

Reviewed Preprint

v1 • July 13, 2026

Not revised

Fourfold lifespan extension in *C. elegans* daf-2 mutant males

✉ For correspondence:

sunlab@uab.edu

Competing interests: No

competing interests declared

Funding: See [page 11](#)

Reviewing editor: Detlef Weigel,
Max Planck Institute for Biology
Tübingen, Germany

© 2026, Russell et al. This article is
distributed under the terms of the
[Creative Commons Attribution
License](#), which permits unrestricted
use and redistribution provided that
the original author and source are
credited.

Mike Russell, Michelle Lin, Alexander Tate Lasher, Steven N Austad, Liou Y Sun ✉

Department of Biology, University of Alabama at Birmingham, Birmingham, United States

eLife Assessment

This **useful** study reports on lifespan extension in *C. elegans* males that carry a mutation in a gene for an insulin receptor; while the observations are striking, the strength of evidence is currently **incomplete**. The central claim of male-specificity is undermined by the absence of direct hermaphrodite healthspan comparisons, and the reliance on a single mutant allele leaves open the possibility that background mutations, rather than daf-2 loss-of-function, drive the phenotype. Methodological details critical for reproducibility are also lacking, particularly regarding male housing density, censoring of plate-leaving animals, and the adequacy of replication for the key epistasis experiment. The work could be substantially strengthened by a targeted set of additional experiments and fuller engagement with the existing literature on sex-specific aging in *C. elegans*.

<https://doi.org/10.7554/eLife.111931.1.sa3>

Abstract

Aging is a universal biological process driven by conserved genetic networks that balance somatic maintenance with growth and reproduction. The insulin/insulin-like growth factor I signaling pathway is a central architect of this balance, and inhibiting its activity has long been established as a primary mechanism to extend lifespan across diverse species. However, our understanding of this pathway's limits has been constrained by a historical focus on hermaphroditic models, which inherently link longevity extensions to a rigid trade-off involving immense reproductive costs and restricted somatic growth. Here we show that the latent potential of this canonical aging pathway is profoundly amplified by male-specific biology. We demonstrate that a classic insulin receptor mutation extends male survival to an unprecedented extreme, vastly surpassing the established benchmarks of the field. We reveal that this extraordinary lifespan expansion remains strictly dependent on the canonical FOXO transcription factor, yet it fuels a male-specific metabolic reprogramming that uncouples aging from stunted growth—massively accumulating neutral lipids to support sustained somatic preservation. These findings establish biological sex as a primary determinant of longevity potential and provide a new framework for identifying hidden, sex-specific mechanisms capable of promoting extreme healthy longevity.

Introduction

Aging is a universal biological process and is among the greatest risk factors for chronic disease (MacNee et al., 2014 [DOI](#)). While decades of research have uncovered conserved genetic pathways that regulate lifespan, the mechanisms that determine why individuals age differently remain unresolved. Among these regulators, the insulin/insulin-like growth factor I (IGF-I) signaling (IIS) pathway was the first discovered and remains one of the most powerful central determinants of longevity (Kenyon, 2011 [DOI](#)). Initial studies from Cynthia Kenyon and colleagues (1993) [DOI](#) demonstrated that a single point mutation in the gene coding for DAF-2, a *Caenorhabditis elegans* homolog of the mammalian insulin or IGF-I receptor (Kimura et al., 1997 [DOI](#)), effectively doubled lifespan. Follow up studies in *Drosophila* (Clancy et al., 2001 [DOI](#); Tatar et al., 2001 [DOI](#)) and mice (Bokov et al., 2011 [DOI](#); Holzenberger et al., 2003 [DOI](#)) have also reported lifespan extension in mutants with interruptions along the IIS pathway. Further, mutations in the human *IGF1R* gene

conferring reduced IGF-IR function have been identified in families with exceptional longevity (Suh et al., 2008 [DOI](#)). Together these position the IIS pathway as a potent, evolutionarily conserved mediator of longevity.

Almost as common as the lifespan extensions in IIS mutants are sex-specific differences in their lifespans. In the *Drosophila* and mouse studies above, significant longevity benefits were only achieved in one sex. Despite the commonality of these observations, very little work has been carried out on the influence of sex on IIS mutations in *C. elegans*. Worm studies largely employ hermaphrodites, leaving the impact of sex unstudied. This omission overlooks evidence suggesting that sex is a fundamental regulator of physiology. Profound differences in metabolism, reproductive strategy, and stress response between the sexes could fundamentally alter the output of core aging pathways (Austad & Fischer, 2016 [DOI](#)). Here, we investigate the influence of biological sex on the well documented *daf-2* mutant *C. elegans* model of longevity. We show that while *daf-2* hermaphrodites exhibit the canonical twofold lifespan extension, *daf-2* males experience a fourfold increase, coupled with a similar extension of healthspan. This work reveals that sex-specific biology can dramatically amplify the output of a conserved aging pathway, establishing sex as a primary determinant of longevity potential.

Results

Male *daf-2* Mutants Live Twice as Long as Hermaphrodite *daf-2* Mutants

To determine if the *daf-2(e1370)* mutation's effect on lifespan was dependent on sex, we carried out three independently replicated lifespan analyses. Hermaphrodite *daf-2* mutants displayed notable extensions in lifespan over hermaphrodite WTs, consistent with previous reports (Kenyon et al., 1993 [DOI](#)). Surprisingly, male *daf-2* mutants markedly outlived hermaphrodite WTs, male WTs, and even hermaphrodite *daf-2* mutants (Fig. 1A-C [DOI](#)). Hermaphrodite *daf-2* median lifespan (14.5 days) was comparable to that of the hermaphrodite WTs (14 days), however mean lifespan was 153.1% of the WT mean lifespan (14.7 days vs 22.5 days). Male *daf-2* median lifespan was 408.3% of the WT male median (18 days vs 73.5 days), and mean lifespan was 360.3% of the WT males (19.4 days vs 69.9 days). The male WT median lifespan was 128.6% of the hermaphrodite WT median (18 days vs 14 days), and the male WT mean lifespan was 132% of the hermaphrodite WT mean (19.4 days vs 14.7 days). Male *daf-2* median lifespan was a marked 506.9% of the hermaphrodite *daf-2* median (73.5 days vs 14.5 days) and male *daf-2* mean lifespan was 310.7% of the hermaphrodite *daf-2* mean (69.9 days vs 22.5 days). Hermaphrodite *daf-2* overall survival was significantly improved over hermaphrodite WTs (logrank $P < 0.0001$), and hazard ratio was significantly reduced (HR=0.4753; $P < 0.0001$). Survival at the 75th and 90th percentiles of life were significantly improved in hermaphrodite *daf-2* over hermaphrodite WTs ($P < 0.0001$ each). Male *daf-2* overall survival was significantly improved over WT males (logrank $P < 0.0001$), and hazard ratio was significantly reduced (HR=0.0185; $P < 0.0001$). Survival at the 75th and 90th percentiles of life were significantly improved in male *daf-2* over male WT males ($P < 0.0001$ each). Interestingly, male WT overall survival was significantly improved over hermaphrodite WT (logrank $P < 0.0001$) and hazard ratio was significantly reduced (HR=0.4424; $P < 0.0001$). Survival at the 75th and 90th percentiles of life were significantly improved in male WT males over hermaphrodite WT males ($P < 0.0001$ each). Male *daf-2* overall survival was significantly improved over hermaphrodite *daf-2* survival (logrank $P < 0.0001$) and hazard ratio was significantly reduced (HR=0.0585; $P < 0.0001$). Survival at the 75th and 90th percentiles of life were significantly improved in male *daf-2* over hermaphrodite *daf-2* ($P < 0.0001$ each). Results for statistical analyses of the pooled survival analyses are presented in Table 1 [DOI](#).

