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Lifespan extension in *Caenorhabditis elegans* by complete removal of food

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Summary

A partial reduction in food intake has been found to increase lifespan in many different organisms. We report here a new dietary restriction regimen in the nematode *Caenorhabditis elegans*, based on the standard agar plate lifespan assay, in which adult worms are maintained in the absence of a bacterial food source. These findings represent the first report in any organism of lifespan extension in response to prolonged starvation. Removal of bacterial food increases lifespan to a greater extent than partial reduction of food through a mechanism that is distinct from insulin/IGF-like signaling and the Sir2-family deacetylase, SIR-2.1. Removal of bacterial food also increases lifespan when initiated in postreproductive adults, suggesting that dietary restriction started during middle age can result in a substantial longevity benefit that is independent of reproduction.

Key words: caloric restriction; calorie restriction; dietary restriction; lifespan; stress resistance; worms.

Introduction

Dietary restriction (DR) is the only intervention shown to increase lifespan in yeast, worms, flies, and mammals. DR results in several physiological changes, which may contribute to its effects on longevity, including decreased activity of nutrient and growth factor responsive pathways, increased resistance to a variety of stresses, decreased translation and ribosome biogenesis, and increased autophagy (Weindruch & Walford, 1988; Masoro, 2005). However, despite many advances in the characterization of DR in a variety of model systems, the mechanism(s) by which DR increases lifespan is not well understood.

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The nematode *Caenorhabditis elegans* has proven to be an important model organism for aging-related research, providing a well-characterized paradigm for the role of insulin/IGF-1-like growth factor signaling (IIS) in aging. IIS also regulates the formation of Dauer larvae, an alternative developmental stage induced by high population density, temperature, and lack of food (Golden & Riddle, 1984; Gottlieb & Ruvkun, 1994). Several mutations in the IIS pathway that enhance Dauer formation also lead to increased lifespan (Vanfleteren & Braeckman, 1999). Components of this pathway that have been shown to regulate longevity in worms include the insulin-like receptor *daf-2* (Kenyon *et al*., 1993; Kimura *et al*., 1997), the PI-3 kinase *age-1* (Friedman & Johnson, 1988; Morris *et al*., 1996), proteins orthologous to Akt kinases (*akt-1*, *akt-2*, and *sgk-1*) (Hertweck *et al*., 2004; Hamilton *et al*., 2005; Oh *et al*., 2005), and the FOXO-family transcription factor *daf-16* (Lin *et al*., 1997; Ogg *et al*., 1997). Global gene expression profiling by microarray has further elucidated some of the downstream components of this pathway involved in antimicrobial, oxidative, and other stress responses (McElwee *et al*., 2003; Murphy *et al*., 2003). Additionally, two independent genome-wide RNAi screens have been carried out for genes that influence longevity in worms (Dillin *et al*., 2002; Lee *et al*., 2003; Hamilton *et al*., 2005; Hansen *et al*., 2005). Genes that increase lifespan when knocked down can be grouped into functional categories, the largest being genes important for mitochondrial function and genes involved in IIS.

Both genetic and environmental manipulations have been used to model DR in *C. elegans*, including mutations that decrease feeding rate (*eat* mutants) (Lakowski & Hekimi, 1998), growth in axenic culture (Vanfleteren & Braeckman, 1999), and dilution of the bacterial food source (Klass, 1977; Houthoofd *et al*., 2003). Lifespan extension by these methods does not require *daf-16* and is additive with longevity-enhancing alleles of *daf-2* (Lakowski & Hekimi, 1998; Houthoofd *et al*., 2003), suggesting that pathways other than IIS are likely to mediate the effect of DR. Although important findings have been made using different DR regimens in *C. elegans*, none have been adopted as a standard method for DR. Genetic mimics of DR, such as *eat-2*, could have pleiotropic effects unrelated to food consumption, which might influence experimental outcome. Axenic culture involves growth conditions differing substantially from the agar plate lifespan assay used by many laboratories, making it difficult to compare these DR studies with other interventions. An additional method for DR more closely related to the standard methodology would therefore be of value.

We have developed a novel DR protocol based on the standard agar plate-based lifespan assay. We report here the surprising finding that complete removal of the bacterial food source

during adulthood increases lifespan to a greater extent than partial reduction of food, with median and maximum lifespans reproducibly increased by approximately 50%. Lifespan extension in response to food deprivation is distinct from mutations affecting lifespan in the IIS pathway and independent of the sirtuin protein deacetylase *sir-2.1*. We believe that this is an important new model for genetic and mechanistic studies of DR in *C. elegans*.

