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Original Article

Resistance to Stress Can Be Experimentally Dissociated From Longevity

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Abstract

On the basis of multiple experiments demonstrating that high resistance to stress is associated with long lifespan, it has been proposed that stress resistance is a key determinant of longevity. However, the extent to which high resistance to stress is necessary or sufficient for long life is currently unclear. In this work, we use a genetic approach to disrupt different stress response pathways and examine the resulting effect on the longevity of the long-lived insulin-like growth factor 1 (IGF1) receptor mutant *daf-2*. Although mutation of the heat shock factor gene *hsf-1*, deletion of *sod* genes, deletion of the p38 MAPK kinase gene *pmk-1*, or deletion of the transcription factor gene *egl-27* all resulted in decreased resistance to at least one form of stress and decreased lifespan, the magnitude of change in stress resistance did not correspond to the magnitude of change in lifespan. In addition, we found that deletion of the glycerol-3-phosphate dehydrogenase genes *gpdh-1* and *gpdh-2* or deletion of the DAF-16 cofactor gene *nhl-1* also results in decreased resistance to at least one form of stress but increases lifespan. Overall, our results suggest that while increased stress resistance is associated with longevity, stress resistance, and lifespan can be experimentally dissociated.

Keywords: Aging, Stress resistance, *Caenorhabditis elegans*, *daf-2*, Genetics, Lifespan

Stress may be defined as a relationship between an organism and external or internal factors that act to disrupt homeostasis (1). Organisms have evolved to have a variety of stress response pathways to mitigate the detrimental effects of stress to restore homeostasis. However, if the internal or external stress exceeds an organism's stress resistance capacity this can lead to negative consequences.

A number of experiments have linked stress resistance and aging leading to the proposition that the ability to survive multiple stresses may be a key to longevity (2–6). First, it has been shown that resistance to multiple forms of stress decline with age (7–9). Although the precise mechanisms involved have yet to be defined, it appears that this may be due to a decreased ability to activate stress response pathways in older individuals (9), which results from a genetically programmed event (8,10).

Second, it has been observed that long-lived genetic mutants often exhibit increased resistance to various stresses. For example, *daf-2* worms live more than twice as long as wild-type (WT) worms (11) and exhibit high resistance to heat (12), oxidative (13), osmotic (14), hypoxic (15), ultraviolet (16), and heavy metal stresses (17). However, this raises the question as to whether high resistance to stress is the cause of their increased longevity and, if so, which specific forms of stress resistance can increase lifespan.

Third, it has been shown that exposure to a mild dose or short duration of a normally toxic stress can increase resistance to subsequent exposure to the same stress and, at least in some cases, increase lifespan. The process of increasing stress resistance after a mild exposure to stress, known as hormesis, has been demonstrated for multiple types of stress including heat stress (12,18–21), oxidative stress (19,22), osmotic stress (23), and cold stress (24). In addition, for heat stress (12,19), oxidative stress (25–27), osmotic stress (9), and cold stress (9), exposure to a mild stress has been shown to increase lifespan.

Although these previous studies demonstrate a strong association between stress resistance and aging, to test causation it is necessary to modulate stress resistance and examine the resulting effect on lifespan. The worm *Caenorhabditis elegans* provides the ideal model system to address this question because of the ease of genetic manipulations and the wealth of knowledge on the genetics of stress resistance and aging. *Caenorhabditis elegans* has been used extensively for aging research: the first gene that was shown to increase lifespan was identified in this species (28) and more lifespan-extending genes have been identified in the worm than in any other species (29). Importantly, genes and interventions that increase lifespan in worms have been shown to increase lifespan in other species as well, thereby indicating conservation across species (30).

In this work, we examine the relationship between stress resistance and lifespan in the long-lived, stress-resistant, insulin-like growth factor 1 (IGF1) receptor mutant *daf-2* by genetically modulating different pathways of stress resistance and examining the resulting effect on longevity. Although in some cases, genetic mutations that decreased stress resistance also decreased lifespan, in others, mutations that decreased stress resistance resulted in increased lifespan. Overall, our results suggest that stress resistance can be experimentally dissociated from longevity.

Materials and Methods

Strains

Worms were maintained at 20°C on Nematode Growth Media plates and fed OP50 bacteria. The following strains were used:

WT(N2 bristol);
JVR001 *sod-1(tm783);sod-2(ok1030);sod-3(tm760);sod-4(gk101);sod-5(tm1146)*;
JA1194 *egl-27(we3)*;
MQ1783 *gpdh-1(ok1558);gpdh-2(ok1733)*;
JVR305 *nhl-1(gk15)*;
JVR165 *pmk-1(km25)*;
JVR217 *hsf-1(sy441)*;
JVR120 *daf-16(mu86)*;
CB1370 *daf-2(e1370)*;
JVR015 *daf-2(e1370);sod-1(tm783);sod-2(ok1030);sod-3(tm760);sod-4(gk101);sod-5(tm1146)*;
SD1625 *daf-2(e1370);egl-27(we3)*;
JVR318 *daf-2(e1370);gpdh-1(ok1558);gpdh-2(ok1733)*;
JVR319 *daf-2(e1370);nhl-1(gk15)*;
JVR322 *daf-2(e1370);pmk-1(km25)*;
JVR324 *daf-2(e1370);hsf-1(sy441)*;
JVR326 *daf-2(e1370);daf-16(mu86)*.

