9.53	<2.00E-16
Interaction	-1.32	0.38	-3.51	0.0005
METFO	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-1.15	0.07	-15.87	<2.00E-16
Sex	2.24	0.24	9.28	<2.00E-16
Interaction	-0.04	0.16	-0.25	0.81

THIOT	Cox Proportional Hazards			
	Effect	Stderr	z-value	p-value
Compound	-1.03	0.10	-10.81	<2.00E-16
Sex	1.98	0.19	10.38	<2.00E-16
Interaction	-1.76	0.31	-5.59	2.27E-08

Supplemental Table S.3.4, continued

Supplemental Table S.3.5 Mating assay summary statistics

Compound	Age	Mean Mating Success	SD	Count	SEM
CTRL_DMSO	1	0.59	0.49	111	0.05
75 µM ATRA	1	0.55	0.50	67	0.06
150 µM ATRA	1	0.53	0.50	68	0.06
100 µM PG	1	0.60	0.49	65	0.06
200 µM PG	1	0.56	0.50	68	0.06
50 µM RSVTL	1	0.52	0.50	48	0.07
100 µM RSVTL	1	0.70	0.46	50	0.07
50 µM SULF	1	0.47	0.50	49	0.07
100 µM SULF	1	0.50	0.51	48	0.07
CTRL_DMSO	5	0.17	0.38	95	0.04
75 µM ATRA	5	0.12	0.32	69	0.04
150 µM ATRA	5	0.12	0.32	68	0.04
100 µM PG	5	0.24	0.43	68	0.05
200 µM PG	5	0.16	0.37	67	0.05
50 µM RSVTL	5	0.14	0.35	69	0.04
100 µM RSVTL	5	0.15	0.36	68	0.04
50 µM SULF	5	0.23	0.43	30	0.08
100 µM SULF	5	0.10	0.31	30	0.06
CTRL_DMSO	7	0.04	0.21	113	0.02
75 µM ATRA	7	0.03	0.17	68	0.02
150 µM ATRA	7	0.04	0.21	67	0.03
100 µM PG	7	0.11	0.32	53	0.04
200 µM PG	7	0.03	0.17	33	0.03
50 µM RSVTL	7	0.04	0.21	68	0.03
100 µM RSVTL	7	0.06	0.24	52	0.03
50 µM SULF	7	0.13	0.34	60	0.04
100 µM SULF	7	0.07	0.25	59	0.03
CTRL_H2O	1	0.67	0.47	146	0.04
50 µM GST	1	0.68	0.47	59	0.06
100 µM GST	1	0.53	0.50	72	0.06
35 mM METFO	1	0.66	0.48	127	0.04
70 mM METFO	1	0.63	0.49	126	0.04
25 µM THIOT	1	0.68	0.47	59	0.06
50 µM THIOT	1	0.68	0.47	40	0.08
CTRL_H2O	5	0.09	0.29	123	0.03

Compound	Age	Mean Mating Success	SD	Count	SEM
50 µM GST	5	0.19	0.40	52	0.06
100 µM GST	5	0.08	0.27	53	0.04
35 mM METFO	5	0.28	0.45	78	0.05
70 mM METFO	5	0.14	0.35	99	0.04
25 µM THIOT	5	0.09	0.30	53	0.04
50 µM THIOT	5	0.09	0.29	33	0.05
CTRL_H2O	7	0.07	0.26	107	0.03
50 µM GST	7	0.06	0.25	32	0.04
100 µM GST	7	0.05	0.23	38	0.04
35 mM METFO	7	0.03	0.17	108	0.02
70 mM METFO	7	0.08	0.28	106	0.03
25 µM THIOT	7	0.08	0.27	53	0.04
50 µM THIOT	7	0.05	0.22	40	0.03

Supplemental Table S.3.5, continued

Supplemental Table S3.6 Mating assay GLMM model output

ATRA	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	0.28	0.28	1.00	0.32
75 µM	-0.03	0.32	-0.09	0.93
150 µM	-0.13	0.32	-0.41	0.68
Age 5	-1.90	0.34	-5.59	2.24E-08
Age 7	-3.39	0.50	-6.81	9.95E-12
75 µM: Age 5	-0.41	0.57	-0.72	0.47
150 µM: Age 5	-0.29	0.57	-0.52	0.61
75 µM: Age 7	-0.39	0.91	-0.43	0.67
150 µM: Age 7	0.15	0.81	0.18	0.85
PG	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	0.29	0.25	1.17	0.24
100 µM	0.15	0.32	0.45	0.65
200 µM	-0.03	0.32	-0.08	0.93
Age 5	-1.90	0.34	-5.62	1.89E-08
Age 7	-3.39	0.50	-6.81	1.01E-11
100 µM: Age 5	0.28	0.51	0.55	0.58
200 µM: Age 5	0.01	0.54	0.01	0.99
100 µM: Age 7	0.91	0.71	1.29	0.20
200 µM: Age 7	-0.25	1.16	-0.21	0.83
Intercept	0.34	0.20	1.69	0.09
50 µM	-0.48	0.35	-1.35	0.18
100 µM	-0.36	0.36	-1.00	0.32
Age 5	-1.94	0.34	-5.75	8.69E-09
Age 7	-3.41	0.50	-6.86	6.81E-12
50 µM: Age 5	0.89	0.62	1.42	0.15
100 µM: Age 5	-0.25	0.76	-0.33	0.74
50 µM: Age 7	1.67	0.69	2.43	0.02
100 µM: Age 7	0.80	0.77	1.03	0.30
GST	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	0.72	0.22	3.29	0.001

Treatment	Effect	Stderr	z-value	p-value
50 µM	-0.53	0.32	-1.66	0.10
100 µM	0.08	0.35	0.22	0.82
Age 5	-3.08	0.38	-8.19	2.73E-16
Age 7	-3.14	0.42	-7.51	6.01E-14
50 µM: Age 5	0.49	0.69	0.72	0.47
100 µM: Age 5	0.96	0.60	1.61	0.11
50 µM: Age 7	0.13	0.88	0.15	0.88
100 µM: Age 7	-0.21	0.90	-0.24	0.81
METFO	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	0.75	0.26	2.88	0.004
35 mM	-0.01	0.27	-0.05	0.96
70 mM	-0.17	0.26	-0.65	0.52
Age 5	-3.18	0.37	-8.49	<2.00E-16
Age 7	-3.19	0.42	-7.67	1.77E-14
35 mM: Age 5	1.48	0.49	3.00	0.002
70 mM: Age 5	0.56	0.51	1.11	0.27
35 mM: Age 7	-1.03	0.74	-1.39	0.17
70 mM: Age 7	0.31	0.57	0.54	0.59
THIOT	Generalized Linear Model			
Treatment	Effect	Stderr	z-value	p-value
Intercept	0.74	0.25	2.99	0.003
25 µM	0.09	0.36	0.24	0.81
50 µM	0.06	0.42	0.13	0.90
Age 5	-3.10	0.38	-8.15	3.55E-16
Age 7	-3.14	0.42	-7.52	5.67E-14
25 µM: Age 5	0.23	0.68	0.34	0.74
50 µM: Age 5	0.30	0.81	0.37	0.71
25 µM: Age 7	0.02	0.73	0.03	0.98
50 µM: Age 7	-0.42	0.92	-0.45	0.65

Supplemental Table S3.6, continued

Bridge to chapter 4

In chapter 3, I tested the effects of several pro-longevity chemical compounds on male lifespan and contrasted my findings to previously published hermaphrodite lifespan data. While some compounds had sex-shared effects on lifespan, others had sex-specific effects. In the next chapter, I characterize the effects of two of these compounds, metformin and Thioflavin T, on *C. elegans* aging at the transcriptional level while accounting for sex as a biological variable.

CHAPTER 4: PRO-LONGEVITY INTERVENTIONS GENERATE SEXUALLY DIMORPHIC TRANSCRIPTOMIC SIGNATURES IN AGING *CAENORHABDITIS ELEGANS*

Rose S. Al-Saadi, Erik Johnson, Erik Segerdell, Christine A. Sedore, Patrick C. Phillips

Author contributions

RSA and PCP conceptualized the project. RSA conducted the lifespan assays, performed the RNA-seq sample collection, conducted the statistical analysis and visualization, and wrote the original manuscript. EJ conducted the RNA extractions and sample preparation for sequencing. ES performed the genome alignment and read assignment. CAS contributed to RNA-seq sample collection. PCP supervised the project, reviewed and edited the manuscript, and acquired funding.

Introduction

Sex differences in aging are one of the most ubiquitous yet paradoxical phenomena in the biology of aging; women often live longer than men yet suffer worse health outcomes in older age (Austad and Fischer 2016). The prevalence of such sex differences extends beyond “natural aging” and has been documented in the response to pro-longevity interventions. Several types of interventions have been identified in an effort to extend both lifespan and healthspan, including genetic, pharmacological, and lifestyle interventions. However, many of these result in sexually dimorphic effects on lifespan and healthspan either in the efficacy or the magnitude of the effect (Austad and Bartke 2016; Knufinke et al. 2023; Bartke et al. 2024). Due to historically male-biased experimental paradigms, there remains a need to comprehensively characterize the effects of pro-longevity interventions while accounting for sex as a major biological variable.

The nematode *Caenorhabditis elegans* represents an excellent model to address this need. In addition to their short lifespan and susceptibility to pro-longevity interventions, they display an array of sexual dimorphisms in aging and age-associated behavioral decline (McCulloch and Gems 2007; Liggett et al. 2015; Hotzi et al. 2018; Weng and Murphy 2024). The *Caenorhabditis* Intervention Testing Program (CITP) has leveraged *Caenorhabditis* nematodes as a tool for

screening dozens of chemical and pharmacological interventions and has thus far identified 12 compounds that robustly and reproducibly extend hermaphrodite lifespan (Driscoll et al. 2025). Recently, we explored the effects of a subset of these compounds on male lifespan and healthspan and found that, although they all extended male lifespan, the magnitude of the effect of several of them was sexually dimorphic, and only two of the tested compounds, metformin and sulforaphane, extended healthspan (Al-Saadi and Phillips 2025). Whether these compounds extend lifespan through shared or distinct molecular pathways in the two sexes remains unknown.

Two compounds, metformin and Thioflavin T (ThioT), are of particular interest due to their clinical relevance and their dramatic effects on male lifespan and healthspan. Metformin, a biguanide medication, is the second most prescribed drug in the United States and is used to treat type-2 diabetes, polycystic ovarian syndrome (PCOS), and gestational diabetes. In addition to its benefits in regulating blood glucose levels, its use has been correlated with a decreased risk of age-associated diseases including cardiovascular diseases, stroke, and cancer (Lv and Guo 2020; Zhu et al. 2024). Metformin has also been shown to extend lifespan in model organisms including mice (Anisimov et al. 2011; Martin-Montalvo et al. 2013) and *C. elegans* (Onken and Driscoll 2010; Onken et al. 2022). Additionally, while the effects of metformin on *C. elegans* lifespan are shared between the two sexes, it extends male reproductive success in late life as well (Al-Saadi and Phillips 2025). Characterizing the sex-specific effects of metformin on the *C. elegans* transcriptome therefore holds potential for highlighting functional targets of this drug.

ThioT is a fluorescent amyloid-binding dye that binds protein fibrils and has been traditionally used to stain protein aggregates in the context of neurodegenerative diseases. Due to its ability to slow aggregation in vitro, it has been hypothesized to extend lifespan through promoting protein homeostasis (Porat et al. 2006; Alavez et al. 2011). Indeed, in *C. elegans,* it has been shown to significantly extend lifespan in both hermaphrodites and males (Alavez et al. 2011; Al-Saadi and Phillips 2025). Out of seven different pro-longevity interventions tested in males, ThioT was the most efficacious in extending their median lifespan, leading to a 77% increase in median lifespan compared to 60% in hermaphrodites (Alavez et al. 2011; Al-Saadi and Phillips 2025). However, ThioT did not demonstrate any obviously positive healthspan effects on males. So, again,

transcriptomics holds the potential to elucidate both shared and distinct sex-specific effects across these two particularly efficacious longevity-extending compounds.

Here, we use a transcriptomics approach to characterize the effects of metformin and ThioT on aging trajectories and examine how sex modulates these effects in *C. elegans*. We additionally describe the first transcriptomic profile of aging *C. elegans* males. We find that metformin has sexually dimorphic effects on the aging transcriptome while ThioT produces a strong sex-shared signature of upregulating metabolism and detoxification pathways. This study provides the first comprehensive characterization of the effects of sex and pro-longevity intervention on aging in the classic aging model *C. elegans* and contributes to the growing effort of addressing biological sex as an important modulator of aging.

Materials and methods

C. elegans strains and maintenance

The strain used in this study, *C. elegans* N2_PD1073 strain, was provided by the *Caenorhabditis* Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The strain was maintained on 60 mm of standard NGM media seeded with *E. coli* OP50-1. Hermaphrodites and males were maintained in separate populations to eliminate male effects on hermaphrodite lifespan. To generate male stocks, 4–5 hermaphrodites and 12–16 males from a mixed population were transferred twice weekly to fresh plates to maintain a high proportion of males. Hermaphrodites were transferred twice weekly to fresh plates.

Compound treatment

The compound treatment protocol utilized here is based on a previously described protocol, with slight modifications (Lucanic et al. 2017; Al-Saadi and Phillips 2025). Briefly, the compounds were dissolved in water to generate a 1.68 M working stock and 52.5 mM final concentration for metformin and 1.2 mM working stock and 37.5 µM final concentration for ThioT. Small petri dishes containing 4 mL of standard NGM and seeded with *E. coli* OP50-1 were treated with 125 µL of each compound and allowed to dry at room temperature for two days then stored at 4ºC in dark containers due to the photosensitivity of ThioT.

Lifespan assays

The lifespan assay protocol used here is based on the protocol described in chapter 2 and previous work (Lucanic et al. 2017). To generate males for lifespan assays, on Mondays, 20 L4 males and hermaphrodites per plate were picked onto medium NGM plates and allowed to reach adulthood and mate. On Tuesdays, the hermaphrodites were picked onto a new medium NGM plate, allowed to lay eggs for 4 hours, then removed. On Thursdays, 50 L4 males per plate are picked to small NGM plates to prevent them from mating with hermaphrodites. On Fridays, adult males were transferred to treated plates to start the lifespan assay.

