Highlights

- The role of insulin signalling in high glucose mediated damage was studied in *C. elegans*
- Oxidative stress and glycation are downstream targets of INS-7
- INS-7 signalling affects the neuronal system and longevity via SOD-3 and GLOD-4.
- Blocking of *ins-7* decreases ROS and AGEs under high glucose conditions.
- Thereby the neuronal system and lifespan is protected from glucose mediated damage.

Article

Reduction in *ins-7* gene expression in non-neuronal cells of high glucose exposed *Caenorhabditis elegans* protects from reactive metabolites, preserves neuronal structure and head motility, and prolongs lifespan

Michael Mendler[a], Christin Riedinger[a], Andrea Schlotterer[a,e], Nadine Volk[a], Thomas Fleming[a], Stephan Herzig[b/c/d], Peter P. Nawroth[a/c/d] and Michael Morcos[a,e]

Affiliations

a. Department of Medicine 1 and Clinical Chemistry, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

b. Institute for Diabetes and Cancer IDC, Helmholtz Center Munich, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

c. Joint Heidelberg IDC Translational Diabetes Program, Heidelberg University Hospital, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

d. German Center for Diabetes Research (DZD)

e. 5th Medical Department, University Medical Center, University of Heidelberg, Mannheim, Germany

Corresponding author

Michael Mendler, MD

Department of Medicine I and Clinical Chemistry

University of Heidelberg

Im Neuenheimer Feld 410

69120 Heidelberg, Germany

Phone: +49 6221 5637941, Fax: +49 6221 564233

E-mail: michael.mendler@med.uni-heidelberg.de

Word count abstract: 178

Word count main text including figure legends: 3,886

Abstract

Background: Glucose derived metabolism generates reactive metabolites affecting the neuronal system and lifespan in *C. elegans*. Here, the role of the insulin homologue *ins-7* and its downstream effectors in the generation of high glucose induced neuronal damage and shortening of lifespan was studied.

Results: In *C. elegans* high glucose conditions induced the expression of the insulin homologue *ins-7*. Abrogating *ins-7* under high glucose conditions in non-neuronal cells decreased reactive oxygen species (ROS)-formation and accumulation of methylglyoxal derived advanced glycation endproducts (AGEs), prevented structural neuronal damage and normalised head motility and lifespan. The restoration of lifespan by decreased *ins-7* expression was dependent on the concerted action of *sod-3* and *glod-4* coding for the homologues of iron-manganese superoxide dismutase and glyoxalase 1, respectively.

Conclusions: Under high glucose conditions mitochondria-mediated oxidative stress and glycation are downstream targets of *ins-7*. This impairs the neuronal system and longevity via a non-neuronal/ neuronal crosstalk by affecting *sod-3* and *glod-4*, thus giving further insight into the pathophysiology of diabetic complications.

Keywords Insulin action; diabetic neuropathy; glycation/AGE; oxidative stress/ROS; diabetic complications; longevity; *C. elegans*; neuronal function

Abbreviations

AGE	advanced glycation end-product
AU	arbitrary units
C. elegans	*Caenorhabditis elegans*
ctrl	control
DAF	abnormal *dauer* formation
DN	diabetic neuropathy
E. coli	*Escherichia coli*
FCCP	carbonyl cyanide-*4*-(trifluoromethoxy)phenylhydrazone
FOXO	forkhead box O
GFP	green fluorescent protein
GLOD	glyoxalase domain containing
HG	high glucose
k.o.	knock-out
MG	methylglyoxal
NAC	N-acetylcysteine
NGM	nematode growth medium
RNAi	RNA interference
ROS	reactive oxygen species
S	standard
SOD	superoxide dismutase
WT	wild-type

1. Introduction

Diabetic neuropathy (DN) is a burden to patients with diabetes mellitus (Kerner et al., 2014; Sen et al., 2015). The link between diabetes and neuropathy is not fully understood; there is, however, a link between the insulin/ insulin receptor signalling and neurodegeneration, the latter also being an integral part of the ageing process (Cohen and Dillin, 2008). The insulin/insulin receptor signalling is highly conserved throughout all species including the nematode *Caenorhabditis elegans* (*C. elegans*) (Holzenberger, 2009; Kenyon et al., 1993; Tatar et al., 2001). In *C. elegans*, the insulin receptor homologue (abnormal dauer formation-2, *daf-2*) is critically linked to lifespan and metabolic function (Porte et al., 2005; Tissenbaum, 2012). In *daf-2* knock out-mutants, lifespan is extended by two- to threefold, however, restoration of insulin receptor expression exclusively in neurons is sufficient to normalise lifespan (Wolkow et al., 2000), which emphasizes the link between the neuronal system and the whole organism. INS-7 is a well characterised insulin/IGF-1-like peptide in *C. elegans*, acting as a DAF-2 agonist. In a feedback loop, *ins-7* expression is inhibited in animals with reduced DAF-2 activity (Murphy et al., 2003).

High glucose (HG) culture conditions, comparable to blood glucose levels of poorly treated diabetic patients, are known to decrease *C. elegans'* lifespan and to induce neuronal damage (Lee et al., 2009; Schlotterer et al., 2009; Schulz et al., 2007). We chose the model system *C. elegans* as an easily accessible system to analyse mechanisms contributing to the generation of neuronal damage found under HG conditions. Because of their critical role in high-glucose induced damage, generation and neutralisation of ROS were also analysed. In low concentrations, ROS contribute to cell-protective signalling pathways by a mechanism known as mitohormesis. In higher concentrations, however, they induce cell damage (Schieber and Chandel, 2014). Protein modification by the reactive glycolysis by-product methylglyoxal (MG) leads to enhanced generation of ROS and reduced glyoxalase-1 activity (Mendler et al., 2015), enhancing MG formation in a self-perpetuating mechanism. With regard to downstream mechanisms being involved in the pathophysiological changes, we were especially interested in the iron/ manganese superoxide dismutase SOD-3, a known downstream target of INS-7/DAF-2 signalling (Murphy et al., 2003). Hence, the role of INS-7 and its downstream targets under HG conditions with respect to neuronal damage and lifespan were analysed in this study.