e1370 is a point mutation that affects one exon toward the 3′ end of most isoforms of *daf-2*. *mu86* is a 10,980 bp deletion affecting all transcripts of *daf-16* and is a null mutant. *sy441* is a point mutation that affects exon 7 of 8 exons in *hsf-1*. *sod-1(tm783);sod-2(ok1030);sod-3(tm760);sod-4(gk101);sod-5(tm1146)* worms have deletions in all five *sod* genes and have no detectable superoxide dismutase (SOD) activity (26). *ok1558* is a 1,227 bp deletion that disrupts exon 2 and 3 of 4 exons and results in a frame shift in *gpdh-1*. *ok1733* is a 1,702 bp deletion that affects the final exons of all transcripts of *gpdh-2*. *gk15* is a 1,409 bp deletion that disrupt multiple exons toward the 3′ end of *nhl-1* transcripts. *km25* is a 375 bp deletion that disrupts the transcriptional and translation start sequence in *pmk-1* resulting in a null mutant. *we3* has been reported to be a strong loss of function mutation (31).

Generation of Double Mutants

To generate *daf-2* double mutants, we crossed WT males to *daf-2* hermaphrodites. The resulting *daf-2* males were crossed to the mutant of interest. Males from this cross were mated again to the mutant of interest to increase the likelihood of obtaining a homozygous mutant. The hermaphrodite offspring were selfed and the resulting eggs/L1s were transferred to 25°C. Worms that were homozygous for the *daf-2* mutation were expected to form dauers. As a result, approximately 20 dauers were picked, maintained an extra 2 days at 25°C to ensure that they would not develop to adulthood. Worms that remained dauer were transferred to 16°C and allowed to develop to adulthood. Worms were then singled and genotyped for the mutation of interest. For homozygous mutants, DNA was sent for sequencing to confirm the presence of the *daf-2* point mutation. Because the *daf-16* mutation prevents dauer formation, we first generated *daf-2–/–;daf-16+/–* worms and then selfed to generate double homozygotes.

Lifespan

Lifespan assays were performed with day 1 young adult worms on plates containing 25 µM fluorodeoxyuridine (FUdR) to reduce the development of progeny. This concentration of FUdR has been shown to prevent progeny from developing to adulthood after the first transfer, while having minimal effects on lifespan (32). Worms were scored every 2 days. Worms that crawled up the side of the dish, exhibited internal hatching of progeny or exhibited externalization of internal organs were censored. Lifespan assays included five replicates with at least 40 worms per replicate at the outset.

Heat Stress Assay

Heat stress assays were performed at 37°C. Worms were transferred to a seeded Nematode Growth Media plate and placed directly into a 37°C incubator. Survival was checked at 2, 4, 6, 7, 8, and 10 hours. Three replicates using a minimum of 20 worms per replicate were performed.

Oxidative Stress Assay

Resistance to oxidative stress was assessed by exposing worms to 4 mM paraquat beginning on day 1 of adulthood. Survival was monitored daily until all of the worms had died. Three replicates using a minimum of 20 worms per replicate were performed.

Bacterial Pathogen Stress Assay

Resistance to bacterial pathogen stress was measured by exposing worms to *Pseudomonas aeruginosa* as described previously as the slow kill assay (33). In this assay, which we have referred to as the bacterial ingestion assay, worms are thought to die from the over colonization of the intestine. We performed three replicates using a minimum of 20 worms per replicate.

Osmotic Stress Assay

Resistance to osmotic stress was assessed by exposing worms to Nematode Growth Media plates containing 700 mM NaCl. We examined turgidity at 24 and 48 hours, and survival at 48 hours. A loss of turgidity was defined as an observable loss of pressure in the body of the worm, rendering the animal unable to move. Three replicates using a minimum of 20 worms each were measured.

Anoxia Assay

To measure resistance to anoxia, worms were placed on Nematode Growth Media plates seeded with OP50 bacteria and placed in anoxic biobags (BD Bio-Bag Type A environmental chambers; Becton,

Dickinson and Company, Franklin Lakes, NJ) according to the manufacturer's instructions. After 72 or 96 hours of anoxia, worms were taken out of the biobags and allowed to recover for 24 hours before survival was assessed. Four replicates using a minimum of 20 worms were assessed for each duration.

Stress Resistance in Aged Worms

Worms were aged to day 10 of adulthood on plates containing 25 μM FUdR. Worms were transferred to fresh FUdR plates after 3 days. On day 10, only worms that appeared healthy and mobile were selected for the stress assays. Stress assays were performed as described earlier with the following modifications. We did not perform the bacterial pathogen stress assay at the aged time point because bacterial consumption is known to decline markedly with age and this would be predicted to markedly influence survival in this assay. For the osmotic stress assay, we used plates containing 600 mM NaCl and assessed survival at 24 hours. For anoxia, we performed a single time point at 96 hours. In the anoxia assay, we quantified both survival and mobility. Mobility was defined as the ability of the entire worm to move a small distance after a gentle prod.

Experimental Design and Statistical Analysis

All experiments were performed in a way that the experimenter was blinded to the genotype of the strains being tested. Statistical significance of lifespan, heat stress assay, bacterial pathogen stress assay, and oxidative stress assay were assessed using the log-rank test. For simple comparisons between two groups a Student's *t* test was used to assess statistical significance. For the osmotic stress assay and anoxia assay, the percentage survival for each replicate is plotted and represents a minimum of 20 worms.