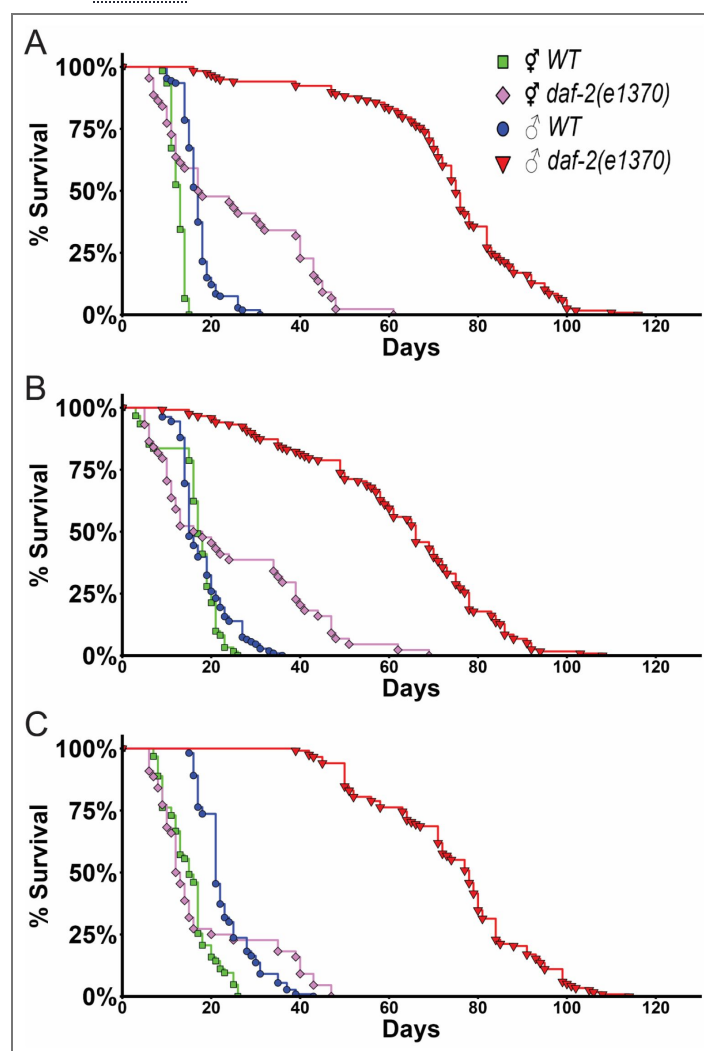
Table 1. Survival statistics for the aggregated lifespan data from Figure 1.

Note these are visualized in Figure S1.

Group	Median Lifespan	% Median Difference	Mean Lifespan	% Mean Difference	Logrank p-value	Cox Proportional Hazard		Maximal Lifespan	
						Hazard Ratio	p-value	75th p-value	90th p-value
♀ WT (n=185)	14 days	103.6	14.7 days	153.1	<0.0001	1	<0.0001	<0.0001	<0.0001
♀ daf-2(e1270) (n=132)	14.5 days		22.5 days			0.4753			
♂ WT (n=325)	18 days	408.3	19.4 days	360.3	<0.0001	1	<0.0001	<0.0001	<0.0001
♂ daf-2(e1370) (n=353)	73.5 days		69.9 days			0.0185			
♀ WT (n=185)	14 days	128.6	14.7 days	132.0	<0.0001	1	<0.0001	<0.0001	<0.0001
♂ WT (n=325)	18 days		19.4 days			0.4424			
♀ daf-2(e1270) (n=132)	14.5 days	506.9	22.5 days	310.7	<0.0001	1	<0.0001	<0.0001	<0.0001
♂ daf-2(e1370) (n=353)	73.5 days		69.9 days			0.0585			

Figure 1. Loss of *daf-2* extends male lifespan fourfold.

Kaplan-Meier survival curves for first (A), second (B) and third (C) independent replicates. N=44-118 per group in all panels. Statistical analysis is presented in Table 1.



Male *daf-2* Mutants are Longer and Accumulate More Lipids in Adulthood

Male *daf-2* mutants were observed to be more optically opaque than male WTs during adulthood. To investigate this observation, oil red o (ORO) staining was carried out to visualize neutral lipid accumulation in adult animals. Image analysis revealed a significant effect of genotype ($P=0.0006$) on body length, and a significant interaction effect of genotype X age ($P_x=0.0450$) on body length. Post-hoc analyses of these data revealed male *daf-2* mutants were significantly longer than male WTs at day 14 of adulthood (Fig. 2A). Quantitative analysis of ORO staining revealed a significant effect of genotype ($P<0.0001$) and a significant interaction effect of genotype X age ($P_x<0.0001$) on the percentage of the worm body stained with ORO. Post-hoc analyses of these data revealed that significantly less of the *daf-2* male body stained for ORO compared to WT males as day 1 adults, however at day 14 and day 20 *daf-2* males had significantly greater percentages of their body staining for ORO (Fig. 2B, 2D). Quantitative analysis of the mean ORO stain intensity revealed a significant effect of genotype ($P<0.0001$) and a significant interaction effect of genotype X age ($P_x<0.0001$) on mean ORO intensity. Post-hoc analysis revealed significantly reduced ORO stain intensity compared to WT males at day 1, but significantly greater ORO stain intensity at day 14 and day 20 of adulthood (Fig. 2C, 2D). Together, these indicate that *daf-2* male *C. elegans* accumulate more lipids in adulthood.

