Cell Metabolism
Article

Glucose Shortens the Life Span of *C. elegans* by Downregulating DAF-16/FOXO Activity and Aquaporin Gene Expression

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DOI 10.1016/j.cmet.2009.10.003

SUMMARY

Many studies have addressed the effect of dietary glycemic index on obesity and diabetes, but little is known about its effect on life span itself. We found that adding a small amount of glucose to the medium (2%) shortened the life span of *C. elegans* by inhibiting the activities of life span-extending transcription factors that are also inhibited by insulin signaling: the FOXO family member DAF-16 and the heat shock factor HSF-1. This effect involved the downregulation of an aquaporin glycerol channel, *aqp-1.* We show that changes in glycerol metabolism are likely to underlie the life span-shortening effect of glucose and that *aqp-1* may act cell nonautonomously as a feedback regulator in the insulin/IGF-1-signaling pathway. Insulin downregulates similar glycerol channels in mammals, suggesting that this glucose-responsive pathway might be conserved evolutionarily. Together, these findings raise the possibility that a low-sugar diet might have beneficial effects on life span in higher organisms.

INTRODUCTION

Diets of industrialized countries tend to have high glycemic index (GI) values. The high-GI diets, which are enriched in processed carbohydrates or sugars, are easily metabolized to glucose and raise blood glucose level very quickly. High-GI diets have been linked to obesity, type 2 diabetes, and cardiovascular diseases (Aston, 2006; Venn and Green, 2007). How high-GI diets may accelerate the onset and progression of these diseases is not clear. Possible mechanisms include direct metabolic effects, changes in body weight, and alterations in hormonal regulatory systems. The hormone insulin may mediate at least some of the effects of the high-GI diets on human health. Blood insulin levels rise rapidly after the consumption of high-GI foods and then fall quickly (Aston, 2006; Venn and Green, 2007). This dramatic fluctuation in insulin levels may lead to insulin resistance and eventually to type 2 diabetes, although further research on the molecular effects of insulin fluctuations is required.

The insulin-signaling pathway is evolutionarily well conserved from *C. elegans* to mammals (Barbieri et al., 2003; Katic and Kahn, 2005). In mammals, insulin and its close homolog IGF-1 bind to tyrosine-kinase receptors and result in the inhibition of the FOXO transcription factor, an important transcriptional regulator of many cellular processes such as metabolism, stress responses, and apoptosis (Barbieri et al., 2003; Katic and Kahn, 2005; Salih and Brunet, 2008).

The insulin/IGF-1-signaling pathway has been shown to regulate the life span of many organisms (Barbieri et al., 2003; Katic and Kahn, 2005; Kenyon, 2005). Reducing the activity of this pathway—for example, by mutation of the *C. elegans daf-2* insulin/IGF-1 receptor gene (Kimura et al., 1997)—slows the aging process and doubles life span (Kenyon et al., 1993). This extended life span requires the activity of the FOXO transcription factor DAF-16 and the heat shock transcription factor HSF-1 (Henderson and Johnson, 2001; Hsu et al., 2003; Kenyon et al., 1993; Lee et al., 2001; Lin et al., 1997, 2001; Morley and Morimoto, 2004; Ogg et al., 1997). In addition, DAF-16 and HSF-1 contribute to the longevity of wild-type animals cultured on bacteria under standard laboratory conditions, as reducing either *daf-16* or *hsf-1* gene activity accelerates the rate of tissue aging and shortens life span (Garigan et al., 2002; Herndon et al., 2002; Kenyon et al., 1993; Lin et al., 2001). Although the connection between the insulin/IGF-1-signaling pathway and aging in *C. elegans* has been well established, our current knowledge of the effect of glucose on the *C. elegans* insulin/IGF-1-signaling pathway and on life span is very limited.

In this study, we tested whether glucose feeding affected the life span of *C. elegans*. As recently shown by Schulz et al. in an independent study (Schulz et al., 2007), we found that a glucose-enriched diet significantly shortened the life span of *C. elegans*. We then found that glucose decreased life span by inhibiting the DAF-16 and HSF-1 transcription factors. In addition, we found that the downstream aquaporin gene *aqp-1*, which encodes a glycerol channel, was responsible for the life span-shortening effect of a glucose-containing diet. In mammals, insulin represses the expression of genes encoding similar aquaporin glycerol channels (Kishida et al., 2001; Kuriyama et al.,

2002). Genetic depletion of one of these aquaporin channels in mice causes obesity and insulin resistance, pointing to the importance of these glycerol channels in mammalian glucose metabolism (Hara-Chikuma et al., 2005; Hibuse et al., 2005). The role of glycerol channels in glucose metabolism seems to be conserved in *C. elegans*, since we found that *C. elegans aqp-1* was also a glucose-regulated downstream target of DAF-16 and HSF-1. Furthermore, we showed that *aqp-1*, in turn, influenced DAF-16 activity. This positive feedback loop may act cell nonautonomously to adjust the level of DAF-16/FOXO activity among the tissues. It is possible that, like many other pathways that affect aging (Barbieri et al., 2003; Guarente, 2007; Katic and Kahn, 2005; Kenyon, 2005; Piper and Bartke, 2008; Stepanyan et al., 2006), the glucose-responsive pathway might be conserved from *C. elegans* to mammals. If so, then low-sugar diets might have beneficial effects on mammalian aging. Surprisingly, dietary glucose could completely suppress the long life span of *daf-2(−)* insulin/IGF-1 receptor mutants in *C. elegans*, suggesting that individuals with an impaired insulin receptor might benefit disproportionately from a low-sugar diet.

RESULTS

Dietary Glucose Shortens the Life Span of *C. elegans*

To ask whether glucose might influence the life span of *C. elegans*, which are normally fed a diet of *E. coli* OP50 bacteria, we added 2% glucose to culture plates containing normal growth (NG) medium and a lawn of bacteria. We found that glucose addition decreased life span by approximately 20% (Figure 1A). This life span shortening required glucose treatment during adulthood, as feeding only during development had no effect on adult life span (Figure 1B).

We confirmed that the animals ingested glucose, as glucose-treated worms contained ~50% more glucose than did control animals (Figure 1C). To test whether an indirect effect of glucose addition might influence life span, we asked whether increased osmolarity or glucose-fed bacteria could shorten the life span of *C. elegans*. Neither of these treatments appeared to have any effect. First, 2% L-glucose or D-sorbitol did not affect life span (Figures 1D and 1E), whereas as little as 0.1% (5.6 mM) D-glucose was sufficient to decrease life span significantly (Figure 1F). Second, when we cultured worms on NG medium (no added glucose) plated with bacteria prefed 2% glucose, the animals were not short lived (Figure 1G), arguing against the possibility that a metabolite of glucose produced by the bacteria was responsible for the shorter life span. Consistent with this, animals were short lived when cultured on 2% glucose plates containing killed bacteria (Figure 1G). Likewise, 2% glucose plates seeded with ΔPTS mutant bacteria, which are defective in glucose uptake (Deutscher et al., 2006), decreased the life span of the animals (Figure 1H). Taken together, these data suggest that glucose consumption directly decreases the life span of *C. elegans*. However, we note that we cannot rule out the possibility that the sensory perception of glucose plays a role in this pathway. While this study was in progress, Schulz et al. reported independently that glucose shortens *C. elegans*' life span and described analogous control experiments (Schulz et al., 2007).