Results

The IGF1 receptor gene *daf-2* was one of the first genes that was shown to influence longevity (11) and since the IGF1 pathway has been one of the most well-studied pathways of longevity. Multiple groups have examined stress resistance in *daf-2* worms and found that these mutants are resistant to a variety of stresses (12–17). Although this association between stress resistance and longevity suggests the possibility that resistance to stress contributes to the long life of *daf-2* worms, to determine causation it is necessary to experimentally modulate resistance to stress and examine the resulting impact on longevity.

As an initial step, we first sought to confirm that *daf-2(e1370)* mutants are resistant to stress. We examined sensitivity to 37°C heat stress, oxidative stress on plates containing 4 mM paraquat, bacterial pathogen stress induced by *P aeruginosa*, osmotic stress on plates containing 700 mM NaCl, and anoxic stress. In each case, we found that *daf-2* worms have markedly increased resistance to stress compared to WT worms (Supplementary Figure 1A–F). The increase in stress resistance in *daf-2* mutants was associated with a marked increase in lifespan (Supplementary Figure 1G) (11). In addition, we found that both the increase in stress resistance and the increase in lifespan are dependent on canonical IGF1 signaling as a mutation in *daf-16(mu86)* completely prevents the increase in stress resistance and lifespan in *daf-2* worms (Supplementary Figure 1).

Disruption of Heat Shock Factor Decreases Heat Stress Resistance and Lifespan in *daf-2* Mutants

To assess the contribution of heat stress resistance to *daf-2* longevity, we crossed *daf-2* mutants to *hsf-1(sy441)* mutants. *hsf-1* encodes heat shock factor 1, which is a transcription factor that responds to heat stress by inducing the expression of various heat shock proteins. Although *hsf-1* mutants have an equivalent heat stress survival compared to WT worms, the *hsf-1* mutation significantly decreased heat stress resistance in *daf-2* worms (Figure 1A). To determine whether the disruption of *hsf-1* would also affect sensitivity to other types of stress, we also examined sensitivity to oxidative stress, bacterial pathogen stress, osmotic stress, and anoxia. In each case, we found that *daf-2* stress resistance was not decreased by the *hsf-1* mutation (Figure 1B–F). Having shown that the *hsf-1* mutation could specifically decrease heat stress resistance in *daf-2* worms, we examined the resulting effect on lifespan. As has been observed previously (34), we found that *daf-2;hsf-1* worms have decreased lifespan compared to *daf-2* worms (Figure 1G). However, we also observed a significant decrease in *hsf-1* lifespan compared to WT worms, even though resistance to heat stress was not impacted.

Loss of SOD Activity Abolishes Resistance to Oxidative Stress and Heat Stress Resistance but Only Mildly Decrease Lifespan in *daf-2* Mutants

The role of oxidative stress resistance in *daf-2* longevity was assessed by crossing *daf-2* mutants to a superoxide dismutase quintuple mutant (*sod-1(tm783);sod-2(ok1030);sod-3(tm760);sod-4(gk101);sod-5(tm1146); sod-12345* for short). *sod-12345* worms have no SOD activity and as a result have markedly increased sensitivity to oxidative stress (26). Examination of sensitivity to oxidative stress revealed that loss of SOD activity decreased oxidative stress resistance in *daf-2* worms such that *daf-2;sod-12345* worms survived markedly shorter than even WT worms when exposed to 4 mM paraquat (Figure 2B). The loss of SOD activity also completely reverted *daf-2* heat stress resistance back to WT (Figure 2A) and decreased resistance to bacterial pathogen stress, and osmotic stress (Figure 2C–E). Although deletion of the five *sod* genes significantly decreased *daf-2* lifespan, *daf-2;sod-12345* worms still exhibited a markedly elongated lifespan compared to WT worms (Figure 2G). The fact that *daf-2* lifespan is still greatly increased despite *daf-2*'s enhanced resistance to heat and oxidative stress being completely abolished by the *sod* gene deletions indicates that increased heat stress resistance and oxidative stress resistance do not account for *daf-2* longevity.

Disruption of the p38 MAPK PMK-1 Reverts Bacterial Pathogen Resistance to WT but Only Mildly Decreases Lifespan in *daf-2* Mutants

To determine the contribution of bacterial pathogen stress resistance to the long lifespan of *daf-2* worms, we disrupted *pmk-1* (*pmk-1(km25)* deletion mutant). *pmk-1* encodes a p38 MAP kinase that has shown to be important for resistance against bacterial pathogens (35–37). As previously reported (36), we found that deletion of *pmk-1* reverted *daf-2* resistance to *P aeruginosa*-mediated bacterial pathogen stress to WT (Figure 3C). We also observed that the loss of *pmk-1* reduced resistance to heat stress, oxidative stress, osmotic stress, and anoxic stress in *daf-2* worms with little or no impact on WT worms (Figure 3A, B, D–F). Despite the marked decrease in resistance to bacterial pathogens, *daf-2;pmk-1* worms only exhibited a mild decrease in mean lifespan and no decrease in maximum lifespan (Figure 3G). This indicates that increased bacterial pathogen resistance is not required for *daf-2* longevity.