Male *daf-2* Mutants Retain Motility and Resist Oxidative Stress

A key question regarding extreme lifespan extension is the preservation of health. Past work has demonstrated that the motility of *daf-2(e1370)* mutants is impaired, at least in early hermaphrodite adulthood (Bansal et al., 2015; Gems et al., 1998; Mulcahy et al., 2013; Roy et al., 2022). This is undesirable, as it may represent periods of poor health. *daf-2(e1370)* hermaphrodites are known to resist oxidative stress, which is among the hypothesized mediators of their longevity (Bansal et al., 2015; Dues et al., 2019; Honda & Honda, 1999). We evaluated if the dramatically extended lifespan in our male *daf-2* mutants coincided with improvements in motility and stress resistance. Thrashing assay analysis revealed a significant effect of genotype ($P<0.0001$) and a significant interaction effect of genotype X age ($P_x<0.0001$) on motility. Post-hoc analysis revealed that while thrashing rates were comparable at day 1 of adulthood, day 10 *daf-2* males displayed significantly greater thrashing rates compared to WTs (Fig. 3A). Hydrogen peroxide stress testing revealed that a significantly greater proportion of *daf-2* males survived 5mM and 10mM concentrations of hydrogen peroxide compared to WT males ($P<0.0001$ each; Fig. 3B). These data indicate that the male *daf-2* longevity is not simply a state of protracted frailty.

Functional DAF-16 is Required for the Extreme Male *daf-2* Mutant Longevity

It has previously been demonstrated that improvements in wild-type male *C. elegans* longevity is not the result of reduced DAF-2 function (Gems & Riddle, 2000). It remains untested however, if the significant gains in male longevity conferred by *daf-2* mutation proceed through canonical signaling pathways or through multiple distinct mechanisms. To determine if the dramatic extension in male lifespan functions through canonical DAF-2-DAF-16 pathway as in hermaphrodites, we evaluated the lifespan of male *daf-2(e1370)/daf-16(mu86)* double mutants. The lifespan of male WTs, male *daf-16*, and male *daf-2/daf-16* mutants were generally comparable with each other and notably shorter than the male *daf-2* mutants (Fig. 4). Median lifespan of male *daf-16* mutants was 94.7% of the male WT median (18 days vs 19 days) and mean lifespan was 98.4% of the male WTs (18.9 days vs 19.2 days). Median lifespan of male *daf-2/daf-16* mutants was 84.2% of the male WTs (16 days vs 19 days) and mean lifespan was 93.2% of the male WTs (17.9 days vs 19.2 days). Median lifespan of male *daf-2/daf-16* mutants was 88.9% of the male *daf-16* mutant lifespan (16 days vs 18 days) and mean lifespan was 94.7% of the male *daf-16* mutants (17.9

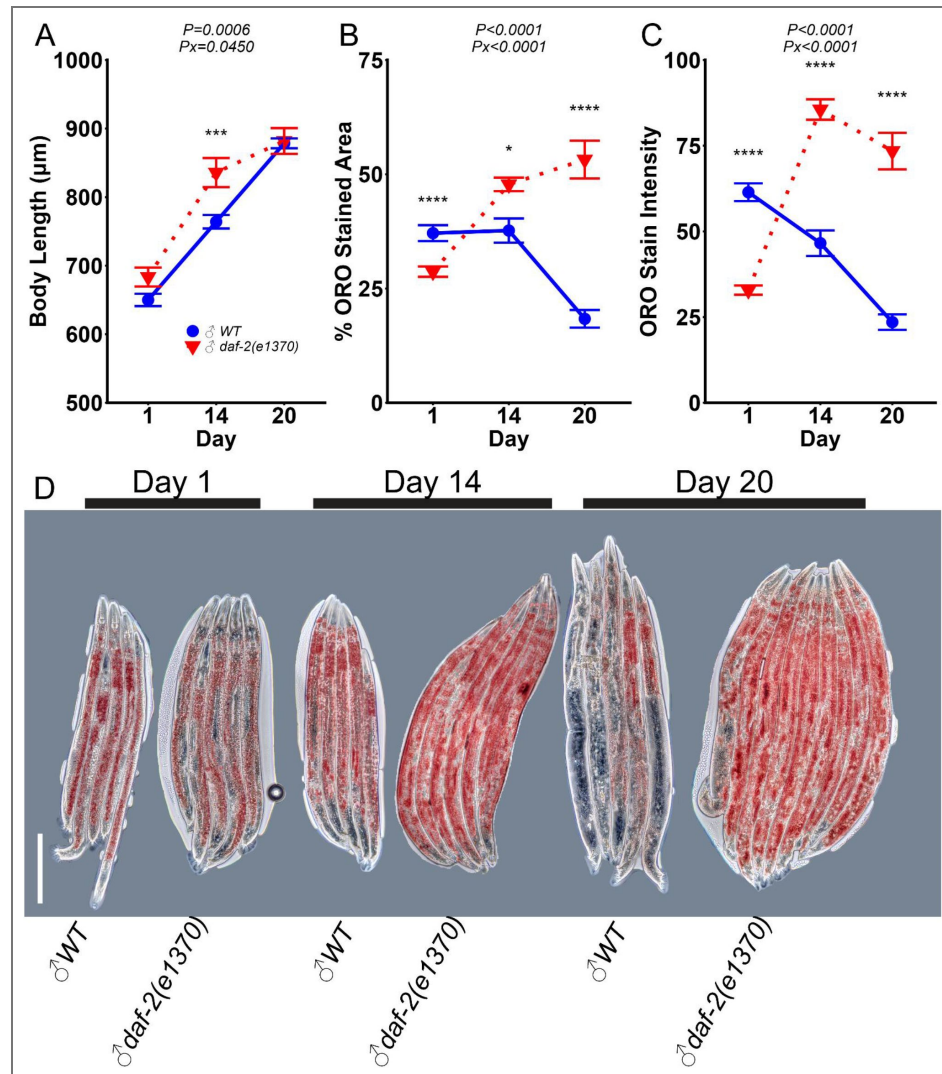


Figure 2. Long-lived *daf-2* mutant males accumulate and retain lipids in age.

Quantification of body length (A). Quantification of the percentage of the worm body stained with oil red O (ORO) (B). Quantification of the average ORO stain intensity within the worm body (C). Representative images of stained worms (D). Scale bar represents 200μm. Statistical significance assessed by two-way ANOVA. Data presented as mean ± SEM. P represents the main effect of genotype; Px represents the genotype X age interaction effect. *P<0.05; ***P<0.001; ****P<0.0001 as determined by Tukey-HSD pairwise comparisons. N=13-41 per group.