2. Material and methods

2.1 Quantification of gene expression

Gene expression was quantified by quantitative real-time PCR (Lightcycler, Roche Diagnostics, Mannheim, Germany) following total RNA isolation (RNeasy Micro Kit, Qiagen, Hilden, Germany) and reverse transcription (First Strand cDNA Synthesis Kit for RT-PCR, Roche Diagnostics, Mannheim, Germany). The following primers were used (Thermo Electron, Ulm, Germany): *ins-7*: 5'- TAT TGT CTT AAT CAT GCC CTC TT-3' and 5'- ATC AAT ACG GTA AGT ACT CTC AA-3', *sod-3*: 5'-ATG CCA CCT ACG TGA ACA ATC-3' and 5'-TCG CGC TTA ATA GTG TCC ATC-3', *tbg-1* (reference sequence): 5'-TGA TGA CTG TCC ACG TTG GA-3' and 5'-CGT CAT CAG CCT GGT AGA ACA-3' (Schumacher et al., 2005). *cat-1*: 5´-ATT GAT TGG GAC GAG AAC CC-3´ and 5´-GGA GCT TAT GCC TTT CTT CC-3´. mRNA was quantified by the second derivative maximum method.

2.2 *C. elegans* maintenance

Wild-type (N2 Bristol), the *ins-7* mutant strain RB1388 [*ins-7(ok1573)*], the *sod-3* mutant strain VC433 [*sod-3*(*gk235*) X.], and the *glod-4* mutant strain VC343 *[glod-4*(*gk189*) III.] were kindly provided by the *Caenorhabditis Genetics Center*, University of Minnesota, Minneapolis, MN, USA). NW1229 [*Prgef-1::GFP(evIs111)*] carrying a pan-neuronal GFP reporter has been described before (Altun-Gultekin et al., 2001). *C. elegans* were daily treated with 150 µl 0.4 M sorbitol (control) or 0.4 M glucose (HG) and cultured on an *Escherichia coli* (*E. coli*) OP50 lawn (*Caenorhabditis Genetics Center*, University of Minnesota, Minneapolis, MN, USA). The glucose treatment leads to glucose concentrations of 14 mM in whole worm extracts as published before (Schlotterer et al., 2009). FCCP and NAC were added to the plates in a concentration of 50 µM and 0.1 mM, respectively. Strains were cultured on nematode growth medium (NGM) plates (diameter 60 mm), and were transferred to plates containing 300 µg/ml 5-fluorodesoxyuridine on day 1 of adulthood (Sigma-Aldrich, Taufkirchen, Germany). For the evaluation of RNAi the feeding method was used and *C. elegans* were cultured on NGM plates containing 1 mM isopropyl-ß-D-1-thiogalactopyranoside (IPTG) and 25 µg/ml carbenicillin, seeded with *E. coli* HT115 expressing specific RNAi for *ins-7*, *sod-3*, *glod-4* or control RNAi, and transferred daily to fresh plates. The bacterial strains were provided by the Fire Lab *C. elegans* Vector Kit (Principal Investigator: Andrew Fire) (Addgene, Cambridge, MA, USA). The *cad-1* RNAi expressing bacteria were kindly provided by Prof. Ralf Baumeister, Institute for Biology 3, University of Freiburg, Germany and Prof. Björn Schumacher, Institute for Genome Stability in Ageing and Disease, University of Cologne, Germany. In lifespan assays nematodes were regarded as dead if they did not move after repeated stimulus. Nematodes were excluded if they crawled away from the plate or contained internally hatched nematodes. Experiments were performed at least in triplicates with 60 nematodes each.

2.3 Evaluation of neuronal damage

Structural damage was evaluated in the pan-neuronal GFP-expressing strain NW1229 (Altun-Gultekin et al., 2001) using fluorescence microscopy followed by a blinded evaluation procedure employing a four-staged classification scheme as described before (Mendler et al., 2012). Briefly, at least 20 nematodes were scored by at least 3 scientists. Score 0 was attributed to animals without any damage; score 1: minor damage, meaning a thinning of nerve cords and visible cell bodies because of cell shrinking; score 2: major damage, meaning disruption or loss of nerve cords and commissures. Nematodes with an extended loss of neuronal structure due to preparation artefacts and dead animals were excluded (score 3). To assess head motility, nematodes were recorded on video at day 12 of adulthood (Moticam 1000, Beyersdörfer, Mandelbachtal, Germany) and a worm tracking software (WormTracker 4.0, Thomas Bornhaupt, Neustadt/ Weinstraße, Germany) was used for the calculation of relative head motility.

2.4 Quantification of ROS and MG-derived AGEs

MG-derived AGEs were detected by immunostaining with a mouse primary antibody (AGE06B, Biologo, Kronshagen, Germany) and visualised by an Alexa Fluor-labelled goat secondary antibody (Invitrogen A11002, Thermo Fisher, Rockford, IL, USA). ROS were detected by oxidation of O_2^--sensitive hydroethidine. ROS and AGEs were quantified by confocal laser scanning microscopy as described before followed by calculation of mean pixel intensities with ImageJ software (Abramoff et al., 2004) as described before (Morcos et al., 2008). The method of ROS quantification was shown before to give similar results compared with measuring H_2O_2 production (Mendler et al., 2015).