Results

We developed a protocol for DR in *C. elegans* by first determining the effect on lifespan from feeding animals decreased amounts of bacterial food. We used killed [either by ultraviolet (UV) radiation or heat, see Experimental procedures] *Escherichia coli* rather than live *E. coli* for these experiments, because this method allows for more careful control of the amount of food placed on the plates, and because live bacterial food shortens lifespan independent of food concentration (Garigan *et al.*, 2002). Abundant UV-killed *E. coli* was fed to wild-type (N2) hermaphrodites during development from eggs to adulthood. Beginning on the second day of adulthood, worms were fed either a control diet of abundant food, or a diet reduced by a fixed percentage of the control diet, for the remainder of the worms' lifespan. Remarkably, the greatest increase in lifespan was observed in adult worms maintained in the complete absence of food, with a 50% increase in median and maximum lifespan relative to control-fed animals (Fig. 1A, $P = 6 \times 10^{-13}$). Other reduced bacterial feeding regimens tested, including a 90% reduction in UV-killed *E. coli* ($P = 1 \times 10^{-12}$), were less effective in increasing longevity. Starved worms appeared morphologically normal under a dissecting microscope, and showed no obvious signs of lethargy relative to fed worms, even after more than 2 weeks in the absence of bacterial food. We have termed the complete removal of food during adulthood dietary restriction through food deprivation (DR-FD).

To understand the effects of age and development on lifespan extension by DR in worms, we initiated DR-FD at different age-points. *C. elegans* undergo four larval stages during development from egg to adult. As expected, if transfer to media lacking bacterial food occurred prior to the fourth larval stage (L4), the worms' development was arrested and maturation was prevented (data not shown). If transfer occurred at L4, the worms showed only a modest increase in lifespan relative to control-fed animals (Fig. 1B, $P = 0.02$), and a substantially shorter lifespan than animals subjected to DR-FD starting at the second day of adulthood ($P = 5.5 \times 10^{-8}$). Transfer to media without food at the eighth day of adulthood resulted in significant lifespan increase relative to control fed animals (Fig. 1B, $P = 2 \times 10^{-6}$) and a lifespan not significantly different than animals transferred to DR-FD at the second day of adulthood ($P = 0.13$). Thus, onset of DR-FD at any point in early adulthood results in substantial lifespan extension.

DR increases lifespan and thermotolerance independent of IIS

We next considered the possibility that prolonged starvation could induce a Dauer-like state during adulthood, and that this results in longer lifespan. Since longevity-enhancing mutations in the IIS pathway, such as *daf-2(e1370)*, require functional *daf-16* for lifespan extension (Lin *et al.*, 1997; Ogg *et al.*, 1997), we tested whether lifespan extension from DR-FD also requires *daf-16* to slow aging. *Daf-16*(*m27*) mutants were short-lived relative to N2 animals in the presence of food ($P = 0.001$), but DR-FD significantly increased the median lifespan of *daf-16(m27)*