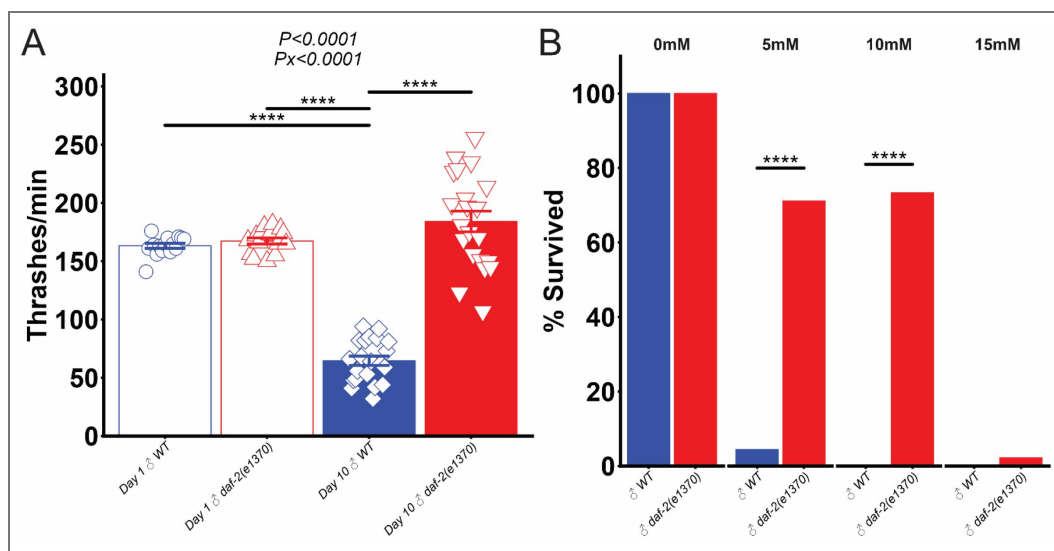


Figure 3. Long-lived *daf-2* mutant males retain motility and resist oxidative stress.

Thrashing assay (A) and survival following a 4-hour incubation at the indicated hydrogen peroxide concentrations in day 1 males (B). Data presented as mean $\pm$ SEM with points representing individual animals (A) or as percentage surviving after incubation (B). P represents the main effect of genotype; Px represents the genotype X age interaction effect. **** $P < 0.0001$ as determined by Tukey-HSD pairwise comparisons (A). **** $P < 0.0001$ as determined by fisher exact test (B). N=15-21 per group (A) or N=27-46 per group (B).

days vs 18.9 days). Overall survival and hazard ratios were comparable in male *daf-16* mutants compared to male WTs (logrank $P=0.3716$, $HR=1.1593$, $P=0.3310$), but survival at the 90th percentile of life was reduced ($P=0.0029$).

Overall survival was not different in male *daf-16/daf-2* mutants compared to male WTs (logrank $P=0.1877$); however hazard ratio was significantly greater in male *daf-2/daf-16* mutants ($HR=1.3443$; $P=0.0437$). Survival at the 75th percentile of life was reduced in male *daf-2/daf-16* mutants compared to male WTs ($P=0.0322$) and while a trend for reduced survival at the 90th percentile was also observed, this failed to reach statistical significance ($P=0.0883$). Overall survival was not different between male *daf-2/daf-16* and male *daf-16* mutants, and while a trend for greater hazard ratios was observed in *daf-2/daf-16* mutants, this failed to reach statistical significance ($P=0.0812$). Survival at the 75th percentile of life was reduced in male *daf-2/daf-16* mutants compared to male *daf-16* mutants ($P=0.0203$), but no differences were observed in the survival at the 90th percentile of life. The results of statistical analyses are presented in Table 2. Together, these data indicate that the extreme lifespan of male *C. elegans* without functional DAF-2 requires functional DAF-16.

Discussion

Our current study reveals that the physiological plasticity inherent in the IIS signaling architecture is far more expansive than the benchmarks established over the past three decades. This work builds upon the seminal findings of Gems and Riddle, who first identified the sex-dependent lifespan effects of IIS and noted that *daf-2* mutant males possess a significant survival advantage over their wild-type counterparts (Gems & Riddle, 2000). These earlier reports focused primarily on the behavioral and environmental modulators of male lifespan such as the high energetic costs of mating and density-dependent stress. Our data here reveals a far more focused genetic expansion. By identifying a 110-day “longevity ceiling,” we demonstrate that the male IIS output is not merely a variation of the hermaphrodite program, but a distinct, fourfold amplification of somatic maintenance.

Interestingly, the 110-day longevity expansion is entirely dependent on functional DAF-16, yet it exhibits a striking “effect asymmetry.” In hermaphrodites, DAF-16 activation via loss of DAF-2 activity yields an approximately twofold lifespan extension (Kenyon et al., 1993; Ogg et al., 1997); in males, the same DAF-16 activation produces a nearly fourfold increase. This discrepancy suggests that DAF-16 operates within a unique male-specific chromatin environment, potentially binding to an entirely different network of “unknown targets.” This permissive chromatin landscape allows male DAF-16 to unlock latent survival pathways that remain dormant or suppressed in the hermaphrodite situation. In this vein, our work is consistent with previous reports that ablation of the *daf-2(e1370)* germ line precursor cells extends hermaphrodite *daf-2(e1370)* lifespan to a level comparable to our mutant males (Hsin & Kenyon, 1999). These benefits were dependent on functional DAF-16 and DAF-12, and thus it was proposed that the mechanism for this improvement was that the germline produces signals that impair DAF-16 and DAF-12 activity, shortening lifespan. Our data presented here build off this proposed mechanism and implicate oocytes as the origin for these signals.

Figure 4. Functional *daf-16* is required for the extreme *daf-2* mutant male lifespan.

Kaplan-Meier survival curves for WT, *daf-16(mu86)* and *daf-2(e1370) daf-16(mu86)* double mutant (*daf-2/daf-16*) males, as well as aggregated *daf-2(e1370)* male appended from figure 1 for visual reference. Statistical analysis is presented in Table 2.

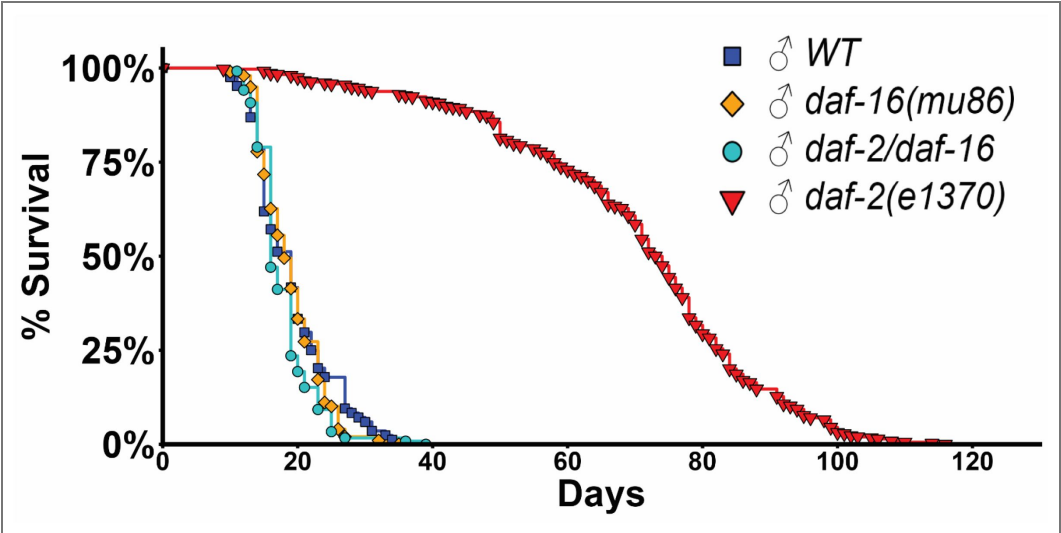


Table 2. Survival statistics for the lifespan data presented in Figure 4.