To generate hermaphrodites for lifespan assays, on Mondays, 20 L4 hermaphrodites per plate were picked onto medium NGM plates and allowed to reach adulthood. On Tuesdays, the hermaphrodites were picked onto a new medium NGM plates, allowed to lay eggs for 4 hours, then removed. On Thursdays, 50 L4 hermaphrodites per plate were picked to small NGM plates to prevent them from mating with any spontaneous males and to mimic the male lifespan assay setup conditions. On Fridays, adult hermaphrodites were moved to treated plates to start the lifespan assay. All nematodes were transferred to new plates every other day until the hermaphrodites stopped producing progeny, followed by weekly transfer until all individuals were dead.

Lifespan statistical analysis

All the statistical analyses described in this study were conducted in R version 4.5.1 (R Core Team, 2024). To test for the effect of compound treatment, sex, and FUdR supplementation on lifespan, we used a mixed effects Cox-Proportional Hazards (CPH) model followed by planned comparisons where compound treatment, sex, FUdR, and their interaction were used as fixed effects, and biological and technical replicates were used as random effects. The coxme package version 2.2-22 (Therneau 2024) was used to fit the CPH models and the survival package version 3.8-3 was used to create the Kaplan-Meier curves (Therneau and Grambsch 2000; Therneau 2024). The emmeans package version 1.11.2 (Lenth 2025) was used to conduct planned comparisons.

Transcriptomics

The same protocol for the lifespan assays was used to generate the RNA-seq samples, with approximately 50 nematodes per age and per treatment, and 2–4 independent biological replicates. RNA extractions were performed using the KAPA mRNA HyperPrep kit and sequenced on the Illumina Novaseq 6000 with the SP 100 cycle at the Genomics and Cell Characterization Core Facility at the University of Oregon. The subread package was used to align the resulting reads to the *C. elegans* WBcel235 genome and the featureCount program was used to map unique reads (Liao et al. 2013; Liao et al. 2014). Scripts modified from CITP were used to conduct the differential expression analysis (Sedore et al. 2025). The edgeR package version 4.7.3 was used to conduct differential expression analyses where genes with low reads (less than 5–10) within age, sex, and compound treatment groups were filtered followed by library size normalization (Robinson et al. 2009). A negative binomial generalized linear model followed by a quasi-likelihood F-test were used to test for differentially expressed genes. A design matrix that tests for the effect of groups (defined by the interaction of sex by age by compound treatment) followed by planned comparisons were also used. Differential expression was determined based on a log2 fold change that is greater than or equal to 1 and an FDR that was less than or equal to 0.05. The clusterProfiler package version 4.17 was used to conduct the KEGG pathway enrichment analysis (Yu et al. 2012).

Results

FUdR supplementation impacts the response to pro-longevity interventions in a sex-specific manner

Metformin and ThioT have been shown previously to extend hermaphrodite and male longevity (Onken and Driscoll 2010; Alavez et al. 2011; Onken et al. 2022; Al-Saadi and Phillips 2025). However, one caveat is hermaphrodite lifespan assays were conducted on 5-Fluoro-2'-deoxyuridine (FUdR)-supplemented media while male lifespan assays were conducted on standard, non-FUdR media, as FUdR is not needed to block offspring production in males as it is in hermaphrodites. Therefore, we first tested the effect of metformin treatment on lifespan in the presence and absence of FUdR in both sexes. We treated populations of hermaphrodites and

males with either water (control), 52.5 mM metformin, or 37.5 μM ThioT and measured their survival. In hermaphrodites, we found that metformin treatment in the presence of FUdR extended median lifespan by 29% ($p < 0.0001$) but had no effect on median lifespan in the absence of FUdR (Fig. 4.1a). In males, however, metformin decreased median lifespan by 12% in the presence of FUdR ($p < 0.001$) and extended median lifespan by 21% in the absence of FUdR ($p < 0.0001$, Fig. 4.1b). This demonstrates that FUdR supplementation generates a strong sex-by-drug interaction in the response to metformin.

Next, we asked whether the effects of ThioT on lifespan were similarly modulated by FUdR supplementation. We found that hermaphrodites had a robust increase in median lifespan regardless of FUdR supplementation, but the magnitude of response was modulated by FUdR; hermaphrodite median lifespan increased by 59% in the presence of FUdR ($p < 0.0001$) and by 20% in its absence ($p < 0.0001$, Fig. 4.1c). Similarly, ThioT increased the median lifespan of males by 29% in the presence of FUdR ($p < 0.0001$) and by 57% in its absence ($p < 0.0001$, Fig. 4.1d). So, similar to metformin, FUdR enhances longevity extension in hermaphrodites while moderating it in males. Thus, we find that any positive effects of metformin on lifespan are both sex- and FUdR-dependent while ThioT's effects on lifespan are universal, but the magnitude of the effect is dependent on FUdR. Due to the potentially confounding effects of FUdR (especially given its negative effects in males), we conducted the rest of the experiments in this study on non-FUdR media only.

Transcriptomic signatures of aging are sexually dimorphic

Next, we sought to characterize the transcriptional changes associated with aging and with compound treatment in both hermaphrodites and males. We treated populations of hermaphrodites and males with either water (control), 52.5 mM metformin, or 37.5 μM ThioT starting at day 1 of adulthood and collected samples for whole-animal RNA sequencing at two adult life stages: two-days (young) and twelve-days of adulthood (old). Using principal component analysis, we found that the samples separate by sex on PC1 and by age on PC2, with treatments clustering mostly together, more so in older than younger samples (Supplemental Fig. S4.1).

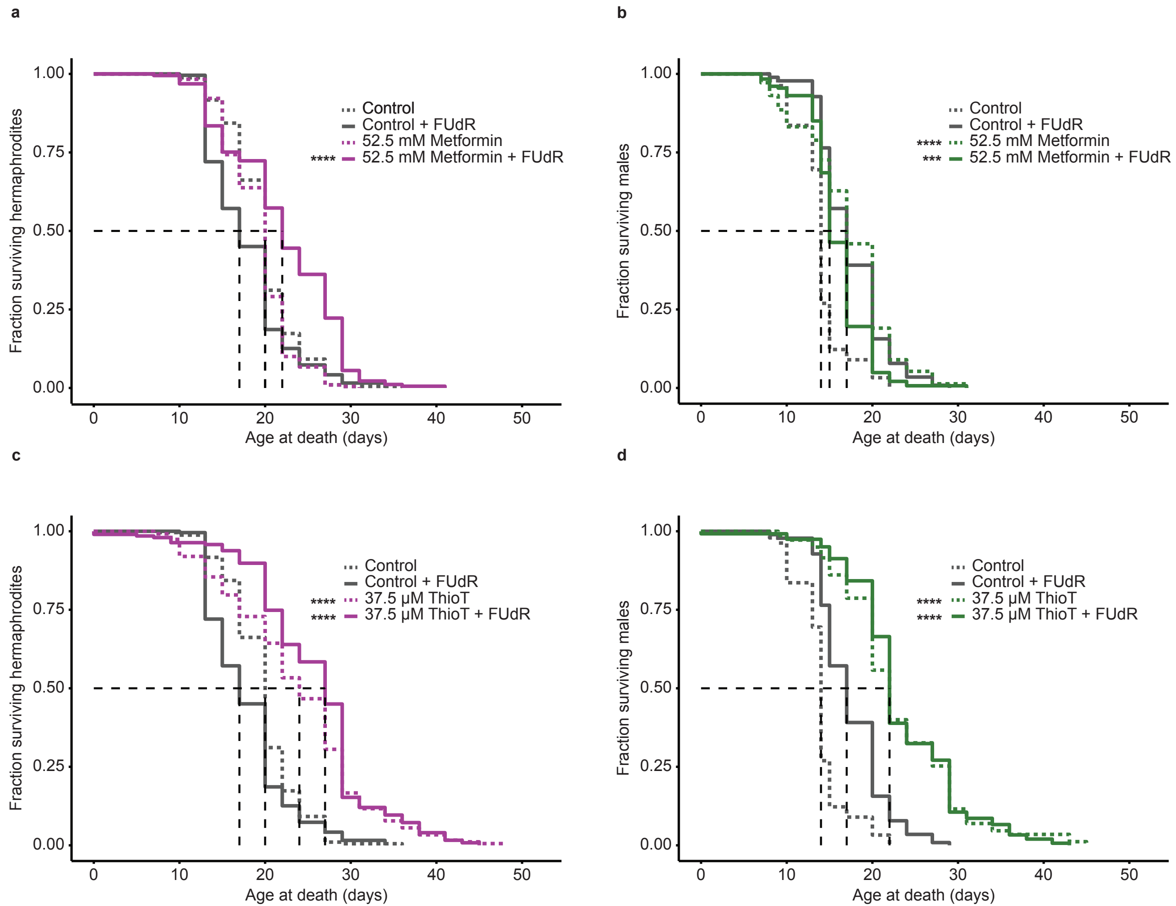


Fig. 4.1 FUdR interacts with pro-longevity compounds in a sex-dependent manner. Kaplan-Meier curves showing survival of (a) *C. elegans* hermaphrodites treated with metformin or (c) ThioT and (b) males treated with metformin or (d) ThioT. The dashed lifespan curves represent assays where FUdR was not supplemented while the solid lines represent assays where FUdR was supplemented. The gray lines represent survival on the negative control (water) in both sexes while the purple and green lines represent hermaphrodite and male survival on the tested compound, respectively. Each line represents two biological replicates with total n = 100-216. The black dashed lines denote the age at which 50% of the population has died. The asterisks denote *p*-values from a Cox Proportional Hazards model where ***p<.001, ****p<.0001. For additional information and the output of the CPH model, see Supplemental Table S4.1

To determine whether sex affects transcriptional changes associated with "natural aging", we compared the transcriptional changes of our control hermaphrodite to our control male samples. Aging significantly impacts transcriptional profiles in both sexes: old hermaphrodites had a total of 3421 upregulated and 1472 downregulated genes compared to young hermaphrodites. We will refer to these differentially expressed genes due to aging as "aging-DEGs" (Fig. 4.2a). Males similarly had a drastic change in their transcriptional profile over age with a total of 2685

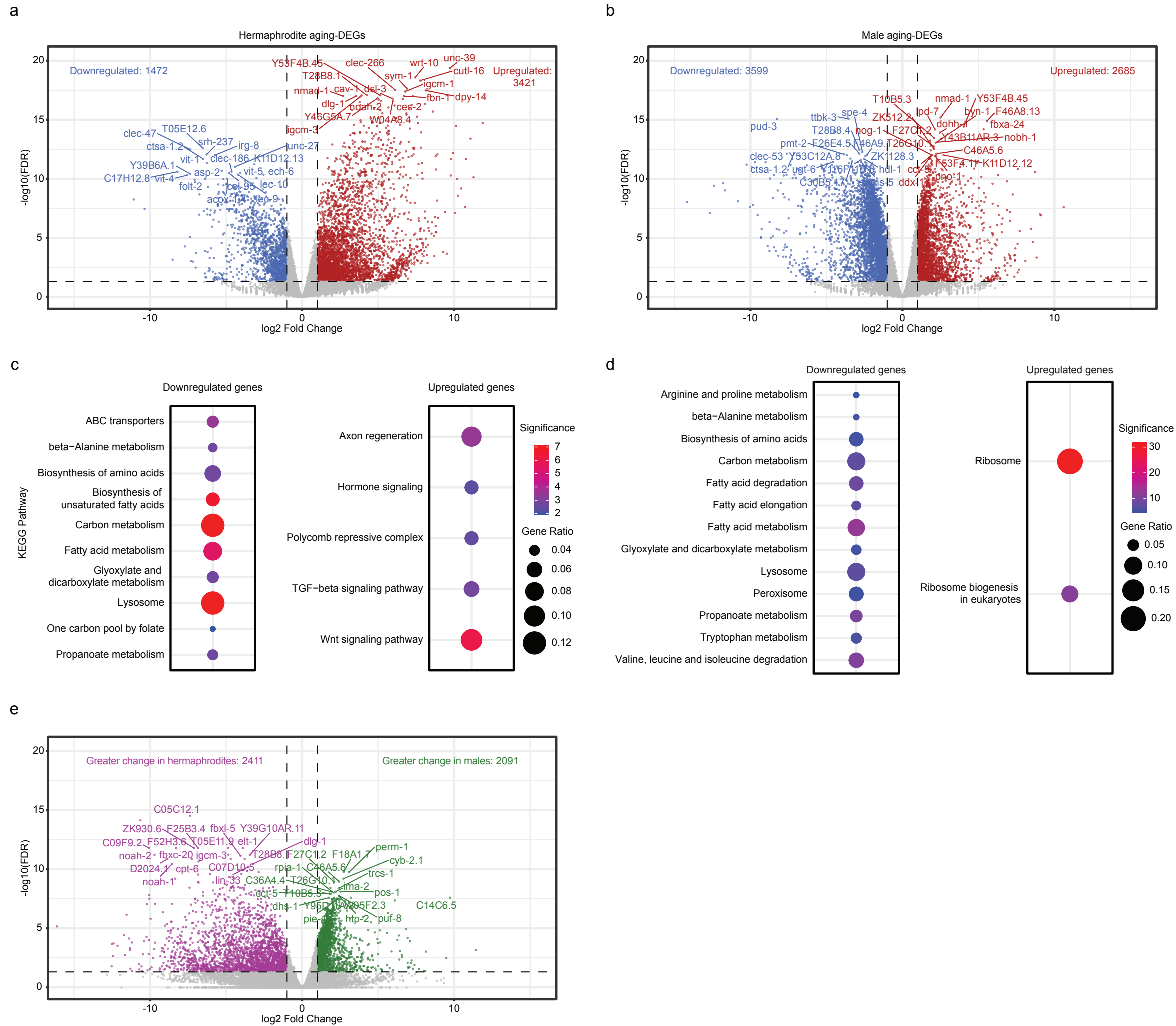


Fig. 4.2 Hermaphrodites and males have distinct transcriptomic profiles associated with aging. (a, b) Volcano plots showing differentially expressed genes between days 2 and 12 of adulthood in (a) hermaphrodites and (b) males. Significantly upregulated genes are shown in red, significantly downregulated genes are shown in blue, and non-significant genes are shown in gray. (c, d) KEGG pathway enrichment plots for (c) hermaphrodite aging-DEGs and (d) male aging-DEGs. The colors denote "Significance" which represents the -log10 of the p-value and the dot size represents gene ratio, or the number DEGs divided by the total number of genes in a KEGG category. (e) Volcano plots showing genes that display a greater change in expression with age in hermaphrodites (purple) and males (green). A log2 fold change of at least 1 and an FDR of 0.05 or less was used as the threshold for DEGs

upregulated and 3599 downregulated genes (Fig. 4.2b). To determine the molecular pathways impacted by aging, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) to conduct an enrichment analysis and found that in both sexes, downregulated genes were enriched for metabolic pathways including the amino acid, fatty acid, and carbon metabolism pathways (Fig. 4.2c, d). However, older hermaphrodites tend to upregulate genes found within major signaling pathways such as the TGF-beta, Wnt, and hormone signaling, while males upregulate genes enriched for ribosomal function (Fig. 4.2c, d).

