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Dietary deprivation extends lifespan in *Caenorhabditis elegans*

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Summary

Dietary restriction (DR) is well known as a nongenetic intervention that robustly extends lifespan in a variety of species; however, its underlying mechanisms remain unclear. We have found in *Caenorhabditis elegans* that dietary deprivation (DD) during adulthood, defined as removal of their food source *Escherichia coli* after the completion of larval development, increased lifespan and enhanced thermotolerance and resistance to oxidative stress. DD-induced longevity was independent of one *C. elegans* SIRTUIN, *sir-2.1*, which is required for the effects of DR, and was independent of the *daf-2*/insulin-like signaling pathway that independently regulates longevity and larval diapause in *C. elegans*. DD did not significantly alter lifespan of *fem-1(hc17); eat-2(ad465)* worms, a genetic model of DR. These findings suggest that DD and DR share some downstream effectors. In addition, DD was detrimental for longevity when imposed on reproductively active young adults, suggesting that DD may only be beneficial in the absence of competing metabolic demands, such as fertility. Adult-onset DD offers a new paradigm for investigating dietary regulation of longevity in *C. elegans*. This study presents the first evidence that long-term DD, instead of being detrimental, can extend lifespan of a multicellular adult organism.

Keywords

ad libitum; aging; *Caenorhabditis elegans*; calorie restriction; dietary restriction; reproduction

Introduction

Lifespan is regulated by diverse genetic and environmental factors (Kenyon, 2005; Kirkwood, 2005; Masoro, 2005). One of the most robust environmental manipulations of lifespan is dietary restriction (DR) (Masoro, 2005). DR has been shown to extend lifespan in many species, ranging from invertebrates to mammals. In addition, DR enhances resistance to a variety of stresses, such as heat and oxidative stress, and delays onset of age-related diseases in murine models for human cancer and diabetes (Masoro, 2005). Several genetic components of the DR longevity pathway have been identified. Foremost among these are the NAD-dependent protein deacetylase, SIR2 and other SIRTUIN family members (Guarente, 2005; Sinclair, 2005), and TOR (target of rapamycin), a protein kinase that coordinates cell growth in response to nutrient availability (Vellai *et al*., 2003; Kapahi *et al*., 2004; Kaeberlein *et al*., 2005). In *Drosophila*, insulin-like signaling converges with DR and may also be a downstream like target of DR

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(Clancy *et al*., 2002). However, DR and insulin-like signaling appear to be independent in *Caenorhabditis elegans* (Lakowski & Hekimi, 1998; Houthoofd *et al*., 2003).

The degree of calorie reduction can be a critical factor modu-lating the effect of DR on lifespan (Everitt *et al*., 1982; Houthoofd *et al*., 2002). In rodents, DR, often referred to as calorie restriction, is normally achieved by relatively precise control of food or calorie intake (Masoro, 2005). DR to the level of 20–50% reduction of the *ad libitum* (AL) calorie intake extends lifespan, while further caloric restriction is detrimental (Everitt *et al*., 1982). Furthermore, imposing DR during development can be detrimental (Houthoofd *et al*., 2002). In yeast, worms, and flies, uncertainty over the amount of food intake has made DR problematic (Houthoofd *et al*., 2005). Historically, DR in these model systems is generally imposed by dilution of the caloric food source with low-calorie media (Klass, 1977; Houthoofd *et al*., 2002; Magwere *et al*., 2004). As observed for rodents, dilution studies have also shown that extreme caloric deprivation during development is detrimental (Klass, 1977; Henderson *et al*., 2006). DR has also been imposed using genetic mutations that interfere with nutrient uptake (Klass, 1977;Lakowski & Hekimi, 1998;Houthoofd *et al*., 2003; Kapahi *et al*., 2004; Kaeberlein *et al*., 2005). However, in some cases, longevity induced by these genetic mutations can be variable (Vellai *et al*., 2003; Walker *et al*., 2005; Henderson *et al*., 2006).

Previous studies in *C. elegans* imposed DR by food dilution prior to the completion of larval development (Houthoofd *et al*., 2002). However, adverse effects on development could arise even when DR is imposed during larval development, as suggested by findings that extreme food dilutions is usually detrimental (Avery & Horvitz, 1990; Henderson *et al*., 2006). This possibility, in combination with the uncertainty inherent in food dilution protocols and variable effects from genetic mutations that impose DR, led us to examine whether a new dietary regimen could affect longevity. Here we report that a dietary deprivation (DD) regimen, in which the food source *Escherichia coli* is removed from adult worm cultures, could prolong adult lifespan by up to 45%. Because this regimen involves removal of the food source, the problem of controlling food intake, which has hampered interpretation of past studies, is alleviated. Using this unambiguous method for dietary manipulation of longevity, we have investigated the genetic pathways necessary for lifespan extension by DD.

Results

Dietary deprivation extends lifespan

To examine the effects of DD on lifespan, we first used the *C. elegans* strain, *fem-1(hc17),* which is a temperature-sensitive mutant developing into semifertile adults at 20 °C and sterile adults at 25 °C. Lifespan analyses can be facilitated by utilizing sterile adult *fem-1(hc17)* populations, which have lifespans similar to those of wild-type animals (Nelson *et al*., 1978). We transferred sterile *fem-1(hc17)* adults on adult day 2 or older to agar plates with or without bacteria (*E. coli* OP50), which represent the AL or DD conditions, respectively. Both DD and AL media contained a mixture of 5-fluoro-2′-deoxyuridine (FUdR) and ampicillin to minimize egg formation and bacterial growth. Mean and maximum lifespan of control AL populations were similar to published results (Kenyon *et al*., 1993). In contrast contrast, the DD regimen significantly increased mean and maximum adult lifespan by up to 45% (Fig. 1).

To assess the temporal requirements for initiation of DD, adult worms were maintained on AL conditions for different periods of time and then transferred to DD conditions for the remainder of their lives. The optimal effect of DD was observed when food was removed at adult day 2. Imposition of DD at later ages, in day 4 or 8 adults, elicited progressively weaker effects on longevity (Fig. 1; Table 1).

Dietary deprivation did not slow the rate of aging-related functional declines

By assessing age-related functional declines in AL- and DD-treat treated worms, we next examined whether DD prolonged healthspan, rather than merely enhancing survival at old age. Two standard measures of functional aging in *C. elegans* are declines in pharynx pumping and locomotion (Johnson, 1987; Herndon *et al*., 2002; Glenn *et al*., 2004; Huang *et al*., 2004; Chow *et al*., 2006). In both AL- and DD-treated animals, pharynx-pumping rates progressively declined with age, although DD-treated animals maintained significantly higher pumping rates at all ages than AL-treated animals (Fig. 2A). The rate of decline of pharynx pumping with advancing age was similar in DD- and AL-treated animals (Fig. 2B). Similarly, spontaneous locomotion also declined progressively during aging of AL- and DD-treated animals, and DD-treated animals exhibited significantly greater spontaneous locomotion than AL-treated animals at all ages examined (Fig. 2C). As observed for pharynx pumping, locomotor activity declined at similar rates in DD- and AL-treated animals (Fig. 2D). This suggests that DD did not significantly slow the rate of aging in these tissues.