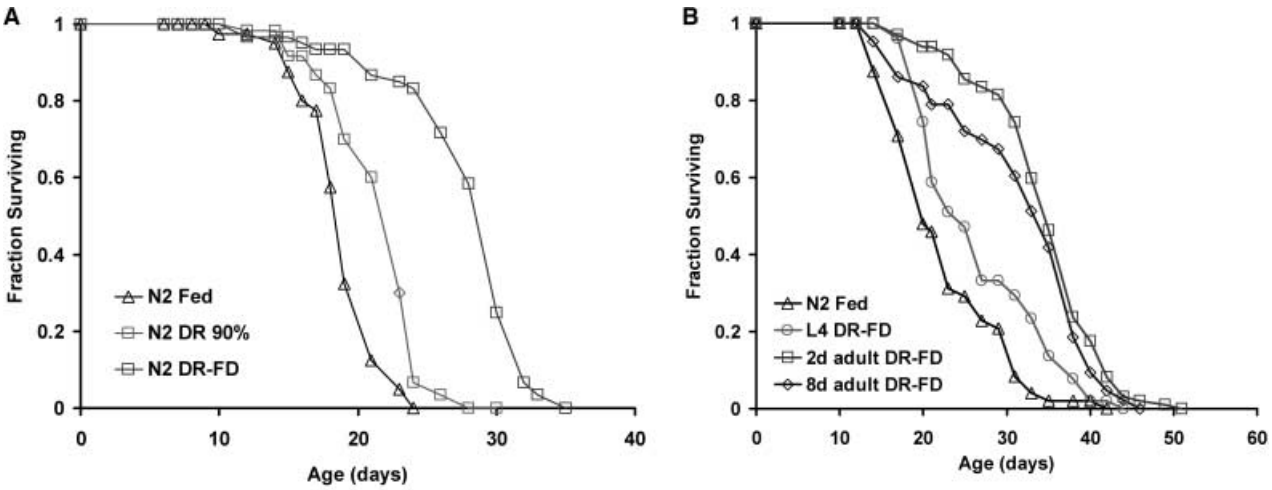

Fig. 1 Absence of bacterial food source during adulthood increases lifespan. (A) A 90% reduction in bacterial food (DR 90%, $n = 60$) increases the lifespan of adult N2 by approximately 20% relative to control fed (N2 Fed, $n = 40$) animals, and complete absence of food (N2 DR-FD, $n = 60$) increases lifespan by approximately 50%. Median lifespans: N2 Fed, 19 days; DR 90%, 23 days; N2 DR-FD, 30 days. (B) Lifespan extension from DR-FD is decreased when DR-FD is initiated at the L4 stage (L4 fed, $n = 48$, DR-FD, $n = 51$) compared to DR-FD initiated at the second day of adulthood (2d adult DR-FD, $n = 97$) or the eighth day of adulthood (8d adult DR-FD, $n = 43$). Median lifespans: N2 Fed, 20 days; L4 DR-FD, 23 days; 2d adult DR-FD, 35 days; 8d adult DR-FD, 35 days.

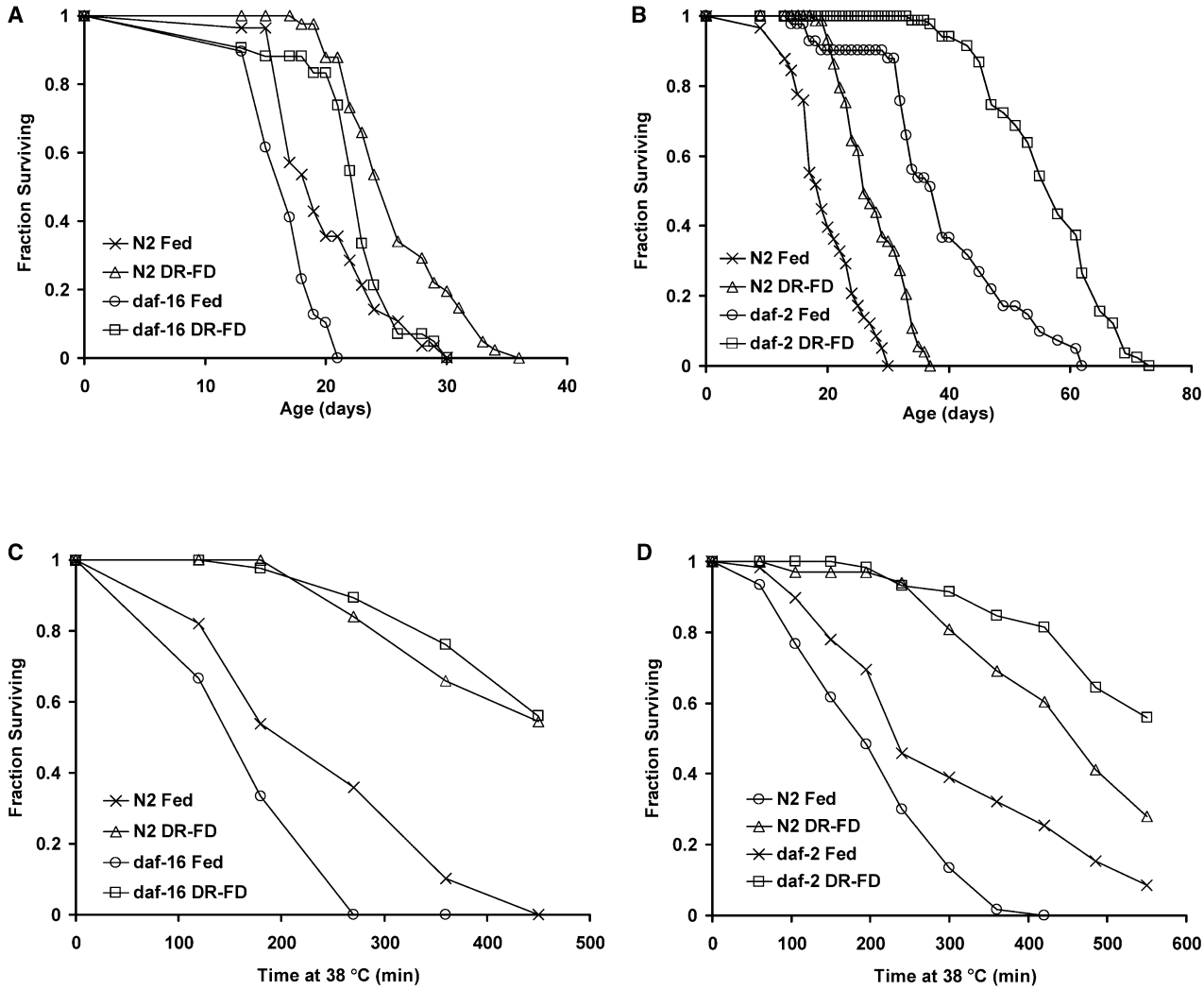

Fig. 2 DR-FD does not require *daf-16* and is additive with *daf-2*. (A) DR-FD increases the lifespan of *daf-16(m27)* mutants. Median lifespans: N2 Fed, 19 days; N2 DR-FD, 26 days; *daf-16* Fed, 16 days; *daf-16* DR-FD, 23 days. (B) DR-FD further increases the long lifespan of *daf-2(e1370)* mutants. Median lifespans: N2 Fed, 19 days; N2 DR-FD, 26 days; *daf-2* Fed, 39 days; *daf-2* DR-FD, 58 days. (C) DR-FD enhances thermotolerance of *daf-16(m27)* mutants. (D) DR-FD further enhances the thermotolerance of *daf-2(e1370)* mutants.

