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Altered bacterial metabolism, not coenzyme Q content, is responsible for the lifespan extension in *Caenorhabditis elegans* fed an *Escherichia coli* diet lacking coenzyme Q

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Summary

Coenzyme Q_n is a fully substituted benzoquinone containing a polyisoprene tail of distinct numbers (n) of isoprene groups. *Caenorhabditis elegans* fed *Escherichia coli* devoid of Q_8 have a significant lifespan extension when compared to *C. elegans* fed a standard 'Q-replete' *E. coli* diet. Here we examine possible mechanisms for the lifespan extension caused by the Q-less *E. coli* diet. A bioassay for Q uptake shows that a water-soluble formulation of Q_{10} is effectively taken up by both *clk-1* mutant and wild-type nematodes, but does not reverse lifespan extension mediated by the Q-less *E. coli* diet, indicating that lifespan extension is not due to the absence of dietary Q per se. The enhanced longevity mediated by the Q-less *E. coli* diet cannot be attributed to dietary restriction, different Qn isoforms, reduced pathogenesis or slowed growth of the Q-less *E. coli*, and in fact requires *E. coli* viability. Q-less *E. coli* have defects in respiratory metabolism. *C. elegans* fed Q-replete *E. coli* mutants with similarly impaired respiratory metabolism due to defects in complex V also show a pronounced lifespan extension, although not as dramatic as those fed the respiratory deficient Q-less *E. coli* diet. The data suggest that feeding respiratory incompetent *E. coli*, whether Q-less or Q-replete, produces a robust life extension in wild-type *C. elegans*. We believe that the fermentation-based metabolism of the *E. coli* diet is an important parameter of *C. elegans* longevity.

Keywords

aging; *Caenorhabditis elegans*; clk-1; coenzyme Q or ubiquinone; dietary restriction; respiratory defective *Escherichia coli*

Introduction

Ubiquinone (coenzyme Q or Q) is found in both prokaryotes and eukaryotes where it functions as an essential component of the respiratory electron transport chain (Gennis & Stewart, 1996; Dutton *et al*., 2000). In addition to its role in respiration, Q is also found

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widely distributed throughout cellular membranes where in its reduced form it scavenges lipid peroxyl radicals (Bentinger *et al*., 2007). Q may also play a part in cell signaling as it acts as a pro-oxidant generating the superoxide free radical, which in turn undergoes dismutation and forms the important signaling molecule, hydrogen peroxide (Linnane *et al*., 2007). Based on its importance in respiration and as an antioxidant, Q is a popular dietary supplement and has shown promise as a therapeutic agent in treating neurodegenerative and cardiovascular disease (Galpern & Cudkowicz, 2007; Pepe *et al*., 2007).

Q is composed of a benzoquinone ring with a polyisoprenoid side chain of varying length. The length of the isoprenoid side chain varies among organisms. For example, *Escherichia coli* produce Q_8, *Caenorhabditis elegans* produce Q_9, and humans produce Q_{10} (Olson & Rudney, 1983). The length of the isoprenoid side chain in Q is determined by the specific long-chain polyisoprenyl diphosphate synthase in each organism (Kawamukai, 2002). The eukaryotic biosynthetic pathway has been elucidated primarily in *Saccharomyces cerevisiae*. In this organism, nine genes designated *COQ1–COQ9* are required for production of Q_6 (Tran & Clarke, 2007). Homologues of *COQ1–COQ8* have been identified in *C. elegans* and humans based on sequence homology. The yeast *coq* mutant strains fail to grow on media containing a nonfermentable carbon source, and this lack of growth is rescued by provision of exogenous Q_6 in the medium (Jonassen *et al*., 1998; Do *et al*., 2001; James *et al*., 2005). Restoration of growth of yeast *coq* mutants on medium containing a nonfermentable carbon source by supplementation with exogenous Q_6 constitutes strong evidence that the growth defect is caused solely by the absence of Q.

As would be expected from its essential role in respiratory metabolism, Q is required for normal development. The *C. elegans clk-1* mutant has a defect in Q_9 biosynthesis, accumulates the intermediate demethoxyubiquinone-9, and relies on the Q supplied via a diet of *E. coli* for development to adulthood and fertility (Jonassen *et al*., 2001; Miyadera *et al*., 2001; Burgess *et al*., 2003; Hihi *et al*., 2003). *clk-1* mutants fed *E. coli* strains lacking Q from the time of hatching stop growing during the L2 larval stage (Jonassen *et al*., 2001), and after about 1 week will eventually develop into sterile adults (Burgess *et al*., 2003). Similarly, *clk-1* dauer larvae fed the Q-less diet recover to adulthood but are completely sterile (Jonassen *et al*., 2001). The growth arrest and sterility phenotypes of the *clk-1* mutants fed Q-less *E. coli* are very reproducible and can be assessed quantitatively over a period of several days (Jonassen *et al*., 2001; Burgess *et al*., 2003). The *clk-1* mutants also experience growth arrest in axenic media (Braeckman *et al*., 1999). Provision of Q-replete *E. coli* rescues the growth on axenic media and also restores fertility in *clk-1* mutants developing from dauer larvae. Mice homozygous for a *clk-1* null mutation show embryonic lethality, lack Q_9, and accumulate demethoxyubiquinone-9 and are not rescued by dietary Q supplementation (Levavasseur *et al*., 2001; Nakai *et al*., 2001).

Caenorhabditis elegans serves as a powerful model system to identify genetic and environmental factors that influence aging (Houthoofd & Vanfleteren, 2007). For example, worms harboring mutations in the *daf-2* gene of the insulin/IGF-like signaling pathway exhibit a profound life extension and stress-resistant phenotypes that are dependent on the product of the *daf-16* gene, a FOXO-family transcription factor (Kenyon, 2005). Many other classes of genes modulating aging have been identified in *C. elegans* by RNAi screens (Hamilton *et al*., 2005). Dietary restriction extends *C. elegans* lifespan (Walker *et al*., 2005), as does treatment with a variety of drugs and natural products such as vitamin E (Collins *et al*., 2006), and plant extracts such as blueberry polyphenols (Wilson *et al*., 2006).

Several lines of evidence suggest that Q has a conserved function in aging in organisms ranging from worms to mammals. *clk-1* mutant worms fed the standard Q-replete OP50 *E. coli* live longer than wild-type animals (Lakowski & Hekimi, 1996; Ewbank *et al*., 1997)

and the Q acquired from their diet is present at decreased levels compared to wild-type worms (Jonassen *et al*., 2001). Wild-type worms fed an *E. coli* diet devoid of Q have an extended adult lifespan (Larsen & Clarke, 2002). The long-lived phenotype of worms fed the Q-less *E. coli* diet is independent of *daf-16* and augments the long life of *daf-2* mutants, *daf-2;daf-12* double mutants and even *clk-1* mutants, provided the Q-less diet is administered after the *clk-1* animals have reached the L4 larval stage of development (Larsen & Clarke, 2002). Heterozygous mice harboring one *clk-1* null allele show normal growth and reproduction, and although Q content in young mice is normal, a significant decline in liver Q content is observed with age (Liu *et al*., 2005). Intriguingly, *clk-1* heterozygous mice have a longer lifespan than their wild-type littermate controls (Liu *et al*., 2005). These observations led to the hypothesis that decreased intake of dietary Q in nematodes, or decreased *de novo* biosynthesis of Q in worms and mice, can cause lifespan extension. The mechanisms responsible for this lifespan extension are not known.

Here we describe a bioassay for the uptake and assimilation of added Q. Supplementation of plate or liquid growth media with NovaSOL® Q_{10} restores fertility of the *clk-1* mutants fed Q-less *E. coli*. We show both *clk-1* mutant and N2 wild-type nematodes transport this exogenous Q_{10} into their mitochondria. We then use this water-soluble Q formulation to test whether the lifespan extension produced by feeding the Q-less *E. coli* diet can be reversed. Surprisingly, supplementation of the Q-less *E. coli* diet with NovaSOL Q_{10} does not reverse the lifespan extension, indicating that other attributes of the *E. coli* 'Q-less' diet are responsible for the lifespan extension. The results presented here suggest that the respiratory deficiency of the *E. coli* diet can produce lifespan extension of wild-type *C. elegans.*

Results

Development of a bioassay for Q uptake

To evaluate the role of Q in modulating nematode lifespan, it is crucial to ascertain whether the Q provided exogenously is assimilated. *C. elegans clk-1* mutants fed Q-less *E. coli* (GD1; Table 1) show arrested or greatly slowed development and are sterile (Jonassen *et al*., 2001; Burgess *et al*., 2003). GD1 *E. coli* harbor a deletion of the *ubiG* gene, encoding an *O*-methyl-transferase in the coenzyme Q biosynthetic pathway, and fail to synthesize Q_8 (Hsu *et al*., 1996). Provision of Q-replete *E. coli* (e.g. OP50 or GD1/pAHG, harboring a plasmid expressing *ubiG*) rescues growth arrest and restores fertility. However, our initial attempts to rescue *clk-1* mutant sterility by adding either purified Q_{10} or Q_9 to plate or liquid media (from 0.5 to 50 μM final concentration added as a suspension in ethanol) failed to rescue sterility of either the *clk-1*(*qm30*) or the *clk-1*(*e2519*) mutants fed GD1. Various additives (present at the indicated final concentrations) were tested during the preparation of plate and liquid growth media to help solubilize or disperse the added Q, including Triton X-100 (1%), dimethylsulfoxide (2%), ethanol (1%), glycerol (1%), or Cremophor EL (1%) (Sigma-Aldrich, St Louis, MO, USA). In addition, a suspension of Q_{10} in Tween 80 was prepared as described (Ishii *et al*., 2004). While none of these additives appeared to harm development of N2 wild-type *C. elegans*, only a few progeny were sporadically produced by the *clk-1* mutants in response to these Q supplementations (data not shown).