To test how sex regulates age-associated gene expression changes more directly, we quantified the effect of age by sex interaction on gene expression changes and found a subset of aging-DEGs whose change in expression magnitude is sex-biased. We identified 2091 aging-DEGs that display a greater change in males and 2411 that display a greater change in hermaphrodites (Fig. 4.2e). This demonstrates that sex impacts age-associated transcriptional changes and could partially underlie sex differences in the response to pro-longevity interventions.

Transcriptomic signatures of metformin treatment are sexually dimorphic

To investigate how pro-longevity interventions affect animals at the transcriptional level and how sex modulates this effect, we next compared the metformin-treated samples to the control samples at days 2 and 12 in both hermaphrodites and males. In young hermaphrodites, metformin induced few transcriptional changes—only 32 upregulated and 30 downregulated genes—which we will refer to as METFO-DEGs (Fig. 4.3a). In old hermaphrodites, however, treatment with metformin induced marked transcriptional changes, with 542 upregulated and 1596 downregulated genes (Fig. 4.3b). KEGG enrichment analysis revealed that metformin primarily downregulated genes in young hermaphrodites enriched for fatty acid synthesis, metabolism, and degradation, and amino acid metabolism and degradation pathways (Fig. 4.3c). In old hermaphrodites, pathways related to ribosomal function were upregulated and axon regeneration and cell structure related pathways were downregulated (Fig. 4.3c).

In young males, most of the METFO-DEGs are upregulated genes (85 genes), and only four were downregulated (Fig. 4.3d). In old males, the number of METFO-DEGs increases to 862 upregulated and 214 downregulated genes (Fig. 4.3e). KEGG enrichment analysis revealed that,

Fig. 4.3 Metformin elicits sexually dimorphic transcriptional changes. (a, b, d, e) Volcano plots showing differentially expressed genes in metformin-treated individuals compared to controls at days (a) 2 and (b) 12 of adulthood in hermaphrodites and days (d) 2 and (e) 12 in males. Significantly upregulated genes are shown in red, significantly downregulated genes are shown in blue, and non-significant genes are shown in gray. (c) KEGG pathway enrichment plots for hermaphrodite METFO-DEGs. The colors denote “Significance” which represents the -log10 of the p-value and the dot size represents gene ratio, or the number DEGs divided by the total number of genes in a KEGG category

unlike hermaphrodites, metformin treatment in old males only led to enrichment of specific pathways. These upregulated pathways include fatty acid, porphyrin, sphingolipid, and amino acid metabolism, lysosome, and cytochrome P450 related pathways (Fig. 4.3f). Overall, we find that metformin treatment has a strong sex-specific transcriptomic signature on aging *C. elegans* nematodes.

Transcriptomic signatures of ThioT treatment are shared across ages and sexes

Next, we investigated whether the effects of ThioT on the aging transcriptome were, like metformin, sexually dimorphic. While metformin had a gradual effect on DEGs with fewer early in life and many more later, ThioT induced largescale transcriptional responses in young hermaphrodites, with 1409 upregulated and 172 downregulated genes (THIOT-DEGs, Fig. 4.4a). In older hermaphrodites, these numbers climbed even higher to 2827 upregulated and 2589 downregulated genes (Fig. 4.4b). Additionally, KEGG enrichment analysis revealed that ThioT treatment upregulated genes in both young and old hermaphrodites were enriched for cytochrome P450 and other drug metabolism pathways while the downregulated genes did not share a specific enrichment signature (Fig. 4.4c). In young hermaphrodites, ThioT treatment additionally upregulated gene enriched for Wnt signaling, glutathione metabolism, and longevity regulating pathways while downregulated genes were enriched for fatty acid synthesis, elongation, and metabolism pathways and lysosomal pathways (Fig. 4.4c).

ThioT treatment in young males led to a more muted transcriptional response than in hermaphrodites, with 318 upregulated genes and 29 downregulated genes (Fig. 4.4d). The transcriptional response increased substantially in older males, while maintaining the bias toward upregulated genes, with 1469 upregulated and 318 downregulated genes in these samples (Fig. 4.4e). KEGG enrichment analysis showed upregulated genes in both young and old males were enriched for cytochrome P450 and other drug metabolism and glutathione metabolism pathways (Fig. 4.4f). Additionally, young males had upregulated genes enriched for worm-specific longevity regulating pathways and downregulated genes enriched for biosynthesis and metabolism of fatty acids and lysosomal activity (Fig. 4.4f). Older males had upregulated genes enriched for amino acid and retinol metabolism pathways while the downregulated genes were not enriched for any specific pathways (Fig. 4.4f). Overall, we see that ThioT treatment produces

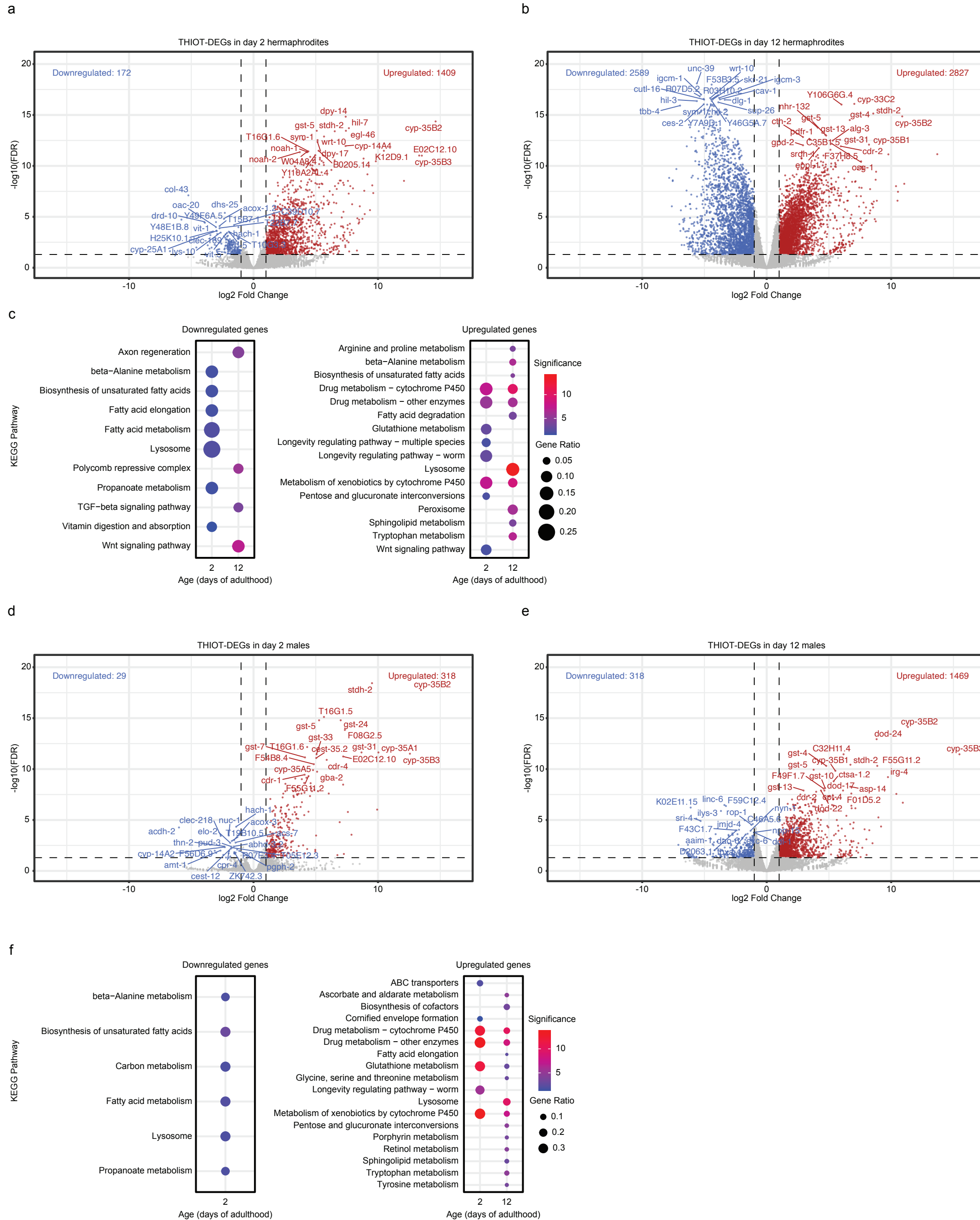


Fig. 4.4 ThioT elicits sexually dimorphic and sex-shared transcriptional changes. (a, b, d, e) Volcano plots showing differentially expressed genes in ThioT-treated individuals compared to controls at days (a) 2 and (b) 12 of adulthood in hermaphrodites and days (d) 2 and (e) 12 in males. Significantly upregulated genes are shown in red, significantly downregulated genes are shown in blue, and non-significant genes are shown in gray. (c, f) KEGG pathway enrichment plots for (c) hermaphrodite and (f) male THIOT-DEGs. The colors denote “Significance” which represents the -log10 of the p-value and the dot size represents gene ratio, or the number DEGs divided by the total number of genes in a KEGG category

a strong, sex-shared transcriptomic signature for upregulation of cytochrome P450 genes, drug metabolism and glutathione metabolism. This striking phenotype correlates with the sex-shared effect of ThioT on longevity and provides a potential mechanism of action of this compound via the induction of detoxification pathways.

Pro-longevity treatments enhance age-associated gene expression changes in a sex-specific manner

After establishing the effects of pro-longevity interventions at specific life stages on gene expression, we next examined how these interventions change aging-DEGs, specifically whether they enhance or reduce the change in gene expression and whether these changes are enriched for specific molecular pathways. To determine how compound interventions affect aging trajectories at the transcriptional level, we quantified the effect of age by compound interaction on gene expression changes and detected a subset of aging-DEGs whose expression change is enhanced or reduced by these treatments. In hermaphrodites, we detected 360 genes whose expression change with aging was enhanced by metformin treatment and 512 genes whose expression change was reduced (Fig. 4.5a). Males had fewer aging-DEGs impacted by metformin treatment, with 107 genes that had enhanced change in expression in old males and only 16 with reduced expression in old males treated with metformin with no specific pathways enriched in either direction (Fig. 4.5b). The genes whose expression is enhanced by metformin treatment in hermaphrodites are enriched for ribosome, oxidative phosphorylation, and fatty acid biosynthesis pathways, while the genes whose expression was reduced were not enriched for any specific pathways (Fig. 4.5c).

ThioT, on the other hand, led to a much more drastic change in aging-DEGs, where hermaphrodites had 1744 genes whose expression change with aging was enhanced and 2537 whose gene expression change was reduced by ThioT treatment (Fig. 4.5d). In males, 475 aging-DEGs had enhanced expression change with ThioT treatment and 224 genes had reduced change in gene expression (Fig. 4.5e). Enhanced genes in both sexes were enriched for amino acid, fatty acid, and carbon metabolism and lysosome-related pathways (Fig. 4.5f, g). Reduced genes, however, differed between the sexes; genes whose expression was reduced by ThioT in hermaphrodites were enriched for Wnt signaling, TGF-beta signaling, polycomb repressive