2.5 Determination of superoxide dismutase activity

C. elegans were harvested and washed using M9 buffer (3 g/l KH_2PO_4, 6 g/l Na_2HPO_4, 5 g/l NaCl, 1 ml/l 1 M $MgSO_4$). After centrifugation an equal volume of zirconium oxide beads (0.5 mm) and a two-fold volume of homogenisation buffer (10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF) plus a protease inhibitor cocktail (Complete, Roche Diagnostics, Mannheim, Germany)) were added to the pellet. Homogenisation was performed with a Bullet Blender Blue (Next Advance Inc., Averill Park, NY, USA) at speed 10 for 2x2 min. Homogenates were centrifuged at 16,000 x *g* for 1 min, and the supernatants were assayed for protein concentration (BCA Protein Assay Kit, Thermo Fisher Scientific, Rockford, IL, USA). Superoxide dismutase activity was determined after filtration (Amicon Ultra-0.5 filter, Merck Millipore, Schwalbach, Germany) with a commercially available assay (Abcam, ab65354, Cambridge, UK).

2.6 Statistical analyses

Statistical analyses were performed by StatView 5.0 (SAS Institute, Cary, NC, USA) and Excel 2010 (Microsoft, Redmond, WA, USA). Unpaired Student's *t*-test was used to analyse differences between two groups. Analysis of variance (ANOVA) was used for comparisons of multiple groups.

3. Results

3.1 *ins-7* is upregulated under high glucose conditions and mediates glucotoxic damage

Under high glucose (HG) conditions of 14 mM corresponding to blood glucose levels seen in poorly controlled diabetic patients (Schlotterer et al., 2009) the mRNA expression of the *C. elegans* insulin homologue *ins-7* was increased by 33%±17% ($p<0.05$) (Fig. 1a). To evaluate the functional role of *ins-7* in the formation of HG-mediated damage, RNAi directed against *ins-7* was used for further experiments. RNAi treatment decreased the expression of *ins-7* by 94% measured by quantitative real-time PCR (Fig. 1b). This must be due to the effect of the RNAi on non-neuronal cells, as neuronal cells are highly refractory to RNAi treatment and cannot significantly be affected by an RNAi approach (Fig. S1). Under HG conditions *C. elegans'* mean lifespan was decreased from 21.4±0.7 days to 17.4±0.7 days ($p<0.001$). Abrogating *ins-7* in non-neuronal cells by specific RNAi prolonged lifespan under HG conditions from 17.4±0.7 days to 21.6±0.5 days ($p<0.001$) (Fig. 1c). This life prolonging effect was also proved for *ins-7* mutant *C. elegans* compared to WT in order to exclude model specific effects (Fig. S2a+b). Likewise, some neuronal damage caused by HG conditions could be reversed by *ins-7* RNAi. While under HG conditions structural damage was increased from 0.36±0.21 in controls to 1.41±0.10 under HG conditions ($p<0.05$), additional treatment with *ins-7* RNAi decreased neuronal damage from 1.41±0.10 to 0.42±0.22 ($p<0.05$) (Fig. 1d). ~~Neuronal function, as evaluated by quantification of~~ Head motility was decreased under HG conditions from 0.120±0.013 mm/s to 0.078±0.014 mm/s ($p<0.05$) and was increased by *ins-7* RNAi treatment from to 0.078±0.014 mm/s to 0.133±0.017 mm/s ($p<0.05$) (Fig. 1e). This indicates that HG mediates its deleterious effects at least partly by inducing *ins-7* likely in non-neuronal cells.

3.2 Downstream mechanisms of an *ins-7* knock-down

Under HG conditions *ins-7* RNAi treatment decreased ROS formation from 29.0±2.1 AU/pixel to 17.5±0.4 AU/pixel ($p<0.01$) (Fig. 2a) and normalised the accumulation of MG-derived AGEs from 22.9±0.3 AU/pixel to 12.7±0.4 AU/pixel ($p<0.001$) (Fig. 2b). Comparable results were obtained, when *ins-7* mutant *C. elegans* were used for the determination of ROS accumulation (Fig. S2c). Further, the effect of the mitochondrial uncoupling agent FCCP and the antioxidant NAC were studied. FCCP treatment of WT *C. elegans* decreased lifespan under S conditions from 21.1±0.3 days to19.6±0.5 days ($p<0.05$) but increased lifespan from 18.2±0.5 days to 20.5±0.2 days ($p<0.05$) under HG conditions to control levels. FCCP treatment in *ins-7* mutant *C. elegans* did not affect lifespan under S or HG conditions (Fig. 3a). Similar results were obtained when *C. elegans* were

treated with NAC. The lifespan of WT *C. elegans* was unchanged under S conditions but increased from 18.2±0.5 days to 22.4±0.1 days (p<0.001) under HG conditions. NAC treatment had no effect on the lifespan in *ins-7* mutant *C. elegans* (p=0.24) (Fig. 3b). This is consistent with the hypothesis that HG-mediated *ins-7* induction mediates its effects via impaired mitochondrial ROS detoxification.

3.3 Decreasing *ins-7* expression restores expression and activity of superoxide dismutase

To explain the ~~neuro-~~protective effects of *ins-7* depletion on ROS accumulation under HG conditions, *sod-3* expression was determined. In WT *C. elegans* the expression of *sod-3* was decreased under HG conditions from 100% to 65±8% (p<0.05) (Fig. 3c). In *ins-7* mutant *C. elegans* *sod-3* expression was unchanged under S conditions when compared to WT (p=0.10). However, it increased to 173±20% (p<0.001) under HG conditions when compared to WT (Fig. 3c). Similar results were obtained when non-neuronal cells were targeted by using *ins-7* mRNAi (Fig. S2d). Accordingly, superoxide dismutase activity, expressed as % inhibition rate, decreased under HG conditions from 56.9±5.6% to 34.8±5.9% (p<0.05) in WT *C. elegans*, but increased in *ins-7* mutant *C. elegans* under HG conditions when compared with WT under HG conditions from 34.8±5.9% to 75.3±1.7% (p<0.05) (Fig. 3d).