It seemed possible that the lack of rescue might lie with the efficiency of the uptake of the exogenously provided Q. Micelles of the hydrophobic compound, vitamin E, significantly increased its short-term bioavailability in healthy human patients (Back *et al*., 2006). In addition, a recent study showed that the degree of *in vitro* dissolution of exogenous coenzyme Q presented in various formulations correlated well with the level of uptake by Caco-2 human intestinal cells (Bhagavan *et al*., 2007). Thus, nematode growth media (NGM) were supplemented with NovaSOL Q, a water-soluble formulation containing Q_{10} as a neutral lipid core component packaged in 30-nm-diameter micelles. The *clk-1* mutants

fed Q-less *E. coli* on media supplemented with Q_{10} in this manner grew to adulthood and were fertile (Fig. 1). Although the NovaSOL Q supplementation provides only a partial rescue of fertility, the brood size of the *clk-1(e2519)* and *clk-1(qm30)* mutants was similar to those of animals fed the standard Q_8-replete OP50 *E. coli* diet (Jonassen *et al*., 2001; Hihi *et al*., 2003). Plate media containing identical amounts of micelle particles but with medium-chain triglycerides as the neutral core component in place of Q_{10} (NovaSOL vehicle) failed to rescue growth arrest or restore fertility (Fig. 1). In order to determine the minimum amount of Q_{10} required to rescue the *clk-1* mutants from sterility, the growth medium was supplemented with decreasing amounts of NovaSOL Q. Supplementation with Q_{10} at concentrations as low as 10 μg mL^{-1} (11.6 μM) sustained the growth and fertility of *clk-1(e2519)* animals fed Q-less *E. coli* (data not shown).

Q_{10} supplementation fails to rescue the Q-less *E. coli* mutant

It was possible that the water-soluble formulation of Q_{10} also rescued the respiratory growth defect of the GD1 *E. coli*. In this event, the exogenous Q may rescue the *clk-1* mutants not necessarily by providing Q to the worms, but rather because the altered Q-less metabolism of the *E. coli* had been repaired. To determine whether this was the case serial dilutions of GD1 *E. coli* were plated onto succinate defined medium (SDM) plates containing succinate as a carbon source, where Q-dependent respiration is needed for growth. These plates were supplemented with either NovaSOL® Q or NovaSOL® vehicle control. As shown in Fig. 2(C and D), GD1 *E. coli* fail to grow on SDM under any of the conditions tested, indicating that the presence of the Q_{10} supplement fails to restore respiratory metabolism in the GD1 *E. coli* mutant. Because addition of Q_6 to liquid media with vigorous aeration restores growth of yeast *coq* mutants (Jonassen *et al*., 1998; Do *et al*., 2001; James *et al*., 2005), we tested whether NovaSOL Q or addition of Q_4, Q_6, Q_9, or Q_{10} would rescue growth of GD1 *E. coli* in liquid SDM. However, no rescue was observed (data not shown). In contrast, *clk-1* mutants fed AN120, a respiratory defective *E. coli* strain lacking complex V but with normal levels of Q_8 (Butlin *et al*., 1971), showed normal development and fertility (data not shown). These results identify Q as the essential component required for growth and fertility of the *clk-1* mutant, and indicate that defective respiration or altered metabolites in the Q-less *E. coli* diet are not responsible for the *clk-1* mutant growth arrest.

Caenorhabditis elegans mitochondria contain the exogenously provided Q_{10}

To determine the extent of uptake of the exogenously supplied Q_{10}, mitochondria were isolated as described in the Experimental procedures from N2 and *clk-1(qm30)* nematodes fed the Q-less *E. coli* supplemented with either NovaSOL Q or NovaSOL vehicle control. The *clk-1(qm30)* mutants cultured in liquid media with vehicle control remain sterile but eventually reach adulthood after 6–7 days in culture. Lipids were extracted from worm lysates and isolated mitochondria, and the quinone content was determined by reverse phase high-performance liquid chromatography (HPLC) with an electrochemical detector. Significant amounts of Q_{10} were present in samples of worm lysate and mitochondria prepared from animals grown in medium supplemented with Q_{10}, but were absent in mitochondria isolated from animals grown in medium supplemented with vehicle control (Fig. 3). While the majority of the exogenously supplied Q_{10} is found in the ‘crude mitochondria’ fraction, a small yet significant portion is present in the gradient purified mitochondria. Thus, both *clk-1* mutant and N2 wild-type nematodes are able to transport this exogenous Q_{10} into their mitochondria.

Q_{10} supplementation does not decrease lifespan of *C. elegans* fed a Q-less *E. coli* diet

After establishing the uptake of exogenous Q, we decided to use this water-soluble formulation to test whether the lifespan extension produced by feeding wild-type *C. elegans* the GD1 Q-less *E. coli* diet can be reversed. Adult *C. elegans* fed a Q-less *E. coli* diet show

significant lifespan extension compared to worms fed a Q_8-replete *E. coli* diet (Larsen & Clarke, 2002). This result implies that the supply of external Q_8 from *E. coli* shortens worm lifespan. If Q is indeed the critical component affecting nematode lifespan, then supplementation of the GD1 Q-less *E. coli* diet with exogenously added Q would be expected to restore the short lifespan. To identify whether Q is the missing component responsible for the lifespan extension observed with the Q-less diet, we assayed lifespan with adult N2 worms fed the GD1 Q-less diet on NGM plates supplemented with 150 μg NovaSOL Q_{10} per milliliter. N2 worms were fed OP50 or GD1 with or without addition of NovaSOL Q_{10}. A vehicle control with a neutral core containing medium-chain triglycerides instead of Q_{10} was also tested. Surprisingly, the NovaSOL Q_{10} supplement failed to restore the short lifespan (Fig. 4 and Table 2). In fact, both NovaSOL Q_{10} and the vehicle control supplement produced a slight but significant lifespan extension. Results were similar when N2 worms were fed the vehicle or Q_{10}-supplemented diets for either one or two generations (data not shown). The data indicate that the lack of Q is not the critical factor responsible for lifespan extension mediated by the *E. coli* GD1 diet.

Diets containing Q_8, Q_9, Q_{10} isoforms do not influence worm lifespan

Differences in polyisoprenoid tail length of Q_n isoforms among various species are well known. *C. elegans* produce Q_9 while *E. coli* synthesize Q_8. It is important to determine whether the endogenous Q_8 isoform produced by *E. coli* might be the dietary component detrimental to lifespan. To determine this we measured the adult lifespan of N2 worms fed *E. coli* engineered to produce Q_7, Q_8, Q_9 or Q_{10} (Table 1). The designated Q_n isoform is the predominant species in each of these *E. coli* strains (Okada *et al*., 1996, 1997, 1998; Jonassen *et al*., 2003). In order to determine whether the Q isoforms produced might be altered following the extended incubations required for lifespan determination, we determined the quinone content for each of these *E. coli* strains recovered from NGM plates following incubation conditions that simulate a lifespan experiment. As shown in Table 3, the predominance of the major Q isoforms was not affected. N2 worms fed Q_8-, Q_9-, or Q_{10}-producing *E. coli* showed no significant difference in lifespan when compared to worms fed the standard OP50 diet (Fig. 5 and Table 2). A modest but significant increase in lifespan was observed in N2 worms fed Q_7. These results indicate that the shorter lifespan of N2 worms fed OP50 cannot be attributed to the ‘unnatural’ Q_8 isoform provided by their *E. coli* diet.

Caenorhabditis elegans consume Q-replete OP50 and Q-less GD1 *E. coli* at identical rates

To investigate whether *C. elegans* fed the *E. coli* GD1 diet experience dietary restriction, we measured the rate of consumption of OP50 or GD1 *E. coli* by N2 adult worms. There was no discernable difference in the rates of bacteria consumed by N2 worms (Fig. 6), suggesting that differences in the food consumption rate do not account for the effects on worm lifespan. Previous studies have shown that lifespan extension afforded by dietary restriction in nematodes is associated with low brood size (Klass, 1977; Bishop & Guarente, 2007). Since the brood size of N2 worms fed either the GD1 or OP50 *E. coli* diets are not significantly different (Jonassen *et al*., 2001), it seems unlikely that the worms fed the GD1 *E. coli* diet are experiencing a dietary restriction.

