C. elegans as model for the study of high glucose mediated lifespan reduction

Running title: Glucose regulates lifespan in C. elegans

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Objective: Establishing *C. elegans* as a model for glucose toxicity mediated lifespan reduction.

Research Design and Methods: *C. elegans* were maintained to achieve glucose concentrations resembling the hyperglycemic conditions in diabetic patients. The effects of high glucose on lifespan, glyoxalase-1 activity, advanced glycation end products (AGEs) and reactive oxygen species (ROS) formation and on mitochondrial function were studied.

Results: High glucose conditions reduced mean lifespan from 18.5±0.4 to 16.5±0.6 days and maximum lifespan from 25.9±0.4 to 23.2±0.4 days, independent of glucose effects on cuticle or bacterial metabolization of glucose. The formation of methylglyoxal-modified mitochondrial proteins and ROS was significantly increased by high glucose conditions and reduced by mitochondrial uncoupling and complex II inhibition. Overexpression of the methylglyoxal-detoxifying enzyme glyoxalase-1 attenuated the life shortening effect of glucose by reducing AGE accumulation (by 65%) and ROS formation (by 50%) and restored mean (16.5±0.6 to 20.6±0.4 days) and maximum lifespan (23.2±0.4 to 27.7±2.3 days). In contrast, inhibition of glyoxalase-1 by RNAi further reduced mean (16.5±0.6 to 13.9±0.7 days) and maximum lifespan (23.2±0.4 to 20.3±1.1 days). The lifespan reduction by glyoxalase-1-inhibition was independent from the insulin signaling pathway, since high glucose conditions also affected daf-2 knock-down animals in a similar manner.

Conclusions: *C. elegans* is a suitable model organism to study glucose toxicity, in which high glucose conditions limit the lifespan by increasing ROS formation and AGE modification of mitochondrial proteins in a Daf-2 independent manner. Most importantly glucose toxicity can be prevented by improving glyoxalase-1 dependent methylglyoxal detoxification or preventing mitochondrial dysfunction.
Due to its short lifespan, the relative easy in modifying its genome and its simple insulin receptor system (daf-2) (1), *C. elegans* can be used not only to study molecular targets affected by normal glucose concentration, but also by pathological glucose concentrations. It is likely a good model to study mechanisms by which high glucose might reduce lifespan.

Increased glycolytic flux leads to formation of mitochondrial reactive oxygen species (ROS). In turn, ROS formation decreases the activity of glyoxalase-1, an enzyme detoxifying methylglyoxal (MG). Accumulation of MG, a highly reactive dicarbonyl derived from triosephosphates (2), leads to rapid protein modification. MG is an arginine-directed glycation agent and precursor of advanced glycation endproducts (AGEs). It modifies proteins mainly but not exclusively on arginine residues forming methylglyoxal-derived hydroimidazolone (MG-H1) with formation of other minor AGE residues – arginine-derived argpyrimidine and lysine derived \( N^\epsilon \)-(1-carboxyethyl)lysine (CEL) and methylglyoxal-derived lysine dimer (MOLD) (3). MG-derived modification of mitochondrial proteins increases mitochondrial ROS formation, thus reducing lifespan in *C. elegans* (4). The formation of MG-modified proteins in cells is suppressed by the activity of the enzyme glyoxalase-1 which catalyses conversion of MG, with the cofactor glutathione, to S-D-lactoylglutathione. This is further converted to D-lactate by glyoxalase-2 regenerating the glutathione (5). We recently showed that with increasing age, glyoxalase-1 activity decreased in *C. elegans*, leading to an increase of MG accumulation and oxidative stress (4). In these settings, overexpression of a glyoxalase-1 homologue in *C. elegans* decreased mitochondrial AGE accumulation and increased lifespan, while knock-down of the glyoxalase-1 gene increased AGE accumulation and decreased lifespan, pointing to a dependence of lifespan on AGE production (4).