3.4 Enzymatic pathways involved in cellular protection by decreased *ins-7* expression

Corresponding to the data obtained with RNAi treatment, the mean lifespan remained unchanged under HG conditions in *ins-7* mutant *C. elegans* (mean lifespan 15.7±0.8 days (S) versus 14.3±0.8 days (HG); p=0.58) (Fig. 4a). Additional silencing of *sod-3* by RNAi decreased mean lifespan under HG conditions from 14.7±0.8 days to 12.1±0.6 days (p<0.0001) (Fig. 4b, and see also Fig. S2e). The same effect was observed for *ins-7* mutant *C. elegans* with additional silencing of *glod-4* where mean lifespan was decreased from 16.2±1.0 days to 8.4±0.6 days (p<0.0001) under HG conditions (Fig. 4c).

As the *sod-3* and *glod-4* RNAi presumably affects only non-neuronal cells, we reciprocally performed the same experiments with *sod-3* and *glod-4* mutant strains and *ins-7* RNAi. Interestingly, mean lifespan remained unchanged from 13.3±0.5 to 12.7±0.4 p=0.1530 by HG treatment in *sod-3* mutants with ins-7 RNAi (Fig. 4e), indicating a sensitive balance between non-neuronal and neuronal cells that provokes the different results with regard to *sod-3*. In contrast HG treatment decreased the mean life span of *glod-4* mutants from 20.7±0.7 to 14.3±0.6 p<0.0001 with *ins-7* RNAi (Fig 4g), which is the same effect as seen by the inverse experiment. Therefore it makes no difference whether *glod-4* expression is decreased ubiquitously or only in non-neuronal cells.

4. Discussion

In this study, the role of the *C. elegans* insulin-like peptide INS-7 was analysed with respect to its influence on high glucose (HG)-mediated damage. HG increased the expression of *ins-7* which is known to shorten lifespan. Down-regulation of *ins-7* by specific RNAi prevented the HG-mediated neuronal damage and prolonged lifespan to control level, indicating a causal role for *ins-7* in the generation of HG-mediated damage. Which neuronal subpopulations are affected here has to be investigated in future studies as our methods only visualizes the structural integrity of larger neurons only and it will be of interest to consider more aspects of neuronal function than head motility. Under standard culture conditions, down-regulation of *ins-7* had only marginal effects on neuronal damage and lifespan in wild-type *C. elegans*. This finding was not expected, as an increase in lifespan was described under standard culture conditions for the RNAi-sensitive strain *rrf-3*(*pk1426*) and for *fer-15; fem-1* (Murphy et al., 2003). Obviously, these lifespan experiments cannot be transferred one-to-one from to the wild-type (N2 Bristol) that has been used in this study as neuronal cells are not affected by the RNAi treatment in wild-type *C. elegans*. ~~Presumably, the *ins-7* RNAi effect is not strong enough to provoke a phenotype without either augmentation in the *rrf-3(pk1426)* strain or under HG conditions.~~ Presumably, the effect *ins-7* RNAi that is accentuated in glucose treated and therefore metabolically altered nematodes seen here must therefore be provoked by non-neuronal cells and there must be crosstalk between non-neuronal and neuronal cells that causes the influence on neuronal structure and head motility. Future studies have to reveal the mechanisms that are involved in this crosstalk, as this is beyond the scope of this manuscript. Further, with the methods used here, we cannot conclude which neuronal subpopulations are important for the effects observed here, as we primarily observed the structural integrity of larger neurons and displayed relative head motility as one movement parameter. ~~The metabolic alteration by HG treatment makes *C. elegans* therefore susceptible to *ins-7* RNAi treatment.~~

As published before, the accumulation of ROS and AGEs is part of the metabolic changes seen under HG culture conditions (Schlotterer et al., 2009) and accumulation of both could be antagonised in this study by decreasing *ins-7* expression. We showed similar effects for the exogenous treatment with human insulin before (Mendler et al., 2015), but beyond that, in the data presented, ROS and AGEs are not only improved pharmacologically by insulin treatment but *C. elegans'* intrinsic insulin signalling is causally involved in mediating HG dependent damage by the increased expression of *ins-7*. ROS, as they are generated under high glucose conditions, have deleterious effects on cells and organisms (Schieber and Chandel, 2014), whereas low concentrations might induce protective signalling pathways (Schulz et al., 2007; Yee et al., 2014). In line with a role of ROS in neuronal damage, treatment with FCCP and NAC improves lifespan under HG conditions in wild-type *C. elegans*. The lifespan of *ins-7* mutant *C. elegans*, in contrast, was not prolonged, indicating that ROS were sufficiently detoxified by other mechanisms.

Supporting this, we observed an increased mitochondrial superoxide dismutase expression and activity under HG conditions by treatment with *ins-7* RNAi. Mitochondrial superoxide dismutase is a known downstream target of insulin/ insulin receptor-signalling (Honda and Honda, 1999; Murphy et al., 2003) . Decreased *ins-7* expression leads to a *daf-2* dependent translocation of the transcription factor DAF-16, thus modifying the *sod-3* expression pattern (Murphy et al., 2007). Decreased peroxide concentration and improved SOD activity are observed in *daf-2*/ insulin receptor mutant *C. elegans* (Zarse et al., 2012). A critical role of SOD activity in our model was confirmed by showing that the life-prolonging effect of an *ins-7* knock-out is lost, when *sod-3* is down-regulated in non-neuronal cells by RNAi. Interestingly, this effect is not observed, when *ins-7* is down-regulated by RNAi in *sod-3* mutants, which also indicates a sensitive balance between non-neuronal and neuronal cells. Notably, under HG conditions the nematodes have a shorter lifespan when neuronal *sod-3* expression is preserved which is supporting the concept of mitohormesis (Schulz et al., 2007; Yee et al., 2014). Future studies have to investigate the mechanisms of this non-neuronal/ neuronal cross-talk. In contrast we do not see a difference with regard to *glod-4*, where we do not see a different result in the inverse experiment. This is not surprising as the RNAi refractory neuronal tissue is rich in insulin receptor but has only a low glyoxalase activity (Bierhaus et. al 2012) and hence we would not expect a large difference between a global knockout and a non-neuronal one. Therefore even in a global knockout the effect in the non-neuronal cells will dominate.