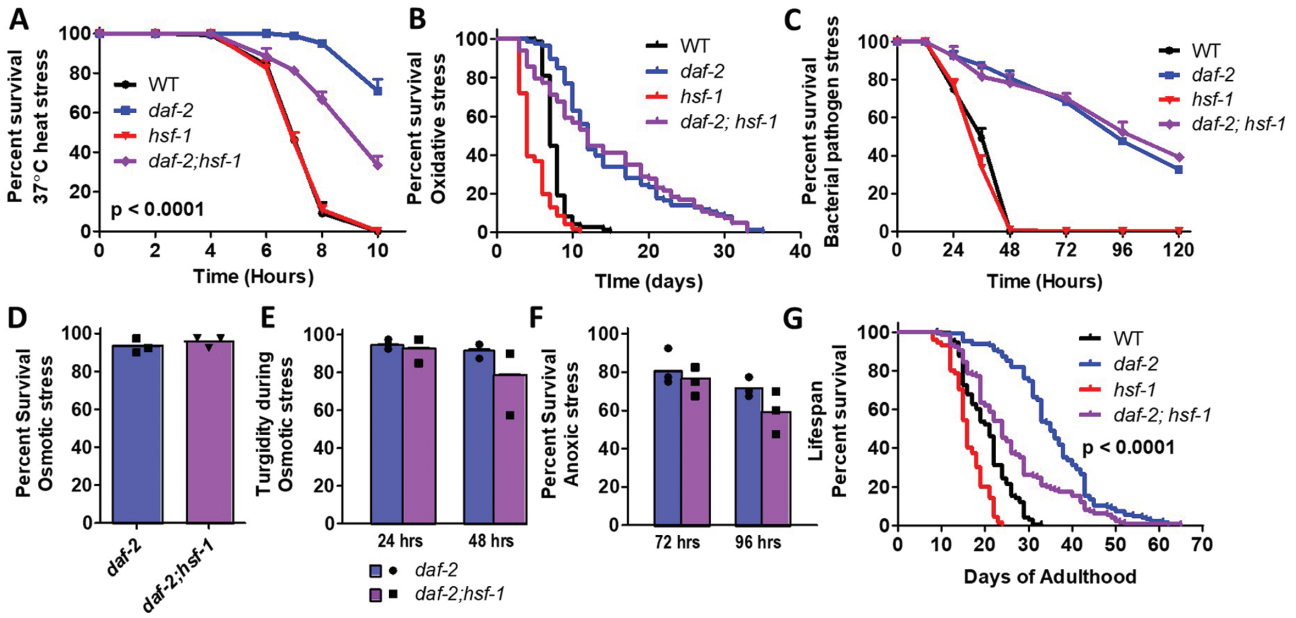

Figure 1. Mutation of *hsf-1* increases sensitivity to heat stress and decreases lifespan in *daf-2* worms. A point mutation in *hsf-1* decreases heat stress resistance in *daf-2* worms (**A**) but does not affect resistance to oxidative stress (**B**), bacterial pathogen stress (**C**), osmotic stress (**D,E**), or anoxic stress (**F**). The *hsf-1* mutation significantly reduces *daf-2* lifespan. Error bars indicate standard error of the mean. *p* Values indicate difference between *daf-2* and *daf-2;hsf-1* worms.

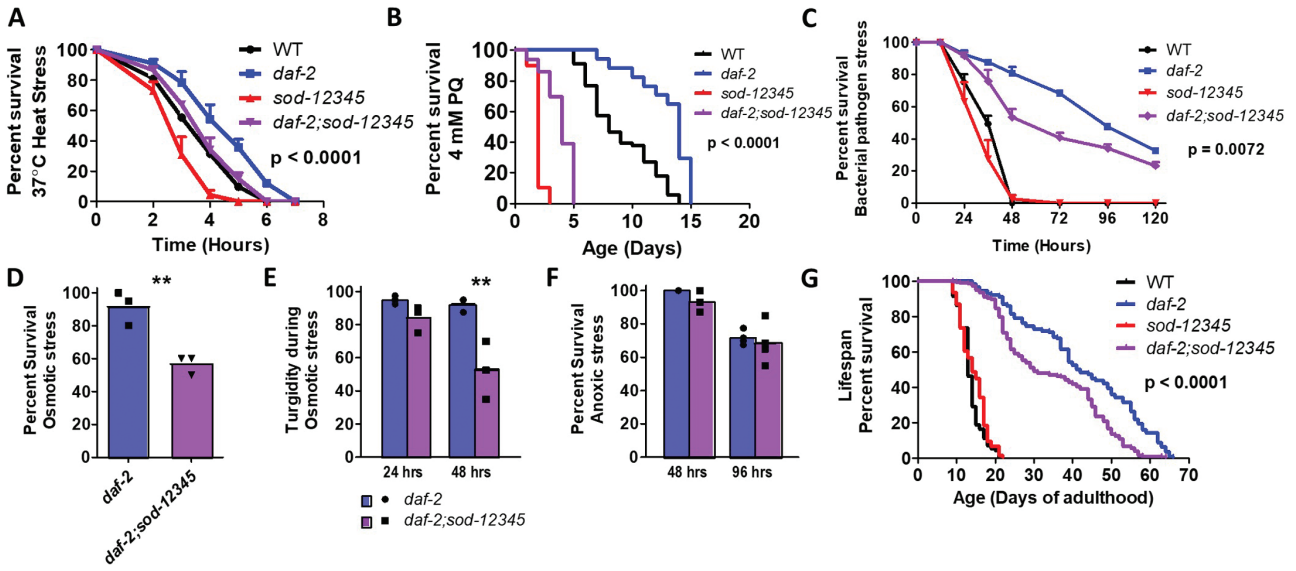

Figure 2. Disruption of all five superoxide dismutase (SOD) genes increases sensitivity to multiple stresses and mildly decreases lifespan in *daf-2* worms. Deletion of all five *sod* genes increases *daf-2* worms' sensitivity to heat stress (**A**), oxidative stress (**B**), bacterial pathogen stress (**C**), and osmotic stress (**D,E**) but does not affect sensitivity to anoxic stress (**F**). Despite the marked reduction in resistance to multiple stresses, the loss of SOD activity results in only a small decrease in *daf-2* lifespan. Error bars indicate standard error of the mean. $^{**}p < .01$. *p* Values indicate difference between *daf-2* and *daf-2;sod-12345* worms.