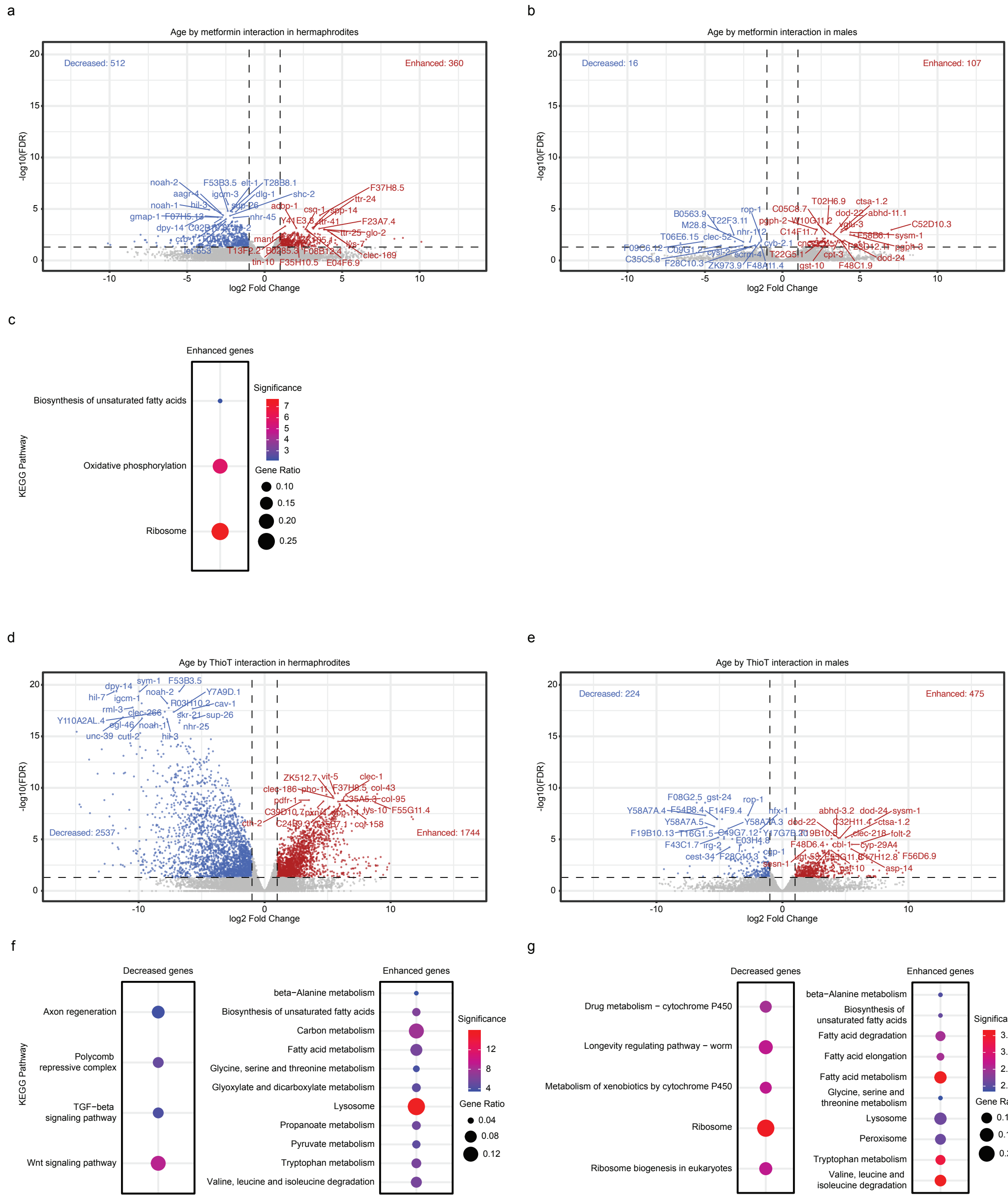


Fig. 4.5 Metformin and ThioT interact with age to modulate gene expression changes. (a, b, d, e) Volcano plots showing genes whose expression change is enhanced or decreased in (a) hermaphrodites and (b) males treated with metformin and (d) hermaphrodites and (e) males treated with ThioT. Significantly enhanced genes are shown in red, significantly decreased genes are shown in blue, and non-significant genes are shown in gray. (c, f, g) KEGG pathway enrichment plots for (c) hermaphrodites treated with metformin and and (f) hermaphrodites and (g) males treated with ThioT. The colors denote "Significance" which represents the -log10 of the p-value and the dot size represents gene ratio, or the number DEGs divided by the total number of genes in a KEGG category

complex, and axon regeneration pathways while in males were enriched for cytochrome P450 worm-specific longevity regulating pathways, and ribosome-related pathways (Fig. 4.5f, g).

Next, we explored how sex modulates these combined effects of aging and pro-longevity interventions on gene expression; in other words, does sex change the way compounds modulate gene-specific aging trajectories? To answer that, we quantified the effect of age by compound by sex interaction on gene expression changes and detected a subset of aging-DEGs with sex-biased compound effects on gene expression changes. For metformin, we found 107 genes whose expression change by metformin was greater in males and 112 whose expression change by metformin was greater in hermaphrodites (Fig. 4.6a). Male-biased genes were enriched for biosynthesis of nucleotide sugars and cell structure related pathways, and no specific pathways were enriched for hermaphrodite-biased genes (Fig. 4.6b). As exemplars of this effect, we looked closer at the nature of the interaction effect of two of these genes, *gmap-1*, a lipid transfer protein, and *T03D8.6*, a predicted glutathione hydrolase (Cox et al. 1981; Njume et al. 2022). In untreated animals, the expression of these genes increased with age in hermaphrodites and decreased in males (Fig. 4.6c, gray lines). Metformin treatment moderately increased the expression in older males and dramatically decreased expression in older hermaphrodites (Fig. 4.6c, blue lines).

In contrast to metformin, ThioT had 769 genes whose expression change was greater in males and 442 whose expression change was greater in hermaphrodites (Fig. 4.6d). Male biased genes were enriched for Wnt signaling, axon regeneration, and cell structure related pathways, while hermaphrodite-biased genes were not enriched for any specific (Fig. 4.6e). When we examined the expression pattern of two of these genes, *dpy-17*, a cuticle collagen (Novelli et al. 2006), and *sup-26*, an RNA binding protein that regulates somatic sex determination and nervous system development (Mapes et al. 2010; Schachtner et al. 2015), we found that expression increased with age in both sexes and ThioT treatment in hermaphrodites but not males reversed the expression dynamic, causing a higher expression in early adulthood and decreased expression in late life (Fig. 4.6f). In general, we see a number of instances in which relative changes in gene expression with age change sign between males and hermaphrodites (Supplemental Fig. S4.2). Metformin treatment produces an array of effects on gene expression changes, with some expression changes in males and hermaphrodites converging with age as in the case of *rml-5* and

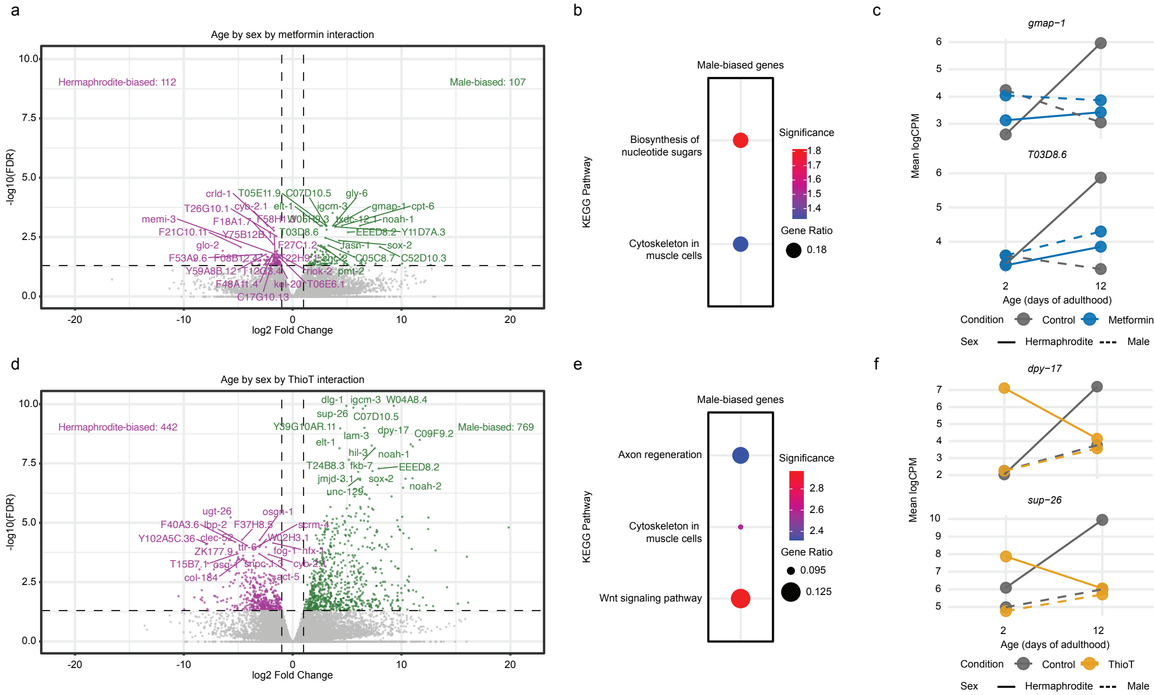


Fig. 4.6 Sex interacts with compound treatments to modulate age-associated gene expression changes. (a, d) Volcano plots showing genes whose expression change by (a) metformin and (d) ThioT is enhanced or decreased in hermaphrodites (purple) and males (green). (b, d) KEGG pathway enrichment plots for individuals treated with (b) metformin or (e) ThioT. The colors denote "Significance" which represents the -log10 of the p-value and the dot size represents gene ratio, or the number DEGs divided by the total number of genes in a KEGG category. (c, f) Line plots for representative genes whose expression change between days 2 and 12 of adulthood by (c) metformin or (f) ThioT is sex biased. Gene expression is measured by the log counts per million (logCPM) per gene. The gray lines represent the control, blue lines represent metformin, and yellow lines represent ThioT treatment. Solid lines represent expression in hermaphrodites and dashed lines represent expression in males

txdc-12.1, some diverging as in the case of *cyb-2.1*, and some plateauing as in *elt-1* and *gmap-1* (Supplemental Fig. S4.2a). ThioT, on the other hand, appears to primarily reverse the directionality of gene expression changes in aging hermaphrodites (Supplemental Fig. S4.2b). Overall, this provides evidence that sex modulates the effects of compound interventions at the transcriptional level; despite having a shared prolonged lifespan phenotype and in the case of ThioT, shared pathways that were differentially expressed, the direction and magnitude of transcriptional changes by compound interventions were sex-biased for several genes, many of which could underlie the biological differences in both longevity differences and sex differences in the response to pro-longevity interventions.

Discussion

Sex differences in aging and the response to pro-longevity interventions have been widely documented, yet knowledge about the mechanisms underlying such differences remains limited. Here, we describe the effects of metformin and ThioT on the aging transcriptome of *C. elegans* and the interplay between sex and these interventions in modulating gene expression changes over age. We find that the effects of pro-longevity interventions are both FUdR- and sex-dependent. While both compounds can extend lifespan, they do so via distinct mechanisms at the molecular level. Additionally, we observe that sex significantly influence the effects of these compounds at the molecular level, leading to a sex-biased expression change in over 4000 genes overall.

C. elegans is a well-established aging model that has been used in numerous studies to explore the effects of aging and different classes of pro-longevity interventions at the physiological, behavioral, and transcriptional levels. However, many of these studies are conducted in the presence of FUdR, a chemotherapy agent that inhibits DNA synthesis, thus disrupting cell division and rendering hermaphrodites sterile (Mitchell et al. 1979; Gandhi et al. 1980). While this significantly reduces the labor and resources associated with lifespan assays by eliminating hermaphrodite self-progeny and eliminating the need for frequent transfers, it has been previously shown that FUdR both directly and indirectly affect *C. elegans* lifespan (Anderson et al. 2016; Wang et al. 2019; McIntyre et al. 2021). Here, we show that FUdR modulates the effects of pro-longevity interventions in a sex-specific manner. In the presence of FUdR, the metformin effect on hermaphrodite lifespan was beneficial while in males it was detrimental. In the absence of FUdR, metformin had no effect on hermaphrodite lifespan and had a beneficial effect on males. While ThioT's effects on lifespan were consistently beneficial in both sexes, the effect size was FUdR-dependent. One potential mechanism to explain these sex differences involves the effect of FUdR on the germline and reproduction. The beneficial effects of FUdR on healthspan and proteostasis in hermaphrodites have been shown to be mediated by oogenesis and oocyte maturation (Angeli et al. 2013). Because males exclusively produce spermatocytes, it is possible that FUdR affects males through distinct mechanisms, contributing to its sexually dimorphic effects on lifespan. Males do not produce self-progeny and do not require FUdR-supplementation; therefore, males provide a significant advantage over hermaphrodites in the

context of aging studies, highlighting their utility as a tool to screen for and characterize pro-longevity compounds in future studies.

Sexual dimorphisms in *C. elegans* at the transcriptional level during development (Kim et al. 2016; Ebbing et al. 2018), in specific tissue types (Purice et al. 2025) and in the context of neuronal aging (Weng and Murphy 2024) have been previously described, but no such studies have characterized sexual dimorphisms of the aging transcriptome at the level of whole animals to date. Here, we characterize sex differences in aging at the transcriptional level and define a set of genes whose expression change over age is sex biased. Although hermaphrodites and males shared similar profiles of downregulated pathways with aging including amino acid and fatty acid biosynthesis and metabolism, the pathways that were upregulated were distinct: males upregulated ribosome-related pathways while hermaphrodites upregulated Wnt signaling, TGF-beta signaling, and hormone signaling pathways, all of which are known for their longevity-regulation role in *C. elegans*. The differential regulation of these longevity-associated pathways, specifically the TGF-beta pathway, could underlie previously described sex differences in aging and in the response of hermaphrodites and males to the classical longevity mutation in this pathway, *daf-2* (Hotzi et al. 2018). Future work could explore the interplay between sex and aging-associated differential expression of longevity-regulating pathways, especially at the single-cell/tissue-specific level.

Mosley et al. (2025) previously characterized the aging transcriptome of hermaphrodite populations ranging from 2–12 days after the L1 larval stage and found, among several other differentially expressed pathways, a downregulation of biosynthesis of amino acids and fatty acids, fatty acid metabolism, glyoxylate and dicarboxylate metabolism, and lysosome related pathways and an upregulation of axon regeneration and Wnt signaling pathways, consistent with our findings here. Similarly, He et al. (2014) characterized the transcriptional profiles of hermaphrodites between the L4 larval stage and day 6 and between day 6 and day 15. They found that beta-alanine metabolism, propanoate metabolism, fatty acid metabolism, one carbon pool by folate, glyoxylate and dicarboxylate metabolism pathways were downregulated in both age comparisons, consistent with our findings here. However, they report an upregulation in L4-D6 and downregulation in D6-D15 of Wnt and TGF-beta signaling pathways. While this is inconsistent with our findings, it is possible that the upregulation early in life is sufficient to

produce an overall upregulation signal in our D2-D12 comparison and that downregulation might not occur until later in the D6-D15 age range. Since these signaling pathways are under dynamic regulation across an animal's lifespan, expanding the number of timepoints in future studies will be necessary to further characterize this dynamic nature. Finally, both studies identified differentially regulated pathways in aging hermaphrodites not identified here including MAPK, an important longevity-regulating signaling pathway. However, this discrepancy could be attributed to the different ages of the nematode samples collected in these studies, with a larger range of ages that includes larval stages in both studies but not included in this study.