Glucose Shortens Life Span by Decreasing DAF-16/FOXO and HSF-1 Activity

Because glucose stimulates insulin secretion in mammals, we wondered whether glucose might shorten the life span of *C. elegans* by influencing components of the insulin/IGF-1-signaling pathway. Insulin/IGF-1 signaling inhibits the transcriptional activity of DAF-16/FOXO (Salih and Brunet, 2008). When insulin/IGF-1 signaling is reduced, life span is doubled, and this life span extension requires *daf-16* (Kenyon et al., 1993). Conversely, when *daf-16* is deleted in otherwise normal animals, the rate of tissue aging is accelerated, and life span is shortened by ~20% (Garigan et al., 2002; Lin et al., 2001). We found that glucose did not further shorten the life span of *daf-16(−)* animals (Figures 2A and S4A).

The long life span produced by inhibiting insulin signaling in *C. elegans* also requires the heat shock transcription factor *hsf-1* (Hsu et al., 2003; Morley and Morimoto, 2004), and as with *daf-16*, reducing *hsf-1* activity accelerates aging and shortens life span (Garigan et al., 2002). We found that glucose did not further shorten the life span of *hsf-1(−)* mutants (Figures 2B and S4B). The effect of glucose on *daf-16(−)* and *hsf-1(−)* animals was specific, because glucose did shorten the life span of animals containing mutations in many other genes that affect life span, including *sir-2.1(−)*, *aak-2(−)*, *nhr-49(−)*, and *daf-12(−)* (Figures S5 and S6, described below). In addition, glucose treatment also completely suppressed the long life span caused by *hsf-1* overexpression, which is *daf-16* dependent (Hsu et al., 2003) (Figure 2C). This genetic specificity implicates *daf-16* and *hsf-1* in the longevity response to dietary glucose and suggests that glucose is not simply toxic to worms in a general, nonspecific way.

During development, the insulin/IGF-1 pathway regulates a hibernation-like state called dauer diapause (Hu, 2007). The dauer is an alternative third larval stage that worms enter in response to food limitation and crowding. Whereas animals carrying weak *daf-2* mutations become long-lived adults, animals carrying stronger mutations become dauers constitutively, as do animals carrying weak *daf-2* mutations but grown at high temperature (Hu, 2007). Like adult life span extension, dauer formation requires *daf-16*. We found that 2% glucose feeding reduced the fraction of the animals that became dauers at 22.5°C in strains carrying reduction-of-function mutations in *daf-2* (Figure 3A). This was true for two different reduction-of-function alleles of *daf-2*, *daf-2(e1368)* and *daf-2(e1370)*. In addition, glucose feeding suppressed dauer formation in wild-type animals grown at a higher temperature, 27°C (Figure 3B). These data are consistent with the hypothesis that glucose treatment upregulates the insulin/IGF-1-signaling pathway, which in turn counteracts the reduced activity of the insulin/IGF-1 receptor in these animals.

Many DAF-16-regulated genes have been identified in microarray and other studies (Halaschek-Wiener et al., 2005; Lee et al., 2003; McElwee et al., 2003, 2004; Murphy et al., 2003; Oh et al., 2006; Ookuma et al., 2003). To ask whether glucose affects the expression of known DAF-16-regulated genes, we carried out microarray analysis. We found that treating wild-type animals with 2% glucose produced a pattern of gene expression that overlapped significantly with that produced by genetic inhibition of *daf-16* activity in *daf-2* insulin/IGF-1 receptor mutants

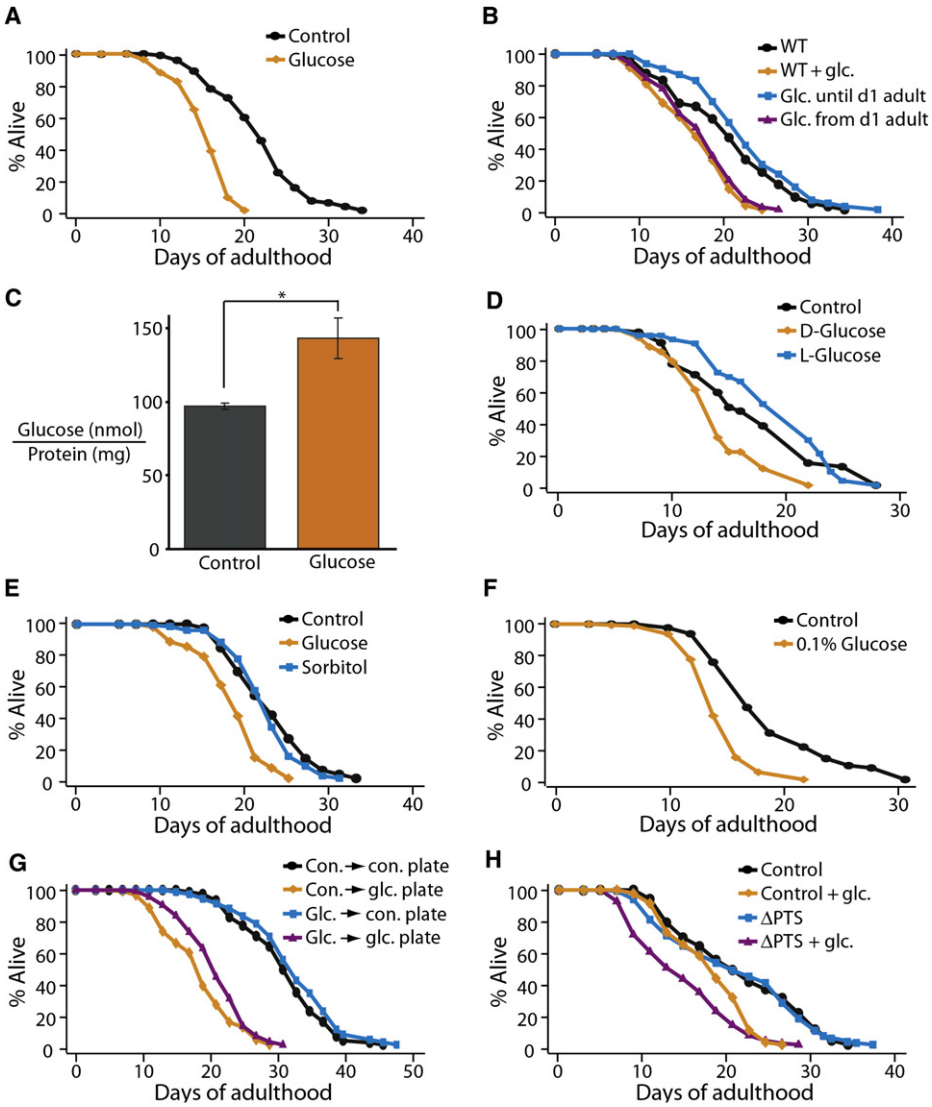

Figure 1. Glucose Feeding Shortens the Adult Life Span of *C. elegans*

(A) A diet containing 2% D-glucose (glucose) decreased the life span of wild-type (N2) animals significantly.

(B) Glucose feeding initiated on day 1 of adulthood (Glc. from d1 adult) shortened the life span of wild-type animals, but glucose feeding during entire larval developmental period (Glc. until d1 adult) did not.

(C) Glucose feeding increased the glucose level inside the animals. Error bars represent SEM (*$p < 0.05$, two-tailed Student's t test).

(D and E) In contrast to D-glucose, diet containing 2% L-glucose (D) or sorbitol (E) did not shorten life span. See Table S1 for additional trials and statistical analysis for these and all other life span data.