Deletion of Glycerol-3-Phosphate Dehydrogenase Genes *gpdh-1* and *gpdh-2* Decreases Resistance to Osmotic Stress but Increases Longevity in *daf-2* Mutants

To examine the role of osmotic stress resistance to *daf-2* longevity, we crossed *daf-2* mutants to worms with deletions in both glycerol-3-phosphate dehydrogenase (GPDH) genes, *gpdh-1(ok1558)* and *gpdh-2(ok1733)*. GPDH-1 and GPDH-2 are needed for the accumulation of glycerol in response to elevated salt concentrations that allow the worm to survive under conditions of osmotic stress (23). Although surprisingly the deletion of *gpdh-1* and *gpdh-2* together did not decrease *daf-2* survival under 700 mM NaCl osmotic stress, *daf-2;gpdh-1;gpdh-2* worms exhibited a marked loss of turgidity in response to osmotic stress compared to *daf-2* worms (Figure 4D and E). In examining resistance to other stresses, we found that the loss of GPDH function caused a mild decrease in resistance to heat stress (Figure 4A), a mild increase in resistance to oxidative stress (Figure 4B), and had no effect on resistance to bacterial pathogen stress (Figure 4C) or anoxia (Figure 4F). In examining the impact on lifespan, we found

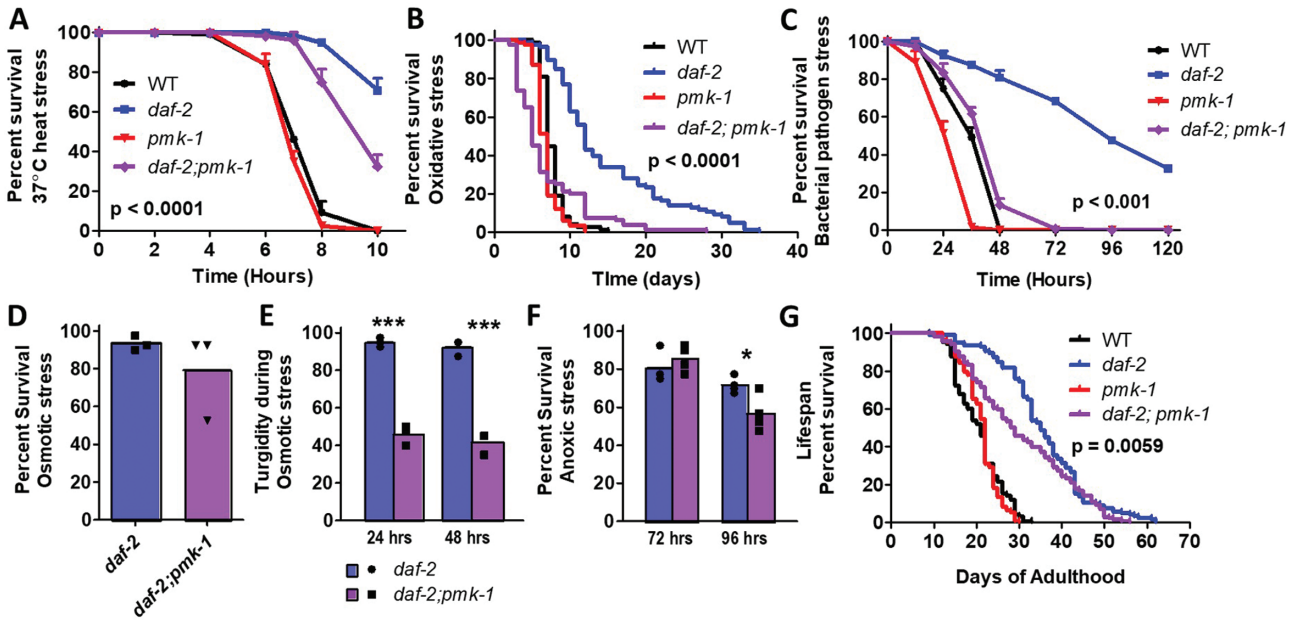

Figure 3. Deletion of *pmk-1* increases sensitivity to multiple stress but only mildly decreases lifespan in *daf-2* worms. A *pmk-1* deletion increases *daf-2* worms' sensitivity to heat stress (**A**), oxidative stress (**B**), bacterial pathogen stress (**C**), osmotic stress (**D**,**E**) and anoxic stress (**F**). Despite the reduction in resistance to multiple stresses, the loss of PMK-1 results in only a small decrease in *daf-2* lifespan. Error bars indicate standard error of the mean. ***$p < .001$. *p* Values indicate difference between *daf-2* and *daf-2;pmk-1* worms.

that deletion of *gpdh-1* and *gpdh-2* markedly increased the already long lifespan of *daf-2* worms and also increased the lifespan of WT worms (Figure 4G). Thus, the *gpdh-1;gpdh-2* mutations have opposite effects on osmotic stress resistance and lifespan.