A diet of nonproliferating *E. coli* produces a lifespan extension greater than that observed with a diet of proliferating *E. coli*, whether Q-replete or Q-less

The Q-less GD1 *E. coli* grow more slowly than either Q-replete OP50 or the rescued strain, GD1/pAHG (Fig. 2). It was previously reported that N2 worms fed nonproliferating OP50 treated with antibiotics live longer than worms fed proliferating OP50 (Garigan *et al*., 2002; Walker *et al*., 2005). Indeed, N2 worms fed OP50 *E. coli* treated with ampicillin (at bacteriostatic concentrations), showed 23% life extension compared to worms fed the

standard OP50 diet (Fig. 7A and Table 2). Based on these results, we hypothesized that the longer lifespan resulting from the GD1 diet might be attributed to the slow growth of the Q-less mutant strain. However, N2 worms fed ampicillin-treated GD1 *E. coli* showed approximately 16% lifespan extension over worms fed either untreated GD1, or GD1 *E. coli* harboring an ampicillin resistance plasmid (Fig. 7B and Table 2). These results suggest that it is unlikely that the lifespan extension can be attributed solely to the slower growth of the GD1 *E. coli*, or is due to differences in bacterial cell density provided on the plate growth media.

Ultraviolet irradiation destroys the GD1-mediated lifespan extension

Because N2 worms fed nondividing OP50 showed a lifespan extension (Gems & Riddle, 2000; Garigan *et al*., 2002; Walker *et al*., 2005), we anticipated that a bactericidal treatment such as ultraviolet (UV) irradiation would also enhance lifespan. However, N2 worms fed UV-treated GD1 showed significantly shorter lifespan than the worms fed untreated GD1 (Fig. 8 and Table 2). The UV treatment completely prevents bacteria from forming colonies when transferred to a new plate, whereas the ampicillin treatment inhibits bacterial growth but allows recovery upon transfer to a media without antibiotic. This indicated that the UV treatment might be destroying a beneficial component present in the GD1 *E. coli*. Additionally, factors produced by metabolically active GD1 *E. coli* may be important for the lifespan extension mediated by the GD1 diet.

N2 worms fed ATP synthase deficient bacteria have extended lifespan

An obvious metabolic difference between GD1 and OP50 *E. coli* is the severely compromised respiratory metabolism in GD1 due to the lack of Q_8. For example, the GD1 *E. coli* fail to grow on minimal media containing succinate as sole carbon source, and this trait is not rescued by the provision of NovaSOL Q_{10} (Fig. 2). To elucidate whether the respiratory defective metabolism of *E. coli* modulates worm lifespan, we decided to test whether providing another strain of *E. coli* unable to grow on succinate might extend worm lifespan. The respiratory electron transport chain of *E. coli* has multiple oxidoreductases that use Q as electron acceptor and multiple terminal electron acceptors that utilize QH_2 as electron donor (Gennis & Stewart, 1996). Because of the versatile respiratory metabolism in *E. coli*, it is difficult to ‘knock out’ respiration per se. Thus, we decided to assay lifespan in worms fed an ATP synthase (complex V) *E. coli* mutant. Although the respiratory chain is intact in this mutant, it cannot utilize the proton motive force generated by respiration and hence is unable to grow on succinate (Butlin *et al*., 1971). Therefore, we fed two independently derived ATP synthase mutants: AN120, which harbors an S373F mutation in *atpA* (*uncA*) encoding the ATP synthase alpha-subunit (Maggio *et al*., 1987); and the 1100 Δbc strain, in which the entire ATP synthase operon has been deleted (Klionsky *et al*., 1983; Stack & Cain, 1994). Importantly, the AN120 mutant has been shown to have similar levels of Q_8 as compared to its parental strain, AN180 (Butlin *et al*., 1971). These ATP synthase defective mutants, or the corresponding parental strains were provided as *E. coli* diets. N2 worms fed the AN120 *atpA* mutant showed 34.9% and 29.7% longer lifespan than N2 fed OP50, or AN180, respectively (Fig. 9 and Table 2). Brood sizes of N2 fed OP50, AN120, or AN180 are not significantly different (306 ± 52, 327 ± 37, and 301 ± 29, respectively; n = 12 for each condition), indicating that this effect is probably not due to dietary restriction. Similarly, N2 worms fed the 1100 Δbc *E. coli* mutant also showed longer lifespans than the worms fed OP50 or 1100, the parental strain of 1100 Δbc (Table 2). Although the lifespan of worms fed either *E. coli* ATP synthase mutant is significantly longer, the degree of the lifespan extension is smaller than that produced by the GD1 diet. This result suggests that although the impaired respiratory metabolism of GD1 appears to be an important factor, additional attributes may be required for the lifespan extension observed with the GD1 diet.

Discussion

A water-soluble formulation of Q_{10} is assimilated by nematodes but not *E. coli*

This study shows that a water-soluble formulation of Q_{10}, NovaSOL Q, is able to rescue the growth defect and restore fertility of the *C. elegans clk-1* mutant. NovaSOL Q contains a neutral lipid core of Q_{10} encapsulated in 30-nm-diameter micelles that readily disperse in aqueous solutions. This enhanced dispersion into micelles is most likely responsible for the cellular uptake of Q_{10} observed in *C. elegans* cultured on medium containing NovaSOL Q, because addition of purified Q to media with a variety of non-ionic detergents such as Triton X-100 failed to rescue the *clk-1* mutants. Micelles of Triton X-100 are about 8.8 nm in diameter (Kumbhakar *et al*., 2004), and may accommodate Q_{10} as a neutral lipid core component less efficiently. Although the NovaSOL Q was able to rescue the sterility and growth arrest of *clk-1* nematodes, it did not restore growth of the Q-less GD1 *E. coli* mutant on media containing succinate. This result identifies Q as the essential component required for growth and fertility of the *clk-1* mutant, and indicates that defective respiration or altered metabolites in the Q-less *E. coli* diet are not responsible for the *clk-1* mutant growth arrest.

Relatively small amounts of exogenous coenzyme Q_{10} in the *clk-1* mitochondria allowed rescue of growth arrest and sterility normally observed with a Q-less *E. coli* diet. Previously, it was shown that the small amounts of coenzyme Q_8 assimilated by *clk-1* animals fed the standard OP50 *E. coli* diet are sufficient to allow for growth and reproduction (Jonassen *et al*., 2002). The maternal effect rescue of *clk-1* phenotypes is attributed to the small contribution of maternally supplied Q in the egg (Burgess *et al*., 2003). In addition, tRNA suppressors present in the *clk-1(e2519)* genetic background supplied only a low level of Q_9 biosynthesis, yet allowed these animals to have brood sizes similar to wild type, and many of the suppressed mutants also had normal lifespans (Branicky *et al*., 2006). Thus, it is evident that amounts of Q much lower than normal can nonetheless function to rescue the *clk-1* mutant growth, fertility and lifespan phenotypes.

Lifespan extension mediated by the GD1 *E. coli* diet is not due to the absence of Q

We report the surprising finding that the long lifespan of *C. elegans* fed the GD1 Q-less *E. coli* diet is in fact not dependent on the absence of Q per se. Supplementation of the Q-less *E. coli* diet with NovaSOL® Q, a water-soluble micelle-based formulation of Q_{10}, did not rescue the long lifespan. Two lines of evidence indicate that the Q_{10} in the NovaSOL Q supplement is taken up by the worms: (i) when added to the GD1 *E. coli* diet this Q_{10} formulation rescues both *clk-1* mutant growth arrest and sterility (Fig. 1); and (ii) the Q_{10} provided is readily detected in isolated mitochondria prepared from either N2 or *clk-1* mutant animals (Fig. 3). We then suspected that the shorter lifespan of N2 worms fed the standard Q_8-replete *E. coli* diet might be due to detrimental effects of the shorter Q_8 isoform, since *C. elegans* normally produce Q_9. However, this was not the case as N2 worms fed a diet of *E. coli* engineered to produce Q_8, Q_9, or Q_{10} isoforms showed lifespans that were no different from the standard Q_8-replete OP50 diet (Fig. 5). These results indicate that the shorter lifespan of N2 worms fed a Q-replete *E. coli* diet cannot be attributed to the content of exogenously supplied Q.

Therefore, other attributes of the Q-less *E. coli* diet must be responsible for the lifespan extension. We considered the possibility that *C. elegans* fed the GD1 *E. coli* diet may experience dietary restriction, which is known to extend *C. elegans* lifespan (Klass, 1977; Bishop & Guarente, 2007). However, N2 young adult worms consume Q-replete and Q-less *E. coli* at equivalent rates (Fig. 3). Moreover, the brood size of N2 worms fed the GD1 *E. coli* diet is the same as worms fed the Q-replete OP50 *E. coli* diet (Jonassen *et al*., 2001). In contrast, dietary restriction regimes are known to decrease *C. elegans* brood size (Klass,

1977; Bishop & Guarente, 2007). Based on these observations, it seems unlikely that the GD1 *E. coli* diet is imposing a dietary restriction, although the experiments presented here do not rule this out. The slow growth of the GD1 *E. coli* might be another potential factor enhancing lifespan, because antibiotic treatment or UV irradiation of Q-replete *E. coli* diets are known to extend N2 worm lifespan (Gems & Riddle, 2000; Garigan *et al*., 2002; Walker *et al*., 2005). The lifespan extension produced by these treatments is thought to result from a decreased toxicity of *E. coli* in the worm intestine, although the exact mechanism accounting for the phenomenon is still unclear. Intriguingly, antibiotic treatment of the GD1 *E. coli* diet further augmented *C. elegans* lifespan, whereas UV irradiation of the GD1 *E. coli* shortened *C. elegans* lifespan (Figs 7 and 8). The opposing effects of these two treatments suggested that the slow growth of GD1 *E. coli* was not the important parameter. The abrogation of lifespan extension by UV treatment of GD1 *E. coli* suggests that the UV irradiation destroyed a beneficial component and/or the metabolic activity of viable GD1 *E. coli* is important.