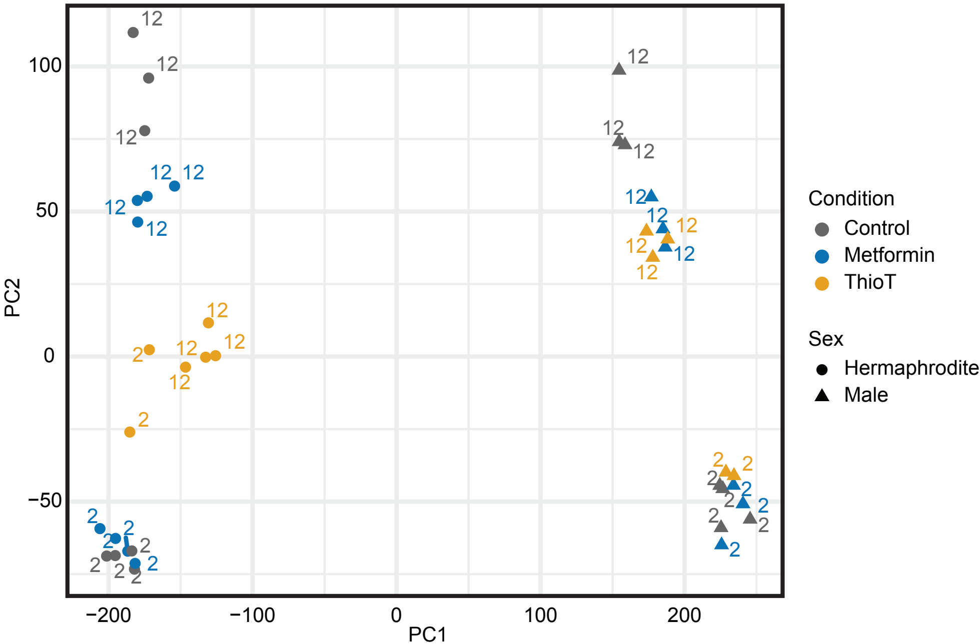
Metformin is thought to extend lifespan through its modulation of the energy and nutrient sensing AMP-activated protein kinase (AMPK) pathway and the mechanistic target of rapamycin complex 1 (mTORC1) (Zhang et al. 2025). Initially, it was thought to upregulate AMPK through the mitochondria by inhibiting complex I of the electron transport chain, leading to an imbalance of AMP/ATP, and triggering a switch to catabolic metabolism (El-Mir et al. 2000; Owen et al. 2000; Zhou et al. 2001; Wheaton et al. 2014; Soukas et al. 2019). However, further studies have shown that metformin can additionally regulate AMPK through the lysosome (Zhang et al. 2016; Chen et al. 2017). Consistent with this, we see an upregulation of lysosome related pathways in both old hermaphrodites and males. However, besides the upregulation of lysosome pathways, older males and hermaphrodites treated with metformin had a distinct transcriptomic landscape that could explain the differences in the lifespan extension reported in Figure 4.1. Interestingly, the positive effects of metformin in the absence of FUdR, which also effects mitochondrial function (Rooney et al. 2014), helps to illustrate that metformin can affect nematode longevity in the absence of obvious mitochondrial stress. Consistent with metformin's effect on nutrient senescing, older males significantly upregulate amino acid and fatty acid metabolism pathways, while older hermaphrodites do not show any enrichment for specific upregulated pathways beside the lysosome. Additionally, older males significantly upregulated drug metabolism pathways related to cytochrome P450 (CYP), a phase 1 detoxification enzyme family. This unique transcriptomic signature of metformin in males suggests that they may be a useful tool for exploring the functional basis of metformin in the absence of FUdR.

Metabolism and detoxification pathways have been previously implicated in aging, with age-associated changes in CYPs have been reported in both rat and human livers (Xu et al. 2019;

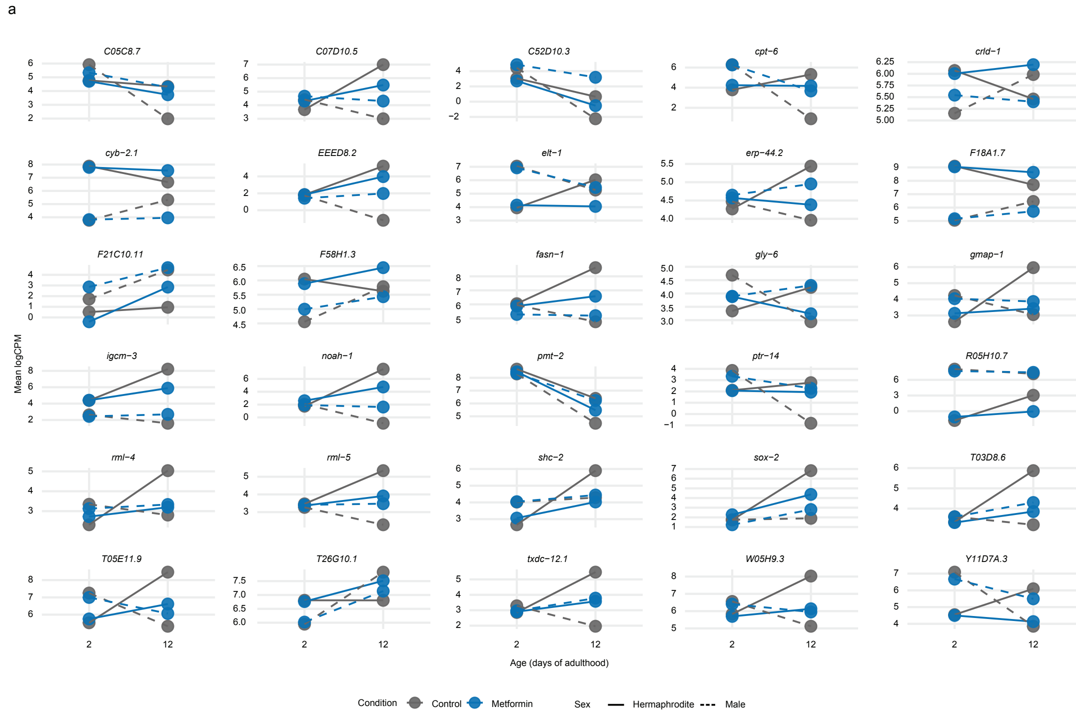
Subash and Prasad 2024). In *C. elegans,* several CYPs have been shown to regulate lifespan, as a mutation in CYP *daf-9* and double mutations in the desaturase *fat-5* and the CYP *cyp-35A2* each extend lifespan. Additionally, several studies reveal the role of CYP14 family in regulating lifespan (Jia et al. 2002; Imanikia et al. 2015; Lim et al. 2024). Consistent with this, we show a strong signature of upregulation of both detoxification and metabolism pathways by ThioT in both sexes. In this study, ThioT extended the lifespan of both hermaphrodites and males, however the magnitude of the extension was much larger in males. Consistent with this observation, we see that ThioT produced a greater number of DEGs in hermaphrodites, potentially disrupting its transcriptome and dampening the effects of the treatment. However, both hermaphrodites and males were enriched for detoxification pathways including several CYPs and the phase 2 detoxification enzymes glutathione-s-transferases (GSTs). While amino acid and fatty acid metabolism are downregulated in both sexes with aging, ThioT treatment reverses that, significantly upregulating metabolic pathways in both sexes. Additionally, hermaphrodites treated with ThioT downregulated Wnt signaling, TGF-beta signaling, polycomb repressive complex, and axon regeneration pathways, all of which were significantly upregulated with aging. Therefore, we hypothesize that ThioT increases hermaphrodite lifespan by specifically targeting hermaphrodite-specific aging-associated pathways and extends lifespan in both sexes by upregulating the detoxification and metabolism pathways.

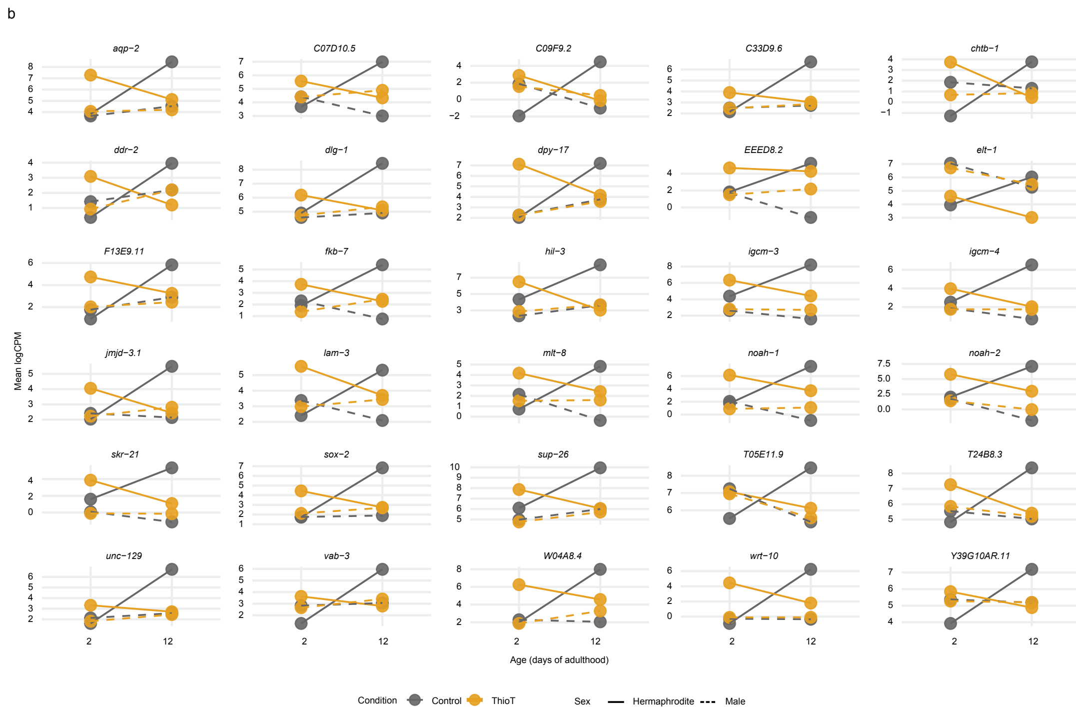
This is further supported by recent findings that the broccoli derivative sulforaphane, which dramatically increases hermaphrodite lifespan, also strongly upregulates the same detoxification pathways (Sedore et al. 2025). Future work could explore the effects of upregulating these pathways in the absence of compound interventions and examining the effects on lifespan. Furthermore, additional samples at different ages could be used to construct an aging clock that can be used to assess the effects of these compounds on the "molecular age" of an animal. Overall, we provide here the first transcriptomic analysis of the effects of pro-longevity interventions on *C. elegans* while accounting for sex as a biological variable modulating this response. Such work is essential to efforts to create pro-longevity interventions that are beneficial for both sexes.

Supplemental Figures



Supplemental Fig. S4.1 Principal component analysis of RNA-seq replicates. Principal component analysis of the biological replicates for the RNA-seq analysis. Circles represent hermaphrodites and triangles represent males. Gray shapes represent control replicates, blue shapes represent metformin-treated replicates, and yellow shapes represent ThioT-treated replicates





Supplemental Fig. S4.2 Top genes whose expression change by compound treatment is sex biased. Line plots for the top 30 genes whose expression change between days 2 and 12 of adulthood by (a) metformin or (b) ThioT is sex biased. Gene expression is measured by the log counts per million (logCPM) per gene. The gray lines represent the control, blue lines represent metformin, and yellow lines represent ThioT treatment. Solid lines represent expression in hermaphrodites and dashed lines represent expression in males

Supplemental Tables

Supplemental Table S4.1 Compound by FUdR by sex CPH model output

ATRA	Cox Proportional Hazards			
Metformin	Effect	Stderr	z-value	p-value
Compound	0.12	0.1	1.26	0.21
FUdR	0.28	0.1	2.79	0.01
Sex	1.62	0.11	14.44	< 2e-16
Compound by FUdR interaction	-1.03	0.14	-7.11	1.12e-12
Compound by sex interaction	-1.34	0.15	-9.08	< 2e-16
FUdR by sex interaction	-1.49	0.16	-9.45	< 2e-16
Full interaction	2.68	0.22	12.17	< 2e-16
ThioT	Effect	Stderr	z-value	p-value
Compound	-0.89	0.11	-8.39	< 2e-16
FUdR	0.27	0.1	2.74	0.01
Sex	1.54	0.11	13.56	< 2e-16
Compound by FUdR interaction	-0.52	0.15	-3.43	0.001
Compound by sex interaction	-1.48	0.17	-8.60	< 2e-16
FUdR by sex interaction	-1.41	0.16	-8.90	< 2e-16
Full interaction	1.66	0.24	7.02	2.29e-12

CHAPTER 5: CONCLUSION

Despite its ubiquitous nature, sex differences in aging have received little experimental attention historically. However, relatively recent efforts emphasized the importance of accounting for sex as a variable in biological research generally and aging research specifically. My dissertation work contributes to these efforts and provides a new experimental paradigm for investigating aging questions in the classical aging model *C. elegans.* In chapter 2, with my co-authors, I investigated sex differences in the response to IIS attenuation and found that, under the same conditions, males lived significantly longer than hermaphrodites in response to DAF-2 degradation. We additionally found that this increase in lifespan was accompanied by a significant improvement in male reproductive success in late life. DAF-2 degradation in the intestine significantly extended male lifespan, consistent with previous reports in hermaphrodites and, unlike in hermaphrodites, neuronal degradation failed to extend lifespan and germline degradation shortened lifespan. This provided further evidence for sexual dimorphism in the response to genetic interventions targeting the IIS pathway and highlighted the neurons and germline as potential mechanistic contributors to these differences.

In chapter 3, I examined whether several compounds that have been shown previously to extend hermaphrodite lifespan have a similar or sex-specific effect on male lifespan. I found that all the tested compounds extended male lifespan, although the effect size for some was sex specific. Additionally, I highlighted the benefits of *C. elegans* males as a screening tool for compound interventions for future studies. In particular, I highlighted the utility of their mating behavior as a complex healthspan metric and the abolished need for FUdR use. I also found that two of these compounds, metformin and sulforaphane, significantly improve male reproductive healthspan in late life.