Group	Median Lifespan	% Median Difference	Mean Lifespan	% Mean Difference	Logrank p-value	Cox Proportional Hazard		Maximal Lifespan	
						Hazard Ratio	p-value	75th p-value	90th p-value
♂ WT (n=84)	19.0 days	94.7	19.2 days	98.4	0.3716	1	0.3310	0.7395	0.0029
♂ daf-16(mu86) (n=99)	18.0 days		18.9 days			1.1593			
♂ WT (n=84)	19.0 days	84.2	19.2 days	93.2	0.1877	1	0.0437	0.0322	0.0883
♂ daf-2/daf-16 (n=119)	16.0 days		17.9 days			1.3443			
♂ daf-16(mu86) (n=99)	18.0 days	88.9	18.9 days	94.7	0.1877	1	0.0812	0.0203	0.6591
♂ daf-2/daf-16 (n=119)	16.0 days		17.9 days			1.2713			

Further, the physical foundation of this 4-fold longevity lies in a post-developmental “metabolic metamorphosis.” We observed a counter-intuitive lipid inversion: *daf-2* mutant males are not born with superior energy reserves—their lipid levels at Adult Day 1 are significantly lower than those of wild-type males. However, by Day 14 and Day 20, this phenotype completely reverses, with *daf-2* mutant males exhibiting massive neutral lipid accumulation. The loss of DAF-2 function has previously been associated with increased lipid accumulation (Kimura et al., 1997 [DOI](#); Murphy et al., 2003 [DOI](#)), and impairments in *daf-2* mutant lipid accumulation results in a partial normalization of lifespan (Wang et al., 2008 [DOI](#)). The increased lipid accumulation through adulthood in our animals may indicate that a pro-longevity lipid profile contributes to the extended life of our animals. This suggests that IIS disruption in males triggers a critical temporal window of metabolic reprogramming, reallocating resources away from early-life energy expenditure and toward late-life somatic preservation.

Importantly, this 4-fold lifespan expansion challenges the canonical premise that extreme longevity requires a compensatory reduction in somatic growth. In classic models of aging, ranging from *daf-2* mutant hermaphrodites to dwarf mice and mammals subjected to caloric restriction, lifespan extension is usually linked to stunted growth or reduced physical size (Bartke, 2017 [DOI](#)). Here, we observe a surprising uncoupling of this growth-longevity axis that challenges the “disposable soma” theory. Rather than suffering the developmental penalties, as implied by the disposable soma theory (Kirkwood, 1977 [DOI](#)) and typical of these established longevity interventions, *daf-2* mutant males undergo a dynamic, age-progressive physical expansion. Quantitative imaging confirms a significant interaction between genotype and age driving this growth divergence, resulting in *daf-2* mutant males growing significantly longer than their wild-type counterparts by adult day 14. This temporal phenomenon, in which 110-day survival coexists with continuous late-life somatic enlargement, dictates a radical reallocation of resources unseen in traditional longevity interventions.

In summary, the male-specific DAF-16/FOXO network acts as a hidden “multiplier,” unlocking a survival potential previously unobserved in metazoans. This provides not only a new blueprint for sex-specific biomarkers of aging but also identifies the maximal plastic limits of healthy lifespan extension through the allosteric modulation of core signaling pathways.

Methods

Strains

The original N2 (WT) animals were a gift from the laboratory of Dr. Haoseng Sun at the University of Alabama at Birmingham. *daf-2(e1370)* (strain #: CB1370) and *daf-16(mu86)* (strain #: CF1038) mutants were acquired from the Caenorhabditis Genetics Center at the University of Minnesota. Double mutant *daf-2(e1370);daf-16(mu86)* animals were generated by mating *daf-2(e1370)* males (generated as detailed below) with *daf-16* hermaphrodites for 48 hours.

Successful transmission of the *daf-2(e1370)* mutation was confirmed by PCR with forward primer (5′-3′) aatccgtaaggcagatgacg and the reverse primer (5′-3′) cgtaaggactgtacccaa, followed by a BspI (New England Biolabs) digestion carried out according to the manufacturer suggested protocol. Successful transmission of the *daf-16(mu86)* mutation was confirmed by PCR with the common forward primer (5′-3′) tcgccttcacatcatccccc, the WT reverse primer (5′-3′) gatgggggcaatctgaggt, and the mutant reverse primer (5′-3′) tcactatctcttacctttagtcgt.

Worm maintenance and Survival Analysis

Male *daf-2(e1370)* mutants were generated by picking spontaneous males from our *daf-2(e1370)* colony and mating them to hermaphrodites to increase the frequency of males in subsequent progeny. Male *daf-16(mu86)* and *daf-2(e1370);daf-16(mu86)* mutants were generated by incubating L4 animals at 30°C for 5-6 hours to increase the frequency of males in subsequent progeny. Animals were grown at 15°C until L4 stage, then shifted to 20°C for survival studies. Animals were maintained on standard NGM plates with palmitic acid barriers for all assays to prevent animals

from leaving the plate (Beydoun et al., 2024 [↗](#)) and desiccating on the plate walls. Hermaphrodites were transferred to new plates every other day until egg-laying ceased and then every three days after that (Park et al., 2017 [↗](#)). The males were transferred every three days as well.

Oil Red O staining

We employed a previously described protocol (Stuhr et al., 2022 [↗](#)) with minor modifications. A stock solution of Oil Red O was prepared by dissolving 0.25g Oil Red O into 49.75ml isopropanol followed by vacuum filtration. A working solution was prepared before staining by diluting the stock solution to a concentration of 60% (v/v) in water. This working solution was agitated overnight in the dark at room temperature to dissolve any aggregates. Animals at the indicated ages were washed off their plates with 1x phosphate buffered saline with 0.01% (v/v) Triton X-100 (PBST), transferred to 1.5ml microcentrifuge tubes, and pelleted by centrifugation. The supernatant was removed and the washing/pelleting steps were repeated two additional times. Washed pellets were incubated in 0.9ml isopropanol at room temperature with agitation for three minutes, pelleted by centrifugation, and then the supernatant was removed. 1ml prepared working Oil Red O staining solution was added to the pellet, and then then agitated in the dark at room temperature for 2 hours. Animals were pelleted, the supernatant was removed and incubated in PBST for 30 minutes at room temperature with gentle agitation. This was repeated for 4 total washes. Stained animals were positioned on a 5% agar pad, oriented with an eyelash pick, and mounted on microscope slides. Brightfield tile scans of whole worms were acquired using a Leica DMI8 microscope with a Leica DMC2900 camera and motorized stage. Images were color deconvoluted in Fiji (Schindelin et al., 2012 [↗](#)) using color deconvolution 1.7 with FastRed FastBlue DAB vectors. The resulting red channels were inverted and subjected to Otsu thresholding without manual adjustment. Regions of interest were manually drawn around the worm bodies and area fraction and mean grey values were used to compare percentage of the worm body stained with Oil Red O and the intensity of the Oil Red O staining, respectively.