Glucose restriction extends *C. elegans* lifespan. Recent studies by Schultz *et al.* (6) using a model of impaired glucose metabolism indicates, that an increase in ROS results in a secondary hormetic increase in stress defense, eventually resulting in reduced net stress levels. In contrast, high glucose concentrations reduce lifespan of *C. elegans*. Since an increased glycolytic flux is likely to cause a long-lasting accumulation of MG-derived AGEs and a steady increase in ROS formation, we hypothesized that *C. elegans* can not only be used for understanding the role of glucose metabolism in normal aging, but most importantly for understanding basic mechanisms of glucose toxicity. Therefore, we studied whether elevation of glucose concentrations in *C. elegans* extracts from 6 to 14 mM (resembling the hyperglycemic conditions in diabetic patients) could limit lifespan in *C. elegans* by accumulation of MG-derived AGEs and impairment of mitochondrial function, and whether detoxifying MG might prevent glucose toxicity and mitochondrial damage.

**RESEARCH DESIGN AND METHODS**

**C. elegans maintenance and lifespan assays.** All nematodes were cultivated on nematode growth medium (NGM) agar as described previously (4) and maintained at 20 °C. Strains used in this study include wild type (N2) and eat-2 (-) mutant (eat-2(ad465)II) provided by the Caenorhabditis Genetic Center, and glyoxalase-1 transgenic (4). Animals were maintained on living *E. coli* (OP50) from a standardized overnight culture with an OD of 1.5, which was added to the surface of the NGM plates. 150 µl of a 400 mM glucose solution was used in order to achieve a concentration of 14 mM in the worms. In experiments using dead bacteria the bacteria were killed by sonication. In experiments using a mitochondrial uncoupler
or a complex III inhibitor, *C. elegans* were cultivated in the presence of 50 µM FCCP (Sigma-Aldrich) or 10 µM myxothiazol (Sigma-Aldrich), respectively.

**Establishment of high glucose conditions.** It was our aim to reach a glucose concentration in a *C. elegans* whole body extract of 10-15 mM, resembling the glucose concentrations in diabetic patients under poor glucose control. Therefore 150 µl of a glucose solution of various concentrations were added to the agar of NGM plates in the absence of *C. elegans* as described above for 1, 3, 5, 10 and 15 days in order to define the time for reaching a steady state of glucose in the agar. A piece of agar was then cut out of the middle of the NGM plate and fluidified using β-agarase (New England Biolabs) as described by the manufacturer. Glucose was measured using a Modular ISE 900 P 800 analyzer (Hitachi) according to the manufacturer’s recommendations.

Wild type *C. elegans* were kept for 5 days under various glucose concentrations in the agar prepared as described above, then harvested and washed. An extract of *C. elegans* was prepared by sonication and analyzed for the glucose concentration using a Modular ISE 900 P 800 analyzer (Hitachi) according to the manufacturer’s recommendations.

**Scanning electron microscopy.** Wild type *C. elegans* were kept under high glucose conditions for 16 days as described above. Worms were then harvested, washed with PBS buffer and incubated with 2.5 % glutaraldehyde overnight. After three times washing with PBS buffer, worms were transferred into microporous specimen capsules and dehydrated in graded ethanol solutions. Specimen were finally dried in a critical point dryer (CPD 030 Critical point dryer, Bal-Tec) and placed onto conductive sticky tape. Subsequently, samples were sputter-coated with a 15 nm gold layer (MED020, Bal-Tec) and imaged in a field emission scanning electron microscope (Leo1530 Gemini).

**Glyoxalase-1 activity.** Glyoxalase-1 activity was assayed as described (4; 8; 9). For the conversion of hemithioacetal to S-D-lactoylglutathione, the change in molar extinction coefficient, \( \Delta \varepsilon_{240} = 2.86 \text{ mM}^{-1} \text{ cm}^{-1} \), was measured after the addition of the *C. elegans* protein extract to hemithioacetal, prepared by pre-incubating methylglyoxal and glutathione. The initial rate of change of absorbance \( \Delta A_{240} \) (AU min\(^{-1}\)) was deduced, and the \( \Delta A_{240} \) for the blank value corrected. The activity of glyoxalase-1 a\( \text{GI} \) is given in units where one unit of glyoxalase-1 activity catalyses the formation of 1 µmol S-D-lactoylglutathione per minute under assay conditions. The standard assay mixture contained 2 mM methylglyoxal and 2 mM glutathione in a sodium phosphate buffer (100 mM, pH 6.6, 37 °C).