Disruption of NHL-1 Decreases Stress Resistance but Increases Lifespan in *daf-2* Mutants

Previous work identified the TRIM-NHL protein NHL-1 as a DAF-16 target gene that acts in the feedback regulation of DAF-16, as measured using a *Psod-3::GFP* reporter strain (38). As with *daf-16* mutations, RNAi against *nhl-1* was found to increase *daf-2* sensitivity to heat stress, oxidative stress, and bacterial pathogen stress (38), but unlike *daf-16*, *nhl-1* RNAi had no impact on *daf-2* lifespan (38). To further explore the role of NHL-1 in *daf-2* stress resistance and longevity, we crossed *daf-2* mutants to an *nhl-1* deletion mutant (*gk15*). Unlike *nhl-1* RNAi, we found that the *nhl-1* deletion did not decrease heat stress resistance in *daf-2* worms (Supplementary Figure 2A) and resulted in increased resistance to oxidative stress (Supplementary Figure 2B). The *nhl-1* deletion did result in decreased resistance to bacterial pathogen stress (Supplementary Figure 2C) and decreased turgidity during exposure to osmotic stress (Supplementary Figure 2E). Finally, we found that *daf-2;nhl-1* worms exhibited a markedly increased lifespan compared to *daf-2* mutants (Supplementary Figure 2G). The fact the *nhl-1* RNAi decreases stress resistance to a greater extent than the *nhl-1* deletion suggests that the *nhl-1* deletion mutant may still express a truncated protein that maintains some function (the deletion disrupts exon 9 of 12 for transcript a and exons 9 and 10 of 13 for transcript b).

Loss of GATA Transcription Factor EGL-27 Decreases Stress Resistance and Lifespan of *daf-2* Mutants

egl-27 encodes a GATA transcription factor that is required for the longevity of *daf-2* worms (39,40). The expression of *egl-27* increases in response to various stresses (including heat, oxidative, and osmotic stress) and loss of *egl-27* decreases resistance to heat stress and oxidative stress in *daf-2* worms (40). Accordingly, we examined stress resistance and lifespan in *daf-2;egl-27(we3)* mutants. As previously observed, we found that *daf-2;egl-27* worms show a mild decrease in resistance to heat and oxidative stress (Supplementary Figure 3A and B). In addition, we found that the *egl-27* mutation also decreases *daf-2* resistance to bacterial pathogen stress, osmotic stress, and anoxia (Supplementary Figure 3C–F). Finally, consistent with previous work, we show that disruption of *egl-27* shortens *daf-2* lifespan (Supplementary Figure 3G).

Resistance to Heat and Oxidative Stress Are Weakly Correlated With Longevity

To further explore the relationship between different types of stress resistance and longevity, we generated correlation plots. We found that heat stress survival ($r^2 = .6464$, $p = .0162$) and anoxia survival ($r^2 = .575$, $p = .0292$) both showed a mild, positive correlation with lifespan (Supplemental Figure S4). However, these significant correlations were primarily driven by the complete reversion to WT stress resistance in *daf-2;daf-16* worms, as the statistical significance was lost when *daf-2;daf-16* was removed. Between different stresses, a positive correlation was observed between oxidative stress survival and bacterial pathogen stress survival ($r^2 = .567$, $p = .031$) and between heat stress survival and anoxia survival ($r^2 = .5648$, $p = .0316$; Supplementary Figures 5–9).

Stress Resistance in Aged Worms

Because we only observed a mild correlation between stress resistance and lifespan in day 1 young adult worms, we decided to examine stress resistance in older worms to see if the relationship with lifespan strengthens with age. Accordingly, we aged worms to day 10 of adulthood and measured resistance to heat stress, oxidative stress, osmotic stress, and anoxic stress (Supplementary Figures 10–16; Table 1). In day 10 adults, we found that heat stress survival

and anoxia survival showed a decreased correlation with lifespan (decreased r^2, increased p value), whereas oxidative stress survival and osmotic stress survival showed an increased correlation with lifespan (increased r^2, decreased p value; Supplementary Figure 17). Only the correlation between oxidative stress survival and lifespan was significant at the aged time point ($p = .0105$).

Discussion

Organisms with enhanced resistance to stress are better able to survive acute exposures to stress. However, it is uncertain whether investing resources into high stress resistance will also be beneficial for natural lifespan. The fact that many long-lived genetic mutants also exhibit high resistance to multiple stresses suggests that having enhanced resistance to stress may promote longevity. However, it is also possible that common genetic pathways control stress resistance and longevity such that there is an associative, rather than a causative, relationship. In this work, we use a genetic approach to test causality. We disrupt pathways associated with specific types of stress resistance in long-lived stress resistant *daf-2* worms and examine the resulting effect on stress resistance and longevity. If enhanced resistance to stress is required for longevity then reducing this stress