Moreover, the life-prolonging effect of an *ins-7* knock-down under HG conditions dependents on glyoxalase 1, which in turn explains the reduced AGE accumulation. This goes along with the findings that glyoxalase 1 activity can be reduced by the accumulation of ROS, namely superoxide and hydrogen peroxide (Antognelli et al., 2014). Vice versa the accumulation of MG following a reduced glyoxalase 1 activity decreases the activity of superoxide dismutase (Choudhary et al., 1997). This vicious circle that augments the deleterious effects of a HG treatment can be intercepted when *ins-7* expression is decreased by RNAi or in *ins-7* mutant *C. elegans*, respectively.

5. Conclusions

Oxidative stress and formation of glycation products are downstream targets of INS-7 under HG conditions and impair the neuronal system and longevity by affecting SOD-3 and GLOD-4. Decreasing the accumulation of ROS and AGEs by decreasing *ins-7* expression prolongs lifespan and protects the neuronal system from glucose-mediated damage. It is sufficient to decrease *ins-7* expression in non-neuronal cells by RNAi to achieve this effect which requires a non-neuronal/ neuronal crosstalk. Our data emphasises the need to target reactive metabolites in order to prevent late complications in diabetes mellitus and it indicates the role of metabolite driven changes in a cellular crosstalk under HG conditions affecting neuronal damage and lifespan.

Funding

This study was supported by the German Research Foundation (DFG), projects 'NA-137' and 'SFB1118, TP C06 Reactive metabolites as cause of diabetic complications' (MMe, CR, TF, SH, PPN) and 'SFB1158', TP A03 (PPN). PPN, TF, and SH are supported by the German Center for Diabetes Research (DZD). NV is supported by DFG research training group (GRK) 1874. PPN is supported by the Dietmar-Hopp-Foundation.

Acknowledgements

We kindly thank Prof. Ralf Baumeister, Institute for Biology 3, University of Freiburg, Germany and Prof. Björn Schumacher, Institute for Genome Stability in Ageing and Disease, University of Cologne, Germany for providing us with the *cad-1* primer sequences and RNAi bacterial strains.

Conflict of interest

All authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

All authors had substantial contributions to conception and design, acquisition of data, analysis and interpretation of the data. All authors drafted or revised the manuscript critically for intellectual content and approved this version to be published.

References

Abramoff, M.D., Magelhaes, P.J., and Ram, S.J. (2004). Image Processing with ImageJ. Biophotonics Int *11*, 36-42.

Altun-Gultekin, Z., Andachi, Y., Tsalik, E.L., Pilgrim, D., Kohara, Y., and Hobert, O. (2001). A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in C. elegans. Development *128*, 1951-1969.

Antognelli, C., Gambelunghe, A., Talesa, V.N., and Muzi, G. (2014). Reactive oxygen species induce apoptosis in bronchial epithelial BEAS-2B cells by inhibiting the antiglycation glyoxalase I defence: involvement of superoxide anion, hydrogen peroxide and NF-kappaB. Apoptosis : an international journal on programmed cell death *19*, 102-116.

Bierhaus A., Fleming T., Stoyanov S., Leffler A., Babes A., Neacsu C., Sauer S.K., Eberhardt M., Schnölzer M., Lasitschka F., Neuhuber W.L., Kichko T.I., Konrade I., Elvert R., Mier W., Pirags V., Lukic I.K., Morcos M., Dehmer T., Rabbani N., Thornalley P.J., Edelstein D., Nau C., Forbes J., Humpert P.M., Schwaninger M., Ziegler D., Stern D.M., Cooper M.E., Haberkorn U., Brownlee M., Reeh P.W., Nawroth P.P. (2012) Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. Nat Med. *18*, 926-933

Choudhary, D., Chandra, D., and Kale, R.K. (1997). Influence of methylglyoxal on antioxidant enzymes and oxidative damage. Toxicology letters *93*, 141-152.

Cohen, E., and Dillin, A. (2008). The insulin paradox: aging, proteotoxicity and neurodegeneration. Nature reviews Neuroscience *9*, 759-767.
Holzenberger, M. (2009). [IGF-1 receptors in the brain control longevity in mice]. Med Sci (Paris) *25*, 371-376.
Honda, Y., and Honda, S. (1999). The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *13*, 1385-1393.
Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461-464.
Kerner, W., Bruckel, J., and German Diabetes, A. (2014). Definition, classification and diagnosis of diabetes mellitus. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association *122*, 384-386.
Lee, S.J., Murphy, C.T., and Kenyon, C. (2009). Glucose shortens the life span of C. elegans by downregulating DAF-16/FOXO activity and aquaporin gene expression. Cell Metab *10*, 379-391.
Mendler, M., Schlotterer, A., Ibrahim, Y., Kukudov, G., Fleming, T., Bierhaus, A., Riedinger, C., Schwenger, V., Herzig, S., Hecker, M., *et al.* (2015). daf-16/FOXO and glod-4/glyoxalase-1 are required for the life-prolonging effect of human insulin under high glucose conditions in Caenorhabditis elegans. Diabetologia *58*, 393-401.
Mendler, M., Schlotterer, A., Morcos, M., and Nawroth, P.P. (2012). Understanding diabetic polyneuropathy and longevity: what can we learn from the nematode Caenorhabditis elegans? Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association *120*, 182-183.
Morcos, M., Du, X., Pfisterer, F., Hutter, H., Sayed, A.A., Thornalley, P., Ahmed, N., Baynes, J., Thorpe, S., Kukudov, G., *et al.* (2008). Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in Caenorhabditis elegans. Aging Cell *7*, 260-269.
Murphy, C.T., Lee, S.J., and Kenyon, C. (2007). Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of Caenorhabditis elegans. Proc Natl Acad Sci U S A *104*, 19046-19050.
Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature *424*, 277-283.
Porte, D., Jr., Baskin, D.G., and Schwartz, M.W. (2005). Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes *54*, 1264-1276.
Schieber, M., and Chandel, N.S. (2014). ROS function in redox signaling and oxidative stress. Current biology : CB *24*, R453-462.
Schlotterer, A., Kukudov, G., Bozorgmehr, F., Hutter, H., Du, X., Oikonomou, D., Ibrahim, Y., Pfisterer, F., Rabbani, N., Thornalley, P., *et al.* (2009). C. elegans as model for the study of high glucose- mediated life span reduction. Diabetes *58*, 2450-2456.
Schulz, T.J., Zarse, K., Voigt, A., Urban, N., Birringer, M., and Ristow, M. (2007). Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab *6*, 280-293.
Schumacher, B., Schertel, C., Wittenburg, N., Tuck, S., Mitani, S., Gartner, A., Conradt, B., and Shaham, S. (2005). C. elegans ced-13 can promote apoptosis and is induced in response to DNA damage. Cell Death Differ *12*, 153-161.
Sen, H.M., Sen, H., Asik, M., Ozkan, A., Binnetoglu, E., Erbag, G., and Karaman, H.I. (2015). The importance of education in diabetic foot care of patients with diabetic neuropathy. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association *123*, 178-181.