(F) 0.1% glucose was sufficient to affect life span. See Figure S2 for other glucose concentrations.

(G) Worms grown on glucose-containing plates (glc. plate) with dead bacteria were short lived whether the bacteria had been previously cultured with glucose (glc. → glc. plate) or not (con. → glc. plate). In contrast, growing bacteria in 2% glucose media prior to killing the bacteria and moving to a control plate without glucose (glc. → con. plate) was not sufficient to affect life span.

(H) Worms fed with glucose-uptake-defective ΔPTS OP50 double mutant bacteria (ΔPTS) (Figure S3) grown on 2% glucose plates were short lived. Finally, since progeny number is negatively correlated with life span in many cases, we measured the effect of glucose feeding on total progeny number. However, glucose feeding decreased the total progeny number (Figure S1).

(hypergeometric probabilities, $p < 10^{-10}$) (Table 1). We also observed changes in expression of several insulin-like genes, including the known DAF-16 target gene *ins-7* (Figure S7A), which affects life span (Murphy et al., 2003; Murphy et al., 2007). Consistent with this, we observed increased *ins-7::GFP* expression in transgenic animals upon addition of glucose (Figures S7B–S7D). In addition, we found that the DNA motif CTTATCA (Budovskaya et al., 2008), which we previously showed to be overrepresented in the upstream sequence of the DAF-16-regulated genes (Murphy et al., 2003), was significantly overrepresented in the promoter regions (2 kb) of the glucose-responsive genes, as well ($p < 10^{-5}$) (Table 1). This sequence was shown recently to bind to DAF-16 (Curran et al., 2009). These findings support the hypothesis that glucose

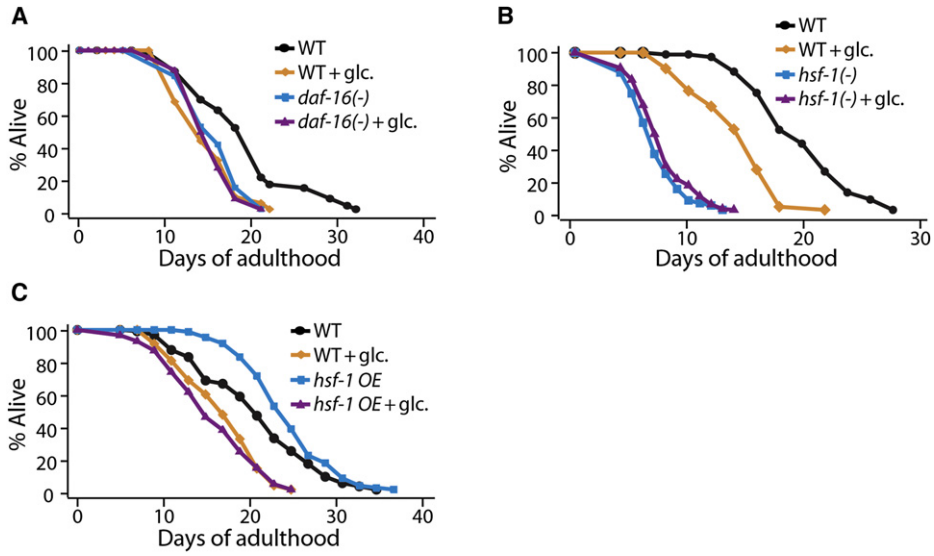

Figure 2. The Life Span-Shortening Effect of Glucose Requires the DAF-16 and HSF-1 Transcription Factors

(A and B) The short life span of the null allele *daf-16(mu86)* (A) (*daf-16(−)*; in this and other figures, *(−)* refers to a reduction or loss-of-function mutation, and the specific allele is given in the corresponding figure legend) or *hsf-1(sy441, RNAi)* (B) animals was not further decreased by glucose feeding. In contrast, wild-type animals (WT) lived significantly shorter on a diet containing 2% glucose.

(C) The long life span caused by overexpression of *hsf-1* (*hsf-1 OE*) was suppressed by adding 2% glucose to the diet. See Figures S3–S5 for the life span data of additional mutants we tested: *daf-16(RNAi)*, *hsf-1(sy441)*, *sir-2.1(ok434)*, *aak-2(ok524)*, *aak-1(RNAi)*, *aak-1(RNAi); aak-2(ok524)*, *nhr-49(gk405)*, and *daf-12(rh61rh411)* animals. Note: The *aak-2* mutations affect subunits of AMP kinase, and *nhr-49* and *daf-12* encode nuclear hormone receptors.

shortens life span by preventing DAF-16 from regulating the expression of specific target genes.

Glucose Completely Prevented *daf-2* Mutations from Extending Life Span

Because the activity of the insulin/IGF-1 pathway is required for growth to adulthood (Hu, 2007), the *daf-2* mutations that extend life span only partially inhibit gene activity. Thus, it was hard to predict what effect glucose might have on the life spans of *daf-2* mutants. We found that glucose almost completely suppressed the life span extension of *daf-2(e1368)* (ligand-binding domain) and *daf-2(e1370)* (tyrosine kinase) mutants (Kimura et al., 1997) back to wild-type levels (Figures 3C and 3D). This dramatic effect, which was seen in multiple trials, suggests

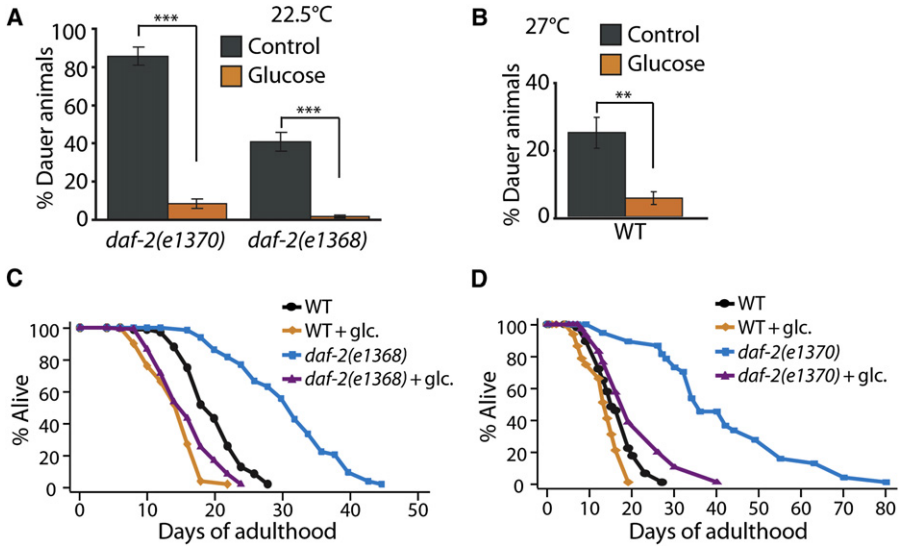

Figure 3. Glucose May Influence Insulin/IGF-1 Signaling to Regulate Dauer Formation and Life Span

(A and B) Glucose feeding significantly decreased dauer formation caused by *daf-2(e1370)* or *daf-2(e1368)* mutation at 22.5°C (A) or by growth at 27°C (B). Error bars are SEM (***$p < 0.001$, **$p < 0.01$, two-tailed Student's t test).