adults by approximately 35%, similar to the percentage increase seen in N2 animals (Fig. 2A, $P = 4 \times 10^{-9}$). Dauer constitutive loss of function mutations in *daf-2* increase lifespan by activating *daf-16* (Lin *et al*., 1997; Ogg *et al*., 1997). When fed a control diet, *daf-2(e1370)* animals had a lifespan 105% longer than control-fed N2 (Fig. 2B, $P = 1.4 \times 10^{-11}$). DR-FD further increased the lifespan of long-lived *daf-2(e1370)* mutants by 49% (Fig. 2B, $P = 1.5 \times 10^{-11}$), resulting in additive median and maximum lifespan extension exceeding 150%, relative to control-fed N2 animals. These findings are consistent with the conclusion that DR-FD increases lifespan through a mechanism distinct from reduced IIS.

In mammals, DR is associated with enhanced stress resistance (Masoro, 1998), and in *C. elegans* longevity-enhancing mutations often lead to stress resistance and thermotolerance (Lithgow *et al*., 1995). We therefore examined whether our DR-FD protocol induces thermotolerance in N2, *daf-16(m27)*, and *daf-2(e1370)* animals. Maintenance of N2 adults in the absence of food for 4 days resulted in a profound increase in survival at 38 °C (Fig. 2C,D, $P < 1 \times 10^{-16}$). This increased thermotolerance, similar to the longevity phenotype, did not require functional *daf-16* (Fig. 2C, $P < 1 \times 10^{-16}$) and was further enhanced by mutation of *daf-2* (Fig, 2D, $P < 1 \times 10^{-16}$). A similar increase in the survival of DR-FD animals at 36 °C was also observed (Fig. S1, Supplementary material; $P < 1 \times 10^{-16}$). These data further support the conclusion that DR acts through a mechanism different from IIS.

Mutation of *eat-2* does not further increase the lifespan of unfed worms

DR-FD is an extreme dietary regimen that might increase lifespan through a mechanism different than traditional DR models. In order to test this possibility, we measured the lifespan

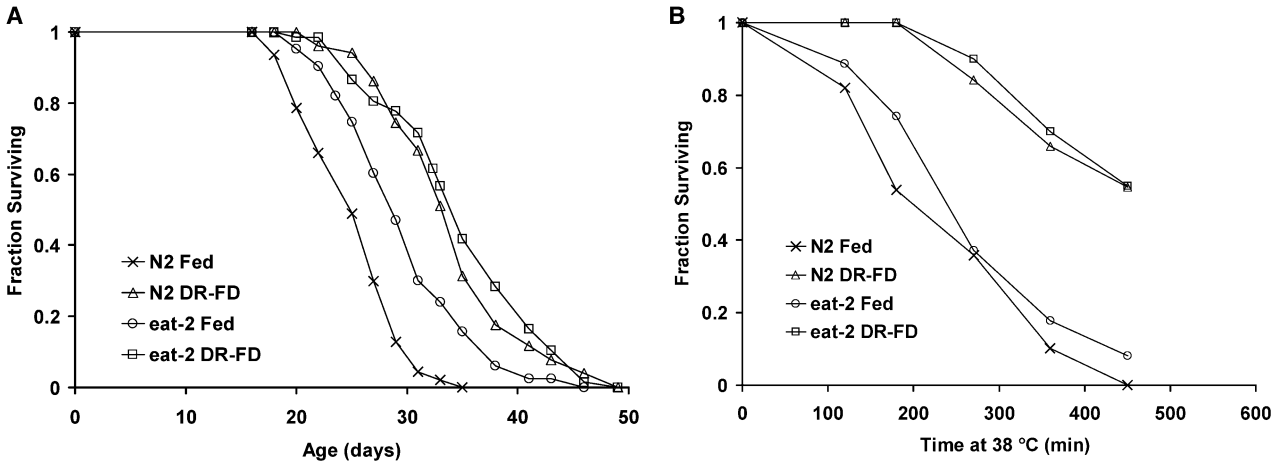

Fig. 3 DR-FD and mutation of eat-2 increase lifespan and thermotolerance by similar mechanisms. (A) DR-FD increases the lifespan of *eat-2(ad465)* mutants, but not beyond that of wild-type. Median lifespans: N2 Fed, 25 days; N2 DR-FD, 35 days; *eat-2* Fed, 29 days; *eat-2* DR-FD, 35 days. (B) DR-FD enhances thermotolerance of *eat-2(ad465)* to the same extent as wild-type.