Tatar, M., Kopelman, A., Epstein, D., Tu, M.P., Yin, C.M., and Garofalo, R.S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science *292*, 107-110.
Tissenbaum, H.A. (2012). Genetics, life span, health span, and the aging process in Caenorhabditis elegans. The journals of gerontology Series A, Biological sciences and medical sciences *67*, 503-510.
Wolkow, C.A., Kimura, K.D., Lee, M.S., and Ruvkun, G. (2000). Regulation of C. elegans life-span by insulinlike signaling in the nervous system. Science *290*, 147-150.
Yee, C., Yang, W., and Hekimi, S. (2014). The intrinsic apoptosis pathway mediates the pro-longevity response to mitochondrial ROS in C. elegans. Cell *157*, 897-909.
Zarse, K., Schmeisser, S., Groth, M., Priebe, S., Beuster, G., Kuhlow, D., Guthke, R., Platzer, M., Kahn, C.R., and Ristow, M. (2012). Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab *15*, 451-465.

Figure captions

Fig. 1. Impairment of lifespan and the neuronal system under high glucose (HG) conditions is mediated by *ins-7*. (a) *C. elegans* were cultured under standard (S) and HG conditions and expression of *ins-7* was quantified in total RNA extracts of WT *C. elegans* at day 5 by RT-PCR and normalised to *tbg-1*. Results are the means and standard errors of 8 independent experiments, each performed in triplicates. *$p<0.05$ for the indicated comparison (calculated using the unpaired Student‘s *t*-test). (b) Expression of *ins-7* could be decreased by 94% with specific RNAi at day 5. (c) Kaplan-Meier graphs of *C. elegans* from a representative experiment out of 3 independent experiments with 60 *C. elegans* each under S (bold lines) and HG conditions (dashed lines) cultivated on control-RNAi (black lines) and *ins-7*-RNAi-feeding plates (grey lines), respectively. ***$p<0.001$ for the indicated comparison (logrank-test). (d) Neuronal structure was visualised by a pan-neuronal-specific GFP reporter under S or HG conditions in *C. elegans* cultivated on control-RNAi or *ins-7*-RNAi feeding plates. Neuronal damage was quantified at day 12 using a four-staged classification scheme as described in methods. Results are the means with standard errors of 3 independent experiments, each with 8 nematodes per group. (e) Functional impairment was quantified by determination of head motility as described in methods. WT *C. elegans* were cultivated under S or HG conditions on control-RNAi or *ins-7*-RNAi feeding plates. Results are the means with standard errors of 3 independent experiments, each with 10 nematodes per group. *$p<0.05$ for the indicated comparison (calculated using the unpaired Student‘s *t*-test).

Fig. 2. Formation of ROS and AGEs (a) WT *C. elegans* were cultivated on control- or *ins-7*-RNAi feeding plates under S and HG conditions and ROS were detected by confocal laser scanning microscopy of ethidium-labeled *C. elegans* at the age of 12 days. (b) MG-derived AGEs were detected by immunostaining and quantified by calculation of mean pixel values at day 12 [arbitrary units (AU)/pixel]. Means and standard error were calculated from 3 independent experiments, each with 30 nematodes per group. Results are the means with standard error of 3 independent experiments. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ for the indicated comparison (calculated using the unpaired Student's *t*-test).

Fig. 3. ROS detoxification (a, b) Effect of the mitochondrial uncoupler FCCP and the antioxidant NAC on lifespan. WT *C. elegans* were cultured on control- or *ins-7*-RNAi feeding plates under standard (S) and high glucose (HG) conditions in the presence and absence of 50 µM FCCP (a) and 1 mM NAC (b), respectively. Shown are the mean lifespans and standard errors of 3 independent experiments. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ for the indicated comparison (calculated using the unpaired Student's *t*-test) (c) *sod-3* mRNA levels were determined by qRT-PCR in WT and *ins-7* mutant *C. elegans* at the age of 12 days, cultivated under standard (S) and HG conditions. mRNA levels were normalised to *tbg-1* expression and expressed relative to S conditions. Means with standard errors of 3 independent experiments performed in triplicates. (d) Superoxide dismutase activity was determined in WT and *ins-7* mutant *C. elegans* at day 12, cultivated under S and HG conditions. Means with standard errors of 3 independent experiments performed in triplicates.

Fig 4. Protection by *ins-7* knock-out is dependent on both *sod-3* and *glod-4*. *Ins-7* mutant *C. elegans* were grown on control (a) *sod-3* RNAi (b) and *glod-4* RNAi feeding plates (c), respectively and were cultured under standard (S, bold lines) and high glucose conditions (HG, dashed lines). Inversely, *sod-3* mutant *C. elegans* were grown on control (d) and *ins-7* RNAi feeding plates (e) and likewise *glod-4* on control (f) and *ins-7* RNAi feeding plates (g). Shown are Kaplan-Meier graphs of one representative experiment out of three with each 60 nematodes.