E. coli respiratory metabolism modulates *C. elegans* lifespan

Wild-type *C. elegans* fed another respiratory deficient strain of *E. coli*, an ATP synthase mutant, have significantly longer lifespan than worms fed the standard *E. coli* diet. The ATP synthase mutant fails to grow on succinate media and is respiratory defective, although it has an intact respiratory chain, with normal content of cytochromes and Q_8 (Butlin *et al*., 1971). We propose that one or more metabolites produced by the respiratory defective *E. coli* enhance *C. elegans* lifespan. It is also possible that respiratory competent *E. coli* produce detrimental metabolites. It is important to note that the lifespan extension provided by axenic media is abrogated by metabolically active, but not by killed, *E. coli* (Lenaerts, I., Walker, G., Van Hoorebeke, L., Gems, D., and Vanfleteren, J., personal communication). Intriguingly, this abrogation required a threshold concentration of *E. coli*. The changes in worm lifespan in response to decreased *E. coli* density could be interpreted as a form of dietary restriction. However, the findings presented here suggest that *E. coli* metabolism itself may modulate nematode lifespan. Thus, the physiology of *E. coli* cultured at high density, rather than *E. coli* 'Calories' per se, may be an important parameter modulating worm lifespan. The respiratory incompetent *E. coli* strains used here may either lack products normally produced by respiratory competent *E. coli* strains, or may produce fermentation products such as acetate, ethanol, lactate, formate and succinate (Bock & Sawers, 1996), that impact *C. elegans* metabolism and, hence, lifespan. This may be a phenomenon specific to *C. elegans* since feeding respiratory incompetent strains of *S. cerevisiae* (both Q-less and Q-replete) to *Drosophila* shortened lifespan as compared to the respiratory competent parental yeast strain (Palmer & Sackton, 2003). Unlike the variety of acids produced by respiratory incompetent *E. coli*, ethanol is the primary fermentation product of respiratory incompetent *S. cerevisiae*.

Q biosynthesis vs. uptake – phenotypes and pharmacology

A growing body of evidence indicates that manipulation of Q content via endogenous synthesis as opposed to dietary intervention produce dramatically different outcomes and may be in part related to intra- and extracellular systems of Q trafficking. Intriguingly, both *C. elegans* and mice heterozygous for a *clk-1* gene disruption show increased lifespan (Liu *et al*., 2005). It is tempting to attribute the enhanced longevity to the decreased levels of endogenously produced Q in these two models. In contrast, although uptake was not monitored, addition of Q and Tween 80 to the growth medium extends lifespan of both wild-type and *mev-1* mutants (Ishii *et al*., 2004). *C. elegans mev-1* mutants suffer from a defect in respiratory Complex II and are hypersensitive to oxidative stress (Ishii *et al*., 1998; Senoo-Matsuda *et al*., 2001). Dietary Q supplementation in this manner also decreases superoxide anion levels in *mev-1* mutants and affects the localization of DAF-16 in both *mev-1* mutants

and in *gas-1* mutants (Kondo *et al*., 2005). *C. elegans gas-1* mutants have a defect in respiratory Complex I (Kayser *et al*., 1999). DAF-16 is normally present in the cytoplasm and moves to the nucleus in response to environmental stressors such as oxidative stress (Henderson & Johnson, 2001). These data suggest that exogenous Q counteracts the stress caused by the *gas-1* and *mev-1* mutations. How can these findings be reconciled with the results presented here? Since Q_{10} is a very hydrophobic molecule, it is likely that the mode of presentation is of critical importance in the experimental outcome. A recent review of pharmacological interventions in *C. elegans* suggests that the variable lifespan effects produced by treatment with superoxide dismutase (SOD) mimetics may be highly sensitive to dosage, uptake, and to conditions of culture, including the degree of imposed stress (Collins *et al*., 2006). Effects of Q supplementation also depend on the species; lifespan of mice fed diets supplemented with Q_{10} showed no alteration (Sohal *et al*., 2006).

The mechanism of Q uptake remains unclear. In *C. elegans* it seems likely that the exogenous Q is ingested, absorbed via the gut, and distributed to the gonad in order to restore fertility. Intracellular transport of exogenous Q in HL-60 cells is sensitive to inhibition by brefeldin-A and requires the endomembrane system (Fernandez-Ayala *et al*., 2005). It is possible that in *C. elegans* cellular uptake of Q relies on this same system. The yolk proteins (vitellogenins) and their receptor, RME-2, have been shown to be important for the proper uptake and transport of cholesterol in *C. elegans* (Matyash *et al*., 2001). Vitellogenins are generated in the intestine, secreted into the body cavity, and then taken up by oocytes in adult hermaphrodites (Kimble & Sharrock, 1983). Q may also bind to the vitellogenins as it is transported from the gut throughout the body of the animal. Once Q is taken into cells it must be assimilated within a variety of organelles including the mitochondrial inner membrane in order to function in respiration. The *C. elegans* homolog of the yeast Coq10p could be involved in its proper delivery to the mitochondria respiratory transport complexes (Barros *et al*., 2005). Providing exogenous Q via the NovaSOL formulation will facilitate further studies elucidating the mechanism of uptake and mode of intracellular transport of Q in *C. elegans*.

Experimental procedures

Culture conditions and strains

Caenorhabditis elegans strains used were the wild-type control N2 (Bristol strain) (Brenner, 1974), and the *clk-1* mutant strains CB4876 *clk-1(e2519)* III and MQ130 *clk-1(qm30)* III (Wong *et al*., 1995). *C. elegans* were cultured in S medium (liquid) [0.1 M sodium chloride, 0.05 M potassium phosphate (pH 6), 5 μg mL^{-1} cholesterol, 0.01 M potassium citrate (pH 6), 3 mM calcium chloride, 3 mM magnesium sulfate, 50 μM EDTA, 25 μM ferrous sulfate, 10 μM manganese chloride, 10 μM zinc sulfate, 1 μM copper sulfate] or on standard NGM plates with various bacteria food sources (Sulston & Hodgkin, 1988).

Escherichia coli strains used in this study are listed in Table 1. The Q-less strain, GD1 (*ubiG*::Kan, *zei*::Tn*10*dTet) (Hsu *et al*., 1996), contains a deletion of the *ubiG* gene required for Q biosynthesis. Succinate defined medium (SDM) contained 0.5% casamino acids supplement and was prepared as described (Poole *et al*., 1989). *E. coli* strains harboring antibiotic resistance were streaked on NGM plates with corresponding antibiotics (e.g. 50 μg mL^{-1} kanamycin and 100 μg mL^{-1} ampicillin). The water-soluble 22% coenzyme Q_{10} solubilisate and vehicle control, hereafter referred to as NovaSOL Q and NovaSOL vehicle control, respectively, were kindly provided by AQUANOVA AG (Darmstadt, Germany).

Escherichia coli growth assay

GD1 and GD1:pAHG, which harbors the *E. coli ubiG* gene on a plasmid and produces Q_8 (Hsu *et al*., 1996), were inoculated in liquid Luria-Bertani and grown overnight. Serial dilutions were plated onto Luria-Bertani, SDM alone, SDM supplemented with NovaSOL Q, and SDM supplemented with NovaSOL vehicle control. NovaSOL Q was added to the SDM at a concentration of 682 μg solubilisate/milliliter medium to achieve a final Q_{10} concentration of 150 μg mL^{-1}. NovaSOL vehicle control was added at a concentration of 532 μg solubilisate/milliliter medium. *E. coli* growth was assessed after an overnight incubation at 37 °C. All media in which the GD1 and GD1:pAHG strains were cultured contained 50 μg mL^{-1} kanamycin.

Brood size determination

Eggs were isolated from gravid N2, *clk-1(e2519)*, and *clk-1(qm30)* worms with an alkaline hypochlorite treatment as described (Sulston & Hodgkin, 1988). The eggs were placed on NGM supplemented with either NovaSOL Q or NovaSOL vehicle control. The concentrations of the NovaSOL Q and NovaSOL vehicle control were the same as those used in the supplemented SDM plates. All plates contained a lawn of GD1. Individual worms that developed to the L4 larval stage were transferred daily to fresh plates, and the number of eggs laid by each worm was recorded.