Dietary deprivation enhances stress resistance

Extension of lifespan by genetic and nongenetic manipulations is often associated with increased resistance to stress (Finkel & Holbrook, 2000). Therefore, we measured resistance to oxidative and thermal stress for worms under DD. DD enhanced resistance to paraquat-induced oxidative stress compared to AL (Fig. 3A). In *C. elegans*, effects on oxidative stress can also be tested using a genetic mutant strain, *mev-1(kn1)* (Melov *et al*., 2000; Wilson *et al*., 2006). The *mev-1(kn1)* strain contains a mutant version of mitochondrial *succinate dehydrogenase cytochrome b*, which is associated with increased superoxide production, oxidative stress, and shortened lifespan (Ishii *et al*., 1998; Senoo-Matsuda *et al*., 2001). DD significantly increased *mev-1(kn1)* adult lifespan (Fig. 3B). This beneficial effect of DD in *mev-1(kn1)* worms could be due to either increased resistance to oxidative stress, reduced production of free radicals, or through independent effects that are beneficial to *mev-1* animals. In addition, DD increased thermotolerance compared to AL in wildtype animals (Fig. 3C).

Dietary deprivation and dietary restriction have overlapping effects

Next we compared DD to other DR regimens. It has been demonstrated that intermittent fasting extends lifespan in rodents onstrated (Goodrick *et al*., 1982, 1990; Anson *et al*., 2005). To compare DD to intermittent fasting, we subjected animals to alternating feeding and fasting regimens. We found that strict DD was superior in extending lifespan to any regimen with varied refeeding periods (Fig. 4A). Imposition of DD for only the first 10–15 days of adulthood could prolong lifespan, but to a lesser extent than the strict DD regimen over the adult lifespan. Interestingly, interrupting the DD regimen with AL feeding on adult days 5–10, and reimposing DD after day 10, negated the benefits of the DD regimen. This suggests that early to mid-adulthood is a critical period for DD to induce the full benefit on longevity.

To compare DD with genetically imposed DR, we examined the effect of DD on lifespan of *fem-1(hc17);eat-2(ad465)* animals, which have reduced food intake due to a mutational defect in pharynx pumping (Lakowski & Hekimi, 1998). DD fails to significantly extend lifespan of long-lived *fem-1(hc17; eat-2(ad465)* adults (Fig 1 and Fig 4C), suggesting that DD and *eat-2* may affect longevity through a similar pathway.

We next examined the requirement for *sir-2.1*, a member of the conserved SIRTUIN family of proteins that regulate responses to DR in many organisms (Tissenbaum & Guarente, 2001; Guarente, 2005; Wang & Tissenbaum, 2006). We found that DD could extend lifespan of *sir-2.1(ok434)* animals, which lack one of the four *C. elegans* sirtuins, and the only one that has been implicated in regulating DR-induced longevity in *C. elegans* (Frye, 2000; Tissenbaum & Guarente, 2001). Together, these results suggest that, although DD prolongs lifespan through

DR, as evidenced by lack of effect on long-lived *fem-1(hc17);eat-2(ad465)* adults, this effect does not apparently require *sir-2.1*. This may reveal a role for additional genes in mediating DD-induced longevity in *C. elegans*.

Lifespan extension by dietary deprivation is independent of insulin-like signaling

When the food source is scarce, *C. elegans* larvae can survive for long periods as diapaused dauer larvae (Albert *et al*., 1981; Riddle *et al*., 1981). An insulin-like signaling pathway regulates both dauer diapause and lifespan (Kenyon, 2005). To investigate the relationship between insulin-like signaling and DD-induced longevity, we evaluated the effects of DD in long-lived *daf-2(e1370)* worms, lacking DAF-2 insulin-like receptor activity. We also examined DD in short-lived *daf-16(mgDf50)* worms, lacking DAF-16/FOXO activity, which is the major downstream target of DAF-2/insulin-like signaling (Lin *et al*., 1997; Ogg *et al*., 1997). Mean and maximum lifespans of these two strains were significantly increased by DD (Fig. 5 A,B; Table 2), suggesting that lifespan extension by DD does not depend solely on DAF-2/ insulin-like signaling or DAF-16 and thus probably does not induce a dauer-like state in adult animals.

Reproductive status is critical for dietary deprivation to extend lifespan

In adult animals, a major sink for metabolic resources is reproduction. Adult *C. elegans* nematodes produce between 150 and 250 progeny during 3–5 days of reproduction. Therefore, reproduction constitutes a major metabolic demand that could modulate the effect of a DD regimen on parental lifespan. We evaluated the effect of reproductive status on DD-induced longevity by taking advantage of the temperature sensitive nature of *fem-1(hc17)* adult sterility (Spence *et al*., 1990). When cultured at a semipermissive temperature (20 °C), *fem-1(hc17)* worms develop into semifertile adults. These semifertile *fem-1(hc17)* adults were then transferred to 25 °C at adult day 0, and DD was imposed at either day 0, 2, or 4. Mean lifespan was dramatically decreased when DD was initiated on adult day 0, when the fertile adults were beginning to lay eggs. However, DD still increased lifespan of fertile *fem-1(hc17)* adults when imposed on adult day 2 or 4, when egg laying had markedly declined (Fig. 6; Table 1). This observation was confirmed in *sir-2.1(ok434)* fertile adults, which showed no benefits, or slightly reduced lifespan, when DD was imposed on fertile adult day 0 (Table 3). For the sterile adults raised from eggs grown at restricted temperature (25 °C), DD significantly extended mean and maximum lifespan of these animals when initiated on adult day 0 compared to AL (Fig. 6; Table 3). These findings suggest that DD is detrimental in animals that are capable of reproduction and suggest that DD can extend lifespan only when reproductive function is minimized.

Discussion

Lifespan is modulated by genetic and environmental factors, including diet and metabolic status (Kenyon, 2005; Kirkwood, 2005; Masoro, 2005). Here we have described an effective paradigm to study dietary regulation of longevity. We have demonstrated that removal of food, or dietary deprivation, in nonreproductive adults, is a robust dietary intervention to extend lifespan in *C. elegans*. The DD regimen offers several advantages to investigate molecular and cellular mechanisms of lifespan regulated by environmental factors. First, it is easy to implement by simply removing the food source, *E. coli*. This simple procedure minimizes complications due to the uncertainty of food intake in current DR regimens in *C. elegans*, which include bacterial dilution, axenic media, or utilizing genetic mutants with feeding defects (Houthoofd *et al*., 2005). It is important to acknowledge for the current DD protocol that we cannot provide conclusive evidence that worms under DD are completely deprived of nutrients since they are maintained on nematode growth medium (NGM) agar, which contains low amounts of soluble protein source (0.25% of Bacto-Pepton) and cholesterol (1 m_M). However,

it is unlikely that these extremely). low concentrations of nutrients can have significant impact on food intake. Second, DD is not a strain-specific phenomenon. We showed that DD extended lifespan of a number of mutant strains. In particular, DD increased lifespan of long-lived *daf 2-(e1370)* animals, as well as two short-lived strains, *mev-1(kn1)* and *daf-16(mgDf50)*). DD elicited a number of beneficial effects, which are commonly associated with longevity, such as enhanced stress resistance (Kenyon, 2005). These findings demonstrate that the DD regimen in *C. elegans* can be a powerful approach for identifying molecular and cellular mechanisms of lifespan regulation by dietary intake.