Figure 1

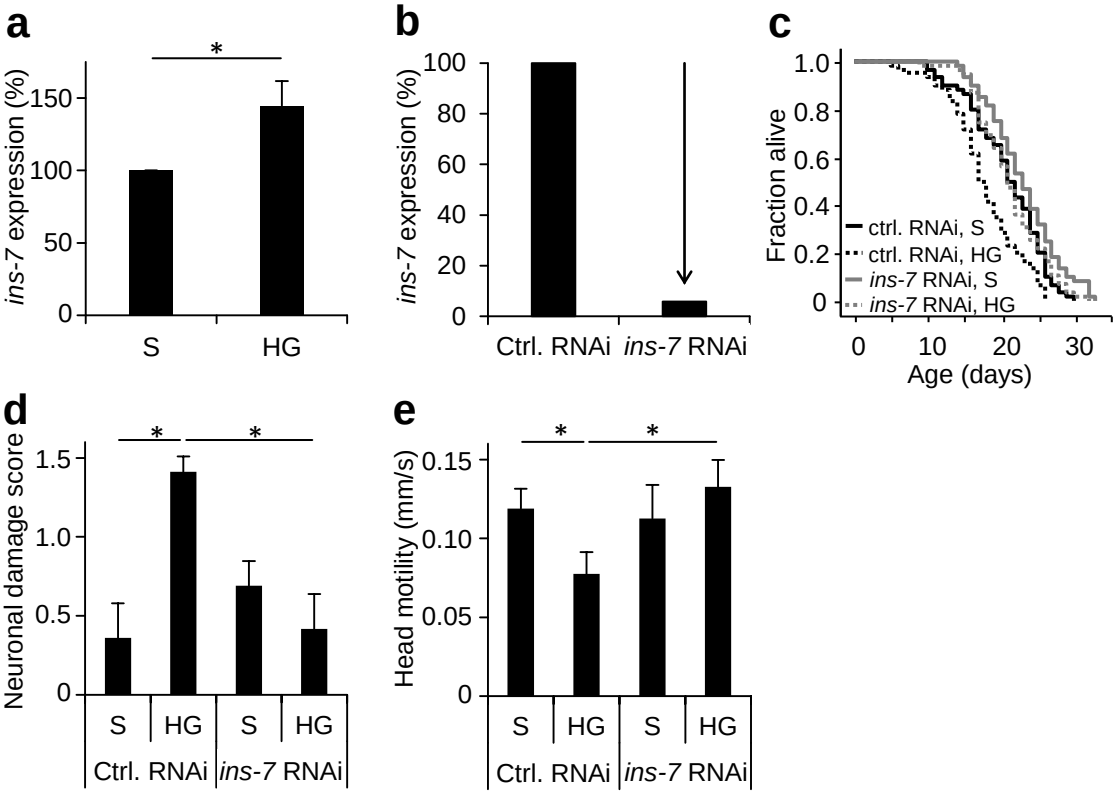

Fig.1

Figure 2

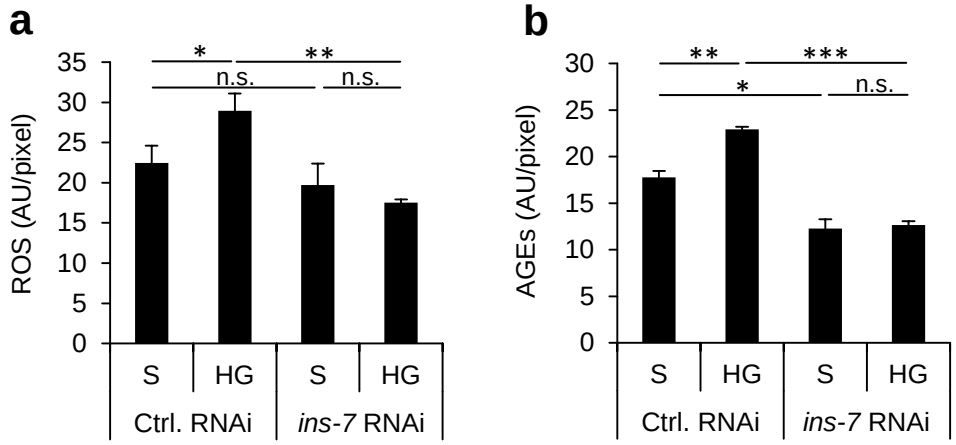

Fig.2