(C and D) The extreme longevity caused by some *daf-2*/insulin-IGF-1 receptor mutations was completely suppressed by glucose feeding. Glucose feeding suppressed the longevity caused by *daf-2(e1368)* (C) and *daf-2(e1370)* (D) mutations.

that glucose can suppress the life span-extending effect of even very high levels of DAF-16 activity. This finding suggested that glucose would also suppress the extended life span of *ins-7(RNAi)* animals, and we found that this was the case (Figure S7E).

Glucose May Shorten Life Span by Altering *aqp-1* Expression

To better understand how glucose affects life span, we tested the functions of genes whose expression changed in response to glucose. To do this, we outcrossed available mutants to our wild-type (N2) strain and measured life span (Figures 4A and S10). Of seven mutants that we tested, one, an *aqp-1(–)* deletion mutant, behaved remarkably like *daf-16(–)* and *hsf-1(–)* mutants: *aqp-1* mutants were short-lived, and their short life span was not further decreased by glucose (Figure 4A). This finding suggested that glucose shortens life span by downregulating the expression of *aqp-1*. Consistent with this interpretation, we found that overexpression of a functional *aqp-1::GFP* fusion (Huang et al., 2007), which rescued the short life span of the *aqp-1* deletion mutant (Figure 4B), partially prevented glucose from shortening life span (Figure 4C). This observation is significant because it argues that reduction of *aqp-1* activity is required for glucose to shorten life span. Together, these findings suggest that glucose shortens life span by inhibiting *aqp-1* activity.

Because of the similarities among the life span phenotypes of *daf-16*, *hsf-1*, and *aqp-1* mutants, we hypothesized that *aqp-1* is a particularly important transcriptional target of both DAF-16 and HSF-1 in animals fed glucose. We performed quantitative RT-PCR to compare the levels of *aqp-1* transcripts in wild-type, *daf-16(–)*, *hsf-1(–)*, and *daf-16(–) hsf-1(–)* animals with and without glucose. We found that glucose feeding downregulated *aqp-1* in wild-type animals, consistent with our microarray data (Figure 4D and Table 1). In addition, *aqp-1* was repressed in *daf-16(–)*, *hsf-1(–)*, and *daf-16(–) hsf-1(–)* animals compared to wild-type, suggesting that normal expression of *aqp-1* requires these transcription factors (Figure 4D). In contrast to the effects of dietary glucose on wild-type animals, glucose feeding did not significantly affect *aqp-1* expression in these mutants (Figure 4D). In addition, we found that *aqp-1(–)* mutation did not further decrease the short life span caused by *daf-16(–)*, *hsf-1(–)*, or *daf-16(–) hsf-1(–)* mutations (Figures 4E, 4F, and S11). Together, these data strongly suggest that glucose, *aqp-1*, *hsf-1*, and *daf-16* all act in the same pathway, in which glucose shortens life span by downregulating the activities of DAF-16 and HSF-1, which in turn downregulates *aqp-1* expression.

Glycerol May Play an Important Role in Life Span Shortening

aqp-1 encodes a glycerol channel (Huang et al., 2007), which is noteworthy because mammalian glycerol channels have been implicated in glucose homeostasis (Hara-Chikuma and Verkman, 2006). Because the AQP-1 channel may alter glycerol metabolism, we wondered what effect glycerol itself might have on life span. To investigate this, we measured the life span of animals grown on normal culture plates supplemented with 1% glycerol (a molarity equivalent to 2% glucose) and a lawn of *E. coli* bacteria. We found that glycerol feeding decreased the life span of wild-type animals by 23%–36% (Figure 5A), which was similar to the effect of glucose feeding. We then tested whether glycerol treatment affected the life span of *daf-16(–)*, *hsf-1(–)*, or *aqp-1(–)* mutants. Similar to glucose treatment, we found that adding glycerol to the diet of these mutants had little or no effect on their already short life span (Figures 5B–5D). In addition, we found that glycerol feeding repressed *aqp-1* expression (Figure 5E).

The similarity between the effects of glucose and glycerol on the life span of wild-type, *daf-16(–)*, *hsf-1(–)*, and *aqp-1(–)* animals suggested that glucose and glycerol may act in the same way (or in the same pathway) to shorten life span. One possibility was that glucose and glycerol have similar effects on life span because they are metabolically interchangeable in *C. elegans*. We examined this possibility by measuring glucose and glycerol in the glucose-fed or glycerol-fed animals. We found that glucose feeding increased both glucose and glycerol in the animals (Figures S12A and S12B). However, glycerol-fed animals contained increased levels of glycerol but not glucose (Figures 5F and 5G). These data suggest that dietary glucose is metabolized to glycerol and that this glycerol may in turn decrease the life span of the animal.

AQP-1 Is a Feedback Regulator of Insulin/IGF-1 Signaling

The *aqp-1* transgene is expressed in the pharynx and intestine (which behaves as the entire endoderm of the animal, including its adipose tissue) (Huang et al., 2007). We examined levels of glucose and glycerol in *aqp-1* mutants with or without dietary glucose, but we did not find any significant difference between whole wild-type versus *aqp-1(–)* animals (Figures S12A and S12B). Thus, presumably, *aqp-1* influences the distribution of glycerol within the animal (something that we cannot measure with current technology).

The fact that loss of *aqp-1* caused a life span phenotype that was similar to that caused by loss of *daf-16* itself was striking and stimulated us to test the possibility that AQP-1 might be acting in a regulatory pathway. Specifically, we hypothesized that AQP-1 action might affect the transcription of DAF-16- and/or HSF-1-regulated genes. To test this hypothesis, we measured mRNA levels of 80 DAF-16 and/or HSF-1 target genes using quantitative RT-PCR analysis. We found that a significant fraction of the genes that are downregulated by mutations in *daf-16* and/or *hsf-1* (8 out of 44) were also significantly downregulated by the *aqp-1* mutation (Figures 6A and S13). One *daf-16* and *hsf-1* target (out of 36 tested) was upregulated by the *aqp-1* mutation (Figures 6B and S14). This was the insulin-like peptide *ins-7*, which was also regulated by glucose, as mentioned above, and is known to influence life span (Murphy et al., 2003, 2007).

The finding that *aqp-1* regulates *ins-7* suggested that *aqp-1* might influence DAF-16 target gene expression in a cell-nonautonomous fashion. To test this idea, we crossed the *aqp-1* mutation into a strain carrying a GFP reporter fusion to the promoter of a direct DAF-16 target gene, *sod-3* (Honda and Honda, 1999). Interestingly, we found that the level of *sod-3::GFP* in the head muscle was decreased by *aqp-1* mutation or glucose feeding (Figures 6C–6F). Based on the transgene experiments (Huang et al., 2007) *aqp-1* seems to be expressed only in the intestine and pharynx and not in the muscle, although it remains possible