of *eat-2(ad465)* mutants in the presence and absence of bacterial food. As previously reported (Lakowski & Hekimi, 1998), *eat-2(ad465)* animals had a lifespan significantly increased relative to N2 animals in the presence of food (Fig. 3A, $P = 1 \times 10^{-5}$). DR-FD further increased the lifespan of *eat-2(ad465)* animals by 21% ($P = 5 \times 10^{-6}$). The lifespan of unfed *eat-2(ad465)* animals was not significantly different than that of N2 animals subjected to DR-FD ($P = 0.45$), however. Similar to the longevity phenotype, *eat-2(ad465)* conferred a modest increase in thermotolerance in the presence of food, but not in animals subjected to DR-FD ($P = 0.64$). These data are consistent with the model that *eat-2(ad465)* and DR-FD affect longevity and thermotolerance by a similar mechanism, but *eat-2(ad465)* represents a less severe degree of DR relative to DR-FD.

Reduced fecundity is not necessary for lifespan extension from DR

In addition to its association with enhanced stress resistance, increased lifespan is often associated with reduced fecundity (Partridge *et al.*, 2005). In the nematode, fecundity correlates negatively with the amount of bacterial food source (Klass, 1977), leading us to test whether decreased reproduction might account for increased lifespan in response to DR-FD. N2 adults were maintained in the presence of food without 5-fluorodeoxyuridine (see Experimental procedures) and allowed to lay viable eggs. By the 10th day of adulthood all of the animals had ceased egg production, and half were transferred to media lacking food while the rest continued to be fed UV-killed bacteria. DR-FD significantly increased the lifespan of postreproductive hermaphrodites (Fig. 4, $P = 7.6 \times 10^{-7}$) to an extent comparable to that of DR-FD initiated at the second day of adulthood (compare to Figs 1–3, 5). This result indicates that decreased reproduction is not necessary for lifespan extension from DR-FD in worms.

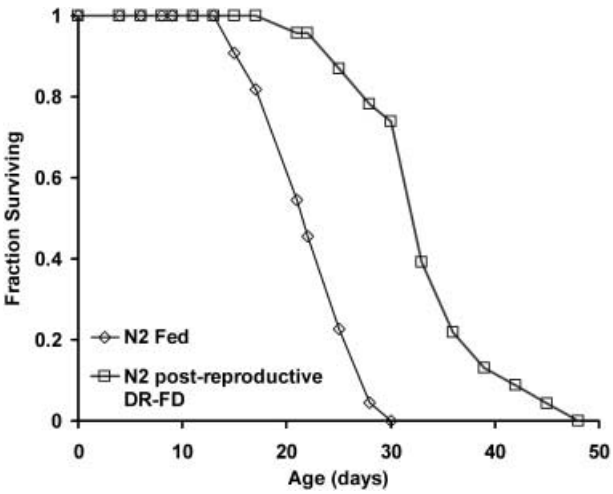

Fig. 4 Lifespan extension from DR-FD is independent of fecundity. DR-FD initiated at the 10th day of adulthood, after N2 hermaphrodites no longer produce eggs (N2 postreproductive DR-FD), increases lifespan by approximately 50% relative to control-fed (N2 Fed) animals. 5-Fluorodeoxyuridine was not added to the media. Median lifespans: N2 Fed, 22 days; N2 postreproductive DR-FD, 33 days.

DR-FD increases lifespan by a mechanism independent of *sir-2.1*

One model that has been proposed as an evolutionarily conserved mechanism for lifespan extension by DR is activation of the Sir2-family protein deacetylases (sirtuins) (Guarente & Picard, 2005), based on observations that elevated expression of sirtuins increases lifespan in yeast (Kaeberlein *et al.*, 1999), worms (Tissenbaum & Guarente, 2001), and flies (Rogina & Helfand, 2004). The role of sirtuins in aging and the putative link to DR has remained controversial, however, and in yeast there is evidence that lifespan extension by DR may not be mediated