In this study, we investigated the genetic requirements for DD-induced longevity. We have found that DD was unable to extend lifespan of a DR mutant *fem-1(hc17); eat-2(ad465)*, which has reduced food consumption, indicating that the effects of DD and DR may have some overlap (Lakowski & Hekimi, 1998). However, DD did extend lifespan of the *C. elegans sirtuin* mutant, *sir-2.1(ok434)*, suggesting that lifespan extension induced by DD is *sir2.1*-independent. It has been demonstrated that SIRTUINs are required for DR to extend lifespan in several species ranging from yeast and worms to fruit flies (Tissenbaum & Guarente, 2001; Wood *et al*, 2004; Guarente, 2005). However, the role of SIRTUINs in DR remains controversial. Sir2, a ever, yeast SIRTUIN, has been shown to mediate DR response in some experimental conditions but not in others (Kaeberlein *et al*., 2004; Guarente, 2005). The discrepancy has been suggested to be due to differences in strain background, DR protocols, or sirtuin gene redundancy (Guarente, 2005). Considering that DD-induced longevity was independent of *sir-2.1*, we suggest that *sir-2.1* function might be critical for lifespan extension by mild, but not by extreme calorie deprivation. However, further work needs to be done to determine whether the other three *C. elegans* sirtuins, *sir-2.2*, *−2.3*, and *−2.4*, are also dispensable for DD-induced longevity (Frye, 2000).

We also presented two pieces of evidence showing that DD probably does not increase longevity by inducing a dauer-like state in adults. First, adult worms under DD have higher pharynx-pumping rate and locomotor activity than AL-treated animals. Dauer larvae cease pharynx pumping and have reduced locomotor activity, inconsistent with our observations. Second, motor DD-induced longevity was independent of components of the insulin-like signaling pathway, which are involved in dauer formation (Riddle *et al*., 1981; Antebi, 2004). This evidence further demonstrates that the worms under DD are not in a dauer-like state, although we cannot exclude that similar downstream genes may be required for DD-induced longevity and dauer longevity.

Reproduction has been shown to be critical in regulating lifespan in *C. elegans* and *Drosophila melanogaster* (Partridge & Prowse, 1997; Hsin & Kenyon, 1999; Sgro & Partridge, 1999; Arantes-Oliveira *et al*., 2002). However, the relationship between reproduction and lifespan is far from clear. For example, in *Drosophila melanogaster*, dietary deprivation of yeast reduces, both fecundity and lifespan when applied to adults (Good & Tatar, 2001). However, deprivation of yeast only in the third instar larvae does not slow aging, although it reduces fecundity (Tu & Tatar, 2003). DD in *C. elegans* appeared to be detrimental when imposed on adult day 0, suggesting that the effects of DD could interact with reproduction. It is possible that DD delays aging by shifting energy use from reproduction to somatic maintenance, which is consistent with the disposable soma theory of aging (Drenos & Kirkwood, 2005). This type of energy shift has been suggested to be the mechanism by which DR extends lifespan (Drenos & Kirkwood, 2005; Kirkwood, 2005).

As far as we are aware, our study is the first to report that a multicellular adult organism can survive under long-term food deprivation conditions, which contrasts with the conventional view that DR is beneficial only up to a certain degree of deprivation. This effect may be limited depending on the particular metabolic demands imposed on a particular organism. For example,

DD was detrimental in animals during reproductive periods, and starvation is detrimental in organisms with life histories that differ from that of *C. elegans* (Masoro, 2005; Henderson *et al*., 2006). Further studies of the DD paradigm in *C. elegans* should reveal mechanisms and genetic pathways governing longevity under different environmental conditions. Some of the mechanisms should be evolutionarily conserved and therefore applicable to other species.

Experimental procedures

Dietary deprivation assay

Caenorhabditis elegans stocks were obtained from the Caenorhabditis Genetics Center and maintained at 15 °C. For lifespan assays, worms were allowed to produce progeny for 6 h at 25 °C; the progeny developed from the L1 to L4 stage within 48 h (Duhon *et al*., 1996). We defined day 0 of adulthood as the first day following the L4 molting to adult. After hood being fed with *E. coli* OP50 during larval development, adult animals were transferred to 3.5 cm Petri dishes with or without a lawn of *E. coli* on NGM agar, which represented *ad libitum* (AL) or dietary deprivation (DD), respectively. NGM agar contains Bacto-Peptone (2.5 g L^{-1}), NaCl (3 g L^{-1}), cholesterol (5 mg L^{-1}), $CaCl_2$, (1 mM), $MgSO_4$ (1 mM), KH_2PO_4 (25 mM, pH 6.0) and agar (21 g L^{-1}). DD and AL plates were pretreated with 0.25 mL of 250 μg mL^{-1} 5-fluoro-2′-deoxyuridine (FUDR) and ampicillin (Walker *et al*., 2005). The number of dead worms was recorded once every 2 or 3 days by touch and movement analysis. Surviving worms were transferred to fresh plates once every 6–8 days.

Pharynx-pumping rate assay

Pharynx-pumping rates (the number of contractions of the pharynx terminal bulb in 1 min) were assayed at room temperature as previously described with modifications (Huang *et al*., 2004). For pumping rate assays, worms were allowed to equilibrate to room temperature with light for at least 1 h and pumping rates were measured. This was recorded as the 0-h data point. Then 10–12 worms from each group were transferred to a 6-cm NGM plate with bacteria, and the pumping rate was recorded at 1, 2, and 5 h after transfer. Each assay was performed with at least 6 worms and was repeated at least twice.

Spontaneous locomotion assay

This assay was performed at room temperature as previously described with modifications (Glenn *et al*., 2004). At adult days 4, 6, and 8, worms subjected to either AL or DD from adult day 1 were washed with 0.5 mL of S-basal buffer containing 250 μg mL^{-1} of Amp to remove any residual bacteria and air-dried for 1 h on bacteria-free NGM agar plates. Then 20 worms were transferred to the center of a 6-cm NGM bacteria-free plate at room temperature. The distance each worm traveled from the center was measured at 0.5, 1, 2, and 5 h after the transfer by using concentric circles on a transparent film with 1 mm difference in diameter from the center. The average value was calculated based on the total number of worms inside the plate. All experiments were performed with three independent populations.

Oxidative stress assay

Paraquat CL tetrahydrate (Chem Service, West Chester, PA, USA) was dissolved in 5 μg mL^{-1} FUdR/amp solution to final concentrations of 10 and 20 mM. A 0.25 mL of each paraquat solution was added on the top of 5 mL agar medium in a 3.5-cm Petri dish with or without a lawn of *E. coli*. Therefore, the final concentrations of paraquat were 2.5 and 6.5 he μM plate $plate^{-1}$, respectively (Yanase *et al*., 2002). Adult day 3 worms were then transferred onto the AL or DD plates treated with paraquat. The number of dead worms was counted every other day until all had died.

Thermotolerance assay

At adult day 7, 11, and 12, worms under AL or DD were transferred onto 6 cm Petri dishes containing NGM agar without bacterial lawn. The plates were sealed to maintain moisture and kept at 35 °C. After 17 h, the number of dead worms was recorded after the plates were cooled down to room temperature for 1 h.

Statistical methods

Lifespan data are expressed as mean or maximum ± SEM. Statistical significance was determined either using a one-way analysis of variance (ANOVA) with *post hoc* Fisher's test for comparisons of mean lifespan and functional parameters, or using parisons Student's *t*-test for comparison of maximum lifespan (defined as 10% survival) with assistance from STATVIEW software (SAS Institute, Inc.). The level of significance was accepted as $P < 0.05$.