**Staining of mitochondria and MG-H1.** Staining of mitochondria for MG-H1 and confocal microscopy were performed as described (4). Briefly, mitochondria were stained by incubation with 50 µM MitoTracker Deep Red FM (Molecular Probes) according to the manufacturer’s protocol. MG-H1 was detected using a mouse antibody (Biozol) and visualized by a Texas Red-labelled rabbit anti-mouse second antibody (Biozol) as described previously (4). Immunohistology was performed according to the protocol of Ruvkun (for details see http://www.wormatlas.org/anatmeth/finneyruvkun.pdf). Formation and colocalization of MG-H1 with mitochondria was quantified by using the image processing and analysis software ImageJ (10) with the Intensity Correlation Analysis plug-in (11) as described previously (4).

**Staining of ROS.** Staining of ROS, confocal microscopy and analysis with
ImageJ were performed as described previously (4). Briefly, *C. elegans* were washed, incubated with 3 µM of dihydroethidium for 30 min and analyzed for fluorescence intensity by confocal microscopy. Raw data from confocal microscopy was then analyzed using ImageJ.

**RNAi.** For RNAi experiments, the feeding technique was used as described previously (4; 12; 13). Individual colonies of *E. coli* HT115 containing plasmids of interest (Y55D5A.5 (daf-2) and C16C10.10 (glyoxalase-1)) were inoculated in LB broth containing ampicillin (100 mg/ml) (Sigma-Aldrich, München, Germany) and grown overnight at 37 °C. Bacteria (150 µl) were then seeded onto NGM plates containing carbenicillin (0.025 g/l) and IPTG (0.25 g/l) and allowed to dry overnight. *E. coli* containing only the control vector were grown in parallel and seeded on control plates.

Twenty L4 stage hermaphrodites (one generation) were placed on each plate and incubated overnight at 20 °C. The worms were then transferred on a fresh RNAi plate and left to lay eggs for 24 hours. The old worms were removed from the plates and the eggs were incubated at 20 °C until worms were in L4 stage. These worms were used for lifespan observation. Furthermore, in order to use active RNAi *E. coli* and to prevent mixture of generations, worms were transferred by picking on a new NGM-IPTG/carbenicillin plate seeded with the appropriate *E. coli* clone every day.

**Determination of lifespan.** Age-synchronized worms were obtained as described before (4). Lifespan studies without RNAi were performed as described (4; 7) on NGM plates containing 300 µg/ml 5-fluorodeoxyuridine (Sigma-Aldrich, Munich, Germany) to prevent progeny production. Lifespan studies using RNAi were performed on NGM plates containing carbenicillin (0.025 g/l) and IPTG (0.25 g/l) and animals were transferred to new plates daily. In all experiments, the pre-fertile period of adulthood was used as t = 0 for lifespan analysis. Animals that did not move after repeated stimulus were regarded as dead. Animals that crawled away from the plate or contained internally hatched worms were excluded. One hundred worms were used for each experiment; all experiments were performed at least 3 times.

**Statistical analysis.** Statistical analysis was performed using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA). In experiments in which only two groups were compared (Fig. 1-5), unpaired t tests were used to determine significance. If multiple groups were run (Table 1 and Fig. 6), the data were compared across the groups using analyses of variance (ANOVA); additional between-group comparisons were made using Fisher’s protected least significant difference (PLSD) post hoc tests.