Table 1. Genes Differentially Regulated by Glucose Treatment

Genes upregulated by glucose treatment

Transcript/Gene	Brief description	DAF-16 binding sites[a] (bp) GTAAAC/TA	DAF-16 and GATA transcription factor binding site (bp) CTTATCA
K09H11.7	Haloacid dehalogenase-like hydrolase	−4863	
C53A3.2[b]	Haloacid dehalogenase-like hydrolase	−4908, −4873, −4189	−4550, −1559
C18H9.6	Unknown	−4875	−430
F44E7.2	Haloacid dehalogenase-like hydrolase	−2485, −1801, −1766	−2124, −636
K02G10.7 (*aqp-8*)[c]	Major intrinsic protein (MIP), aquaporin	−2919, −2080	−230
Y40B10A.6	O-methyltransferase domain	−2021, −390	
F21C10.9	Unknown	−2937, −1952, −1637, −373	
K11D12.4	Choline or carnitine O-acyltransferase	−3785, −826, −380	−4348, −2735
F21F8.4[b]	Putative aspartyl (acid) protease	−3035	4371, −4035, −3162, −2287, −1531, −1484
C17C3.1d	Lipid storage	−4547, −2753,−2033	
Y69A2AR.4 (*smf-3*)[c]	Solute carrier family 11		−3187, −207, −194
F10D2.11	UDP-glucoronosyl/glucosyl transferase	−843, −366	4076, −3374, −2136, −12
Y53F4B.6	Growth, size, and locomotor behavior		
Y40B10A.2	O-methyltransferase domain	−2335	−1378
F15H10.1 (*col-12*)	Collagen	−4123, 3192, −1405	−3147
C29E6.5 (*nhr-43*)[c]	Nuclear hormone receptor	−4528, −3392	−1002, −943, −231
F31F4.5	Pseudogene		
F09F3.9	Choline or carnitine O-acyltransferase	−1395, −967, −42	−988
Y80D3A.7 (*ptr-22*)	Patched superfamily	−4270, −2815	−3331
F44G3.2	Plant ATP-guanido phosphotransferase	−2651, −1490	−4345
F15B9.1 (*far-3*)	Fatty acid- and retinol-binding protein 3	−1712, −421, −409	−4749, −979
C54D10.1 (*cdr-2*)[c]	Cadmium-responsive gene 2	−4690, −2245, −2095, −2038, −1540, −1024	
C05E11.4 (*amt-1*)[b]	Ammonium transporter family	−4905, −3889, −3671, −3440, −3246, −2591, −1606, −1327, −1240, −1118	−1789, −1461

Genes downregulated by glucose treatment

Transcript/Gene	Brief description	DAF-16 binding sites[a] (bp) GTAAAC/TA	DAF-16 and GATA transcription factor binding site (bp) CTTATCA
ZK6.7a[b]	Lipid storage, chromosome segregation, pathogen response	−2695, −859	−4312, −3979, −80
C14C6.5	ShTK and CC domains		
C50F7.5	Unknown	−3658, −135	−1107, −1084,−240, −209
T20B12.4	Unknown		
F48D6.4a[b]	Unknown	−4869, −4476, −4316, −1427	−3835, −403
F32A5.5b[b] (*aqp-1*)[c]	Aquaporin	−172	−2364, −2285, −112
F48D6.4c[b]	Unknown	−4869, −4476, −4316, −1427	−3835, −403
ZK6.7b[b]	Lipid storage, chromosome segregation, pathogen response	−2695, −859	−4312, −3979, −80
Y4C6B.6[b]	Glucosyl-ceramidases	−3890, −3363, −2443, −1909	−155, −70
F48D6.4b[b]	Unknown	−4869, −4476, −4316, −1427	−3835, −403
F15E11.14[b]	Unknown	−2707	−394
F27C8.4[b]	Downstream target of a VHL-1 pathway	−4923, −2524, −992	−2810, −1883, −1007, −75
Y37A1B.12 (*tor-1*)	May bind ATP	−113, −59	−4039
T08A9.9	Similar to bactericidal amoebapores	−4817, −3857, −348	−1039, −132
F59B2.11	Unknown	−4056	
C25B8.3a (*cpr-6*)[c]	Cysteine protease domain	−4500, −2119	−1972
C23H5.8a[c]	Unknown	−4036	−72

See Figure S8 for fold changes of these genes and Table S3 for fold changes of all the genes in microarray analysis.

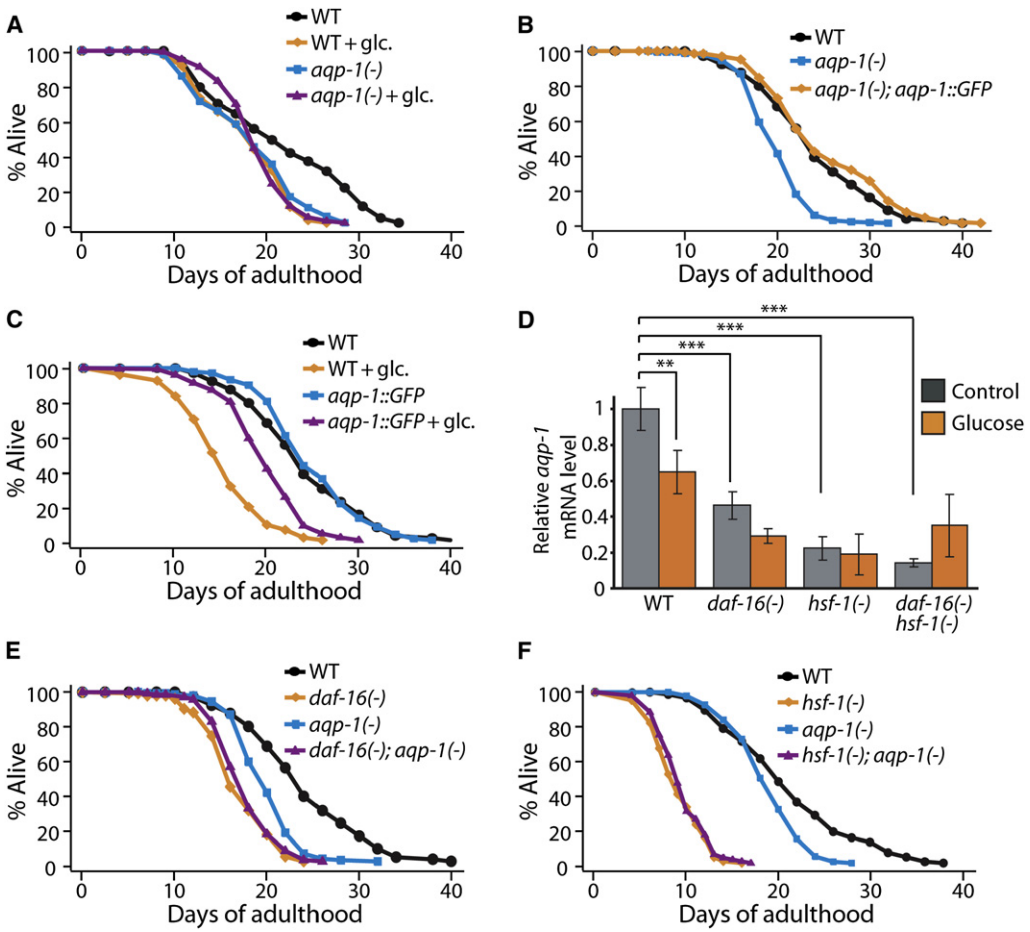

Figure 4. Downregulation of *aqp-1*, a Glucose-Responsive DAF-16/HSF-1 Target Gene, Is Responsible for the Effect of Glucose on Life Span

(A) The short life span caused by *aqp-1(–)* (*aqp-1(tm339)*) mutation was not further shortened by a diet containing 2% glucose, whereas the life span of wild-type animals (WT) was shortened by a 2% glucose diet.

(B) A transgene expressing *aqp-1::GFP* completely rescued the short life span of *aqp-1(–)* animals.

(C) *aqp-1::GFP* transgenic animals were partially resistant to the life span-shortening effect of glucose diet, indicating that reduction of *aqp-1* levels is required for glucose to shorten life span.