In chapter 4, I, along with my co-authors, explored the effects of two of the most efficacious compounds form chapter 3, metformin and ThioT, on *C. elegans* aging at the transcriptional level. We conducted an RNA-seq analysis on young and old individuals of both sexes and asked whether sex, compound interventions, and their interaction have a significant effect on age-associated gene expression changes. We provided the first transcriptomic profile of old *C. elegans* males at a whole-animal scale and described several genes whose change in expression

with age is sex biased. When we compared the transcriptomic profiles of compound-treated aging hermaphrodites and males, we found that metformin resulted in sex-specific signatures on the aging transcriptome while ThioT resulted in mostly sex-shared signatures. These results highlighted several molecular pathways that are differentially modulated by metformin and ThioT, most notably metabolism and detoxification pathways upon ThioT treatment. We postulated that some of these transcriptional changes could underlie the sex-specific response to pro-longevity interventions.

Taken together, this dissertation describes sex differences in aging and the response to both genetic and chemical pro-longevity interventions in an aging model system. This work emphasizes the importance of accounting for sex in aging studies and proposes males as a complementary tool for studying aging mechanisms and for screening and characterizing pro-longevity interventions. Translational research is built upon basic knowledge, and this work contributes to the basic knowledge needed for the development of interventions that benefit both sexes, an essential goal of the aging field.

REFERENCES CITED

Gems D. 2022. The hyperfunction theory: An emerging paradigm for the biology of aging. Ageing Res Rev. 74:101557. https://doi.org/10.1016/j.arr.2021.101557

Williams GC. 1957. Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution. 11:398–411. https://doi.org/10.1126/sageke.2001.1.cp13

Kirkwood TBL. 1977. Evolution of ageing. Nature. 270(5635):301–304. https://doi.org/10.1038/270301a0

Blagosklonny MV. 2006. Aging and Immortality: Quasi-Programmed Senescence and Its Pharmacologic Inhibition. Cell Cycle. 5(18):2087–2102. https://doi.org/10.4161/cc.5.18.3288

Magalhães JP de, Church GM. 2005. Genomes Optimize Reproduction: Aging as a Consequence of the Developmental Program. Physiology. 20(4):252–259. https://doi.org/10.1152/physiol.00010.2005

Mc Auley MTM. 2025. The evolution of ageing: classic theories and emerging ideas. Biogerontology. 26(1):6. https://doi.org/10.1007/s10522-024-10143-5

López-Otín C et al. 2023. Hallmarks of aging: An expanding universe. Cell. 186(2):243–278. https://doi.org/10.1016/j.cell.2022.11.001

López-Otín C et al. 2013. The Hallmarks of Aging. Cell. 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Kennedy BK et al. 2014. Geroscience: Linking Aging to Chronic Disease. Cell. 159(4):709–713. https://doi.org/10.1016/j.cell.2014.10.039

Austad SN. 2011. Sex Differences in Longevity and Aging. In: Handbook of the Biology of Aging. Elsevier; p 479–495 https://linkinghub.elsevier.com/retrieve/pii/B9780123786388000233

Rochelle TL, Yeung DKY, Bond MH, Li LMW. 2015. Predictors of the gender gap in life expectancy across 54 nations. Psychology, Health & Medicine. 20(2):129–138 http://www.tandfonline.com/doi/abs/10.1080/13548506.2014.936884. https://doi.org/10.1080/13548506.2014.936884

Dattani S et al. 2023. https://ourworldindata.org/life-expectancy

Yan BW et al. 2024. Widening Gender Gap in Life Expectancy in the US, 2010-2021. JAMA Internal Medicine. 184(1):108–110 https://doi.org/10.1001/jamainternmed.2023.6041

Zarulli V et al. 2018. Women live longer than men even during severe famines and epidemics. Proc Natl Acad Sci USA. 115(4) https://pnas.org/doi/full/10.1073/pnas.1701535115. https://doi.org/10.1073/pnas.1701535115

Luy M. 2003. Causes of Male Excess Mortality: Insights from Cloistered Populations. Population & Development Rev. 29(4):647–676 https://onlinelibrary.wiley.com/doi/10.1111/j.1728-4457.2003.00647.x. https://doi.org/10.1111/j.1728-4457.2003.00647.x

Lindahl-Jacobsen R et al. 2013. The male–female health-survival paradox and sex differences in cohort life expectancy in Utah, Denmark, and Sweden 1850–1910. Annals of Epidemiology. 23(4):161–166 https://linkinghub.elsevier.com/retrieve/pii/S104727971300029X. https://doi.org/10.1016/j.annepidem.2013.02.001

Lemaître J-F et al. 2020. Sex differences in adult lifespan and aging rates of mortality across wild mammals. Proceedings of the National Academy of Sciences. 117(15):8546–8553 https://www.pnas.org/doi/full/10.1073/pnas.1911999117. https://doi.org/10.1073/pnas.1911999117

Austad SN, Fischer KE. 2016. Sex Differences in Lifespan. Cell Metab. 23(6):1022–1033 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4932837/. https://doi.org/10.1016/j.cmet.2016.05.019

Oksuzyan A, Juel K, Vaupel JW, Christensen K. 2008. Men: good health and high mortality. Sex differences in health and aging. Aging Clin Exp Res. 20(2):91–102 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629373/. https://doi.org/10.1007/bf03324754

Gordon EH et al. 2017. Sex differences in frailty: A systematic review and meta-analysis. Experimental Gerontology. 89:30–40 https://linkinghub.elsevier.com/retrieve/pii/S0531556516304442. https://doi.org/10.1016/j.exger.2016.12.021

Rajan KB et al. 2021. Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020–2060). Alzheimer's Dement. 17(12):1966–1975. https://doi.org/10.1002/alz.12362

Bello-Lujan LM, Serrano-Sanchez JA, Gonzalez-Henriquez JJ. 2022. Stable Gender Gap and Similar Gender Trend in Chronic Morbidities between 1997–2015 in Adult Canary Population. IJERPH. 19(15):9404 https://www.mdpi.com/1660-4601/19/15/9404. https://doi.org/10.3390/ijerph19159404

Daly RM et al. 2013. Gender specific age-related changes in bone density, muscle strength and functional performance in the elderly: a-10 year prospective population-based study. BMC Geriatr. 13:71 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3716823/. https://doi.org/10.1186/1471-2318-13-71

Milan-Mattos JC et al. 2019. Effects of natural aging and gender on pro-inflammatory markers. Braz J Med Biol Res. 52(9):e8392 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6694726/. https://doi.org/10.1590/1414-431x20198392

Mauvais-Jarvis F et al. 2020. Sex and gender: modifiers of health, disease, and medicine. Lancet. 396(10250):565–582. https://doi.org/10.1016/s0140-6736(20)31561-0

Hägg S, Jylhävä J. 2021. Sex differences in biological aging with a focus on human studies. eLife. 10:e63425. https://doi.org/10.7554/elife.63425

Marais GAB et al. 2018. Sex gap in aging and longevity: can sex chromosomes play a role? Biol Sex Differ. 9(1):33. https://doi.org/10.1186/s13293-018-0181-y

Connallon T, Beasley IJ, McDonough Y, Ruzicka F. 2022. How much does the unguarded X contribute to sex differences in life span? Evol Lett. 6(4):319–329. https://doi.org/10.1002/evl3.292

Gentilini D et al. 2012. Age-dependent skewing of X chromosome inactivation appears delayed in centenarians' offspring. Is there a role for allelic imbalance in Healthy Aging and Longevity? Aging Cell. 11(2):277–283. https://doi.org/10.1111/j.1474-9726.2012.00790.x

Roberts AL et al. 2022. Age acquired skewed X chromosome inactivation is associated with adverse health outcomes in humans. eLife. 11:e78263. https://doi.org/10.7554/elife.78263

Muyle A, Bachtrog D, Marais GAB, Turner JMA. 2021. Epigenetics drive the evolution of sex chromosomes in animals and plants. Philos Trans R Soc B. 376(1826):20200124. https://doi.org/10.1098/rstb.2020.0124

Delanoue R et al. 2023. Y chromosome toxicity does not contribute to sex-specific differences in longevity. Nat Ecol Evol. 7(8):1245–1256. https://doi.org/10.1038/s41559-023-02089-7

Brown EJ, Nguyen AH, Bachtrog D. 2020. The Y chromosome may contribute to sex-specific ageing in *Drosophila*. Nat Ecol Evol. 4(6):853–862. https://doi.org/10.1038/s41559-020-1179-5

Bruhn-Olszewska B et al. 2025. The effects of loss of Y chromosome on male health. Nat Rev Genet. 26(5):320–335. https://doi.org/10.1038/s41576-024-00805-y

Weber MT, Mapstone M, Staskiewicz J, Maki PM. 2012. Reconciling subjective memory complaints with objective memory performance in the menopausal transition. Menopause: J North Am Menopause Soc. 19(7):735–741. https://doi.org/10.1097/gme.0b013e318241fd22

Piasecki J et al. 2024. Sex Differences in Neuromuscular Aging: The Role of Sex Hormones. Exerc Sport Sci Rev. 52(2):54–62. https://doi.org/10.1249/jes.0000000000000335

Bove R et al. 2014. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. Neurology. 82(3):222–229. https://doi.org/10.1212/wnl.0000000000000033

Gurvich C, Hoy K, Thomas N, Kulkarni J. 2018. Sex Differences and the Influence of Sex Hormones on Cognition through Adulthood and the Aging Process. Brain Sci. 8(9):163. https://doi.org/10.3390/brainsci8090163

Rocca WA et al. 2007. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 69(11):1074–1083. https://doi.org/10.1212/01.wnl.0000276984.19542.e6

Mishra S, Chung H, Waller M, Mishra G. 2021. Duration of estrogen exposure during reproductive years, age at menarche and age at menopause, and risk of cardiovascular disease events, all-cause and cardiovascular mortality: a systematic review and meta-analysis. BJOG: Int J Obstet Gynaecol. 128(5):809–821. https://doi.org/10.1111/1471-0528.16524

Shadyab AH et al. 2017. Ages at menarche and menopause and reproductive lifespan as predictors of exceptional longevity in women. Menopause. 24(1):35–44. https://doi.org/10.1097/gme.0000000000000710

Maupas E. 1899. La mue et l'enkystement chez les nématodes. Arch Zool exp gén,(3e Série). 7:563–628

Nigon V. 1949. Les modalites de la reproduction et le determinisme due sexe chez quelques nematodes libres. Ann Sci Nat. 11:1–132

Dougherty EC et al. 1959. Axenic cultivation of *Caenorhabditis briggsae* (Nematoda: *Rhabditidae*) with unsupplemented and supplemented chemically defined media.

Brenner S. 2009. In the Beginning Was the Worm …. Genetics. 182(2):413–415. https://doi.org/10.1534/genetics.109.104976

Brenner S. 1974. THE GENETICS OF *CAENORHABDITIS ELEGANS*. Genetics. 77(1):71–94. https://doi.org/10.1093/genetics/77.1.71

Kimble J, Hirsh D. 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev Biol. 70(2):396–417. https://doi.org/10.1016/0012-1606(79)90035-6

Sulston JE, Schierenberg E, White JG, Thomson JN. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol. 100(1):64–119. https://doi.org/10.1016/0012-1606(83)90201-4

Ellis HM, Horvitz HR. 1986. Genetic control of programmed cell death in the nematode *C. elegans*. Cell. 44(6):817–829. https://doi.org/10.1016/0092-8674(86)90004-8

Hedgecock EM, Sulston JE, Thomson JN. 1983. Mutations Affecting Programmed Cell Deaths in the Nematode *Caenorhabditis elegans*. Science. 220(4603):1277–1279. https://doi.org/10.1126/science.6857247

Fire A et al. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 391(6669):806–811. https://doi.org/10.1038/35888

Lee RC, Feinbaum RL, Ambros V. 1993. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell. 75(5):843–854. https://doi.org/10.1016/0092-8674(93)90529-y

Wightman B, Ha I, Ruvkun G. 1993. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. Cell. 75(5):855–862. https://doi.org/10.1016/0092-8674(93)90530-4

Chalfie M et al. 1994. Green Fluorescent Protein as a Marker for Gene Expression. Science. 263(5148):802–805. https://doi.org/10.1126/science.8303295

The *C. elegans* Sequencing Consortium. 1998. Genome Sequence of the Nematode *C. elegans*: A Platform for Investigating Biology. Science. 282(5396):2012–2018. https://doi.org/10.1126/science.282.5396.2012

Klass MR. 1983. A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. Mech Ageing Dev. 22(3–4):279–286. https://doi.org/10.1016/0047-6374(83)90082-9

Friedman DB, Johnson TE. 1988. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics. 118(1):75–86. https://doi.org/10.1093/genetics/118.1.75

Johnson TE. 1990. Increased Life-Span of *age-1* Mutants in *Caenorhabditis elegans* and Lower Gompertz Rate of Aging. Science. 249(4971):908–912. https://doi.org/10.1126/science.2392681

Kenyon C et al. 1993. A *C. elegans* mutant that lives twice as long as wild type. Nature. 366(6454):461–464 https://www.nature.com/articles/366461a0. https://doi.org/10.1038/366461a0

Kenyon C. 2011. The first long-lived mutants: discovery of the insulin/IGF-1 pathway for ageing. Philos Trans R Soc Lond B Biol Sci. 366(1561):9–16 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3001308/. https://doi.org/10.1098/rstb.2010.0276

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. 1997. *daf-2*, an Insulin Receptor-Like Gene That Regulates Longevity and Diapause in *Caenorhabditis elegans*. Science. 277(5328):942–946 https://www.science.org/doi/10.1126/science.277.5328.942. https://doi.org/10.1126/science.277.5328.942

Kishimoto S, Uno M, Nishida E. 2018. Molecular mechanisms regulating lifespan and environmental stress responses. Inflamm Regen. 38(1):22. https://doi.org/10.1186/s41232-018-0080-y

Paradis S, Ruvkun G. 1998. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev. 12(16):2488–2498. https://doi.org/10.1101/gad.12.16.2488

Morris JZ, Tissenbaum HA, Ruvkun G. 1996. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature. 382(6591):536–539. https://doi.org/10.1038/382536a0