Mitochondria purification

Mitochondria were isolated from young adult worms as described (Curran *et al*., 2004). L1 larvae were prepared by treating gravid adult nematodes with sodium hypochlorite, rinsing the liberated eggs, and allowing the eggs to hatch overnight in S medium without *E. coli.* Fresh cultures were then started with starved L1 larvae from N2 or *clk-1(qm30)* at 2000 larvae/milliliter of S medium supplemented with either NovaSOL Q or with NovaSOL vehicle control at the concentrations stated above. These nematodes were fed GD1 and allowed to develop into young adults at 20 °C. The animals were cleaned via sucrose floatation and suspended in STEG buffer [250 mM sucrose, 5 mM Tris-HCl (pH 7.4) and 1 mM EGTA] containing 1 mM PMSF and protease inhibitor mixture (PIC, Roche Diagnostics, Indianapolis, IN, USA). The worm suspension was homogenized with a Kontes ground glass tissue grinder, and unbroken worm bodies were removed by centrifugation at 750 *g* for 10 min. An aliquot of the 750 *g* supernatant was saved as the ‘total lysate’, and the remainder was then centrifuged at 12 000 *g* for 10 min. An aliquot of the 12 000 *g* supernatant was saved as the ‘post mitochondrial supernatant’. The 12 000 *g* pellet was resuspended in STEG buffer without protease inhibitors, and centrifuged at 750 *g* for 10 min, and the supernatant centrifuged at 12 000 *g* for 10 min. A portion of this pellet was saved as ‘crude mitochondria’.

An enriched mitochondrial fraction, which we designated ‘pure mitochondria’, was generated by resuspending the remaining ‘crude mitochondria’ and loading onto a discontinuous sucrose gradient composed of layers of 1.2 M, 1.3 M, 1.6 M, and 1.8 M solutions of sucrose. This gradient was centrifuged at 25 000 *g* for 90 min, and the band migrating at the 1.3 M and 1.6 M sucrose interface was resuspended in STEG buffer and saved as ‘pure mitochondria’. All worm lysate fractions were stored at −80 °C until use. Protein concentrations were measured by the bicinchoninic acid assay (Pierce, Rockford, IL, USA). Enzyme assays were performed as described (Jonassen *et al*., 2003). The enzymatic rates reported were calculated by subtracting signal produced in the absence of substrate from the signal produced when substrate was present at various time points. The band migrating at the 1.3 M and 1.6 M sucrose interface was enriched in succinate-cytochrome *c* reductase (mitochondria-specific) activity, while the mannosidase II (Golgi-specific) and

glucose 6-phosphatase (endoplasmic reticulum-specific) activities in this band were lower than those present in the 'crude mitochondria' pellet (Table 4).

Isolation and quantification of quinones

Aliquots of the total lysate, post mitochondrial supernatant, crude mitochondria, and pure mitochondria from N2 and *clk-1(qm30)* worms were subjected to lipid extraction and analyzed for Q content (Jonassen *et al*., 2002). Q_6 was used to gauge quinone recovery in the lipid extracts and was added to all standards and worm lysate samples. Standards and samples were extracted by adding 0.5 mL water, 9 mL methanol, and 6 mL petroleum ether to each. The mixtures were vortexed for 1 min, centrifuged at 910 *g*, and the top layer of petroleum ether was removed from each mixture and saved in a separate vial. Fresh petroleum ether (6 mL) was added to each vial containing the aqueous phase and vortexed for 1 min The vials were subjected to centrifugation as before and the second petroleum ether layer removed. The process was repeated once more, and the three pooled petroleum ether fractions were dried under nitrogen and resuspended in 150 μL methanol.

The rhodoquinone-9 standard was purified from whole *Ascaris suum* tissue with the lipid extraction procedure described above, and rhodoquinone-9 was isolated by normal phase thin layer chromatography (Whatman, Florham Park, NJ, USA, cat. no. 4861-830) developed with 1 : 1 benzene/chloroform. The Q_6 and Q_{10} standards were from Sigma-Aldrich (St. Louis, MO, USA), and Q_9 was a gift from Dr Youssef Hatefi.

The quinones present in extracted standards and samples were separated and quantified by HPLC connected to an electrochemical detector as described (Jonassen *et al*., 2002), with the following exceptions: the precolumn electrode was the only electrode used and was set at +650 mV to oxidize all quinones, and a Gilson 118 UV/Vis detector was utilized to detect quinones as they eluted from the column. The amounts of rhodoquinone-9, Q_9, demethoxyubiquinone-9, and Q_{10} in the standards and samples was normalized to the amount of Q_6 recovered in the individual lipid extracts.

The content of Q isoforms was determined for OP50 and in the KO229 *E. coli* strains designed to produce predominantly Q_7, Q_8, Q_9, or Q_{10}. In order to determine whether incubation on NGM plate media affected the predominant Q isoform, each strain was subjected to incubation conditions that would simulate the conditions of a lifespan experiment. Each *E. coli* strain was applied to an NGM plate, and after incubation at 37 °C overnight, the plates were stored at 4 °C for 4 weeks, followed by 1 week at 20 °C. Each plate was then rinsed with M9, and *E. coli* recovered from five NGM plates were collected in one tube. Preparation of *E. coli* lipid extracts and determination of the quinone content was performed as described (Jonassen *et al*., 2003).

Food consumption assay

The rate of bacterial consumption by adult N2 *C. elegans* was measured as previously described (Jones *et al*., 1996). Young adult worms were cultured in S medium containing equivalent amounts of either GD1 or OP50. Aliquots were taken from these cultures at 30-min intervals, and the OD_{600} of each aliquot was measured after briefly allowing the worms to settle on ice.

Preparation of UV irradiated bacteria

NGM plates with *E. coli* strains (OP50 or GD1) were irradiated with UV light for 5 min with a DNA Transfer Lamp (FOTODYNE, Inc., Hartland, WI, USA). Following irradiation, at least one of the treated plates was checked to verify complete loss of colony formation when replica plated to Luria-Bertani plate media.

Lifespan analysis

All measurements were performed at 20 °C, with the zero time point taken at the late fourth larval stage. During the egg-laying period hermaphrodites were transferred daily to new plates. These animals were transferred to freshly streaked bacterial plates every 4 days after the egg-laying period. Animals that did not respond when prodded by a platinum wire pick were counted as dead. If an animal was suspected dead, the worm was straightened out with the pick and checked to determine whether it remained straight (dead) or it had moved (alive) the next day. Worms showing abnormal death such as vulva explosion, hatched progeny inside the hermaphrodite or climbing up the plate wall were excluded from the lifespan analysis. Statview 5.0.1 (SAS) software was used to construct lifespan curves and to perform statistical analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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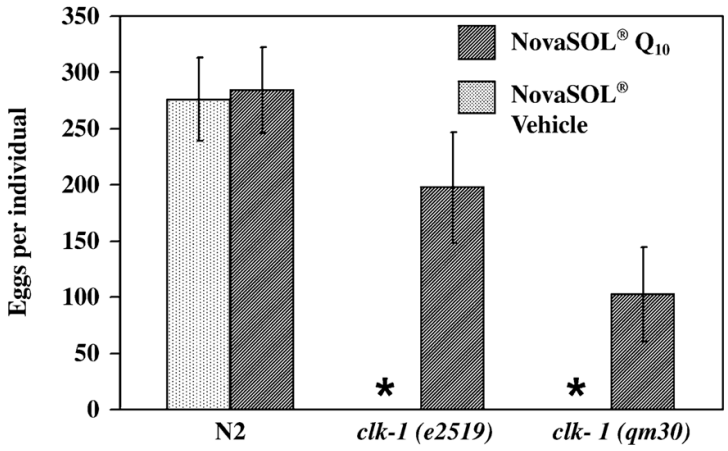

Fig. 1.

Supplementation with NovaSOL Q restores fertility of *Caenorhabditis elegans clk-1* mutants fed Q-less *Escherichia coli*. The number of eggs laid per individual were determined for N2, *clk-1 (e2519)*, and *clk-1 (qm30)* worms grown from the time of hatching on plates supplemented with NovaSOL Q (150 μg Q_{10} mL^{-1} plate media; dark cross-hatched bars), or with the NovaSOL vehicle control (light stippled bar). A lawn of the Q-less *E. coli* strain, GD1, was present on all plates. *clk-1* mutants on nematode growth medium (NGM) plates with vehicle control arrested as L2 larvae for the duration of the brood size determination (*, 9 days). $n = 12$ for all conditions except for N2 worms grown on NGM with vehicle control where $n = 10$.