(D) Glucose feeding or mutation of *daf-16* or *hsf-1* lowered *aqp-1* mRNA levels. Panel shows quantitative RT-PCR analysis of *aqp-1* mRNA level in wild-type, *daf-16(–)* (*daf-16(mu86)*), *hsf-1(–)* (*hsf-1(sy441, RNAi)*), or *daf-16(–) hsf-1(–)* animals with (orange) or without (gray) glucose treatment. Error bars represent SEM (***$p < 0.001$, **$p < 0.01$, two-way ANOVA followed by post hoc Bonferroni tests).

(E and F) In contrast to its life span-decreasing effect in wild-type animals, the *aqp-1(–)* mutation did not affect the short life span of *daf-16(–)* (E) or *hsf-1(–)* (F) animals.

that the *aqp-1* transgene does not exactly reproduce the expression pattern of endogenous *aqp-1*. Thus, these data imply that *aqp-1* mutation affects the transcriptional activity of DAF-16 in other tissues in a nonautonomous manner.

In otherwise wild-type animals, increasing DAF-16/FOXO levels in the intestine increases DAF-16/FOXO activity in other tissues in a process that appears to involve feedback regulation of *ins-7* (Libina et al., 2003; Murphy et al., 2007). Since *aqp-1* mutations increased *ins-7* expression, we wondered whether the *aqp-1* mutation would also influence this FOXO-to-FOXO signaling among tissues. To test this idea, we increased the level of DAF-16 in the intestine of otherwise wild-type animals using an intestine-specific *daf-16* transgene (*Pges-1::GFP::daf-16*) (Libina et al., 2003) and assayed the level of *sod-3::GFP* in other tissues. In previous experiments, we showed that intestinal DAF-16 overexpression leads to a dramatic increase in head-muscle *sod-3::GFP* expression (Libina et al., 2003), as long as the head muscles are *daf-16(+)*. We found that in the presence of glucose,

[a] We found that 37 of the 40 glucose-responsive transcripts (93%) contain one or more DAF-16 binding elements in their 5 kb upstream regulatory regions. This enrichment is statistically significant ($p < 0.05$, hypergeometric probability) compared to that (78%) of random *C. elegans* genes (Kenyon and Murphy, 2006).

[b] Genes that were upregulated (or downregulated) both by glucose treatment and by inactivation of *daf-16*.

[c] The change in mRNA expression level upon glucose treatment was confirmed by qRT-PCR (Figure S9). The life span of deletion mutants for these genes was measured (Figures 4A and S10).

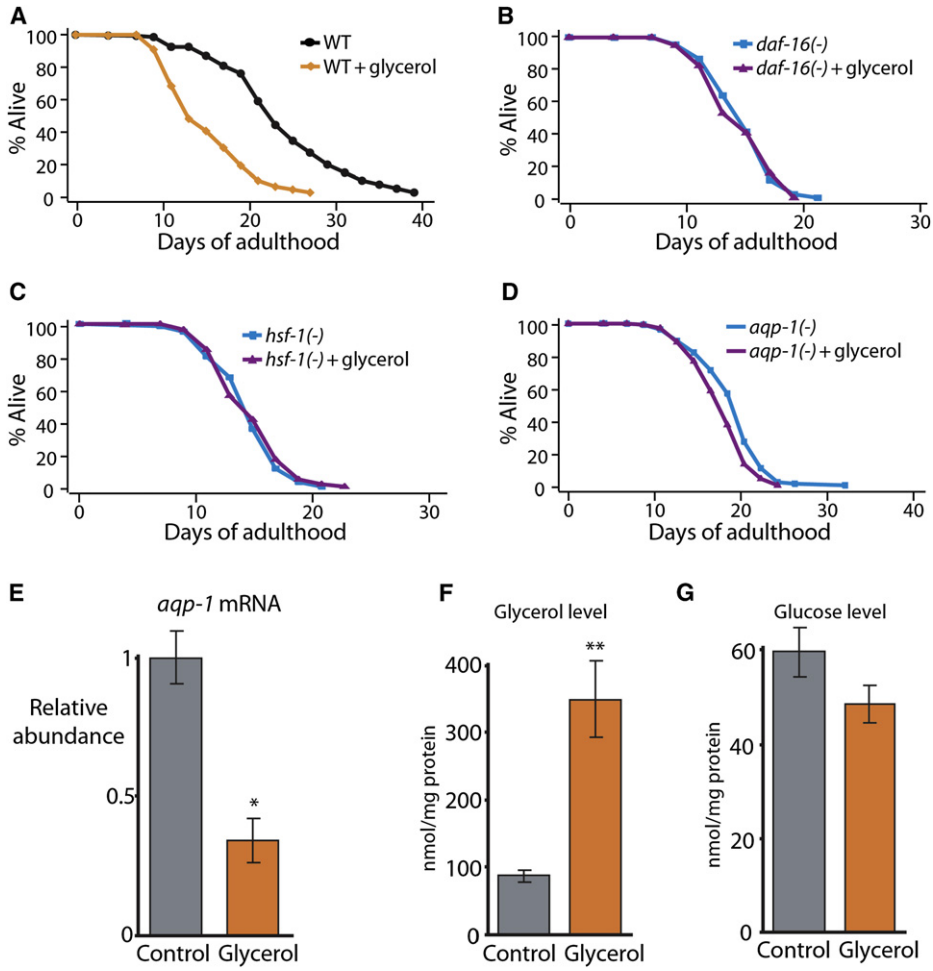

Figure 5. The Glycerol Channel AQP-1 Mediates the Life Span-Shortening Effect of Glycerol Feeding

(A) Diet containing 1% glycerol (glycerol) decreased the life span of wild-type animals significantly.

(B–D) The short life span of *daf-16(mu86)* (B), *hsf-1(sy441)* (C), or *aqp-1(tm339)* (D) animals was not further decreased by glycerol feeding. The life span of *hsf-1(sy441)* mutants was decreased by glycerol feeding in one out of two trials (Table S1). However, the % decrease in life span (−11%) caused by glycerol feeding was much smaller than that of wild-type animals (−36%) in that trial.

(E) Glycerol feeding (Glycerol) significantly decreased the mRNA level of *aqp-1*.

(F and G) Glycerol treatment (Glycerol) increased the glycerol level inside the animals (F) but not the glucose level (G). Error bars represent SEM (*$p < 0.05$, **$p < 0.005$, two-tailed Student's t test). In an effort to confirm the effect of glycerol on life span using genetic models, we measured the life span of mutant animals that have abnormal glycerol levels, but the data were inconclusive at this point. See Figure S17 and the figure legend for detail.

the *aqp-1* mutation reduced this upregulation of *sod-3::GFP* expression in head muscles (Figure 6G). Together, these findings also imply a cell-nonautonomous role for *aqp-1* in the homeostasis of this hormonal signaling system.