Lin K, Dorman JB, Rodan A, Kenyon C. 1997. *daf-16*: An HNF-3/forkhead Family Member That Can Function to Double the Life-Span of *Caenorhabditis elegans*. Science. 278(5341):1319–1322. https://doi.org/10.1126/science.278.5341.1319

Ogg S et al. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature. 389(6654):994–999. https://doi.org/10.1038/40194

Uno M, Nishida E. 2016. Lifespan-regulating genes in *C. elegans*. npj Aging Mech Dis. 2(1):16010. https://doi.org/10.1038/npjamd.2016.10

Vellai T et al. 2003. Influence of TOR kinase on lifespan in *C. elegans*. Nature. 426(6967):620–620. https://doi.org/10.1038/426620a

Jia K, Chen D, Riddle DL. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development. 131(16):3897–3906. https://doi.org/10.1242/dev.01255

Sheaffer KL, Updike DL, Mango SE. 2008. The Target of Rapamycin Pathway Antagonizes *pha-4*/FoxA to Control Development and Aging. Curr Biol. 18(18):1355–1364. https://doi.org/10.1016/j.cub.2008.07.097

Chen D et al. 2013. Germline Signaling Mediates the Synergistically Prolonged Longevity Produced by Double Mutations in *daf-2* and *rsks-1* in *C. elegans*. Cell Rep. 5(6):1600–1610. https://doi.org/10.1016/j.celrep.2013.11.018

Driscoll M et al. 2025. NIA *Caenorhabditis* Intervention Testing Program: identification of robust and reproducible pharmacological interventions that promote longevity across experimentally accessible, genetically diverse populations. GeroScience. 1–26. https://doi.org/10.1007/s11357-025-01627-4

Sedore CA et al. 2025. The broccoli derivative sulforaphane extends lifespan by slowing the transcriptional aging clock. bioRxiv. 2025.05.11.653363. https://doi.org/10.1101/2025.05.11.653363

Onken B et al. 2022. Metformin treatment of diverse *Caenorhabditis* species reveals the importance of genetic background in longevity and healthspan extension outcomes. Aging Cell. 21(1):e13488. https://doi.org/10.1111/acel.13488

Lucanic M et al. 2017. Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects. Nat Commun. 8(1):14256. https://doi.org/10.1038/ncomms14256

Banse SA., Sedore CA, et al. 2024. Antioxidants green tea extract and nordihydroguaiaretic acid confer species and strain-specific lifespan and health effects in *Caenorhabditis* nematodes. GeroScience. 46(2):2239–2251. https://doi.org/10.1007/s11357-023-00978-0

Banse SA et al. 2024. Computer prediction and genetic analysis identifies retinoic acid modulation as a driver of conserved longevity pathways in genetically-diverse *Caenorhabditis* nematodes. https://doi.org/10.7554/elife.104375.1

Reilly DK et al. 2021. Distinct neuropeptide-receptor modules regulate a sex-specific behavioral response to a pheromone. Commun Biol. 4(1):1018. https://doi.org/10.1038/s42003-021-02547-7

Barr MM, García LR, Portman DS. 2018. Sexual Dimorphism and Sex Differences in *Caenorhabditis elegans* Neuronal Development and Behavior. Genetics. 208(3):909–935. https://doi.org/10.1534/genetics.117.300294

Hotzi B et al. 2018. Sex-specific regulation of aging in *Caenorhabditis elegans*. Aging Cell. 17(3):e12724. https://doi.org/10.1111/acel.12724

Weng Y, Murphy CT. 2024. Male-specific behavioral and transcriptomic changes in aging *C. elegans neurons*. iScience. 27(6):109910. https://doi.org/10.1016/j.isci.2024.109910

Purice MD et al. 2025. Molecular profiling of adult *C. elegans* glia across sexes by single-nuclear RNA-seq. Dev Cell. [published online ahead of print]. https://doi.org/10.1016/j.devcel.2025.05.013

Barr M, Garcia LR. 2006. Male mating behavior. WormBook. [published online ahead of print] http://www.wormbook.org/chapters/www_malematingbehavior/malematingbehavior.html. https://doi.org/10.1895/wormbook.1.78.1

Guo X, Navetta A, Gualberto DG, García LR. 2012. Behavioral decay in aging male *C. elegans* correlates with increased cell excitability. Neurobiol Aging. 33(7):1483.e5-1483.23 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3378242/. https://doi.org/10.1016/j.neurobiolaging.2011.12.016

Chatterjee I et al. 2013. Dramatic fertility decline in aging *C. elegans* males is associated with mating execution deficits rather than diminished sperm quality. Exp Gerontol. 48(11):1156–1166. https://doi.org/10.1016/j.exger.2013.07.014

Gandhi S et al. 1980. A simple method for maintaining large, aging populations of *Caenorhabditis elegans*. Mech Ageing Dev. 12(2):137–150. https://doi.org/10.1016/0047-6374(80)90090-1

Wang H, Zhao Y, Zhang Z. 2019. Age-dependent effects of floxuridine (FUdR) on senescent pathology and mortality in the nematode *Caenorhabditis elegans*. Biochem Biophys Res Commun. 509(3):694–699. https://doi.org/10.1016/j.bbrc.2018.12.161

Anderson EN et al. 2016. *C. elegans* lifespan extension by osmotic stress requires FUdR, base excision repair, FOXO, and sirtuins. Mech Ageing Dev. 154:30–42. https://doi.org/10.1016/j.mad.2016.01.004

McIntyre G et al. 2021. Effects of FUdR on gene expression in the *C. elegans* bacterial diet OP50. BMC Res Notes. 14(1):207. https://doi.org/10.1186/s13104-021-05624-6

Mitchell DH, Stiles JW, Santelli J, Sanadi DR. 1979. Synchronous Growth and Aging of *Caenorhabditis elegans* in the Presence of Fluorodeoxyuridine. J Gerontol. 34(1):28–36. https://doi.org/10.1093/geronj/34.1.28

Venz R et al. 2021. End-of-life targeted degradation of DAF-2 insulin/IGF-1 receptor promotes longevity free from growth-related pathologies. eLife. 10:e71335. https://doi.org/10.7554/elife.71335

Zhang Y-P et al. 2022. Intestine-specific removal of DAF-2 nearly doubles lifespan in *Caenorhabditis elegans* with little fitness cost. Nat Commun. 13(1):6339 https://www.nature.com/articles/s41467-022-33850-4. https://doi.org/10.1038/s41467-022-33850-4

Roy C et al. 2022. DAF-2/insulin IGF-1 receptor regulates motility during aging by integrating opposite signaling from muscle and neuronal tissues. Aging Cell. 21(8):e13660. https://doi.org/10.1111/acel.13660

Knufinke M, MacArthur MR, Ewald CY, Mitchell SJ. 2023. Sex differences in pharmacological interventions and their effects on lifespan and healthspan outcomes: a systematic review. Front Aging. 4 https://www.frontiersin.org/journals/aging/articles/10.3389/fragi.2023.1172789/full. https://doi.org/10.3389/fragi.2023.1172789

Bartke A, Hascup E, Hascup K. 2024. Responses to Many Anti-Aging Interventions Are Sexually Dimorphic. World J Mens Health. 42(1):29–38 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10782120/. https://doi.org/10.5534/wjmh.230015

Kenyon CJ. 2010. The genetics of ageing. Nature. 464(7288):504–512 https://www.nature.com/articles/nature08980. https://doi.org/10.1038/nature08980

Tramunt B et al. 2020. Sex differences in metabolic regulation and diabetes susceptibility. Diabetologia. 63(3):453–461 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6997275/. https://doi.org/10.1007/s00125-019-05040-3

Ciarambino T, Crispino P, Guarisco G, Giordano M. 2023. Gender Differences in Insulin Resistance: New Knowledge and Perspectives. Curr Issues Mol Biol. 45(10):7845–7861 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10605445/. https://doi.org/10.3390/cimb45100496

Garratt M. 2020. Why do sexes differ in lifespan extension? Sex-specific pathways of aging and underlying mechanisms for dimorphic responses. NHA. 5(4):247–259 https://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/NHA-190067. https://doi.org/10.3233/nha-190067

Sulston JE, Albertson DG, Thomson JN. 1980. The *Caenorhabditis elegans* male: Postembryonic development of nongonadal structures. Dev Biol. 78(2):542–576. https://doi.org/10.1016/0012-1606(80)90352-8

Molina-García L et al. 2020. Direct glia-to-neuron transdifferentiation gives rise to a pair of male-specific neurons that ensure nimble male mating. eLife. 9:e48361. https://doi.org/10.7554/elife.48361

Nishimura K et al. 2009. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat Methods. 6(12):917–922. https://doi.org/10.1038/nmeth.1401

Zhang L, Ward JD, Cheng Z, Dernburg AF. 2015. The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*. Development. 142(24):4374–4384. https://doi.org/10.1242/dev.129635

Loose JA, Ghazi A. 2021. Auxin treatment increases lifespan in *Caenorhabditis elegans*. Biol Open. 10(5):bio058703. https://doi.org/10.1242/bio.058703

Shi C, Runnels AM, Murphy CT. 2017. Mating and male pheromone kill *Caenorhabditis* males through distinct mechanisms. eLife. 6:e23493. https://doi.org/10.7554/elife.23493

Shi C, Murphy CT. 2014. Mating Induces Shrinking and Death in *Caenorhabditis* Mothers. Science. 343(6170):536–540. https://doi.org/10.1126/science.1242958

Maures TJ et al. 2014. Males Shorten the Life Span of *C. elegans* Hermaphrodites via Secreted Compounds. Science. 343(6170):541–544. https://doi.org/10.1126/science.1244160

Hahm J-H et al. 2015. *C. elegans* maximum velocity correlates with healthspan and is maintained in worms with an insulin receptor mutation. Nat Commun. 6(1):8919. https://doi.org/10.1038/ncomms9919

Banse SA., Jackson EG, et al. 2024. The coupling between healthspan and lifespan in *Caenorhabditis* depends on complex interactions between compound intervention and genetic background. Aging (Albany NY). 16(7):5829–5855. https://doi.org/10.18632/aging.205743

Bansal A, Zhu LJ, Yen K, Tissenbaum HA. 2015. Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. Proc Natl Acad Sci. 112(3):E277–E286. https://doi.org/10.1073/pnas.1412192112

Schedl T, Kimble J. 1988. *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics. 119(1):43–61. https://doi.org/10.1093/genetics/119.1.43

Austad SN, Bartke A. 2016. Sex Differences in Longevity and in Responses to Anti-Aging Interventions: A Mini-Review. Gerontology. 62(1):40–46 https://www.karger.com/Article/FullText/381472. https://doi.org/10.1159/000381472

Kim D, Kim B. 2022. Anatomical and Functional Differences in the Sex-Shared Neurons of the Nematode *C. elegans*. Front Neuroanat. 16:906090. https://doi.org/10.3389/fnana.2022.906090

Ludewig AH et al. 2019. An excreted small molecule promotes *C. elegans* reproductive development and aging. Nat Chem Biol. 15(8):838–845. https://doi.org/10.1038/s41589-019-0321-7

Booth LN et al. 2022. Males induce premature demise of the opposite sex by multifaceted strategies. Nat Aging. 2(9):809–823. https://doi.org/10.1038/s43587-022-00276-y

Olshansky SJ. 2018. From Lifespan to Healthspan. JAMA. 320(13):1323. https://doi.org/10.1001/jama.2018.12621

Huang C, Xiong C, Kornfeld K. 2004. Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. Proc Natl Acad Sci. 101(21):8084–8089. https://doi.org/10.1073/pnas.0400848101

Mulcahy B, Holden-Dye L, O'Connor V. 2012. Pharmacological assays reveal age-related changes in synaptic transmission at the *Caenorhabditis elegans* neuromuscular junction that are modified by reduced insulin signalling. J Exp Biol. 216(3):492–501. https://doi.org/10.1242/jeb.068734

Weng Y et al. 2024. The neuron-specific IIS/FOXO transcriptome in aged animals reveals regulatory mechanisms of cognitive aging. eLife. 13:RP95621. https://doi.org/10.7554/elife.95621

Murphy CT et al. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature. 424(6946):277–283. https://doi.org/10.1038/nature01789

Glenn CF et al. 2004. Behavioral Deficits During Early Stages of Aging in *Caenorhabditis elegans* Result From Locomotory Deficits Possibly Linked to Muscle Frailty. J Gerontol Ser A: Biol Sci Méd Sci. 59(12):1251–1260. https://doi.org/10.1093/gerona/59.12.1251

R Core Team. 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. [published online ahead of print] https://www.R-project.org

Therneau TM. 2024. coxme: Mixed Effects Cox Models. https://CRAN.R-project.org/package=coxme

Therneau TM. 2024. A Package for Survival Analysis in R. https://CRAN.R-project.org/package=survival

Therneau TM, Grambsch PM. 2000. Modeling Survival Data: Extending the Cox Model. Springer.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 67(1). https://doi.org/10.18637/jss.v067.i01

Lenth RV. 2025. emmeans: Estimated Marginal Means, aka Least-Squares Means. CRAN: Contrib Packag. [published online ahead of print] https://rvlenth.github.io/emmeans/. https://doi.org/10.32614/cran.package.emmeans

Holdcroft A. 2007. Gender bias in research: how does it affect evidence based medicine? J R Soc Med. 100(1):2–3 https://doi.org/10.1177/014107680710000102

Zhang S et al. 2020. *Caenorhabditis elegans* as a Useful Model for Studying Aging Mutations. Front Endocrinol. 11:554994. https://doi.org/10.3389/fendo.2020.554994

Andersen EC, Rockman MV. 2022. Natural genetic variation as a tool for discovery in *Caenorhabditis nematodes*. Genetics. 220(1):iyab156. https://doi.org/10.1093/genetics/iyab156

Teterina AA et al. 2022. Genetic diversity estimates for the *Caenorhabditis* Intervention Testing Program screening panel. microPublication Biol. 2022:10.17912/micropub.biology.000518. https://doi.org/10.17912/micropub.biology.000518