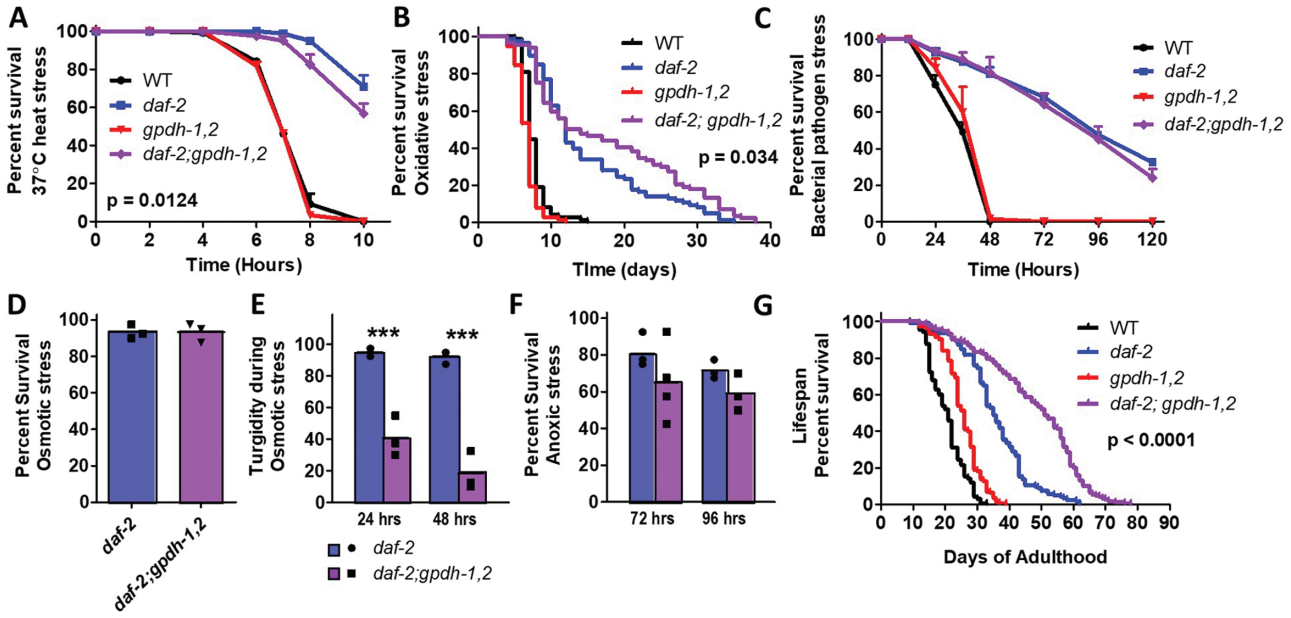

Figure 4. Deletion of *gpdh* genes increases sensitivity to osmotic stress but increases lifespan and resistance to oxidative stress in *daf-2* worms. Deletion of both *gpdh* genes mildly increase *daf-2* worms' sensitivity to heat stress (**A**) and oxidative stress (**B**) but did not affect bacterial pathogen stress sensitivity (**C**). Although *daf-2* worms' survival under osmotic stress was not affected (**D**), *daf-2;gpdh-1;gpdh-2* showed markedly decreased turgidity under osmotic stress (**E**). *daf-2* survival under anoxic stress was not affected by loss of the *gpdh* genes (**F**). Although resistance to specific stresses was decreased by the loss of the *gpdh* genes, lifespan was markedly increased (**G**). Error bars indicate standard error of the mean. ***$p < .001$. p Values indicate difference between *daf-2* and *daf-2;gpdh-1;gpdh-2* worms.

Table 1. Summary of Changes in Stress Resistance and Lifespan Relative to *daf-2*

Strain	Age	Heat stress	Oxidative stress	Bacterial pathogen stress	Osmotic stress	Anoxia	Lifespan
daf-2;daf-16	Day 1	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Day 10	↓↓↓	↓↓↓	ND	↓↓↓	↓↓↓	
daf-2;hsf-1	Day 1	↓↓	=	=	=	=	↓↓
	Day 10	=	↓↓	ND	↓↓	↓↓	
daf-2;sod-12345	Day 1	↓↓↓	↓↓↓	↓	↓↓	=	↓
	Day 10	↓↓	↓↓↓	ND	=	=	
daf-2;pmk-1	Day 1	↓↓	↓↓↓	↓↓↓	↓↓	↓	↓
	Day 10	=	↓↓	ND	↓↓	=	
daf-2;gpdh-1,2	Day 1	↓	↑	=	↓↓	=	↑↑
	Day 10	=	↑	ND	=	=	
daf-2;nhl-1	Day 1	=	↑	↓	↓↓	=	↑↑
	Day 10	=	=	ND	↓↓	↓↓	
daf-2;egl-27	Day 1	↓	=	↓	↓↓	↓	↓
	Day 10	=	↓↓	ND	↓↓	↓↓	

Notes: ↑ Indicates an increase. ↓ Indicates a decrease. Three arrows indicate reversion to wild type. Two arrows indicate a marked increase or decrease. One arrow indicates a significant increase or decrease. = indicates no significant difference. ND indicates not done.

resistance should prevent the increase in lifespan. Within this context, we examined the results of the genetic interventions.

Stress Resistance in Long-lived Mutants

One of the strongest pieces of evidence linking stress resistance and longevity is the observation that long-lived genetic mutants have increased resistance to multiple stresses (41). Although this has been clearly demonstrated for *daf-2* mutants (12–17), it has been less well studied in other long-lived mutants. Long-lived *sod-2* deletion mutants have decreased resistance to oxidative stress and WT survival under conditions of heat and osmotic stress (42). *clk-1* worms have increased resistance to chronic oxidative stress but increased sensitivity to acute oxidative stress (43). *nuo-6* mutants have increased resistance to oxidative stress, osmotic stress, and heat stress, but not anoxia (Senchuk and Van Raamsdonk, unpublished data). *isp-1* worms show increased resistance to oxidative stress, osmotic stress, heat stress, and bacterial pathogen stress (44). However, further increasing resistance to oxidative stress *isp-1* worms through deletion of *sod-3* or *sod-5* was found to decrease lifespan, demonstrating that oxidative stress resistance and longevity can be experimentally dissociated (44). In fact, there are many examples of long-lived mutants that exhibit increased sensitivity to oxidative stress (45). Finally, *eat-2* mutants have increased resistance to oxidative stress but decreased resistance to heat stress, decreased resistance to osmotic stress and normal survival under anoxia (Andrews and Van Raamsdonk, unpublished data). Thus, although there is a general trend that long-lived mutants have increased resistance to stress, this is not true of all long-lived mutants or for all stresses.