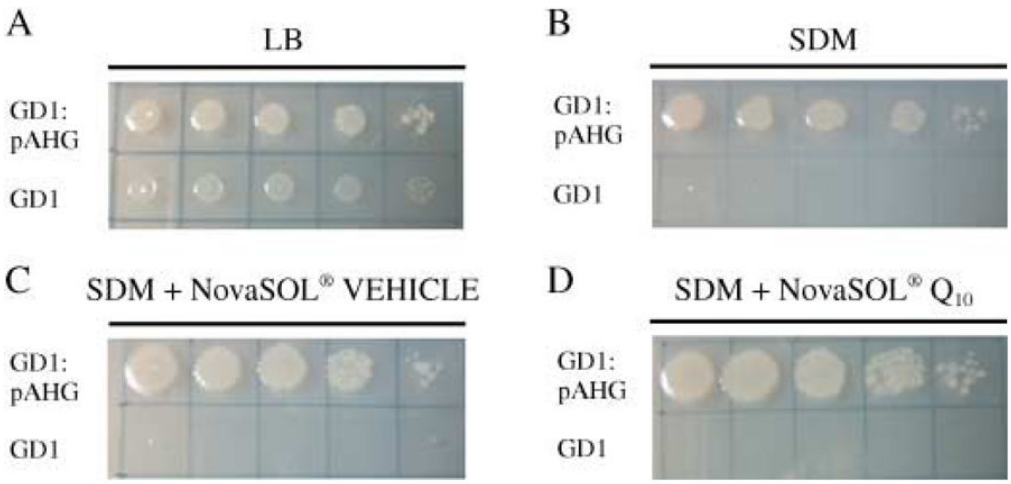

Fig. 2.

NovaSOL Q fails to restore growth of the Q-less *Escherichia coli* strain, GD1, on succinate defined medium (SDM). Serial dilutions of GD1 and the rescued strain, GD1:pAHG were applied to the following plate media: (A) Luria-Bertani, (B) SDM, (C) SDM supplemented with NovaSOL vehicle control, and (D) SDM supplemented with NovaSOL Q. Each plate was incubated overnight at 37 °C.

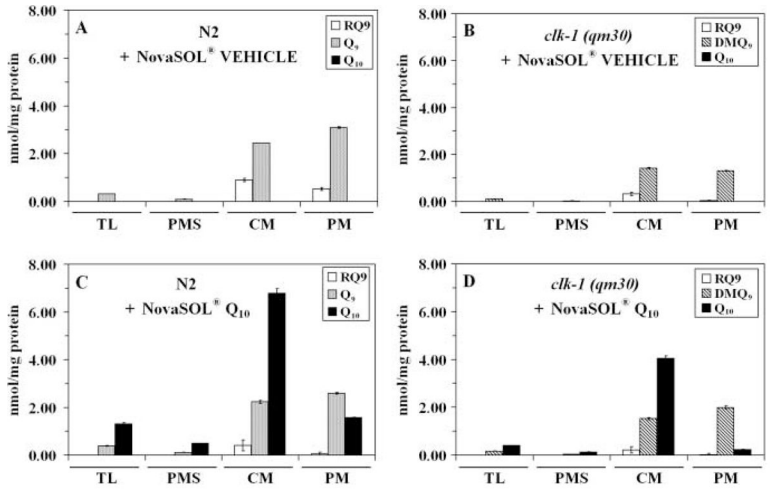

Fig. 3.

Coenzyme Q_{10} is transported from the growth medium into both N2 and *clk-1 (qm30)* worm mitochondria. Measurement of rhodoquinone-9 (RQ9), demethoxyubiquinone-9 (DMQ_9), ubiquinone-9 (Q_9), and ubiquinone-10 (Q_{10}) content in lipid extracts from worm total lysate (TL), post-mitochondrial supernatant (PMS), crude mitochondria (CM), and pure mitochondria (PM). N2 and *clk-1 (qm30)* worms were grown from starved L1 larvae to young adults in S medium supplemented with either NovaSOL vehicle control (A and B, respectively) or in S medium supplemented with NovaSOL Q (C and D, respectively). The Q-less *Escherichia coli* strain, GD1, was the bacterial food source in all cases. Data shown are representative of two independent experiments.

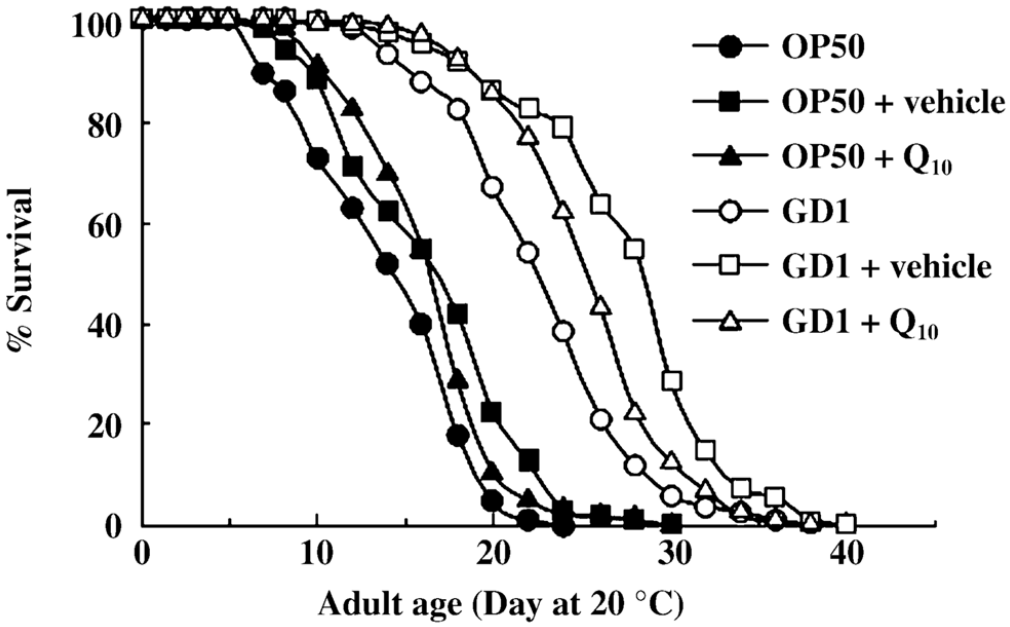

Fig. 4.

Adult *Caenorhabditis elegans* lifespan extension mediated by the GD1 *Escherichia coli* diet is not decreased by supplementation with NovaSOL Q_{10}. Survival curves are shown for N2 worms cultured at 20 °C and fed either Q_8-replete OP50 (black symbols) or Q-less GD1 (white symbols) *E. coli*. Worms were cultured for two generations on unsupplemented nematode growth medium (●,○), medium supplemented with NovaSOL Q_{10} (150 μg Q_{10} mL^{-1}, ▲, △), or vehicle control (■, □). The vehicle control contains medium chain triglycerides as the neutral lipid core in place of Q_{10}. Similar results were obtained for N2 worms fed the each of the stated diet for one generation.

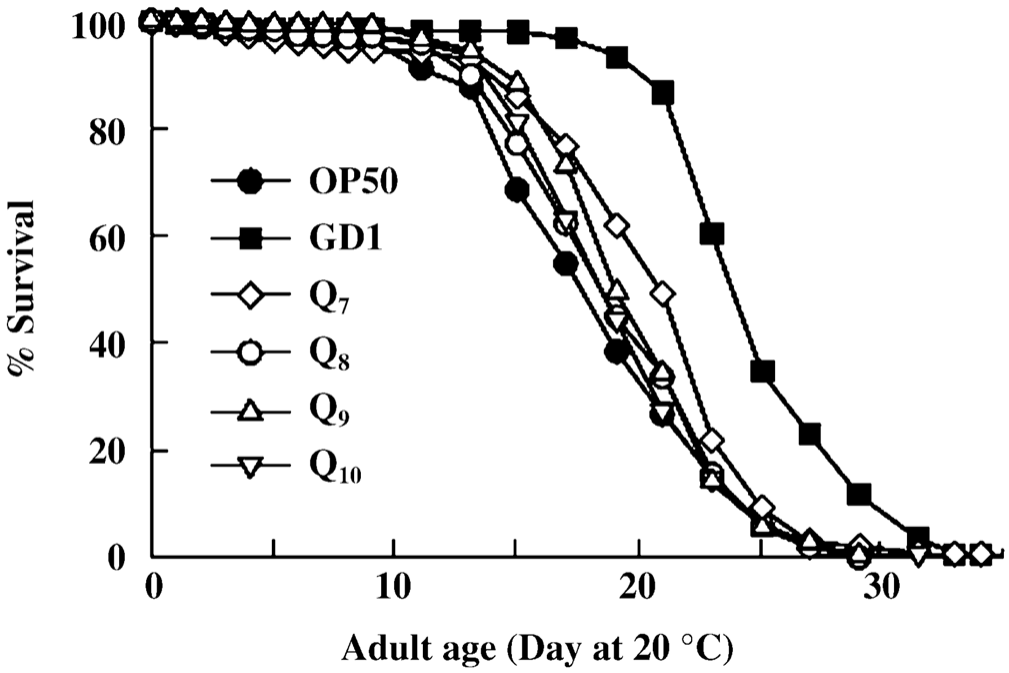

Fig. 5.

Caenorhabditis elegans fed Q_8-, Q_9-, or Q_{10}-producing *Escherichia coli* have similar lifespans. N2 worms were cultured at 20 °C and fed the standard Q_8-replete OP50 *E. coli* diet (●). Alternatively, worms were reared from hatching until the L4 larval stage on OP50 *E. coli* and then were transferred to the designated diets: GD1 Q-less *E. coli* (■), or *E. coli* engineered to produce predominantly Q_7 (◇), Q_8 (○), Q_9 (△), or Q_{10} (▽). Results are representative of two experiments.