**RESULTS**

To establish the potential rate of diffusion of glucose from the medium to the agar, a solution of glucose (400 mM) was added to an agar plate and incubated for a period of 15 days. At consecutive time points, glucose concentration was determined. No significant change in glucose concentration was observed in the agar (data not shown). In all subsequent experiments, the agar plates containing 40 mM glucose resulted in an intracellular concentration of 14 mM (Fig. 1), which is within the range observed in poorly controlled diabetic patients.
experiments, *C. elegans* cultured under high glucose conditions, refers to a concentration of 40 mM, while standard conditions refers to no additional glucose being added to the agar.

Under high glucose conditions, mean lifespan was reduced from 18.5 ± 0.4 to 16.5 ± 0.6 days (*p* < 0.05), and maximum lifespan was reduced from 25.9 ± 0.4 to 23.2 ± 0.4 days (*p* < 0.05) (Fig. 2 and Table 1). Scanning electron microscopy studies excluded non-specific high glucose effects on the cuticle of the worms, since there was no morphological difference in the appearance of *C. elegans* held under high glucose conditions for 16 days and the control group (data not shown). To exclude non-specific osmotic effects, experiments were performed by adding sorbitol as control. Sorbitol affected neither mean (*p* > 0.05) nor maximum (*p* > 0.05) lifespan (Table 1). To exclude non-specific effects due to bacterial metabolization of glucose, control experiments were performed using dead bacteria. In these experiments, the addition of glucose reduced mean lifespan from 29.3 ± 0.1 to 25.1 ± 0.1 days (*p* < 0.01) and maximum lifespan from 40.5 ± 2.5 to 35.5 ± 0.5 days (*p* < 0.05) (Table 1). The increased lifespan compared to animals fed with living bacteria could be caused by caloric restriction due to differential food preferences. These results point to a specific life-shortening effect of glucose. Thus, increasing the total body glucose concentration to 14 mM resulted in a reduction of lifespan not explained by non-specific effects.

Glyoxalase-1 is central in controlling the extent of MG and MG-derived AGE formation as a result of glucose metabolism. After high glucose exposure for 5 days, glyoxalase-1 activity determined in whole body extracts of *C. elegans* was reduced from 0.368 ± 0.004 to 0.211 ± 0.025 mU/µg protein, while sorbitol had no significant effect (Fig. 3).

To test whether glucose-dependent glyoxalase-1 downregulation leads to an increase in AGE modification of mitochondria in *C. elegans* (4; 14), the level of MG-H1 was determined using immunostaining. The staining of MG-H1 in standard (Fig. 4B) and high glucose animals (Fig. 4E) did not differ with respect to localization, only with respect to the intensity. When mitochondria were stained with MitoTracker and counterstained for MG-H1, colocalization of MG-H1 with mitochondria could be detected (Fig. 4C, F). Due to the higher content of MG-H1, staining for colocalization was stronger under high glucose than standard glucose conditions.

Previously we reported a *C. elegans* strain with transgenic stable overexpression of glyoxalase-1 (4). This resulted in a ~200-fold increase of glyoxalase-1 activity (4). These animals with genetically controlled stable glyoxalase-1 expression were also exposed to standard and high glucose conditions. Overexpression of *C. elegans* glyoxalase-1 (4) resulted in a decrease in total and mitochondrial MG-H1 in standard (Fig. 4H-I) as well as in high glucose (Fig. 4K-L) conditions.