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References

Albert PS, Brown SJ, Riddle DL. Sensory control of dauer larva formation in *Caenorhabditis elegans*. J. Comp. Neurol 1981;198:435–451. [PubMed: 7240452]

Anson RM, Jones B, deCabo R. The diet restriction paradigm paradigm: a brief review of the effects of every-other-day feeding. AGE 2005;27:17–25.

Antebi A. Inside insulin signaling, communication is key to long. Sci. Aging Knowledge Environ 2004;2004:pe25. [PubMed: 15190176]

Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C. Regulation of lifespan by germ-line stem cells in *Caenorhabditis elegans*. Science 2002;295:502–505. [PubMed: 11799246]

Avery L, Horvitz HR. Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. J. Exp. Zool 1990;253:263–270. [PubMed: 2181052]

Chow DK, Glenn CF, Johnston JL, Goldberg IG, Wolkow CA. Sarcopenia in the *Caenorhabditis elegans* pharynx correlates with muscle contraction rate over lifespan. Exp. Gerontol 2006;41:252–260. [PubMed: 16446070]

Clancy DJ, Gems D, Hafen E, Leevers SJ, Partridge L. Dietary restriction in long-lived dwarf flies. Science 2002;296:319. [PubMed: 11951037]

Drenos F, Kirkwood TB. Modelling the disposable soma theory of ageing. Mech. Ageing Dev 2005;126:103.

Duhon SA, Murakami S, Johnson TE. Direct isolation of longevity mutants in the nematode *Caenorhabditis elegans*. Dev. Genet 1996;18:144–153. [PubMed: 8934876]

Everitt AV, Porter BD, Wyndham JR. Effects of caloric intake and dietary composition on the development of proteinuria, age-associated renal disease and longevity in the male rat. Gerontology 1982;28:168–175. [PubMed: 7095434]

Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239–247. [PubMed: 11089981]

Frye, RA. Biochem. Biophys. Res. Commun. 273. 2000. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins; p. 793-798.

Glenn, CF.; Chow, DK.; David, L.; Cooke, CA.; Gami, MS.; Iser, WB.; Hanselman, KB.; Goldberg, IG.; Wolkow, CA. J. Gerontol. A Biol. Sci. Med. Sci. 59. 2004. Behavioral deficits during early stages of aging in *Caenorhabditis elegans* result from locomotory deficits possibly linked to muscle frailty; p. 1251-1260.

Good TP, Tatar M. Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. J. Insect Physiol 2001;47:1467–1473. [PubMed: 12770153]

Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider NL. Effects of intermittent feeding upon growth and lifespan in rats. Gerontology 1982;28:233–241. [PubMed: 7117847]

Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mech. Ageing Dev 1990;55:69–87. [PubMed: 2402168]

Guarente L. Calorie restriction and SIR2 genes – towards a mechanism. Mech. Ageing Dev 2005;126:923–928. [PubMed: 15941577]

Hansen M, Hsu AL, Dillin A, Kenyon C. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. PLoS Genet 2005;1:119–128. [PubMed: 16103914]

Henderson ST, Bonafe M, Johnson TE. *daf-16* protects the nematode ematode *Caenorhabditis elegans* during food deprivation. J. Gerontol. A Biol. Sci. Med. Sci 2006;61:444–460. [PubMed: 16720740]

Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M. Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. Nature 2002;419:808–814. [PubMed: 12397350]

Houthoofd K, Braeckman BP, Johnson TE, Vanfleteren JR. Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in *Caenorhabditis elegans*. Exp. Gerontol 2003;38:947–954. [PubMed: 12954481]

Houthoofd K, Braeckman BP, Lenaerts I, Brys K, De Vreese A, Van Eygen S, Vanfleteren JR. Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends lifespan in *Caenorhabditis elegans*. Exp. Gerontol 2002;37:1371–1378. [PubMed: 12559406]

Houthoofd K, Johnson TE, Vanfleteren JR. Dietary restriction in the nematode *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci 2005;60:1125–1131. [PubMed: 16183950]

Hsin H, Kenyon C. Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature 1999;399:362–366. [PubMed: 10360574]

Huang C, Xiong C, Kornfeld K. Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. Proc. Natl Acad. Sci. USA 2004;101:8084–8089. [PubMed: 15141086]

Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 1998;394:694–697. [PubMed: 9716135]

Johnson TE. Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. Proc. Natl Acad. Sci. USA 1987;84:3777–3781. [PubMed: 3473482]

Kaeberlein M, Kirkland KT, Fields S, Kennedy BK. Sir2-independent lifespan extension by calorie restriction in yeast. PLoS Biol 2004;2:E296. [PubMed: 15328540]

Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 2005;310:1193–1196. [PubMed: 16293764]

Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr. Biol 2004;14:885–890. [PubMed: 15186745]

Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell 2005;120:449–460. [PubMed: 15734678]

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild-type. Nature 1993;366:461–464. [PubMed: 8247153]

Kirkwood TB. Understanding the odd science of aging. Cell 2005;120:437–447. [PubMed: 15734677]

Klass MR. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing lifespan. Mech. Ageing Dev 1977;6:413–429. [PubMed: 926867]

Lakowski B, Hekimi S. The genetics of caloric restriction in *Caenorhabditis elegans*. Proc. Natl Acad. Sci. USA 1998;95:13091–13096. [PubMed: 9789046]

Lin K, Dorman JB, Rodan A, Kenyon C. *daf-16*: an HNF-3/forkhead family member that can function to double the lifespan of *Caenorhabditis elegans*. Science 1997;278:1319–1322. [PubMed: 9360933]

Magwere T, Chapman T, Partridge L. Sex differences in the effect of dietary restriction on lifespan and mortality rates in female and male *Drosophila melanogaster*. J. Gerontol. A Biol. Sci. Med. Sci 2004;59:3–9. [PubMed: 14718480]

Masoro EJ. Overview of caloric restriction and ageing. Mech. Ageing Dev 2005;126:913–922. [PubMed: 15885745]

Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ. Extension of lifespan with superoxide dismutase/catalase mimetics. Science 2000;289:1567–1569. [PubMed: 10968795]

Nelson GA, Lew KK, Ward S. Intersex, a temperature-sensitive mutant of the nematode *Caenorhabditis elegans*. Dev. Biol 1978;66:386–409. [PubMed: 700253]

Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in duces *C. elegans*. Nature 1997;389:994–999. [PubMed: 9353126]

Partridge L, Prowse N. The effects of reproduction on longevity and fertility in male *Drosophila melanogaster*. J. Insect Physiol 1997;43:501–512. [PubMed: 12770412]

Riddle DL, Swanson MM, Albert PS. Interacting genes in nematode dauer larva formation. Nature 1981;290:668–671. [PubMed: 7219552]

Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N. A defect in the cytochrome b large zawa subunit in complex II causes both superoxide anion overproduction and abnormal energy metabolism in *Caenorhabditis elegans*. J. Biol. Chem 2001;276:41552–41558.