Thrashing assay

Approximately five worms were placed in 20μL of M9 buffer and allowed to acclimate for a few seconds to the solution before recording video acquisition of body bending movements for one minute. For counting body-bending, the video was slowed down to half speed (0.5x) and movements were recorded by an experimenter blinded to the animal's group membership. Recordings were collected using a Nikon AZ100M dissecting scope with a Nikon DS-Fi2 camera.

Hydrogen Peroxide Stress Assay

We followed a previously published protocol (Huang et al., 2022 [↗](#)) with minor modifications. Between 9 and 16 synchronized day 1 adults were transferred into each well of a 12-well plate containing S-basal buffer supplemented with various concentrations of H₂O₂ (0mM, 5mM, 10mM, and 15mM). Plates were incubated at 20°C for 4 hours. Following incubation 100μl of 1 mg/ml catalase was transferred to neutralize the H₂O₂. The mortality of worms was recorded following neutralization.

Statistical analysis

Lifespan data were visualized using Kaplan-Meier survival curves. Overall survival was statistically compared using logrank tests, and hazard ratios were compared using cox proportional hazard testing. Maximal lifespan was evaluated by comparing the proportion of individuals within each group remaining alive at the 75th and 90th percentiles of survival using the quantile-regression approach previously described (Wang et al., 2004 [↗](#)). Where group means were compared, a two-way ANOVA was used with Tukey HSD post-hoc tests carried out where significant main effects or interaction effects were detected. Where proportions were compared, a fisher exact test was used. For all statistical tests significance was established at P<0.05. All analyses were carried out and all figures were generated using the R programming language (R Core Team, 2025 [↗](#)).

Data availability

All data used to generate the statistical analyses and figures in this manuscript are available from the corresponding author upon reasonable request.

Acknowledgements

The authors would also like to recognize the critical feedback and insightful comments from all members of the Sun Lab that helped conceive this manuscript.

Additional information

Contributions

Liou Y. Sun and Steven N. Austad conceptualized the study. Liou Y. Sun oversaw overall direction and secured funding. Mike Russell, Michelle Lin, and Liou Y. Sun designed experiments. Mike Russell and Michelle Lin conducted experiments and collected data. Alexander Tate Lasher analyzed data. Mike Russell took the lead in writing the manuscript. Mike Russell, Alexander Tate Lasher, Michelle Lin, Liou Y. Sun, and Steven N. Austad edited the manuscript. All authors provided critical feedback that helped shape the research, analysis, and final report presented here.

Funding

This work was supported in part by the National Institute on Aging grants AG048264, AG057734, and AG050225 (L.Y.S.). ATL is supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under award number T32HD071866.

Funding

Funder	Grant reference number	Author
HHS NIH National Institute on Aging (NIA)	AG085793	Liou Y Sun

Author ORCID iDs

Alexander Tate Lasher:  <https://orcid.org/0000-0002-6232-2844>

Liou Y Sun:  <https://orcid.org/0000-0002-9802-6780>

References

- Austad SN, Fischer KE (2016) Sex Differences in Lifespan. *Cell Metab* **23**:1022-1033
<https://doi.org/10.1016/j.cmet.2016.05.019> | PubMed
- Bansal A, Zhu LJ, Yen K, Tissenbaum HA (2015) Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proc Natl Acad Sci U S A* **112**:E277-286
<https://doi.org/10.1073/pnas.1412192112> | PubMed
- Bartke A (2017) Somatic growth, aging, and longevity. *NPJ Aging Mech Dis* **3**:14
<https://doi.org/10.1038/s41514-017-0014-y> | PubMed
- Beydoun S, Sridhar A, Bhandari M, Kitto ES, Leiser SF (2024) Polyethylene glycol as an improved barrier to prevent fleeing in *C. elegans*. *MicroPubl Bio* <https://doi.org/10.17912/micropub.biology.001288> | PubMed
- Bokov AF, Garg N, Ikeno Y, Thakur S, Musi N, DeFronzo RA, Zhang N, Erickson RC, Gelfond J, Hubbard GB, et al. (2011) Does reduced IGF-1R signaling in *Igf1r*^{+/−} mice alter aging?. *PLoS One* **6**:e26891
<https://doi.org/10.1371/journal.pone.0026891> | PubMed

- Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, Partridge L (2001) Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292**:104-106 <https://doi.org/10.1126/science.1057991> | PubMed
- Dues DJ, Andrews EK, Senchuk MM, Van Raamsdonk JM (2019) Resistance to Stress Can Be Experimentally Dissociated From Longevity. *J Gerontol A Biol Sci Med Sci* **74**:1206-1214 <https://doi.org/10.1093/gerona/gly213> | PubMed
- Gems D, Riddle DL (2000) Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* **154**:1597-1610 <https://doi.org/10.1093/genetics/154.4.1597> | PubMed
- Gems D, Sutton AJ, Sundermeyer ML, Albert PS, King KV, Edgley ML, Larsen PL, Riddle DL (1998) Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* **150**:129-155 <https://doi.org/10.1093/genetics/150.1.129> | PubMed
- Holzenberger M, Dupont J, Ducos B, Leneuve P, G  lo  n A, Even PC, Cervera P, Le Bouc Y (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**:182-187 <https://doi.org/10.1038/nature01298> | PubMed
- Honda Y, Honda S (1999) The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J* **13**:1385-1393 <https://doi.org/10.1096/fasebj.13.11.1385> | PubMed
- Hsin H, Kenyon C (1999) Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* **399**:362-366 <https://doi.org/10.1038/20694> | PubMed
- Huang M, Hong M, Hou X, Zhu C, Chen D, Chen X, Guang S, Feng X (2022) H3K9me1/2 methylation limits the lifespan of daf-2 mutants in *C. elegans*. *eLife* **11** <https://doi.org/10.7554/eLife.74812> | PubMed
- 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**:9-16 <https://doi.org/10.1098/rstb.2010.0276> | PubMed
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**:461-464 <https://doi.org/10.1038/366461a0> | PubMed
- 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**:942-946 <https://doi.org/10.1126/science.277.5328.942> | PubMed
- Kirkwood TB (1977) Evolution of ageing. *Nature* **270**:301-304 <https://doi.org/10.1038/270301a0> | PubMed
- MacNee W, Rabinovich RA, Choudhury G (2014) Ageing and the border between health and disease. *Eur Respir J* **44**:1332-1352 <https://doi.org/10.1183/09031936.00134014> | PubMed
- Mulcahy B, Holden-Dye L, O'Connor V (2013) 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**:492-501 <https://doi.org/10.1242/jeb.068734> | PubMed
- Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**:277-283 <https://doi.org/10.1038/nature01789> | PubMed
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**:994-999 <https://doi.org/10.1038/40194> | PubMed
- Park HH, Jung Y, Lee SV (2017) Survival assays using *Caenorhabditis elegans*. *Mol Cells* **40**:90-99 <https://doi.org/10.14348/molcells.2017.0017> | PubMed
- R Core Team (2025) R: A Language and Environment for Statistical Computing. <https://www.R-project.org/>