The increase of mitochondrial immunoreactive MG-H1 was paralleled by an increase in ROS formation (Fig. 5A-D). When dihydroethidium staining was used to visualize ROS generation, a strong increase was seen in *C. elegans* cultured for 15 days in high glucose (Fig. 5B) compared to standard conditions (Fig. 5A). Using transgenic *C. elegans* overexpressing glyoxalase-1, a dramatic reduction in dihydroethidium staining was observed. Under standard conditions, transgenic *C. elegans* had a staining significantly below control (Fig. 5A, C) and even under high glucose conditions, overexpression of glyoxalase-1 reduced staining below wild type controls (Fig. 5B, D).
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To test whether high glucose-induced mitochondrial dysfunction is responsible for increased MG-H1 and ROS formation, experiments were repeated with C. elegans cultured in the presence of the electron transport chain uncoupler FCCP (50 µM) or the complex IIIQo inhibitor myxothiazol (10 µM) for 15 days (Fig. 6A+B). When arbitrary units/pixel were used to quantify MG-H1 generation, a significant increase from 93 ± 4 to 142 ± 12 (p < 0.001) was observed in worms treated with high glucose for 15 days (Fig. 6A). MG-H1 formation in high glucose wild type C. elegans was significantly reduced by FCCP and myxothiazol. FCCP and myxothiazol reduced MG-H1 formation in standard and high glucose treated animals to the same level, which was by about 49% of staining observed in wild type under standard conditions and by about 66% compared to high glucose (Fig. 6A).

A similar result was observed with ROS formation. When arbitrary units/pixel were used to quantify ROS generation, a significant increase from 45 ± 6 to 88 ± 4 (p < 0.001) was observed in worms treated with high glucose for 15 days (Fig. 6B). FCCP and myxothiazol reduced ROS formation in standard and high glucose cultured C. elegans to the same level, which was to 28% below wild type under standard conditions and 63% below high glucose (Fig. 6B). Thus overcoming high glucose-induced mitochondrial dysfunction reduces MG-H1 and ROS formation.

Treatment with either FCCP or myxothiazol enhanced lifespan under both conditions (Table 1). FCCP increased mean lifespan from 18.5 ± 0.4 to 21.7 ± 1.7 days (p < 0.05) and maximum lifespan from 25.9 ± 0.4 to 31.5 ± 0.5 days (p < 0.01), while myxothiazol increased mean lifespan from 18.5 ± 0.4 to 21.1 ± 1.1 days (p < 0.01) and maximum lifespan from 25.9 ± 0.4 to 31.0 ± 1.7 days (p < 0.01) under standard glucose conditions (Table 1). Under high glucose conditions FCCP increased mean lifespan from 16.5 ± 0.6 to 19.5 ± 0.9 days (p < 0.05) and maximum lifespan from 23.2 ± 0.4 to 28.5 ± 1.5 days (p < 0.01), while myxothiazol increased mean lifespan from 16.5 ± 0.6 to 20.4 ± 0.1 days (p < 0.001) and maximum lifespan from 23.2 ± 0.4 to 27.7 ± 1.9 days (p < 0.01).

To determine whether overexpression of glyoxalase-1 can not only reduce MG-H1 and ROS formation, but also counteract the life shortening effect of glucose, glyoxalase-1 overexpressing worms were exposed to glucose (Table 1). Overexpression of glyoxalase-1 prolonged mean lifespan in standard culture conditions from 18.5 ± 0.4 in wild type to 20.3 ± 0.2 in transgenic (p < 0.05) and maximum lifespan from 25.9 ± 0.4 to 29.2 ± 1.5 days (p < 0.05). Under high glucose conditions, mean lifespan of 16.5 ± 0.6 days in wild type was increased to 20.6 ± 0.4 days in transgenic animals (p < 0.001) and maximum lifespan of 23.2 ± 0.4 to 27.7 ± 2.3 days (p < 0.01), thus restoring lifespan under high glucose conditions to the lifespan seen in wild type under standard glucose concentration (Table 1).

In contrast, a decrease of lifespan under standard glucose conditions was seen when RNAi was used to knock-down glyoxalase-1 activity by 75% (4). Under normal glucose conditions mean lifespan of 18.5 ± 0.4 days in wild type was reduced by glyoxalase-1 RNAi to 13.5 ± 1.2 days (p < 0.001). Maximum lifespan was reduced from 25.9 ± 0.4 to 21.0 ± 1.2 days (p < 0.01). In the presence of high glucose mean lifespan was reduced from 16.5 ± 0.6 in wild type to 13.9 ± 0.7 in glyoxalase-1 RNAi treated C. elegans (p < 0.01), and maximum lifespan from 23.2 ± 0.4 to 20.3 ± 1.1 days (p < 0.05) (Table 1).