Sgro CM, Partridge L. A delayed wave of death from reproduction in Drosophila. Science 1999;286:2521–2524. [PubMed: 10617470]

Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation regulation. Mech. Ageing Dev 2005;126:987–1002. [PubMed: 15893363]

Spence AM, Coulson A, Hodgkin J. The product of *fem-1*, a nematode sex-determining gene, contains a motif found in cell cycle control proteins and receptors for cell–cell interactions. Cell 1990;60:981–990. [PubMed: 2317869]

Tissenbaum HA, Guarente L. Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. Nature 2001;410:227–230. [PubMed: 11242085]

Tu MP, Tatar M. Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster*. Aging Cell 2003;2:327–333. [PubMed: 14677635]

Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature 2003;426:620. [PubMed: 14668850]

Walker G, Houthoofd K, Vanfleteren JR, Gems D. Dietary restriction in *C. elegans*: from rate-of-living effects to nutrient sensing pathways. Mech. Ageing Dev 2005;126:929–937. [PubMed: 15896824]

Wang Y, Tissenbaum HA. Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. Mech. Ageing Dev 2006;127:48–56. [PubMed: 16280150]

Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow CA. Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. Aging Cell 2006;5:59–68. [PubMed: 16441844]

Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 2004;430:686–689. [PubMed: 15254550]

Yanase S, Yasuda K, Ishii N. Adaptive responses to oxidative damage in three mutants of *Caenorhabditis elegans* (*age-1*, *mev-1* and *daf-16*) that affect lifespan. Mech. Ageing Dev 2002;123:1579–1587. [PubMed: 12470895]

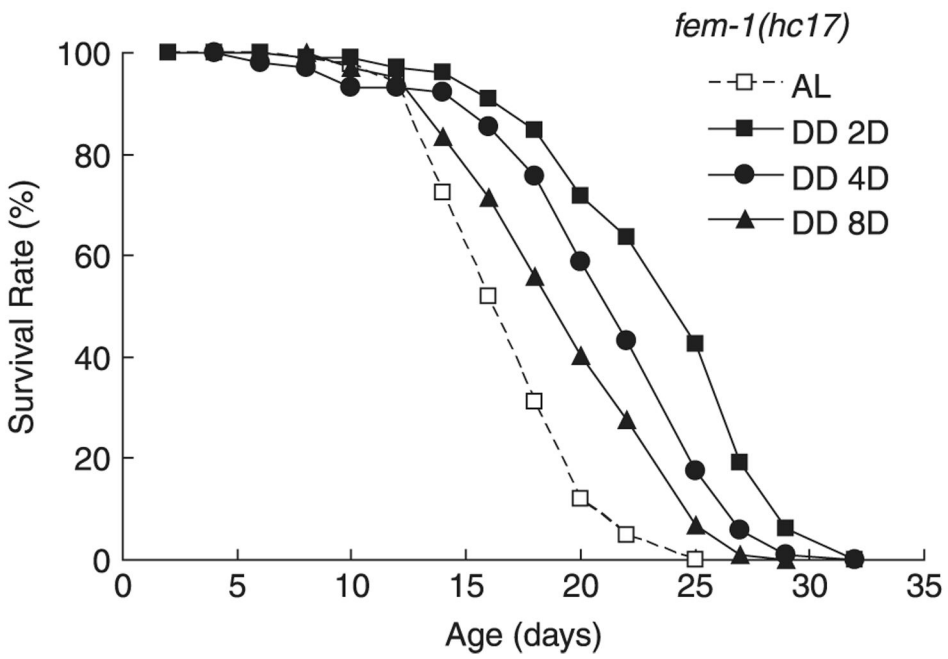

Fig. 1. Extension of adult lifespan by dietary deprivation (DD)

DD was initiated in sterile *fem-1(hc17)* adults on adult day 2 (2D), 4 (4D) or 8 (8D). Lifespan under each DD regimen was extended compared to *ad libitum* (AL) controls, with the most effective extension observed on day 2 DD (42.5% and 41.4% increase mean and maximum; $P < 0.0001$ for both vs. AL controls (see Table 1). For AL conditions, worms were transferred onto agar plates with the same drug supplements as DD, except supplemented with *E. coli* OP50. AL controls that were initiated at adult day 2 were shown here; similar results were obtained when AL was initiated on adult day 4 or 8. *n* = approximately 30 worms tested in triplicate. Graph shows results from one representative of three independent experiments performed by two different individuals.

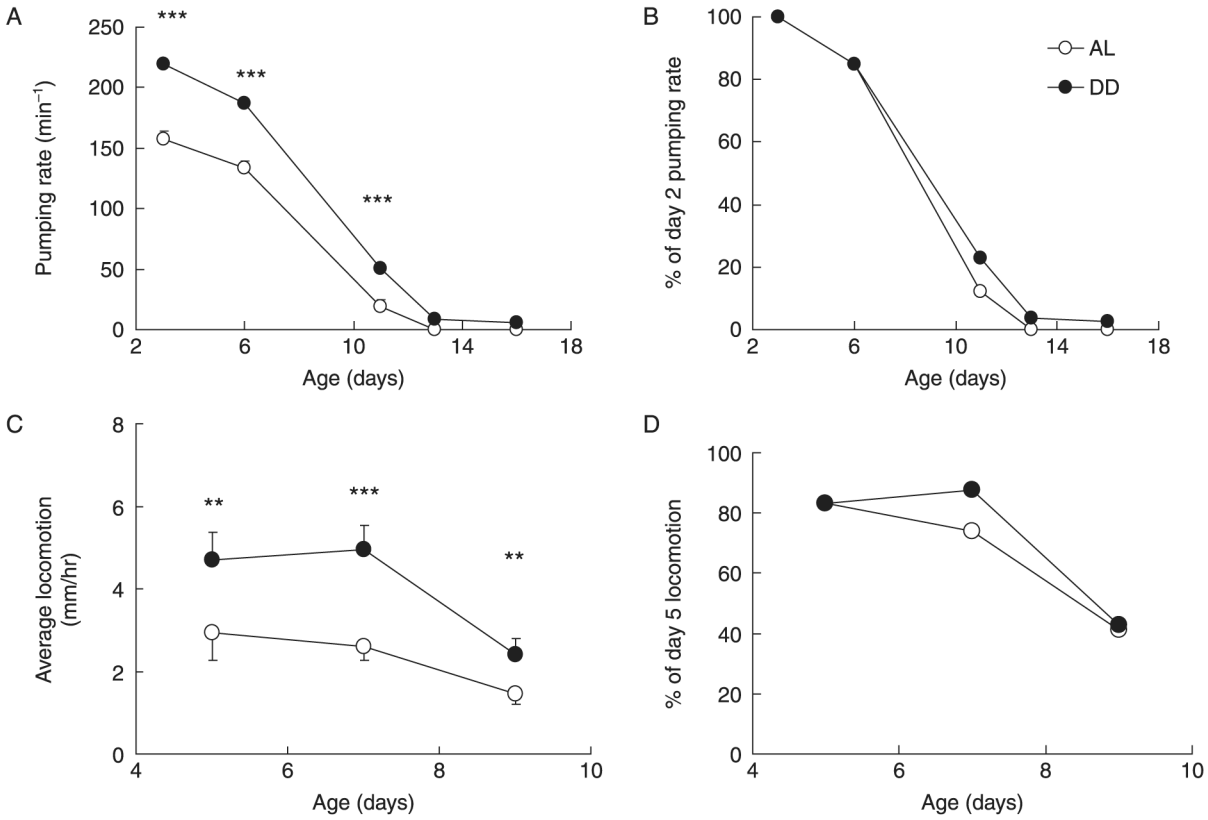

Fig. 2. Effects of dietary deprivation (DD) on aging-related functional decline

DD or *ad libitum* (AL) treatments were initiated on adult day 1 in sterile adult *fem-1(hc17)* worms. (A) Pharynx-pumping rates were measured at indicated ages in DD- or AL-treated worms maintained at 25 °C. Pumping rates were higher in DD worms than age-matched AL worms (n = 6–12 worms/treatment group). (B) Decline in pharynx-pumping rate during aging relative to day 2 pumping rate. By this analysis, the rate of aging-related decline of pharynx pumping was similar in DD- and AL-treated worms. (C) Spontaneous locomotion in DD- or AL-treated worms. Spontaneous locomotion was measured as the average distance moved within 1 h (n = ~60 worms/treatment). (D) Change in spontaneous locomotion at indicated ages, relative to day 5 locomotion rate. Movement declined similarly in DD- and AL-treated worms. Curves are the results from one representative of three experiments; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