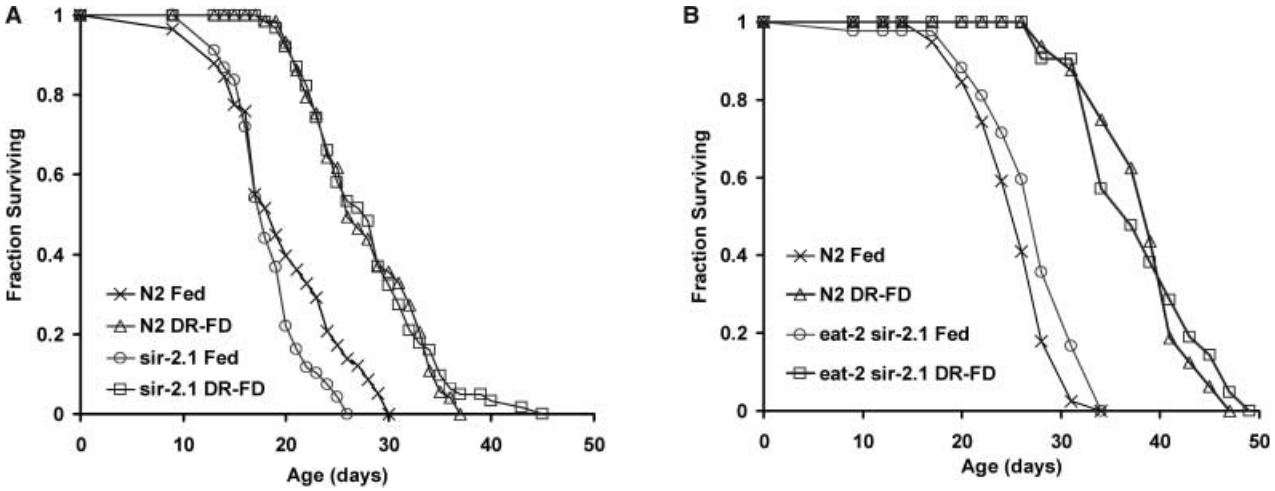

Fig. 5 Lifespan extension from DR-FD is independent of *sir-2.1*. (A) DR-FD increases the lifespan of *sir-2.1(ok434)* deletion mutants to the same extent as N2 animals. Median lifespans: N2 Fed, 19 days; N2 DR-FD, 26 days; *sir-2.1* Fed, 18 days; *sir-2.1* DR-FD, 28 days. (B) DR-FD increases the lifespan if *sir-2.1(ok434) eat-2(ad-465)* double mutants to the same extent as N2 animals. Median lifespans: N2 Fed, 26 days, N2 DR-FD, 39 days; *sir-2.1 eat-2*, 28 days; *sir-2.1 eat-2* DR-FD, 37 days.

by Sir2 (Kaeberlein *et al*., 2004, 2005a,b, 2006). In *C. elegans*, elevated expression of *sir-2.1* increases lifespan in a *daf-16*-dependent manner (Tissenbaum & Guarente, 2001), whereas deletion of *sir-2.1* slightly shortens lifespan and partially blocks lifespan extension resulting from a mutation in *eat-2* (Wang & Tissenbaum, 2006). To determine whether lifespan extension by DR-FD requires *sir-2.1*, we measured the lifespans of *sir-2.1(ok434)* animals, which carry a deletion allele of *sir-2.1*, in the presence and absence of food. As was the case for wild-type animals ($P = 1 \times 10^{-11}$), DR-FD significantly increased the lifespan of *sir-2.1(ok434)* animals by approximately 50% (Fig. 5A, $P < 10^{-16}$). DR-FD also significantly increased the lifespan of *eat-2 sir-2.1* double mutant animals relative to control-fed animals (Fig. 5B, $P = 3 \times 10^{-8}$). Under DR-FD conditions, there was no significant difference in the median lifespans of N2 and *eat-2 sir-2.1* double mutant animals ($P = 0.75$), consistent with the conclusion that *eat-2* and DR-FD act to increase lifespan via a similar mechanism, and that DR-mediated lifespan extension does not require *sir-2.1*.

Discussion

We have described a simple and reproducible method for DR in the nematode. By limiting the amount of bacterial food source provided to adult worms, we have determined that a complete removal of food during adulthood increases both median and maximum lifespan up to 50%. This lifespan extension is comparable in magnitude to lifespan extension from optimal DR in other organisms (Weindruch & Walford, 1988; Kaeberlein *et al*., 2004). This is the first demonstration, to our knowledge, that long-term starvation can result in lifespan extension in *C. elegans* or any other organism.

The importance of optimizing lifespan extension from DR by testing multiple food levels has been demonstrated in both flies and yeast (Clancy *et al*., 2002; Kaeberlein *et al*., 2005a). Our finding that reducing bacterial feeding by 90% gives rise to a lifespan intermediate between control-fed animals and DR-FD animals suggests that DR-FD may be an optimal method for lifespan extension from reduced bacterial feeding. Interpretation of this experiment is complicated somewhat by the fact that at our low bacterial concentrations (such as 90% DR in Fig. 1A) the worms are undergoing a cycle of feeding and fasting where food is abundant on the first day after transfer, but is exhausted prior to the next transfer to fresh bacterial plates. This type of feeding cycle could limit lifespan extension, although we know of no evidence supporting this possibility, and recent studies suggest that feeding-fasting cycles can increase lifespan and reduce disease in rodents (Mattson, 2005).