The reduction of lifespan under high glucose conditions is independent from the pathways activated by caloric restriction, since the effect of glucose was still observed...
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in eat-2 (-) mutants with reduced pharyngeal activity and thus reduced caloric uptake (Table 1). In eat-2 mutants, mean lifespan was reduced from 24.3 ± 0.3 days in standard culture conditions to 21.5 ± 1.4 days in high glucose conditions ($p < 0.05$), and the maximum lifespan from 33.3 ± 0.3 to 29.0 ± 1.5 days ($p < 0.05$). Thus, the proportional reduction of mean and maximum lifespan is 12 % and 13 % in eat-2 mutants, respectively. Both correspond well to a reduction of mean lifespan of 11 % and a reduction of maximum lifespan of 10 % by high glucose in wild type C. elegans. Thus, the glycotoxic effects described here are independent from the eat-2 controlled starvation pathway.

A decline in daf-2 insulin signaling pathway of C. elegans (1) is known to result in activation of the fork-like transcription factor daf-16 and to prolong lifespan (7). This effect, however, was also observed to be diminished under high glucose conditions. Administration of glucose to C. elegans knock-down for daf-2 by RNAi had similar effects as in wild type worms, namely a reduction of mean lifespan of 11 % and a reduction of maximum lifespan of 10 % by high glucose in wild type C. elegans. Thus, the glycotoxic effects described here are independent from the eat-2 controlled starvation pathway.

DISCUSSION

The major purpose of the study was to establish C. elegans as a model for diabetes research in order to understand basic mechanisms underlying glucose effects on cellular and mitochondrial function. Since a total body glucose concentration of 14 mM was sufficient to achieve significant effects on lifespan, this in vivo model organism provides a reliable tool to decipher changes in cellular functions induced by glucose concentrations which are within the range observed in poorly controlled diabetic patients.

As shown here, long-lasting high glucose conditions result in a permanent accumulation of MG-derived modifications of mitochondrial proteins, a steady increase in ROS formation and a significant reduction in lifespan. These effects are independent of osmotic pressure, the pathways activated by caloric restriction in eat-2 mutants, and the insulin like receptor daf-2/daf-16 pathway, one of the central pathways involved in lifespan determination. Since overexpression of the C. elegans homologue of glyoxalase-1 partly reversed these specific glucose effects, while knock-down of glyoxalase-1 further shortened the lifespan, protective enzymes capable of reducing reactive metabolites generated by high glucose, do not only normalise the function of single cells, but determine the lifespan of a whole organism.

In a recent study, Schulz et al. (6) analyzed lifespan extension in C. elegans by glucose restriction. It was concluded that impairment of glucose metabolism initially causes enhanced mitochondrial respiration and increased ROS formation resulting in a short-lasting and nonlethal stress, leading to a secondary and long-lasting increase in antioxidant defences. Therefore, glucose restriction increases C. elegans lifespan by hormetic sustained reduction of net stress levels. This fits well to the previously established role of ROS in activating antioxidant response mechanisms and thereby providing tools for prolonging lifespan. Another study shows that mitochondria of long-lived daf-2 mutants produce increased amounts of ROS compared to wild type mitochondria (15), probably because daf-2 mutants show a higher antioxidant activity by up-regulation of antioxidant enzymes (16-18). Up to now, however, it is not known whether these life enhancing effects of such a glucose restriction would still be evident under
conditions with high extracellular glucose concentrations.

Additional studies are needed to analyze whether the changes in lifespan observed by glucose administration or restriction are mainly depending on extracellular or intracellular effects. Our data suggest that the excess of a certain glucose level leads to an overload and subsequent inhibition of the glyoxalase-1 system. A potential explanation is that the long-lasting high glucose conditions used here results in a sustained excess of ROS, depleting glyoxalase-1, which in turn might result in a breakdown of the mitochondrial integrity by MG dependent protein modification and ultimatively ends in increased ROS formation and lifespan reduction (4).