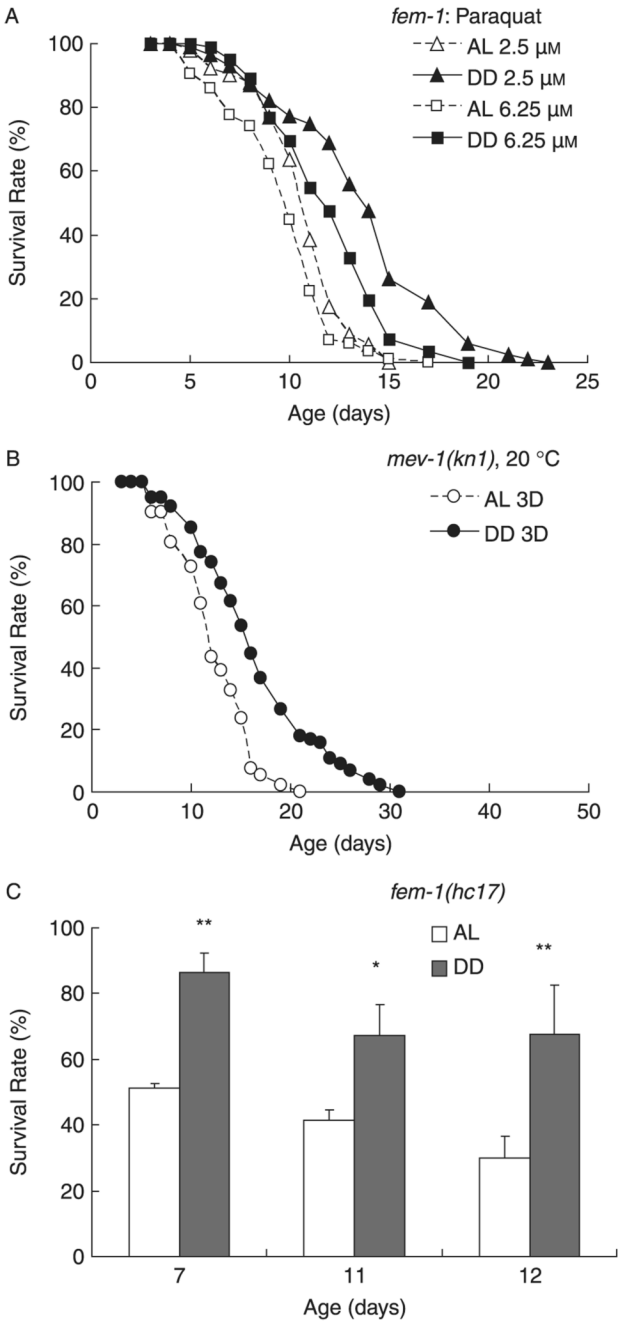

Fig. 3. Dietary deprivation (DD) enhanced stress resistance

(A) DD enhanced resistance to oxidative stress from paraquat. Adult day 3 *fem-1(hc17)* worms were transferred to DD or *ad libitum* (AL) conditions with 2.5 or 6.25 μM paraquat. DD increased mean and maximum survival on both paraquat concentrations, 2.5 μM paraquat, 29.5% increased mean survival, $P < 0.0001$; 6.25 μM paraquat, 26.1% increase, $P < 0.0001$. *n* = approximately 30 worms tested in triplicate. Curves are created from one representative experiment of two independent experiments. (B) DD increased mean and maximum lifespan of *mev-1(kn1)* worms, which experienced increased oxidative stress. DD was initiated at adult day 3; increased mean lifespan by 35.7% ($P < 0.0001$). *n* = approximately 30 worms tested in triplicate of one experiment. (C) DD enhanced survival of *fem-1(hc17)* worms under thermal

stress. Graph shows fractional survival at indicated ages after 17 h at 35 °C. DD enhanced thermotolerance at adult days 7, 11, and 12 by 68.8% (*$P < 0.05$), 62.9% (**$P < 0.01$), and 125% (***$P < 0.001$), respectively.n = approximately 20 worms/treatment tested in quadruplicate. Curves show results from one representative from three independent experiments.

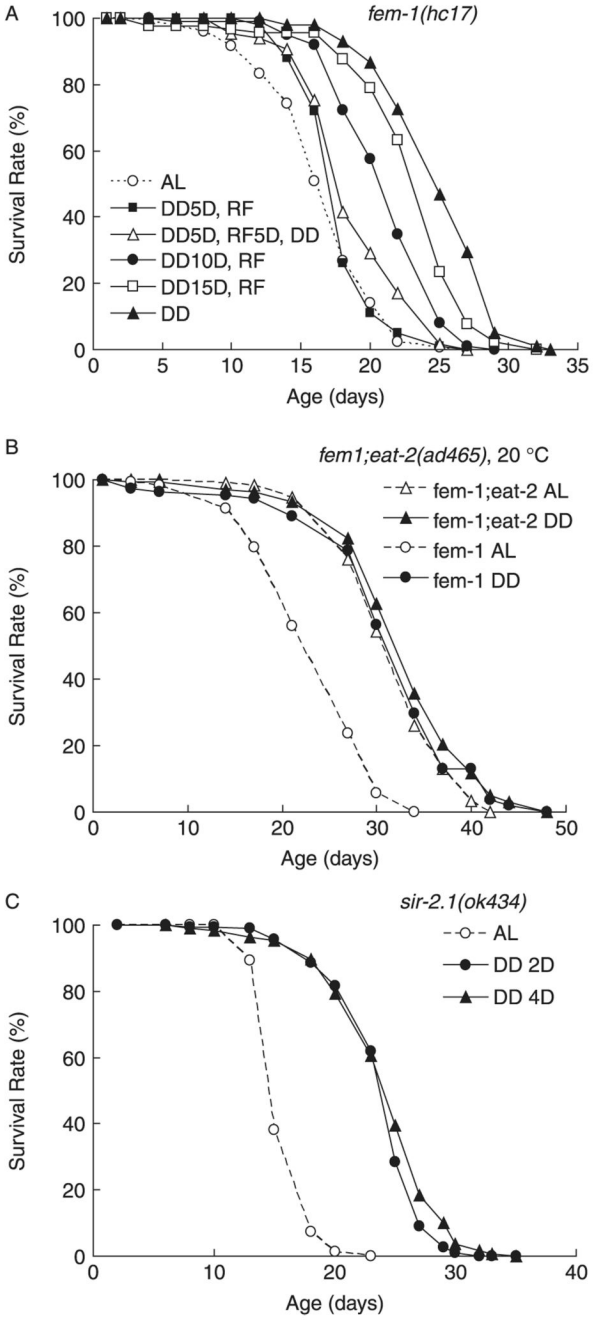

Fig. 4. Effects of re-feeding (RF) and dietary restriction (DR) genetic pathways on dietary deprivation (DD) induced lifespan extension