- Roy C, Molin L, Alcolei A, Solyga M, Bonneau B, Vachon C, Bessereau JL, Solari F (2022) DAF-2/insulin IGF-1 receptor regulates motility during aging by integrating opposite signaling from muscle and neuronal tissues. *Aging Cell* **21**:e13660 <https://doi.org/10.1111/ace1.13660> | PubMed
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**:676-682 <https://doi.org/10.1038/nmeth.2019> | PubMed
- Stuhr NL, Nhan JD, Hammerquist AM, Van Camp B, Reoyo D, Curran SP (2022) Rapid Lipid Quantification in *Caenorhabditis elegans* by Oil Red O and Nile Red Staining. *Bio Protoc* **12**:e4340 <https://doi.org/10.21769/BioProtoc.4340> | PubMed
- Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci U S A* **105**:3438-3442 <https://doi.org/10.1073/pnas.0705467105> | PubMed
- Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS (2001) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**:107-110 <https://doi.org/10.1126/science.1057987> | PubMed
- Wang C, Li Q, Redden DT, Weindruch R, Allison DB (2004) Statistical methods for testing effects on "maximum lifespan". *Mech Ageing Dev* **125**:629-632 <https://doi.org/10.1016/j.mad.2004.07.003> | PubMed
- Wang MC, O'Rourke EJ, Ruvkun G (2008) Fat metabolism links germline stem cells and longevity in *C. elegans*. *Science* **322**:957-960 <https://doi.org/10.1126/science.1162011> | PubMed

Peer reviews

Reviewer #1 (Public review):

Summary:

This paper provides interesting observations about the effects of a classical mutation in the *daf-2* insulin-like receptor in male *C. elegans*. The observations are a contribution in and of themselves; however, the conclusions reached about these observations are not supported by the work presented. Most importantly, male-specific effects on healthspan measures are asserted without direct comparison to hermaphrodites. Perhaps more fundamentally, essential features of the methods and experimental design are lacking, which makes formal assessment of the results impossible, especially given our knowledge of negative male-male interactions, which have gone completely unacknowledged here. Indeed, there is a general lack of context for known sex differences in *C. elegans*, especially in terms of the core elements of longevity, which are presented here as entirely novel but in fact are not.

Major comments:

(1) The main overall criticism of the premise of the paper is that it lacks a clear hypothesis that would lead to explicit experimental tests. Instead, many of the results are observational, and the conclusions reached go beyond the actual experiments conducted. The goal should be explicit and consistent between the introduction/ discussion, and the findings should directly address the goal.

The overall focus appears to be that *daf-2* males have an extended lifespan for reasons that are different from hermaphrodites. This conclusion is apparently based on the observation of lipid reserves in mutant animals. However, none of the healthspan measures were conducted in parallel with identical measures in hermaphrodites. How can the authors then claim that males are unique? This is especially problematic since other studies have demonstrated that *daf-2* hermaphrodites also have altered lipid composition (Vrablik 2015 *Biochim Biophys Acta*; Horikawa 2010 *Mol Cell Endocrin*).

(2) The authors make unwarranted claims about causation from observational data that is correlative in nature. Again, they claim that male longevity is caused by increased lipid reserves. This may in fact be the case, but there is no evidence to show that this is causal, only that lipid reserves are increased in mutant animals. Causation requires an actual experiment, in this case, disrupting lipid maintenance in *daf-2* males (e.g., Lapierre 2013 Autophagy). Their conclusions are consistent with their results, but their conclusions are much too strong given the nature of the evidence, especially given the concerns about proper comparisons to hermaphrodites.

(3) With these concerns in mind, all conclusions related to male-specific effects should be statistically tested using a sex-by-treatment interaction term in the statistical model. This is obviously impossible for the healthspan data, but for lifespan, this can be directly tested using (genotype x sex interaction in the CPH analysis). Further, it is unclear why each of the replicates is shown separately in Figure 1.

It is nice that the authors do not directly pool them, as most longevity studies do, but the replicate effects can be included in a more comprehensive model, which would yield an appropriate "average" effect curve.

(4) There is an inadequate review of pre-existing literature and findings that predate the observations presented here. While this is not an issue in general, the authors present their work as entirely novel when it is not.

In addition to Gems and Riddle (2000), which is tangentially cited in the discussion, the following papers should be cited and discussed in the introduction to clarify what is currently known and what remains to be explored:

Partridge and Gems (2002) Mechanisms of aging: public or private?

McCulloch and Gems (2007) Sex-specific Effects of the DAF-12 Steroid Receptor on Aging in *Caenorhabditis elegans*

Hotzi et al (2018) Sex-specific regulation of aging in *Caenorhabditis elegans*

Al-Saadi et al (2025) Disruption of the insulin signaling pathway in *C. elegans* dramatically increases male longevity and enhances reproductive health late in life

In addition, the authors assert that the study of sex differences is unstudied. If the authors are specifically referring to the sex differences in aging research, they should explicitly state that and revise their language to reflect that it is "understudied" rather than "unstudied". But as stated below, there are many studies that look at sex-specific differences in behavior, physiology, development, etc. This is most important in the context of sexual conflict, of which there are many studies that are directly relevant to the work presented here. The authors are encouraged to review some of these papers.

(5) This is particularly important in the context of how the experiments presented here were actually conducted. The methods are inadequate to assess this, and the results would therefore be impossible to replicate in the absence of additional details. Exactly how many individuals were raised on each plate during the longevity assays (and other work) is critical to understanding the results of this study. This is because males have direct, chemically and physically mediated negative impacts on one another (see many papers from the Brunet and Murphy labs). Further, it is not even clear whether males and hermaphrodites were reared separately from one another. Males are known to leave plates without hermaphrodites, which requires appropriate inclusion of censoring criteria in studies such as these. It is unclear whether and how this was handled. Censoring is an essential feature of any longevity study and so needs to be explicitly described in the statistical methods.