Figure 3

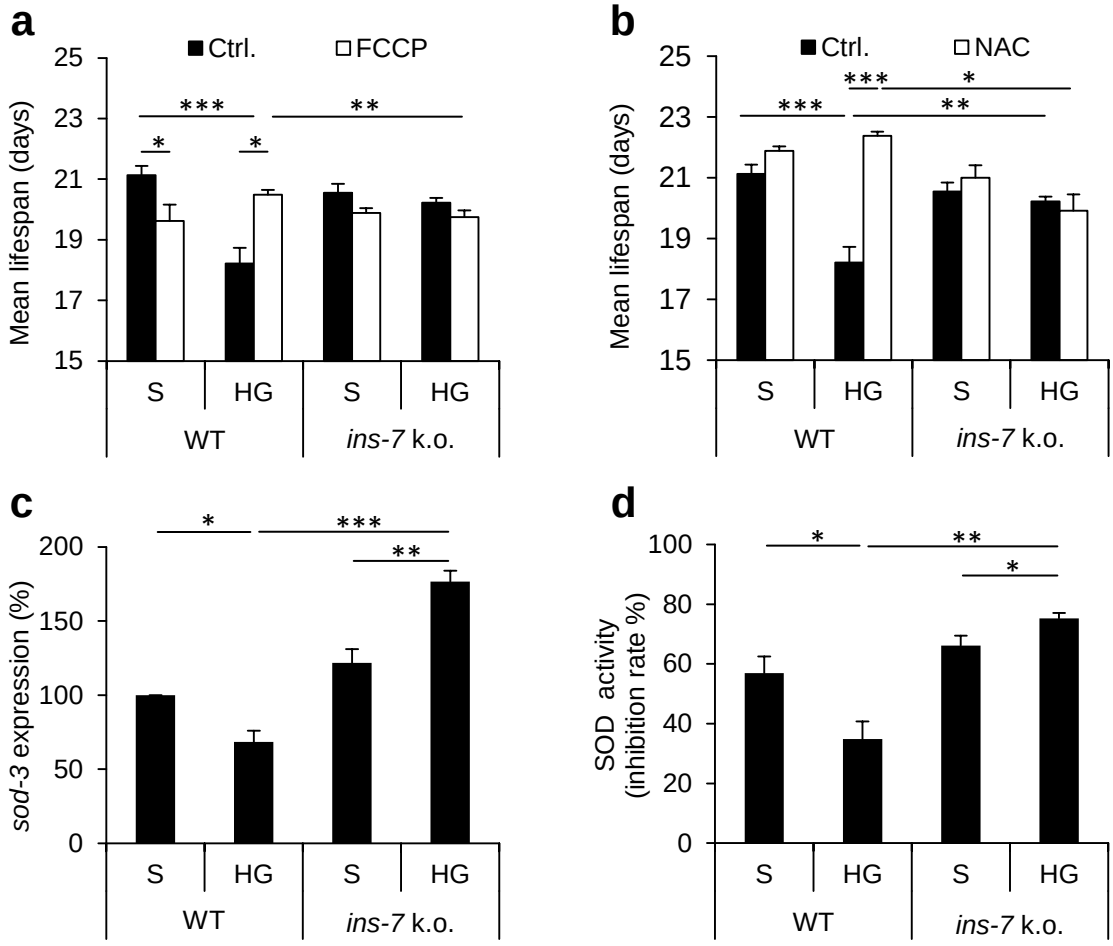

Fig.3

Figure 4

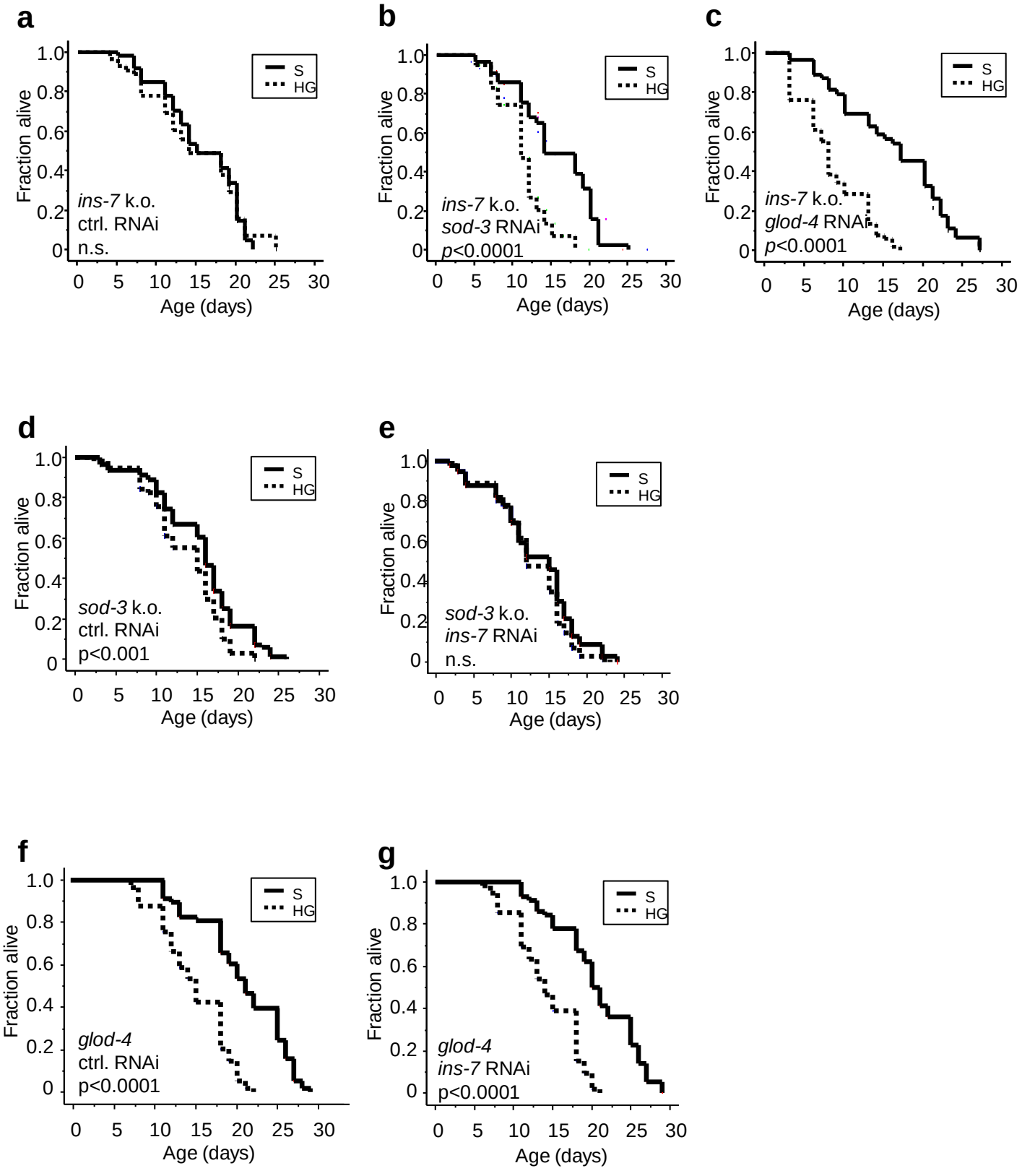

Fig.4