(A) DD was introduced at adult day 1 in sterile *fem-1(hc17)* worms and then either maintained (DD), or worms were transferred to AL conditions after 5 days (DD5D, RF), 10 days (DD10D, RF) or 15 days (DD15D, RF). RF reduced the DD-induced lifespan extension and the severity of reduction correlated with prolonged re-feeding time (1D vs. 5D) of RF ($P < 0.0001$). n = approximately 30 worms tested in triplicate in one experiment. (B) DD did not lengthen mean or maximum lifespan in slow-feeding *fem-1(hc17); eat-2(ad465)* worms, which are subject to DR, although DD significantly increased both mean and maximum lifespan in the *fem-1 (hc17)* single mutant by 28.8% ($P < 0.0001$) and 25.2% ($P < 0.01$), respectively. Approximately

30 worms/treatment were tested in quadruplicate of one experiment. (C) DD initiated at adult day 2 extended both mean and maximum lifespan in *sir-2.1(ok434)* worms by 81.8% ($P < 0.0001$) and 121.8% ($P < 0.0001$), respectively, compared to AL; $n = 30$ worms/treatment tested in 5 replicates in one experiment.

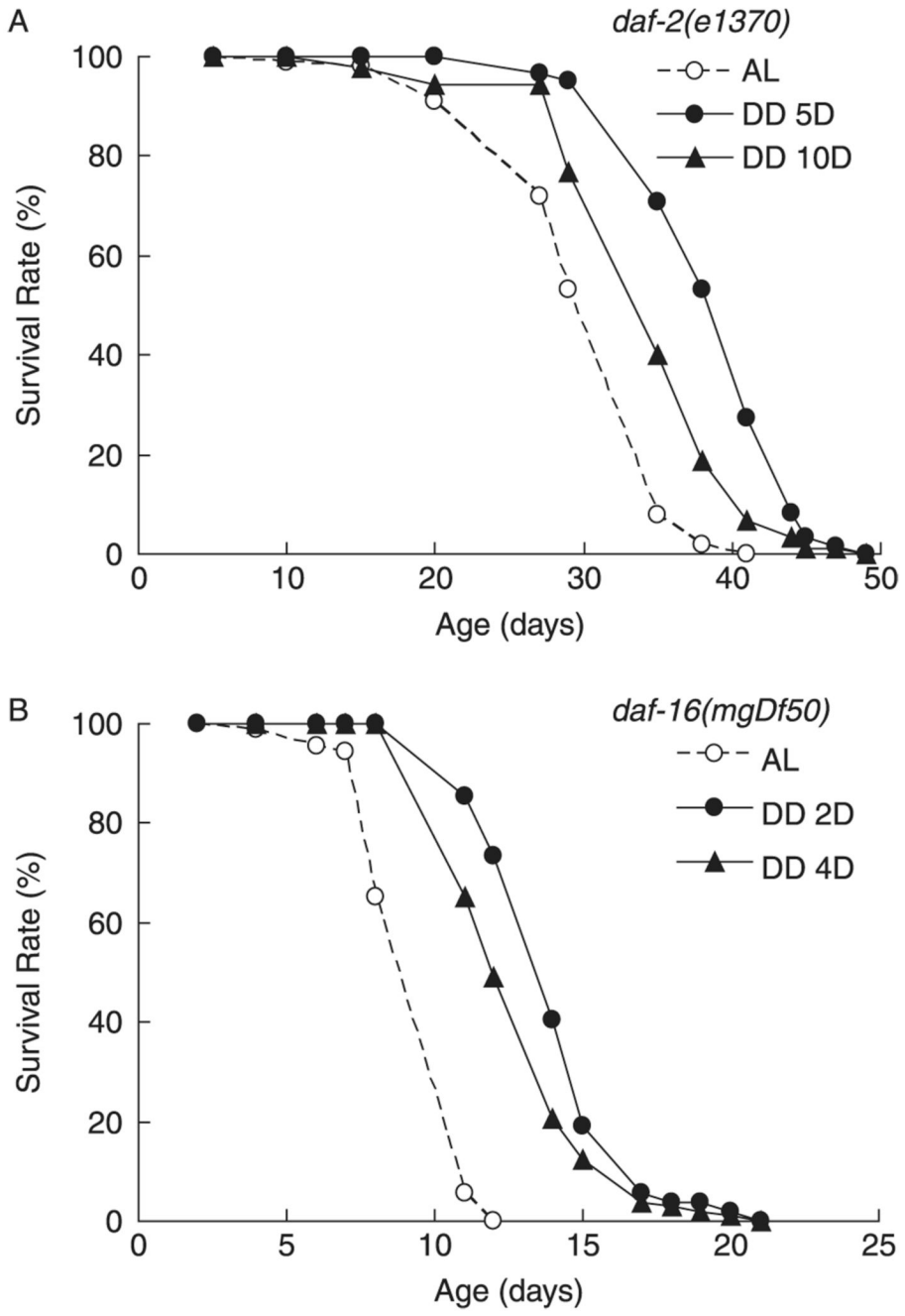

Fig. 5. Dietary deprivation (DD) increased lifespan of strains with defective insulin-like signaling

(A) DD initiated at adult day 5 extended both mean and maximum lifespan of *daf-2(e1370)* worms by 35.2% ($P < 0.0001$) and 30.7% ($P < 0.0001$), respectively, compared to *ad libitum* (AL); n = approximately 30 worms tested in triplicate. Curves are created from one representative experiment of 2 independent experiments. (B) DD initiated at adult day 2 extended both mean and maximum lifespan in *daf-16(mgDf50)* worms by 62.3% ($P < 0.0001$) and 64.4% ($P < 0.0001$), respectively, compared to AL; n = approximately 30 worms tested in quadruplicate in one experiment. DD also extended both mean and maximum lifespan of both strains when initiated at older ages.