Emmons SW. 2014. The development of sexual dimorphism: studies of the *Caenorhabditis elegans* male. Wiley Interdiscip Rev: Dev Biol. 3(4):239–262. https://doi.org/10.1002/wdev.136

Anderson JL, Morran LT, Phillips PC. 2010. Outcrossing and the Maintenance of Males within *C. elegans* Populations. J Hered. 101(suppl_1):S62–S74. https://doi.org/10.1093/jhered/esq003

McCulloch D, Gems D. 2007. Sex-specific Effects of the DAF-12 Steroid Receptor on Aging in *Caenorhabditis elegans*. Ann N York Acad Sci. 1119(1):253–259. https://doi.org/10.1196/annals.1404.018

Honjoh S et al. 2017. The Sexual Dimorphism of Dietary Restriction Responsiveness in *Caenorhabditis elegans*. Cell Rep. 21(13):3646–3652. https://doi.org/10.1016/j.celrep.2017.11.108

Liggett MR, Hoy MJ, Mastroianni M, Mondoux MA. 2015. High-glucose diets have sex-specific effects on aging in *C. elegans*: toxic to hermaphrodites but beneficial to males. Aging (Albany NY). 7(6):383–387. https://doi.org/10.18632/aging.100759

Harrison DE et al. 2014. Acarbose, 17-α-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. Aging Cell. 13(2):273–282. https://doi.org/10.1111/acel.12170

Strong R et al. 2008. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. Aging Cell. 7(5):641–650. https://doi.org/10.1111/j.1474-9726.2008.00414.x

Aprison EZ, Ruvinsky I. 2016. Sexually Antagonistic Male Signals Manipulate Germline and Soma of *C. elegans* Hermaphrodites. Curr Biol. 26(20):2827–2833. https://doi.org/10.1016/j.cub.2016.08.024

Benedetti MG et al. 2008. Compounds that confer thermal stress resistance and extended lifespan. Exp Gerontol. 43(10):882–891. https://doi.org/10.1016/j.exger.2008.08.049

Qi Z et al. 2021. Sulforaphane promotes *C. elegans* longevity and healthspan via DAF-16/DAF-2 insulin/IGF-1 signaling. Aging (Albany NY). 13(2):1649–1670. https://doi.org/10.18632/aging.202512

Alavez S et al. 2011. Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. Nature. 472(7342):226–229. https://doi.org/10.1038/nature09873

Inoue H, Nishida E. 2010. The DM Domain Transcription Factor MAB-3 Regulates Male Hypersensitivity to Oxidative Stress in *Caenorhabditis elegans*. Mol Cell Biol. 30(14):3453–3459. https://doi.org/10.1128/mcb.01459-09

Zajitschek SR et al. 2020. Sexual dimorphism in trait variability and its eco-evolutionary and statistical implications. eLife. 9:e63170 https://doi.org/10.7554/eLife.63170

Zucker I, Beery AK. 2010. Males still dominate animal studies. Nature. 465(7299):690–690. https://doi.org/10.1038/465690a

Beery AK, Zucker I. 2011. Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev. 35(3):565–572. https://doi.org/10.1016/j.neubiorev.2010.07.002

Health NI of. 1994. NIH guidelines on the inclusion of women and minorities as subjects in clinical research. Fed Regist. 59:1408–1413

Health NI of. 2015. Consideration of sex as a biological variable in NIH-funded research (NOT-OD-15-102). https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html

Macchiarini F et al. 2021. Chapter 10 - NIA Interventions Testing Program: A collaborative approach for investigating interventions to promote healthy aging. In: Musi" ["Nicolas, Hornsby"] "Peter J., editors. Handbook of the Biology of Aging (Ninth Edition). Ninth Edition. Academic Press; p 219–235 (Handbooks of Aging). https://www.sciencedirect.com/science/article/pii/B978012815962000010X. https://doi.org/10.1016/b978-0-12-815962-0.00010-x

Barardo D et al. 2017. The DrugAge database of aging-related drugs. Aging Cell. 16(3):594–597. https://doi.org/10.1111/acel.12585

Strong R et al. 2020. Rapamycin-mediated mouse lifespan extension: Late-life dosage regimes with sex-specific effects. Aging Cell. 19(11):e13269. https://doi.org/10.1111/acel.13269

Harrison DE et al. 2024. Astaxanthin and meclizine extend lifespan in UM-HET3 male mice; fisetin, SG1002 (hydrogen sulfide donor), dimethyl fumarate, mycophenolic acid, and 4-phenylbutyrate do not significantly affect lifespan in either sex at the doses and schedules used. GeroScience. 46(1):795–816. https://doi.org/10.1007/s11357-023-01011-0

Miller RA et al. 2019. Glycine supplementation extends lifespan of male and female mice. Aging Cell. 18(3):e12953. https://doi.org/10.1111/acel.12953

Strong R et al. 2022. Lifespan benefits for the combination of rapamycin plus acarbose and for captopril in genetically heterogeneous mice. Aging Cell. 21(12):e13724. https://doi.org/10.1111/acel.13724

Harrison DE et al. 2019. Acarbose improves health and lifespan in aging HET3 mice. Aging Cell. 18(2):e12898. https://doi.org/10.1111/acel.12898

Strong R et al. 2016. Longer lifespan in male mice treated with a weakly estrogenic agonist, an antioxidant, an α-glucosidase inhibitor or a Nrf2-inducer. Aging Cell. 15(5):872–884. https://doi.org/10.1111/acel.12496

Harrison DE et al. 2021. 17-a-estradiol late in life extends lifespan in aging UM-HET3 male mice; nicotinamide riboside and three other drugs do not affect lifespan in either sex. Aging Cell. 20(5):e13328. https://doi.org/10.1111/acel.13328

Miller RA et al. 2020. Canagliflozin extends lifespan in genetically heterogeneous male but not female mice. JCI Insight. 5(21):e140019. https://doi.org/10.1172/jci.insight.140019

Miller RA et al. 2024. Lifespan effects in male UM-HET3 mice treated with sodium thiosulfate, 16-hydroxyestriol, and late-start canagliflozin. GeroScience. 46(5):4657–4670. https://doi.org/10.1007/s11357-024-01176-2

Phillips CM et al. 2005. HIM-8 Binds to the X Chromosome Pairing Center and Mediates Chromosome-Specific Meiotic Synapsis. Cell. 123(6):1051–1063. https://doi.org/10.1016/j.cell.2005.09.035

Rollins JA et al. 2017. Assessing Health Span in *Caenorhabditis elegans*: Lessons From Short-Lived Mutants. J Gerontol Ser A: Biomed Sci Méd Sci. 72(4):473–480. https://doi.org/10.1093/gerona/glw248

Yuan S, Larsson SC. 2023. Epidemiology of sarcopenia: Prevalence, risk factors, and consequences. Metabolism. 144:155533. https://doi.org/10.1016/j.metabol.2023.155533

Livingston G et al. 2024. Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. Lancet. 404(J Gerontol A Biol Sci Med Sci 73 2018):572–628. https://doi.org/10.1016/s0140-6736(24)01296-0

Al-Saadi RS, Phillips PC. 2025. Pro-longevity compounds extend *Caenorhabditis elegans* male lifespan and reproductive healthspan. bioRxiv. 2025.06.29.662228. https://doi.org/10.1101/2025.06.29.662228

Zhu L et al. 2024. Metformin as anticancer agent and adjuvant in cancer combination therapy: Current progress and future prospect. Transl Oncol. 44:101945. https://doi.org/10.1016/j.tranon.2024.101945

Lv Z, Guo Y. 2020. Metformin and Its Benefits for Various Diseases. Front Endocrinol. 11:191. https://doi.org/10.3389/fendo.2020.00191

Anisimov VN et al. 2011. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY). 3(2):148–157. https://doi.org/10.18632/aging.100273

Martin-Montalvo A et al. 2013. Metformin improves healthspan and lifespan in mice. Nat Commun. 4(1):2192. https://doi.org/10.1038/ncomms3192

Onken B, Driscoll M. 2010. Metformin Induces a Dietary Restriction–Like State and the Oxidative Stress Response to Extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS ONE. 5(1):e8758. https://doi.org/10.1371/journal.pone.0008758

Porat Y, Abramowitz A, Gazit E. 2006. Inhibition of Amyloid Fibril Formation by Polyphenols: Structural Similarity and Aromatic Interactions as a Common Inhibition Mechanism. Chem Biol Drug Des. 67(1):27–37. https://doi.org/10.1111/j.1747-0285.2005.00318.x

Liao Y, Smyth GK, Shi W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 30(7):923–930. https://doi.org/10.1093/bioinformatics/btt656

Liao Y, Smyth GK, Shi W. 2013. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 41(10):e108–e108. https://doi.org/10.1093/nar/gkt214

Robinson MD, McCarthy DJ, Smyth GK. 2009. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26(1):139–140. https://doi.org/10.1093/bioinformatics/btp616

Yu G, Wang L-G, Han Y, He Q-Y. 2012. clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. OMICS: A J Integr Biol. 16(5):284–287. https://doi.org/10.1089/omi.2011.0118

Cox GN, Kusch M, DeNevi K, Edgar RS. 1981. Temporal regulation of cuticle synthesis during development of *Caenorhabditis elegans*. Dev Biol. 84(2):277–285. https://doi.org/10.1016/0012-1606(81)90395-x

Njume FN et al. 2022. A lipid transfer protein ensures nematode cuticular impermeability. iScience. 25(11):105357. https://doi.org/10.1016/j.isci.2022.105357

Novelli J, Page AP, Hodgkin J. 2006. The C Terminus of Collagen SQT-3 Has Complex and Essential Functions in Nematode Collagen Assembly. Genetics. 172(4):2253–2267. https://doi.org/10.1534/genetics.105.053637

Mapes J, Chen J-T, Yu J-S, Xue D. 2010. Somatic sex determination in *Caenorhabditis elegans* is modulated by SUP-26 repression of tra-2 translation. Proc Natl Acad Sci. 107(42):18022–18027. https://doi.org/10.1073/pnas.1004513107

Schachtner LT et al. 2015. *Drosophila* Shep and *C. elegans* SUP-26 are RNA-binding proteins that play diverse roles in nervous system development. Dev Genes Evol. 225(6):319–330. https://doi.org/10.1007/s00427-015-0514-3

Angeli S et al. 2013. A DNA synthesis inhibitor is protective against proteotoxic stressors via modulation of fertility pathways in *Caenorhabditis elegans*. Aging (Albany NY). 5(10):759–769. https://doi.org/10.18632/aging.100605

Ebbing A et al. 2018. Spatial Transcriptomics of *C. elegans* Males and Hermaphrodites Identifies Sex-Specific Differences in Gene Expression Patterns. Dev Cell. 47(6):801-813.e6. https://doi.org/10.1016/j.devcel.2018.10.016

Kim B, Suo B, Emmons SW. 2016. Gene Function Prediction Based on Developmental Transcriptomes of the Two Sexes in *C. elegans*. Cell Rep. 17(3):917–928. https://doi.org/10.1016/j.celrep.2016.09.051

Mosley MC et al. 2025. Similarities and differences in the gene expression signatures of physiological age versus future lifespan. Aging Cell. 24(4):e14428. https://doi.org/10.1111/acel.14428

He K et al. 2014. Dynamic regulation of genetic pathways and targets during aging in *Caenorhabditis elegans*. Aging (Albany NY). 6(3):215–230. https://doi.org/10.18632/aging.100648

Zhang T et al. 2025. The Anti-Aging Mechanism of Metformin: From Molecular Insights to Clinical Applications. Molecules. 30(4):816. https://doi.org/10.3390/molecules30040816

Zhou G et al. 2001. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Investig. 108(8):1167–1174. https://doi.org/10.1172/jci13505

El-Mir M-Y et al. 2000. Dimethylbiguanide Inhibits Cell Respiration via an Indirect Effect Targeted on the Respiratory Chain Complex I*. J Biol Chem. 275(1):223–228. https://doi.org/10.1074/jbc.275.1.223

Wheaton WW et al. 2014. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. eLife. 3:e02242. https://doi.org/10.7554/elife.02242

Owen MR, Doran E, Halestrap AP. 2000. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 348 Pt 3:607–14

Soukas AA, Hao H, Wu L. 2019. Metformin as Anti-Aging Therapy: Is It for Everyone? Trends Endocrinol Metab. 30(10):745–755. https://doi.org/10.1016/j.tem.2019.07.015

Chen J et al. 2017. Metformin extends *C. elegans* lifespan through lysosomal pathway. eLife. 6:e31268. https://doi.org/10.7554/elife.31268

Zhang C-S et al. 2016. Metformin Activates AMPK through the Lysosomal Pathway. Cell Metab. 24(4):521–522. https://doi.org/10.1016/j.cmet.2016.09.003

Rooney JP et al. 2014. Effects of 5′-fluoro-2-deoxyuridine on mitochondrial biology in *Caenorhabditis elegans*. Exp Gerontol. 56:69–76. https://doi.org/10.1016/j.exger.2014.03.021

Xu S-F et al. 2019. Age-associated changes of cytochrome P450 and related phase-2 gene/proteins in livers of rats. PeerJ. 7:e7429. https://doi.org/10.7717/peerj.7429

Subash S, Prasad B. 2024. Age-Dependent Changes in Cytochrome P450 Abundance and Composition in Human Liver. Drug Metab Dispos. 52(12):1363–1372. https://doi.org/10.1124/dmd.124.001608

Lim SYM et al. 2024. CYP14 family in *Caenorhabditis elegans*: Mitochondrial function, detoxification, and lifespan. J Appl Toxicol. 44(11):1647–1656. https://doi.org/10.1002/jat.4597

Imanikia S, Hylands P, Stürzenbaum SR. 2015. The double mutation of cytochrome P450's and fatty acid desaturases affect lipid regulation and longevity in *C. elegans*. Biochem Biophys Rep. 2:172–178. https://doi.org/10.1016/j.bbrep.2015.06.007

Jia K, Albert PS, Riddle DL. 2002. DAF-9, a cytochrome P450 regulating *C. elegans* larval development and adult longevity. Development. 129(1):221–231. https://doi.org/10.1242/dev.129.1.221