Future studies are required to prove whether mechanisms described in *C. elegans* can be translated to the situation in diabetic patients. While total lifespan is an emerging goal of diabetes treatment, it is evident that simply lowering glucose concentrations is not sufficient to normalize life expectancy in patients with diabetes (19). The availability of an *in vivo* model of life expectancy might open new opportunities to study cellular defence systems such as glyoxalase-1 in the context of high glucose and total lifespan. Thus, protecting glyoxalase-1 activity from glucose dependent downregulation might provide a promising therapeutic option in increasing the life expectancy in diabetic patients.

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Table 1 Summary of individual lifespan experiments.

|                          | Mean lifespan in days ± SEM | Max lifespan in days ± SEM | p value versus specific group of mean lifespan | p value versus specific group of max lifespan |
|--------------------------|-----------------------------|----------------------------|-----------------------------------------------|--------------------------------------------|
| WT (standard)            | 18.5 ± 0.4                  | 25.9 ± 0.4                 | <0.05\(a\)                                    | <0.05\(a\)                                 |
| WT (high glucose)        | 16.5 ± 0.6                  | 23.2 ± 0.4                 |                                              |                                            |
| WT + sorbitol            | 17.9 ± 0.2                  | 24.5 ± 0.5                 | n.s. (0.6438)\(a\)                           | n.s. (0.4676)\(a\)                        |
| Glyoxalase-1 transgenic  | 20.3 ± 0.2                  | 29.2 ± 1.5                 | <0.05\(a\)                                    | <0.05\(a\)                                 |
| Glyoxalase-1 transgenic (high glucose) | 20.6 ± 0.4                  | 27.7 ± 2.3                 | <0.001\(b\)                                  | <0.01\(b\)                                 |
| WT + glyoxalase-1 RNAi   | 13.5 ± 1.2                  | 21.0 ± 1.2                 | <0.001\(a\)                                  | <0.01\(a\)                                 |
| WT + glyoxalase-1 RNAi (high glucose) | 13.9 ± 0.7                  | 20.3 ± 1.1                 | <0.01\(b\)                                   | <0.05\(b\)                                 |
| WT + dead bacteria       | 29.3 ± 0.1                  | 40.5 ± 2.5                 | <0.01\(c\)                                   | <0.05\(c\)                                 |
| WT + dead bacteria (high glucose) | 25.1 ± 0.1                  | 35.5 ± 0.5                 | <0.01\(c\)                                   | <0.05\(c\)                                 |
| eat-2 (-) mutant         | 24.3 ± 0.3                  | 33.3 ± 0.3                 |                                              |                                            |
| eat-2 (-) mutant (high glucose) | 21.5 ± 1.4                  | 29.0 ± 1.5                 | <0.05\(c\)                                   | <0.05\(c\)                                 |
| WT + daf-2 RNAi          | 24.2 ± 2.0                  | 35.0 ± 3.0                 |                                              |                                            |
| WT + daf-2 RNAi (high glucose) | 20.6 ± 1.7                  | 30.0 ± 2.0                 | <0.05\(c\)                                   | <0.05\(c\)                                 |
| WT + FCCP                | 21.7 ± 1.7                  | 31.5 ± 0.5                 | <0.05\(a\)                                   | <0.01\(a\)                                 |
| WT + FCCP (high glucose) | 19.5 ± 0.9                  | 28.5 ± 1.5                 | <0.05\(b\)                                   | <0.01\(b\)                                 |
| WT + myxothiazol         | 21.1 ± 1.1                  | 31.0 ± 1.7                 | <0.01\(a\)                                   | <0.01\(a\)                                 |
| WT + myxothiazol (high glucose) | 20.4 ± 0.1                  | 27.7 ± 1.9                 | <0.001\(b\)                                  | <0.01\(b\)                                 |