Redundancy Among Stress Response Pathways

To further explore the relationship between stress resistance and lifespan, we crossed long-lived, stress resistant *daf-2* worms to worms with mutations that affect stress response pathways that have been associated with specific types of stress (eg, *hsf-1*—heat stress, *sod* genes—oxidative stress, *pmk-1*—bacterial pathogen stress, *gpdh* genes—osmotic stress). Although we did observe the predicted decrease the survival when the double mutants were exposed to the specific type of stress that the gene had been associated with, in every case we found that the double mutants also showed altered resistance to other types of stress (summarized in Table 1). For example, deletion of *pmk-1* resulted in increased sensitivity to heat, oxidative, osmotic, anoxic, and bacterial pathogen stresses. Similarly, disruption of *sod* genes caused increased sensitivity to heat, oxidative, osmotic, and bacterial pathogen stress, whereas mutation of *hsf-1* resulted in increased sensitivity to heat, oxidative, osmotic, and anoxic stresses. Because all of the genes we chose to examine impacted resistance to multiple stresses, it was not possible to specifically modulate resistance to just one stress and examine the resulting effect on lifespan. Although it is possible that selecting different genes might have enabled us to diminish resistance to a single type of stress, our data suggest that the stress response pathways may be highly intertwined such that it is difficult to modulate one type of stress resistance without affecting others.

Effect of Modulating Stress Resistance on *daf-2* Lifespan

In examining resistance to heat stress, we found that specifically decreasing heat stress resistance through deletion of *hsf-1* decreased *daf-2* lifespan. Similarly, *pmk-1* and *egl-27* mutations decreased heat stress resistance and lifespan in *daf-2* mutants. As a result, we observed a positive correlation between heat stress survival and lifespan in day 1 adults (but not at day 10). On the other hand, deletion of *gpdh-1* and *gpdh-2* decreased heat stress resistance but increased lifespan indicating that these phenotypes could be experimentally dissociated. In addition, a complete reversion of heat stress resistance to WT resulting from the loss of all five *sod* genes, only marginally decreased *daf-2* lifespan, indicating that enhanced resistance to heat stress is not required for the majority of the lifespan increase in *daf-2* worms.

A role for oxidative stress resistance in lifespan is supported by the fact that an *egl-27* mutation decreased oxidative stress survival and lifespan to similar extents and the fact that deletion of *gpdh-1* and *gpdh-2* or *nhl-1* increased resistance to oxidative stress and increased lifespan. Although oxidative stress survival was not significantly correlated with lifespan in day 1 adult worms, at day 10 of adulthood the relationship was strongly positive (r^2 = .69, p = .01). However, we also observed that deletions in *pmk-1* or all five *sod* genes completely abolished *daf-2* worms' enhanced resistance to oxidative stress but only weakly affected lifespan. This suggests that oxidative stress resistance is not required for the majority of the lifespan increase in *daf-2* worms, or that other stress response pathways can compensate for the disruption of oxidative stress resistance.

Bacterial pathogen stress resistance and lifespan in *daf-2* mutants were found to be decreased to similar extents by an *egl-27* mutation or the disruption of all five *sod* genes. However, deletion of *pmk-1* completely reverted bacterial pathogen stress resistance back to WT but only had a modest impact on lifespan, suggesting that bacterial pathogen resistance is not required for the majority of the lifespan increase in *daf-2* worms. Deletion of *nhl-1* in *daf-2* worms decreased resistance to bacterial pathogen stress but increased lifespan indicating that these factors could be experimentally dissociated. Bacterial pathogen stress survival was not correlated with lifespan.

In examining osmotic stress resistance, we found that *daf-2;sod-12345*, *daf-2;pmk-1*, and *daf-2;egl-27* worms all had decreased resistance to osmotic stress compared to *daf-2* worms and decreased lifespan. In contrast, *daf-2;gpdh-1;gpdh-2* and *daf-2;nhl-1* worms produced the opposite result: decreased resistance to osmotic stress and increased lifespan. As a result, osmotic stress survival showed no correlation with lifespan in day 1 or day 10 adults.

Although *pmk-1* and *egl-27* mutations mildly decreased both anoxia resistance and lifespan, most of the genes that we examined had little or no effect on resistance to anoxia, making it more difficult to draw conclusions about the role of anoxia resistance in longevity. Nonetheless, our data indicated a mild but significant correlation between anoxia survival at day 1 of adulthood and lifespan. However, it should be noted that this relationship was primarily driven by the complete reversion to WT in *daf-2;daf-16* worms.

Overall, our results indicate a weak relationship between stress resistance and aging in *daf-2* worms. As our results do not exclude the possibly that increased stress resistance is the primary driver of longevity in other long-lived mutants, it will be important to examine other long-lived mutants.

Conclusions

In modulating stress resistance in *daf-2* worms, we observed multiple instances in which the genetic manipulation both decreased stress resistance and decreased lifespan. However, the magnitude of those changes was often not correlated and we observed examples in which the same mutation caused decreased stress resistance and increased lifespan. Overall, our results suggest that while stress resistance is correlated with longevity, it is not required. Instead, our

data support a model in which the same genetic pathways contribute to both increased resistance to stress and increased lifespan.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of interest statement

None declared.

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