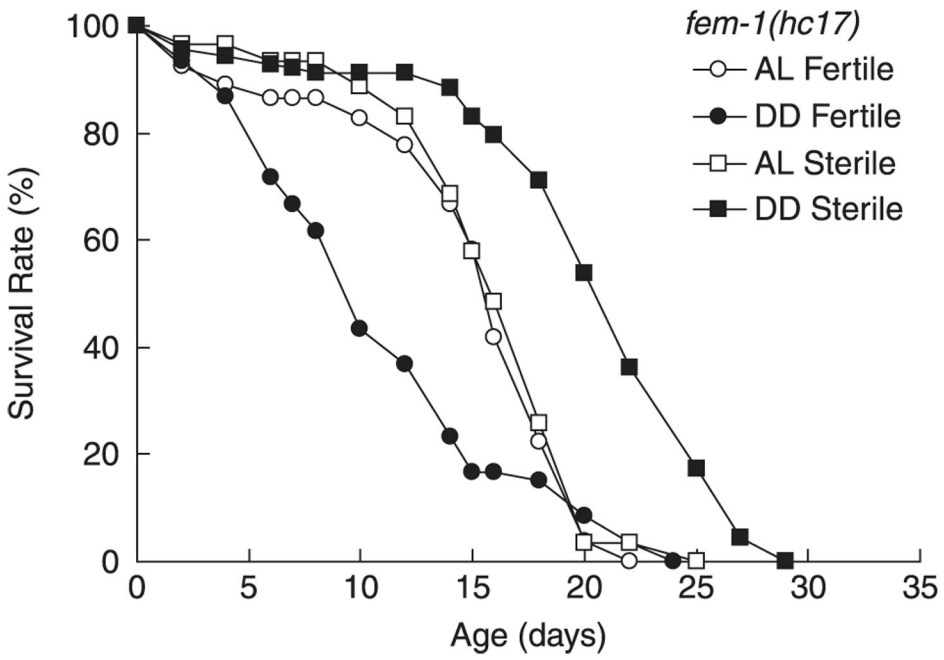

Fig. 6. Role of reproductive status on DD

(A) Lifespan of semifertile and sterile *fem-1(hc17)* adults when DD or AL treatments were initiated at adult day 0. When DD was initiated in fertile *fem-1(hc17)* adults, DD reduced mean lifespan by 29.5% ($P < 0.0001$) but did not statistically alter the maximum lifespan compared to AL (7.2%); n = approximately 30 worms/treatment were tested in triplicate. Curves are created from one representative experiment of two independent experiments.

Table 1

Effects of Dietary Deprivation Regimens on Lifespan

Dietary Regimens	Initiating age (day)	Lifespan (days)				
		n	Mean	Change	Maximum	Change
Effects of DD Initiating Time on Lifespan						
AL	0	89	14.1 ± 0.5		18.0 ± 0.0	
	2	83	15.3 ± 0.4		19.1 ± 0.6	
	4	95	14.6 ± 0.5		20.0 ± 1.2	
	8	90	15.5 ± 0.4		19.5 ± 0.6	
DD	0	69	18.4 ± 0.7***	+30.5%	24.4 ± 1.2**	+35.6%
	2	99	21.8 ± 0.5***	+42.5%	27.0 ± 0.0**	+41.4%
	4	102	19.4 ± 0.5***	+32.9%	24.9 ± 0.1*	+24.5%
	8	102	17.6 ± 0.4**	+13.5%	23.0 ± 1.0*	+18.0%
Effects of Refeeding on DD-Induced Longevity						
AL	1	120	14.7 ± 0.4		19.5 ± 0.5	
DD	1	98	23.1 ± 0.4***		27.0 ± 0.0***	
DD5D,RF	1	100	16.0 ± 0.3†††	−30.7 %	21.7 ± 2.7	
DD5D,RF 5D,DD	1	98	16.9 ± 0.4†††	−26.8%	22.0 ± 0.0†	−18.5%
DD5D,RF1D,DD5D,RF1D,DD	1	100	18.7 ± 0.4†††	−19.0%	23.6 ± 0.9†	−12.6%
DD5D,RF1D,DD	1	105	20.2 ± 0.4†††	−12.6%	25.4 ± 0.4†	−5.9%
DD10D, RF	1	101	19.2 ± 0.4†††	−16.9%	22.8 ± 0.8††	−15.6%
DD15D, RF	1	90	21.0 ± 0.4††	−9.0%	25.5 ± 0.5†	−5.6%

Date are represented as mean ±SEM.

* *P*<0.05

** *P*<0.01

*** *P*<0.0001 *vs.* AL counterparts

† *P*<0.05

†† *P*<0.01

††† *P*<0.0001 *vs.* DD

P-value of mean lifespan calculated by one-way ANOVA and *post-hoc* Fisher's test

P-value of maximum lifespan calculated by *Student's t-test*

AL, ad libitum; DD, dietary deprivation; RF, re-feed.

Table 2

Effects of genetic mutations on DD compared to AL-induced longevity

Mutant	Initiating age (day)	*n*	Lifespan (days) Mean	Change	Maximum	Change
mev-1(kn1)						
AL	3	92	11.2 ± 0.4		15.0 ± 0.0	
	5	92	14.3 ± 0.5		19.0 ± 0.0	
DD	3	101	15.2 ± 0.6***	+35.7%	22.9 ± 2.0*	+52.7%
	5	100	18.20 ± 0.7***	+27.3%	25.9 ± 0.6**	+36.3%
fem-1(hc17)						
AL	1	127	24.1 ± 0.5		30.6 ± 0.7	
DD	1	88	30.8 ± 1.0***	+28.8%	38.3 ± 1.4**	+25.2%
fem-1(hc17); eat-2(ad465)						
AL	1	117	32.3 ± 0.5		39.3 ± 0.9	
DD	1	137	33.4 ± 0.6	+3.4%	40.4 ± 1.2	+2.8%
sir-2.1(ok434)						
AL	2	150	13.7 ± 0.2		15.6 ± 0.6	
	4	144	13.1 ± 0.2		15.6 ± 0.6	
DD	2	191	24.9 ± 0.9***	+81.8%	34.6 ± 7.0*	+121.8%
	4	192	22.9 ± 0.4***	+74.8%	27.5 ± 0.7***	+76.3%
daf-2(e1370)						
AL	5	100	26.4 ± 0.6		30.6 ± 1.6	
	10	73	27.3 ± 0.2		29.0 ± 0.0	
DD	5	62	35.7 ± 0.7***	+35.2%	40.0 ± 0.0*	+30.7%
	10	90	31.1 ± 0.7***	+13.9%	39.0 ± 1.0**	+34.5%
daf-16(mgDf50)						
AL	2	89	7.7 ± 0.1		8.0 ± 0.0	
	6	90	8.3 ± 0.2		9.0 ± 0.8	
DD	2	109	12.5 ± 0.3***	+62.3%	15.0 ± 0.0	+87.5%
	6	111	12.0 ± 0.3***	+11.6%	14.8 ± 0.2**	+64.4%

Data are represented as mean ± SEM.

*$P < 0.05$

**$P < 0.01$

***$P < 0.0001$ vs. AL counterparts.

P-value of mean lifespan calculated by one-way ANOVA and *post hoc* Fisher's test.

P-value of maximum lifespan calculated by Student's *t*-test.

AL, ad libitum; DD, dietary deprivation.

Table 3

Effect of reproduction on DD-induced longevity

Genotype	Initiating age (day)	*n*	Lifespan (days) mean	Change	Maximum	Change
fem-1(hc17)						
AL	0	85	13.2 ± 0.6		18.0 ± 0.0	
	2	80	15.2 ± 0.4		18.3 ± 0.3	
	4	89	14.5 ± 0.4		18.0 ± 0.0	
DD	0	81	9.3 ± 0.8***	−70.5%	16.7 ± 2.2	−7.2%
	2	96	20.3 ± 0.5***	+33.6%	26.0 ± 0.0***	+42.1%
	4	82	17.9 ± 0.7***	+23.4%	23.3 ± 1.3*	+29.4%
sir-2.1(ok434)						
AL	0	150	13.7 ± 0.2		15.6 ± 0.6	
DD	0	160	9.0 ± 0.3***	−34.3%	14.2 ± 0.5	−9.0%

Data are represented as mean ± SEM.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.0001$ vs. AL counterparts.

P-value of mean lifespan calculated by one-way ANOVA and *post hoc* Fisher's test.

P-value of maximum lifespan calculated by Student's *t*-test.

AL, ad libitum; DD, dietary deprivation.