Summary of mean and maximum lifespan and statistical analysis for lifespan experiments. The data were compared across the groups using analysis of variance (ANOVA); additional between-group comparisons were made using Fisher’s protected least significant difference (PLSD) post hoc tests: a-c: p value from a Fisher’s PLSD post hoc test comparing a group versus (a) WT under standard conditions, (b) WT under high glucose conditions, or (c) the corresponding group under standard conditions.
Figure legends

Fig. 1 Glucose concentration in *C. elegans*.
*C. elegans* were cultured on agar having the glucose concentrations of 0, 10, 20, 30, 40, and 50 mM. After 5 days 100 *C. elegans* were harvested and the glucose concentrations in the *C. elegans* whole body extracts were determined. Results are the means and SEM of three independent experiments; n.s. (not significant) describes a *p* value from a t-test > 0.05, * < 0.05, and *** < 0.001 comparing two groups as indicated.

Fig. 2 Effect of high glucose conditions on *C. elegans* lifespan.
Kaplan-Meier graphs of the fraction of *C. elegans* alive. Shown are wild type *C. elegans* cultured under standard and high glucose conditions. Lifespan assays were performed as described in Methods. The results are from a representative experiment out of three independent experiments, each including 100 nematodes; thin line = standard conditions, bold line = high glucose conditions.

Fig. 3 Effect of high glucose conditions on glyoxalase-1 activity.
Quantification of glyoxalase-1 activity in whole body extracts of 5-day-old wild type animals, cultured under standard and high glucose conditions, or sorbitol as control. Results are the means and SEM of three independent experiments; *** describes a *p* value from a t-test < 0.001 comparing wild type under standard conditions versus wild type under high glucose conditions.

Fig. 4 Mitochondrial MG-H1 immunoreactivity in *C. elegans*.
Mitochondria were stained with MitoTracker Deep Red FM (A, D, G, J), and MG-H1 was visualized by immunostaining with a Texas Red-labelled antibody directed against MG-H1 antibody (B, E, H, K). Merged staining is shown in C, F, I, and L. Orange colour indicates colocalization of MG-H1 with mitochondria. Images are shown for 15-day-old wild type animals cultured under standard (A-C) and high glucose conditions (D-F), and 15-day-old transgenic glyoxalase-1 overexpressing animals cultured under standard (G-I) and high glucose conditions (J-L). Shown are animals from a representative experiment out of three independent experiments, each including 100 nematodes.

Fig. 5 Effect of high glucose conditions on formation of ROS.
Shown are ethidium-labelled wild type animals cultured for 15 days under standard (A) and high glucose conditions (B), and transgenic glyoxalase-1 overexpressing animals cultured for 15 days under standard (C) and high glucose conditions (D). Shown are animals from a representative experiment out of three independent experiments, each including 100 nematodes.

Fig. 6 Quantification of MG-H1 and ROS in *C. elegans*.
Formation of MG-H1 (A) and ROS (B) was quantified in 15-day-old wild type animals, without and with 10 μM myxothiazol (myxo) or 50 μM carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and in 15-day-old transgenic glyoxalase-1 overexpressing animals. Each group was cultured under standard and high glucose conditions. The data were compared across the groups using analysis of variance (ANOVA); additional between-group comparisons were made using Fisher’s protected least significant difference (PLSD) post hoc tests. Results are the means and SEM of three independent experiments with 100 nematodes each; * describes a *p* value from a Fisher’s PLSD post hoc test < 0.05, and *** < 0.001 comparing the indicated group versus wild type under standard conditions; ooo describes a *p* value from a Fisher’s PLSD post hoc test < 0.001 comparing the indicated group versus wild type under high glucose conditions.
Glucose regulates lifespan in C. elegans

Fig 1

![Bar graph showing glucose levels in C. elegans](image1)

Fig 2

![Kaplan-Meier plot showing survival](image2)
Glucose regulates lifespan in C. elegans

**Fig 3**

![Bar graph showing glucose levels in C. elegans](image)

**Fig 4**

![Images of C. elegans showing different glucose conditions](image)
Glucose regulates lifespan in C. elegans

Fig 5
Glucose regulates lifespan in C. elegans

Fig 6