DISCUSSION

Glucose Decreases Life Span by Inhibiting DAF-16 and HSF-1

A major finding of this study is that glucose is a potent life span-shortening agent in *C. elegans* that appears to act by downregulating the activities of the life span-extending proteins DAF-16/FOXO and HSF-1. Several lines of evidence lead to this conclusion. First, glucose shortened the life span of wild-type animals, but it did not further decrease the life span of the already short-lived *daf-16(−)* or *hsf-1(−)* mutants. Second, like *daf-16(−)* or *hsf-1(−)* mutations, glucose feeding could suppress the long life span of *daf-2*/insulin/IGF-1 receptor mutants and that of animals overexpressing *hsf-1*. Third, glucose treatment suppressed dauer formation, which is an alternative developmental stage that requires DAF-16 activity. Finally, glucose feeding generated a pattern of gene expression that overlapped significantly with that produced by *daf-16(−)* mutations. Since both DAF-16 and HSF-1 act downstream of and are inhibited by insulin/IGF-1 signaling in *C. elegans*, we propose that glucose may act by stimulating insulin/IGF-1 signaling. Consistent with this hypothesis, glucose stimulates the expression of several insulin genes.

Glucose Dramatically Shortens the Long Life Span of *daf-2* Mutants

We were surprised to find that the long life spans of animals carrying certain reduction-of-function mutations in the *daf-2*

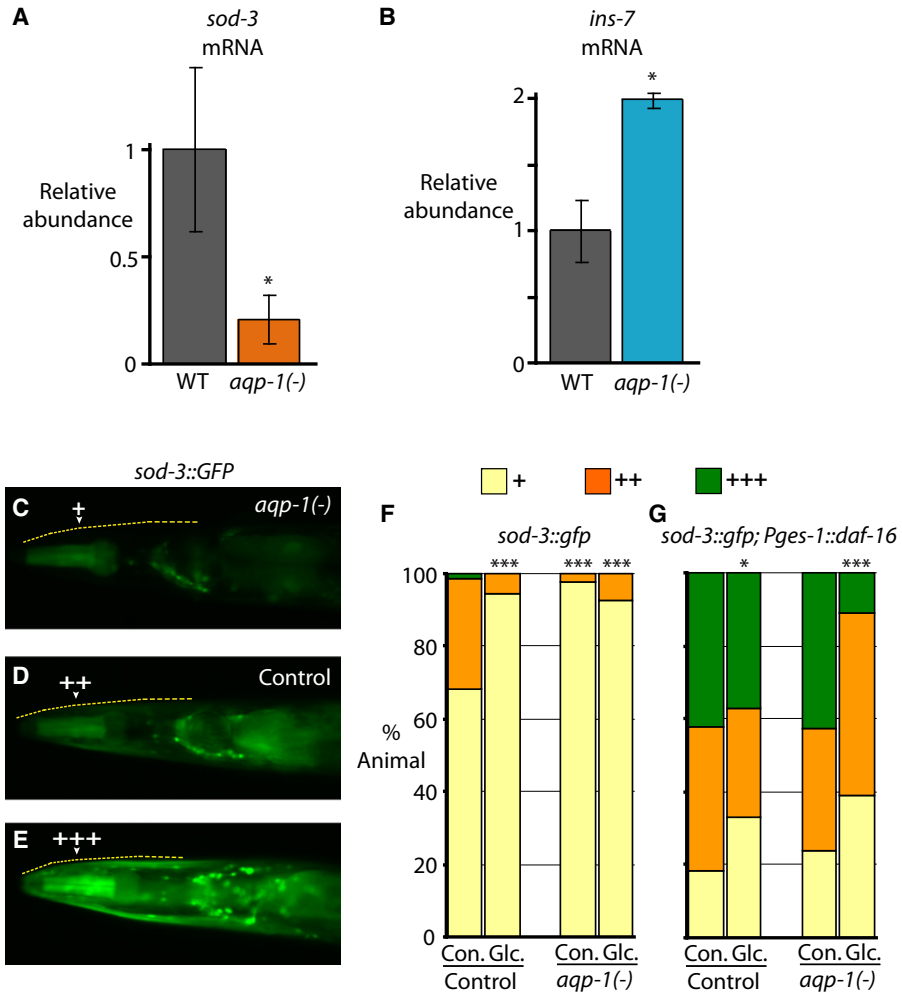

Figure 6. The AQP-1/Glycerol Channel Acts as a Feedback Regulator in the Insulin/IGF-1-Signaling Pathway

(A) *aqp-1* mutation significantly decreased the mRNA level of a direct DAF-16 target gene, *sod-3*.

(B) Expression of the insulin-like peptide gene *ins-7* was induced by *aqp-1* mutation. Error bars represent SEM (*$p < 0.05$, two-tailed Student's t test). These changes in *sod-3* and *ins-7* expression by *aqp-1* mutation were not additive to *daf-16(–)* or *hsf-1(–)* mutations (Figure S16).

(C) *aqp-1* mutant animals that express *sod-3::GFP* generally contain GFP in the pharynx but not in the surrounding head muscles.

(D and E) Some animals that express *sod-3::GFP* in a wild-type or in a *Pges-1::GFP::daf-16* transgenic animal background contain GFP throughout the head and often contain higher levels of GFP in the pharynx and intestine, as well. The upper edge of the animal's head is indicated by the dashed yellow line. Arrowheads indicate head muscles.

(F and G) Quantification of head-muscle *sod-3::GFP* expression for panels (D) and (E) without (F) or with (G) *Pges-1::GFP::daf-16* (*Pges-1::daf-16*) (n : 75, *$p < 0.05$, ***$p < 0.001$, χ^2 test (Tullet et al., 2008).

insulin/IGF-1 receptor gene were drastically reduced by glucose feeding. It is possible that glucose suppresses the high level of DAF-16 activity in these mutants by the same mechanism that it uses to suppress normal DAF-16 activity in the wild-type animals. However, in *daf-2(–)* mutants, loss of *aqp-1* has a much more modest (~20%) effect on life span than does the addition of glucose (Murphy et al., 2003). The physiology of *daf-2* mutants differs from that of wild-type in many ways, and some aspects of this altered physiology must allow glucose to shorten life span independently of *aqp-1*. The mechanism likely involves *daf-16* downregulation, as glucose did not further shorten the life span of *daf-16(–); daf-2(–)* double mutants (Figure S19). The ability of glucose to suppress the life span extensions of these *daf-2* mutants is thought provoking, because it raises the possibility that the more modest (15%–30%) effects that inhibition of insulin/IGF-1 signaling has on the life span of mammals (Russell and Kahn, 2007) might be increased substantially by reducing the glycemic indices of their diets.

Glucose Does Not Appear to Shorten Life Span by Decreasing Respiration

In their recent study, Schulz et al. found that glucose decreases oxygen consumption in wild-type *C. elegans* (Schulz et al., 2007), and we confirmed this finding in our laboratory (Figure S12C). However, we did not find a significant difference between the rates of oxygen consumption in *aqp-1(–)* animals and wild-type (Figure S12C). Because the downregulation of *aqp-1* appears to be necessary and at least partially sufficient for

glucose to shorten life span, these findings argue against the idea that glucose shortens life span by inhibiting respiration.

In addition, Schulz et al. proposed a model suggesting that glucose may shorten life span by inactivating the AMP-dependent kinase (AMPK) (Schulz et al., 2007). This model was mainly based on their findings that the AMPK subunit AAK-2 is inactivated by glucose treatment and that 2-deoxy-glucose (DOG), which acts oppositely to glucose and increases life span, does not lengthen the life span of *aak-2* null mutants (Schulz et al., 2007). This model predicts that glucose would not further decrease the life span of *aak-2* null mutants. However, we found that glucose feeding did further decrease the short life span caused by loss of *aak-2*, to the same extent as wild-type (Figure S5). Thus, our data imply that glucose does not shorten life span by inactivating AMPK. Taken together, these findings argue that whereas DOG may extend life span by activating AMPK and increasing respiration, the effect of glucose on life span is mediated by another pathway.