The methods describe the use of heat shock to induce the production of males, but it is unclear which generation is being used here. Ordinarily, males would be induced, and then male populations would be maintained by forced mating (picking to ensure that there is a high relative frequency of males) for several generations to eliminate any carryover effects of the heatshock itself. Were the heatshock males put directly into the longevity assays? If so, were hermaphrodites subject to identical treatment? This is confusing, and a potentially critical confound is not performed correctly.

<https://doi.org/10.7554/eLife.111931.1.sa2>

Reviewer #2 (Public review):

Summary:

The manuscript presents interesting observations regarding the exceptional longevity and improved healthspan of male *daf-2* mutants. Given the comparatively limited focus on male aging in *C. elegans*, the study provides a potentially useful characterization of sex-specific effects associated with reduced IIS signaling.

Strengths:

The 4-fold increase in lifespan of male *daf-2* mutants is a striking and unexpected observation. The altered fat metabolism between older *daf-2* mutant males and hermaphrodites provides further evidence of sex-specific effects.

Weaknesses:

- (1) A major limitation of the current study is that the conclusions rely primarily on a single *daf-2* allele. It would strengthen the manuscript to validate at least the major observations using an independent *daf-2* allele or through *daf-2* RNAi. This is particularly relevant for the proposed male-specific enhancement of longevity and healthspan, as it remains unclear whether the observed effects broadly reflect reduced IIS signaling or may be influenced by allele-specific effects or background mutations.
- (2) The methods for male lifespan assays require additional detail. Although the authors state that males were generated and transferred every three days, it is not clear whether males were maintained singly or in groups, how many males were placed per plate, or how many were censored by fleeing. These details are particularly important for male aging assays, as male lifespan in *C. elegans* is known to be influenced by social interactions. Factors such as population density can affect survival and healthspan measurements. Clarifying these procedures would improve reproducibility and interpretation of the reported male-specific lifespan effects.
- (3) Because reduced IIS signaling in *daf-2* mutants is known to alter metabolism, physiology, and potentially male-derived signaling, it would be interesting to determine whether the enhanced longevity of *daf-2* males is influenced by altered male-male interactions or resistance to male-associated toxicity. In this context, clarification of whether lifespan assays were performed with grouped or individually maintained males would be valuable. If not already tested, lifespan analysis under isolated single-male conditions could help distinguish intrinsic longevity effects from potential contributions of population-dependent signaling or social interactions.

(4) In Figure 2D, the body-length measurements in WT males appear somewhat unexpected, particularly the apparent increase between Day 14 and Day 20. Since adult worms are not typically expected to exhibit substantial growth at advanced ages, additional clarification regarding the measurement methodology would be helpful, including confirmation that the scale bars and image scaling were applied consistently across conditions.

(5) The use of palmitic acid barriers following Beydoun et al. (2024) is appropriate; however, it would be helpful to clarify whether WT and *daf-2* males exhibited comparable fleeing behavior under these assay conditions. Because male worms are highly prone to plate leaving and censoring, genotype-dependent differences in fleeing behavior could potentially influence survival analyses and the number of censored animals. In addition, as Beydoun et al. primarily characterized these barrier conditions using hermaphrodites, it would be useful to clarify whether comparable barrier effectiveness was observed in male lifespan assays.

(6) Separately, Beydoun et al. (2024) also reported that palmitic acid barrier conditions can influence body-size measurements, whereas PEG-based barriers did not show similar effects on body size. It would therefore be useful to know whether comparable body-length trends were observed under alternative barrier conditions, particularly given the unexpected increase in WT male body length at later ages.

<https://doi.org/10.7554/eLife.111931.1.sa1>

Reviewer #3 (Public review):

This manuscript reports a striking sex-specific effect of the *daf-2(e1370)* mutation on *C. elegans* lifespan. The authors show that male *daf-2* mutants exhibit dramatically extended lifespan relative to wild-type males, wild-type hermaphrodites, and *daf-2* hermaphrodites. The study also demonstrates increased lipid accumulation in these long-lived males, which is increased further over time, improved late-life motility, enhanced oxidative stress resistance, and a requirement for the downstream effector of *daf-2*, *daf-16*, for the longevity phenotype.

The interest of the work is the magnitude and consistency of the lifespan effect. The authors report large increases in both median and mean lifespan in *daf-2* males across independently replicated experiments. This is further supported by healthspan analyses and their finding that male *daf-2* mutants maintain improved motility and stress resistance, which argues against the interpretation that lifespan extension merely reflects prolonged frailty. The genetic epistasis experiment demonstrating loss of the longevity phenotype in *daf-2;daf-16* double mutants provides evidence that the effect depends on canonical insulin/IGF-1 signalling.

The main limitation is that at least the first figure is rather an incremental increase on previous work examining the lifespan of *daf-2* males, although the authors do indeed show that the effects can be much larger (or more 'plastic') than those previously published. While these findings are potentially important, the manuscript would certainly benefit from a more extensive discussion of how the results compare with prior studies of *daf-2* mutants and male longevity, including possible explanations for the apparent discrepancies.

The epistasis experiment shows that this exceptional longevity requires the expression of *daf-16*. However, in contrast to the initial experiments (Figure 1) that show three replicates of the lifespan experiment (the standard in lifespan work in this model), it appears that the *daf-2;daf-16* experiment has only been performed once.

In addition, the lifespan data for hermaphrodite *daf-2* mutants appear somewhat unusual. Although the mean lifespan is increased, the median lifespan is reported to be only modestly greater than that of wild-type hermaphrodites. I know that this mutant can give lifespan

curves that look like this, but either the use of another allele or of the experimental conditions and how these values compare with previously published *daf-2(e1370)* datasets would help readers interpret the magnitude of the male-specific effect.

The lipid phenotype is intriguing. It would be interesting to expand this to examine somatic vs embryonic fat. In addition, I noted that in the methods section, the authors use palmitic acid to stop the male worms 'fleeing' the plates; is it possible to rule out the possibility that the *daf-2* mutants are simply eating and metabolising/storing this fatty acid barrier differently than their wild-type counterparts? This would be worth considering and controlling for, particularly as male *C. elegans* have been shown to have dramatically altered metabolic transcriptional profiles. If indeed this increased lipid is responsible for the extreme longevity of the *daf-2* mutant males, it would be desirable to try to link this mechanistically to the phenotype.

Overall, the evidence convincingly supports the conclusion that male *daf-2(e1370)* mutants are exceptionally long-lived under the conditions tested and that this phenotype requires DAF-16. The work has the potential to make an important contribution to understanding sex-specific regulation of ageing, although further contextualisation within the existing literature would strengthen the manuscript.

<https://doi.org/10.7554/eLife.111931.1.sa0>