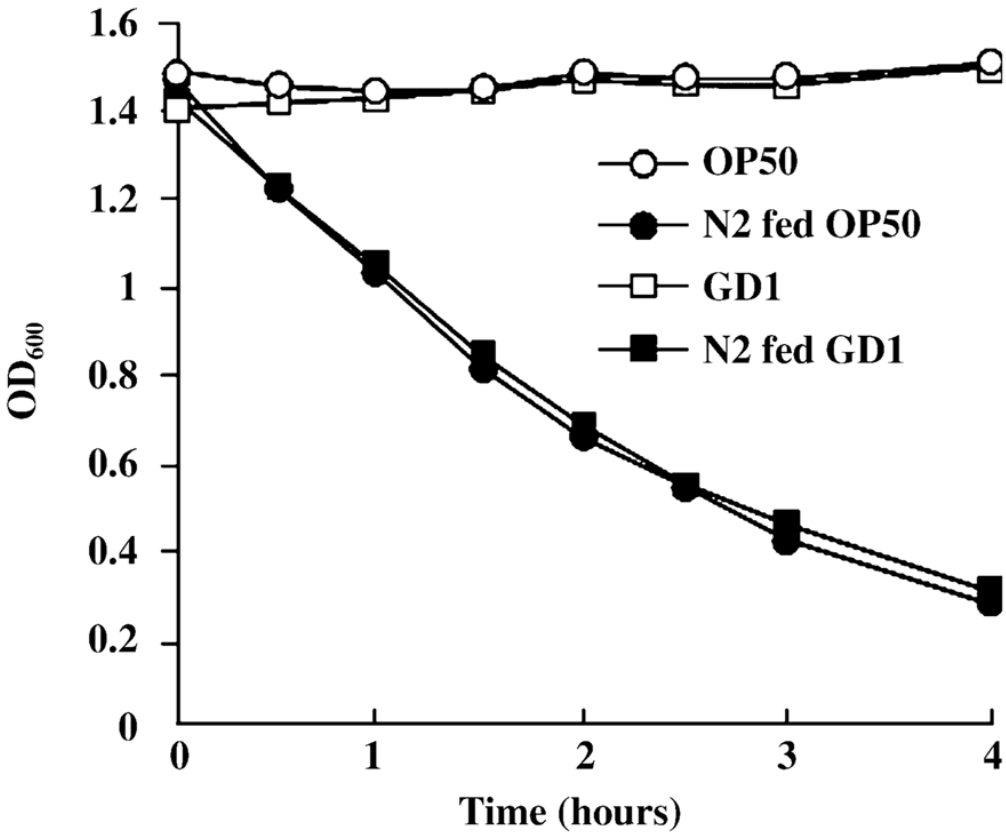

Fig. 6.

Adult *Caenorhabditis elegans* (N2) animals consume the Q-less (GD1) and Q-replete (OP50) *Escherichia coli* at identical rates. The rate of bacterial consumption was measured as previously described (Jones *et al*., 1996). Young adult worms were cultured in S medium containing equivalent amounts of either GD1 or OP50. Aliquots were taken from these cultures at 30-min intervals, and the OD_{600} of each aliquot was measured after briefly allowing the worms to settle on ice.

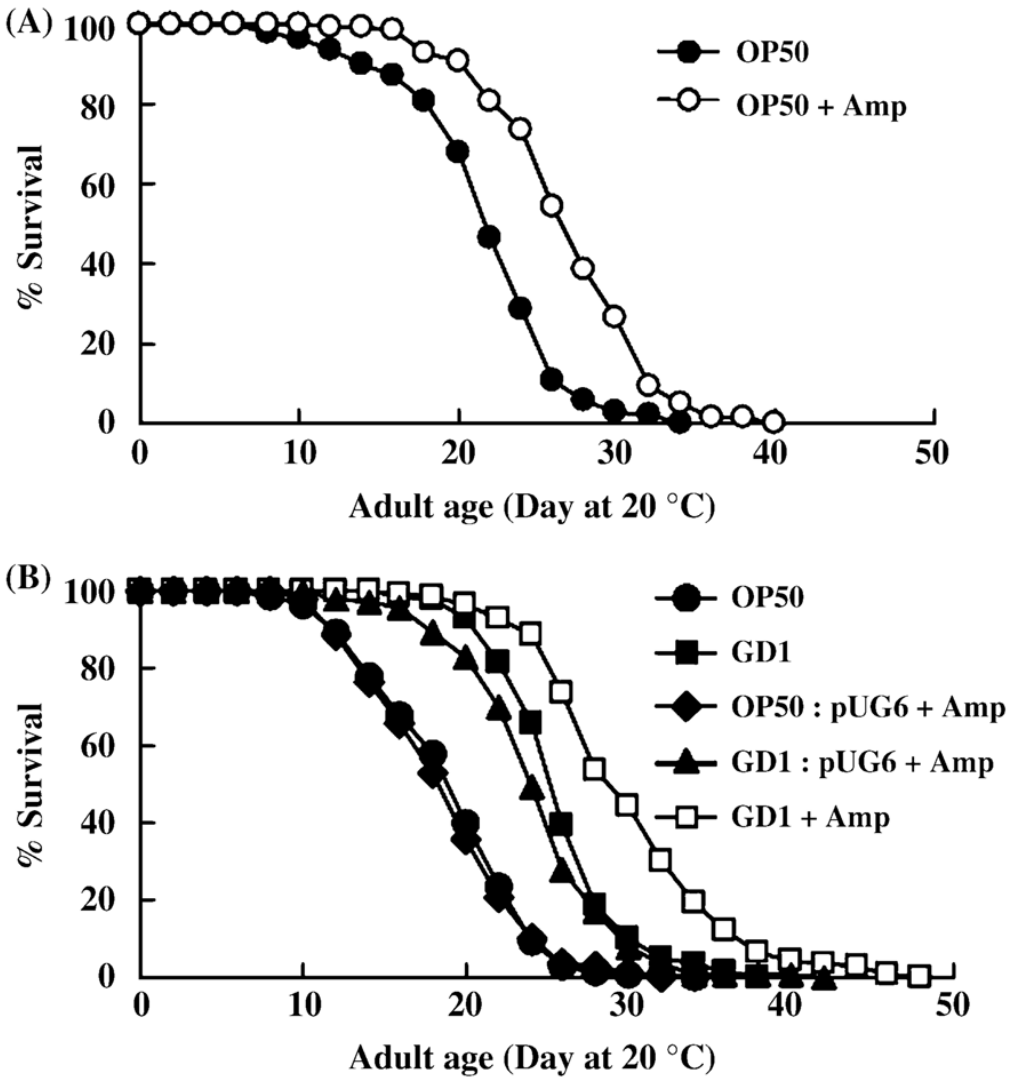

Fig. 7.

A diet of nonproliferating GD1 causes further lifespan extension relative to a diet of proliferating GD1. (A) Adult N2 worms were cultured at 20 °C and fed OP50 on nematode growth medium (NGM) plates with (○) or without ampicillin (●). Worms were cultured on regular NGM plates with OP50 until the L4 larval stage and subsequently transferred to the plates containing either proliferating or nonproliferating bacteria. (B) L4 stage worms were cultured on proliferating OP50 (●,◆), proliferating GD1 (■,▼) or nonproliferating GD1 (□). Bacteria harboring pUG6, an *Escherichia coli* vector conferring ampicillin resistance, are able to grow on NGM + ampicillin plates.

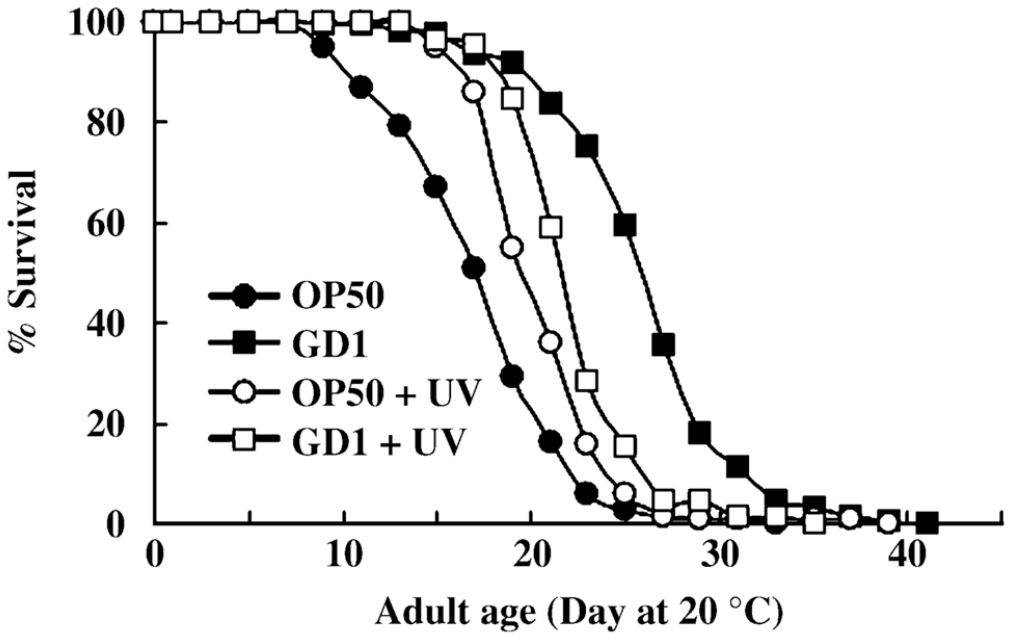

Fig. 8.