It is possible that animals subjected to DR-FD are obtaining nutritional value directly from the nematode growth medium (NGM) plates and that DR-FD is not complete starvation. In addition to agar, the NGM contains salt (3 g L^{-1}), cholesterol (5 mg L^{-1}), and peptone (2.5 g L^{-1}). Additional experiments will be required to determine whether longevity can be further modulated by altering the composition of the media, in the absence of bacterial food. However, the observation that DR-FD of *eat-2(ad465)* animals does not further increase lifespan beyond that of N2 animals under DR-FD supports the idea that DR-FD represents an optimal lifespan extension from DR in *C. elegans*, regardless of whether the animals obtain nutritional value from the NGM.

Our findings that DR-FD increases lifespan to a greater extent than partial reduction of bacterial food source differs from experiments reported by Houthoofd *et al*. (2003) using a reduced bacterial feeding protocol in liquid buffer. In this previous study, it was reported that the longest lifespan was observed at a bacterial concentration of 3×10^9 cells mL^{-1}. The difference is most likely due to the fact that the growth conditions

used by Houthoofd *et al*. (e.g. liquid culture and higher temperature) were suboptimal for reduced bacterial feeding longevity studies, as evidenced by the short lifespans they reported. The average lifespan of N2 animals at the optimal level of bacterial restriction using the method of Houthoofd *et al*. was 19 days, whereas our DR-FD method consistently results in an average lifespan between 30 and 35 days.

Prior observations have suggested that DR increases lifespan by a mechanism distinct from reduced IIS in *C. elegans*. Our observations that lifespan extension and enhanced thermotolerance in response to DR-FD does not require *daf-16*, and is additive with mutation of *daf-2*, are consistent with this hypothesis. An alternative possibility, however, is that DR-FD and reduced IIS act on shared targets, and the additive longevity increase observed when DR-FD is combined with *daf-2(e1370)*, for example, is a result of a more optimal change in the relevant downstream effectors. Further studies will be necessary to differentiate between these possibilities.

Sir2-family proteins have been proposed to mediate the longevity benefits of DR in a wide variety of organisms (Guarente & Picard, 2005). In *C. elegans*, increased expression of *sir-2.1* results in increased lifespan in a *daf-16*-dependent manner, suggesting that *sir-2.1* acts in the IIS pathway (Tissenbaum & Guarente, 2001). However, since DR increases lifespan independently of *daf-16* (Lakowski & Hekimi, 1998; Houthoofd *et al*., 2003), it has been proposed that *sir-2.1* and DR are genetically distinct in worms (Kaeberlein & Kennedy, 2005). Consistent with this hypothesis, we found that deletion of *sir-2.1* has no effect on lifespan extension by DR-FD. It is also of interest that deletion of *sir-2.1* does not shorten lifespan. Thus, lifespan extension from DR-FD cannot be explained as the result of enhanced *sir-2.1* activity or expression, and we find no evidence that *sir-2.1* plays a role in the normal aging process of *C. elegans*.

An important unresolved question in biogerontology is the relationship between reproduction and lifespan (Partridge *et al*., 2005). There are several examples of enhanced longevity in model organisms that correlate with reduced fecundity, leading to speculation that a trade-off exists between reproductive output and maintenance of the aging soma (Holliday, 1989). In mice, severe DR results in loss of fertility through a mechanism that may involve the insulin receptor substrate-2 (Burks *et al*., 2000). In flies, it has been proposed that a reduction in female reproduction accounts for lifespan extension from DR (Rauser *et al*., 2004); however, a direct causal link has yet to be established (Mair *et al*., 2003), and subsequent work has shown that DR can increase longevity in male flies (Magwere *et al*., 2004) or in sterile female flies (Mair *et al*., 2004). Our data suggest that in *C. elegans*, decreased reproduction is not necessary for increased lifespan from DR. Animals maintained on a full diet until they were postreproductive and then subjected to DR-FD had their lifespans extended by nearly the same extent as animals subjected to DR-FD from young adulthood. Thus, although DR results in decreased fecundity in worms, flies, and mice, in *C. elegans* at least, it appears that reduced fecundity is not mechanistically involved in enhanced longevity.

Because DR-FD equates to starvation during adulthood (assuming the contribution from the NGM agar is minimal), it is tempting to think that the mechanism of lifespan extension from DR-FD is likely to be dissimilar from traditional methods of DR. A similar line of reasoning might suggest that DR-FD is likely to be private to *C. elegans* and closely related species. We feel strongly that these conclusions are not supported by experimental evidence, however. In every case examined, DR-FD behaves as predicted if it were acting by a mechanism similar to the *eat-2(ad465)* allele. First, lifespan extension and enhanced thermotolerance from DR-FD is additive with *daf-2(e1370)*. Second, lifespan extension and enhanced thermotolerance from DR-FD is independent of *daf-16(m27)*. Third, the *eat-2(ad465)* allele fails to further increase the lifespan or thermotolerance of animals subjected to DR-FD. While it remains possible that traditional methods of DR, such as mutations in *eat-2* or axenic growth, are fundamentally different from DR-FD, all of the experimental evidence thus far suggests they result in similar phenotypic outcomes and act by a similar mechanism.