Ultraviolet (UV) irradiation of OP50 and GD1 *E. coli* produces opposing effects on worm lifespan. N2 worms were cultured at 20 °C and at the L4 larval stage transferred to plates containing either proliferating bacteria (closed symbols) or UV irradiated bacteria (open symbols). Where indicated, each nematode growth medium (NGM) plate with bacteria was irradiated with UV light by a DNA Transfer Lamp for 5 min. N2 worms were fed either UV irradiated OP50 (○), untreated OP50 (●), UV irradiated GD1 (□), or untreated GD1 (■).

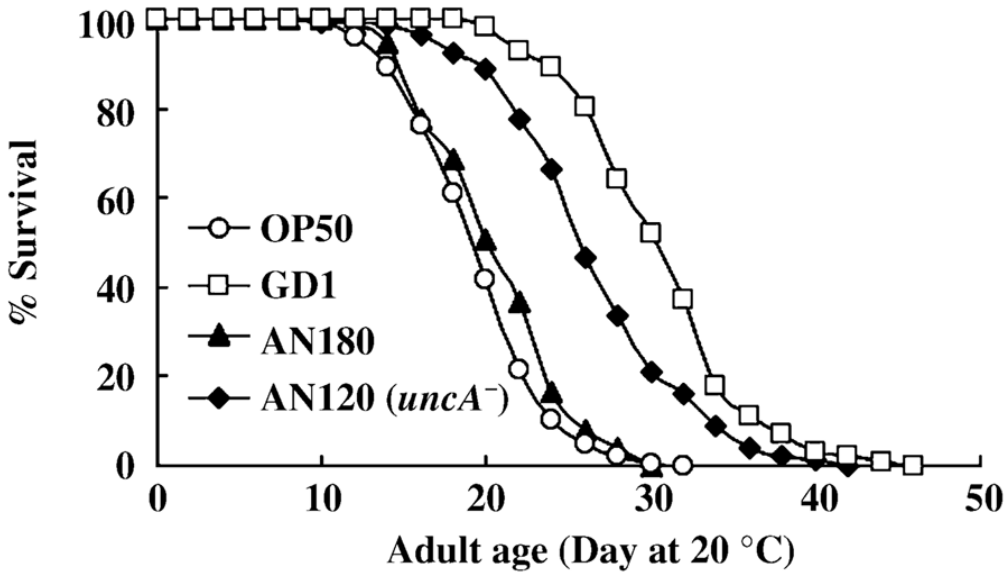

Fig. 9.

Impaired respiratory metabolism is an important factor contributing to the GD1 lifespan extension. N2 worms were reared on OP50 from hatching and at the L4 larval stage N2 worms were transferred to one of the following *Escherichia coli* strains: OP50 (○); GD1 (□); AN120 (◆), harboring a point mutation in the *atpA* gene encoding ATP synthase alpha subunit; or AN180 (▲), the ATP synthase parental strain.

Table 1

Genotype and source of bacterial strains

Strain	Genotype	Reference or source
OP50	*ura*	Sulston & Hodgkin (1988)
GD1	HW272 *ubiG::Kan*^{r}	Hsu *et al*. (1996)
KO229:pMN18 (produce Q_7)	FS1576 *ispB::Cm*^{r} pMN18 plasmid harboring *H. influenzae ispB* homologue	Okada *et al*. (1997)
KO229:pKA3 (produce Q_8)	FS1576, *ispB::Cm*^{r}, pKA3 plasmid harboring *E. coli ispB*	Okada *et al*. (1997)
KO229:pSN18 (produce Q_9)	FS1576 *ispB::Cm*^{r} pSN18 plasmid harboring *Synechocystis* sp. strain PCC6803 *ispB* homologue	Okada *et al*. (1997)
KO229:pLD23 (produce Q_{10})	FS1576 *ispB::Cm*^{r} pLD23 plasmid harboring *G. suboxydans ispB* homologue	Okada *et al*. (1998)
AN180	*argE3 thi-1 str*^{r}	Butlin *et al*. (1971)
AN120	*argE3 thi-1 str*^{r} *atpA401*	Butlin *et al*. (1971)
1100	*bglR thi-1 rel-1 HfrP01*	Humbert *et al*. (1983)
1100 Δbc	1100 deleted for *atpBEFHAGDC*	Klionsky *et al*. (1983), Stack & Cain (1994)

Table 2

Effects of dietary variables on *Caenorhabditis elegans* lifespan. Independent experiments are in groups

Condition	Number of deaths (*n*)[†]	Mean lifespan (days ± SE)[‡]	Maximum (days)	Percentage of mean on OP50
OP50	135	14.48 ± 0.39	22	–
OP50 + vehicle	125	16.77 ± 0.46**	28	115.9
OP50 + Q_{10}	131	16.75 ± 0.35**	28	115.7
GD1	119	23.29 ± 0.47**	36	160.8
GD1 + vehicle	115	27.91 ± 0.53**	38	192.7
GD1 + Q_{10}	123	25.87 ± 0.41**	36	178.7
OP50	152	18.60 ± 0.41	27	–
GD1	160	25.02 ± 0.35**	33	134.5
KO229/Q_7	158	20.63 ± 0.42**	33	110.9
KO229/Q_8	157	19.34 ± 0.38	27	104.0
KO229/Q_9	156	20.13 ± 0.31	27	108.2
KO229/Q_{10}	159	19.37 ± 0.37	29	104.1
OP50	163	22.20 ± 0.39	32	–
OP50 + Amp	136	27.43 ± 0.43**	38	123.6
OP50	178	19.33 ± 0.35	32	–
GD1	174	26.25 ± 0.29**	36	135.8
OP50:pUG6 + Amp	165	19.10 ± 0.37	30	98.8
GD1:pUG6 + Amp	140	24.82 ± 0.39**	40	128.4
GD1 + Amp (non-proliferating)	154	30.53 ± 0.45**	46	157.9
OP50	140	17.73 ± 0.38	31	–
GD1	162	26.46 ± 0.39**	39	149.2
OP50 + UV	138	20.99 ± 0.29**	37	118.4
GD1 + UV	85	22.81 ± 0.37**	33	128.7
OP50	167	20.20 ± 0.32	30	–
GD1	123	31.15 ± 0.47**	44	154.2
AN180	146	21.11 ± 0.35*	28	104.5
AN120 (*atpA*$^-$)	143	27.06 ± 0.47**	40	134.0
1100	160	21.01 ± 0.29	30	104.0
1100 Δbc $^{(atpA\text{-}H-)}$	115	25.70 ± 0.44*	36	127.2

[†]Animals that crawled away, had internally hatched larvae, or had eviscerated gonads were excluded.

[‡]Log rank test from survival analysis *P*-values:

*0.001 < *P* < 0.05;

**$P < 0.001$.

Table 3

Determination of Q isoform content in *Escherichia coli* diets provided on nematode growth medium (NGM) plate media

pmol Q_n isoform per milligram *E. coli* protein					
Strain	**Q_6**	**Q_7**	**Q_8**	**Q_9**	**Q_{10}**
OP50 (Q_8)	ND	192.9 ± 14.6	2751.3 ± 135.2	ND	ND
KO229:pMN18 (Q_7)	165.2 ± 2.8	1219.2 ± 7.3	143.9 ± 1.6	ND	ND
KO229:pKA3 (Q_8)	ND	103.6 ± 3.6	1640.1 ± 48.9	< 10	ND
KO229:pSN18 (Q_9)	ND	< 10	372.1 ± 3.1	1371.9 ± 11.6	ND
KO229:PLD23 (Q_{10})	ND	< 10	26.7 ± 0.7	174.3 ± 3.4	1182.7 ± 6.0

Lipid extracts were prepared from the indicated *E. coli* strains seeded on NGM plate media as described in the Experimental procedures. ND, not detected.

Table 4

Separation of crude mitochondria on a discontinuous sucrose gradient results in a fraction specifically enriched in succinate-cytochrome *c* reductase activity

Subcellular fraction	Succinate-cytochrome *c* reductase activity (mitochondria)	Mannosidase II activity (Golgi)	Glucose 6-phosphatase activity (endoplasmic reticulum)
	nmol cytochrome *c*	nmol para-nitrophenol	nmol phosphate
	reduced/min/mg protein	produced/h/mg protein	produced/min/mg protein
Crude mitochondria	296.6 ± 8.6	73.9 ± 16.4	60.7 ± 7.5
Pure mitochondria	559.4 ± 10.8	44.2 ± 3.3	36.3 ± 11.5

Crude mitochondria isolated from synchronous young adult N2 worms were separated on a discontinuous sucrose gradient (1.2 M, 1.3 M, 1.6 M, and 1.8 M). The band at the interface between 1.3 M and 1.6 M was isolated as the pure mitochondria fraction. Activities represent the average of three assays ± the standard deviation.