We also note evidence suggesting that the mechanism by which DR-FD acts may be conserved in organisms other than *C. elegans*. For example, the observation that DR-FD increases lifespan additively with mutation of *daf*-2 is reminiscent of the additive increase in lifespan seen when dwarf mice are subjected to DR (Bartke *et al*., 2001). Likewise, recent studies have demonstrated that DR increases lifespan independently of Sir2 in yeast (Kaeberlein *et al*., 2004, 2005a,b, 2006), and we find that DR-FD increases lifespan independently of *sir-2.1* in worms. Thus, there is reason to be optimistic that DR-FD, along with traditional methods of DR in *C. elegans*, increases longevity through an evolutionarily conserved mechanism. It is our hope that DR-FD will be of value in future efforts to characterize the genetic and biochemical mechanism(s) by which DR increases lifespan in the nematode, and that these findings may guide efforts to understand the relationship between diet and longevity in humans.

Experimental procedures

Caenorhabditis elegans were propagated at 20 °C on nematode growth medium (NGM) seeded with *E. coli* OP50. N2, *eat-2(ad465)*, *daf-2 (e1370)*, and *daf-16 (m27)* animals were provided by JHT. The *sir-2.1(ok434)* allele is a deletion of the *sir-2.1* gene provided by the Caenorhabditis Genetics Center. The *sir-2.1(ok434) eat-2(ad465)* animals were provided by H. Tissenbaum and have been previously described (Wang & Tissenbaum, 2006). The stock *E. coli* OP50 cultures used for seeding plates were lightly seeded from a single bacterial colony in liquid NGM and allowed to grow overnight at room temperature, then stored at 4 °C. Control plates (NGM agar in 50-mm diameter Petri dishes) were seeded with 200 μL of stock OP50, which were allowed to form a lawn overnight at room temperature. The bacterial food source for all experiments was UV-killed by a 30-min exposure to a UV source, with UV-killing

verified by failure to form colonies upon streaking to luria broth (LB) plates. Plates lacking food were similarly treated with UV.

Lifespan assays were initiated by allowing adult hermaphrodites to lay eggs on NGM containing UV-killed OP50. At L4, eggs were transferred to fresh NGM + UV-killed OP50 supplemented with 50 μM 5-fluorodeoxyuridine (FUDR) to prevent eggs from hatching. Unless otherwise stated, at the second day of adulthood animals were transferred to experimental media: either NGM + UV-killed OP50 +50 μM FUDR (Fed media) or NGM +50 μM FUDR (DR-FD media). For the experiment shown in Fig. 1(A), the DR 90% group was maintained on plates seeded with a 10-fold dilution (20 μL) of OP50 and UV-killed as described above. For the experiment shown in Fig. 1(B), the L4 DR group was transferred to DR-FD media during L4, and the 8d adult DR-FD group was maintained on Fed media until the eighth day of adulthood then transferred to DR-FD media. For the experiment shown in Fig. 4, animals were maintained on NGM + UV-killed OP50 without FUDR; at the 10th day of adulthood, the N2 postreproductive DR-FD group was transferred to NGM without UV-killed OP50 and without FUDR.

Fed animals were transferred to fresh plates every 3 days for the first 2 weeks of each lifespan experiment and then as necessary to prevent depletion of the food source. The viability of each animal was determined every 2–3 days by assaying for movement in response to agitation of the plate or gentle prodding. A Wilcoxon rank sum test was used (MATLAB 'ranksum' function) to determine whether median lifespan differed between groups and to calculate *P*-values. Number of animals, median lifespan, standard error of the mean, and percentage change in median lifespan are reported for data corresponding to each figure in Table S1 (Supplementary material). Every experiment was repeated at least two times with similar results.

Animals that crawled off of the NGM agar surface and failed to return (foraging) were not included in the data analysis. Up to half of the DR-FD animals showed this behavior in a given experiment, whereas, typically less than 5% of fed animals left the agar surface. Since foraging results in a selective loss of animals that are still alive, this loss is likely to result in an underrepresentation of the increase in lifespan in response to DR-FD. No animals that remained on the agar surface were censored from any experiment.

For the thermotolerance experiments shown in Figs 2 and 3, animals were maintained at 20 °C on either Fed or DR-FD media as described until the sixth day of adulthood (4 days without food for the DR-FD group). On the morning of the sixth day of adulthood, animals were transferred to a 38 °C incubator. Viability was assayed periodically by removing one plate at a time (~20 animals per plate) from the incubator and assaying for movement in response to gentle prodding.

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Supplementary Material

The following supplementary material is available for this article:

Fig. S1 Increase in thermotolerance at 36 °C caused by DR-FD.

Table S1 Statistics on survival data for all figures in the manuscript.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1474-9726.2006.00238.x

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