Interestingly, Schulz et al. also proposed that DOG extends life span by increasing the level of reactive oxygen species (ROS) (Schulz et al., 2007). However, Schlotterer et al. recently showed that short-lived, glucose-treated worms also display increased ROS (Schlotterer et al., 2009). How this apparent paradox can be resolved is not yet clear.

Similar Effects of Glucose and Glycerol on Life Span

We found that glucose and glycerol have very similar effects on life span. Both glucose and glycerol significantly decreased the life span of wild-type animals but did not further shorten the life span of *daf-16(−)*, *hsf-1(−)*, or *aqp-1(−)* mutants. This was unexpected. When present as the sole carbon source, glucose and glycerol have opposite effects on intermediary metabolism in yeast (Brisson et al., 2001). For example, growth on glucose triggers catabolite repression, stimulates glycolysis, and inhibits respiration, whereas growth on glycerol disinhibits catabolite repression and stimulates respiration via the conversion of glycerol to pyruvate. Thus, glycerol might have been expected to lengthen, not shorten, the life span of *C. elegans*. To what extent the pathways of carbohydrate metabolism differ between worms and yeast is not known. However, we found that worms fed glucose contain elevated levels of glycerol but not vice versa, suggesting that glycerol may be an important intermediate in the pathway through which glucose shortens life span.

One metabolite whose level could be elevated by glucose and/or glycerol feeding is fat, especially because glycerol is an essential building block of triglyceride. Consistent with this idea, Schulz et al. showed that glucose feeding increases the level of triglyceride in *C. elegans* (Schulz et al., 2007). These results raise the possibility that the increased fat in the glucose-fed animals could shorten life span directly. However, increased fat alone is not sufficient to shorten life span, as some mutants that contain high levels of fat, such as *daf-2* mutants, live long (Kimura et al., 1997). Moreover, since we found that downregulation of the DAF-16/FOXO transcription factor, a condition that does not elevate fat levels (Kimura et al., 1997), mediates the life span-shortening effect of glucose feeding, it seems unlikely that the increased fat level is responsible for the shortened life span caused by glucose feeding.

The fact that we identified a glycerol channel as a mediator for the effect of glucose on life span further lends support to the idea that glycerol plays an important role in the glucose metabolic pathway. We speculate that glucose feeding may increase glycerol level in *C. elegans*, which may downregulate the AQP-1 glycerol channel, and that this in turn may lead to further changes in glycerol metabolism and to short life span. Future studies using traceable glycerol, which does not exist at present, are required to test this model.

The AQP-1 Channel Appears to Act Cell Nonautonomously

In *daf-2* mutants, DAF-16 appears to influence life span by regulating multiple antioxidant, chaperone, immunity, metabolic, and other genes that act in a cumulative way to influence life span (Murphy et al., 2003). Thus, we were surprised to find that loss of a single metabolic gene, *aqp-1,* could apparently account for the effect of glucose on life span (at least in a *daf-2(+)* background). Our finding that *aqp-1* acts as a regulatory gene that is required for the expression of diverse DAF-16-regulated genes provides a possible explanation for this finding. By changing the expression of a variety of genes that individually have smaller effects on life span, loss of *aqp-1* could have a relatively large impact.

The mechanism by which this glycerol channel influences DAF-16 target gene expression is not known, but our findings indicate that AQP-1, which is present only in the intestine and pharynx (Huang et al., 2007), is required for normal *sod-3* expression in the head muscles. Thus, *aqp-1* can function cell nonautonomously. The finding that *aqp-1* activity regulates *ins-7*, which is expressed in the intestine and known to affect life span (Murphy et al., 2003, 2007), suggests a model in which *aqp-1* mutations change the level of glycerol in the intestine, which in turn somehow affects the expression of *ins-7* in that tissue. In addition, since AQP-1 is a glycerol channel, it is also possible that glycerol itself is an intercellular signal that can act on other tissues to influence the expression of DAF-16 target genes.

Roles of Mammalian Glycerol Channels in Glucose Metabolism

In mammals, AQPs 3, 7, 9, and 10 encode glycerol channels (reviewed in Maeda et al., 2008). Among them, AQP7 and AQP9 have interesting features that seem related to those of *C. elegans aqp-1*. First, AQP7 and AQP9 are predominantly expressed in adipocytes and hepatocytes, respectively (Maeda et al., 2008). In worms, *aqp-1* is expressed in the intestine, which performs at least some functions of these two tissues (fat storage and yolk production). (*C. elegans* has no distinct adipose tissue or liver.) The mRNA levels of AQP7 and AQP9 decrease upon insulin secretion (Kishida et al., 2001; Kuriyama et al., 2002). Moreover, both of these proteins influence insulin-dependent physiology: *AQP7* knockout mice exhibit insulin resistance and obesity (Hibuse et al., 2005), whereas *AQP9* deletion decreases blood glucose levels in mice that are obese and diabetic (Rojek et al., 2007). Fourth, recent human studies showed that AQP7 gene expression was significantly reduced in obese women (Ceperuelo-Mallafré et al., 2007) and that AQP7 polymorphisms were associated with risk of obesity and diabetes (Prudente et al., 2007). Together, these studies suggest that mammalian aquaporin/glycerol channels have important roles in glucose

metabolism. We showed that the *C. elegans aqp-1*/glycerol channel is a glucose-responsive gene regulated by the insulin/IGF-1 pathway and that it is crucial for mediating the effect of glucose on life span. These findings suggest that *C. elegans aqp-1* may be a functional homolog of mammalian AQP7 and/or AQP9.

Potential Benefits of Diets with a Low GI on Human Aging

The typical American diet contains high levels of sugar. A typical candy bar contains ~75% sugar, and the National Academy of Sciences Dietary Reference Intake recommends limiting the level of added sugar to 25% of total calories. Although we do not fully understand the mechanism by which glucose shortens the life span of *C. elegans*, the fact that the two mammalian aquaporin glycerol-transporting channels are downregulated by insulin raises the possibility that glucose may have a life span-shortening effect in humans and, conversely, that a diet with a low glycemic index may extend human life span. The finding that FOXO3A variants have now been associated with exceptional longevity in at least seven human cohorts (Willcox et al., 2008; Flachsbart et al., 2009; Anselmi et al., 2009; Pawlikowska et al., 2009; Li et al., 2009) makes this possibility seem realistic. It is particularly interesting to think about low-GI diets in the context of our findings with the *C. elegans* insulin/IGF-1 receptor/*daf-2* mutants. These animals, which are predicted to be somewhat insulin resistant, have very long life spans in the absence of glucose, but not in its presence. In multiple studies, lowering the dietary GI has been shown to benefit humans with insulin resistance associated with the metabolic syndrome or type 2 diabetes (Aston, 2006; Venn and Green, 2007). Our findings raise the unorthodox but nevertheless conceivable possibility that humans with partially defective insulin receptors on low-GI diets may be even more healthy and long-lived than normal people on low-GI diets. Finally, because they block gluconeogenesis, drugs that inhibit glycerol aquaporin channels are now under development pharmaceutically for the treatment of diabetes (Frühbeck et al., 2006). Our findings suggest that while these drugs might be beneficial for curtailing glucose production in diabetics, they may have additional, less beneficial effects.

EXPERIMENTAL PROCEDURES

Strains

See Table S6 for strains that were analyzed in this study.

Life Span Analysis

All life span measurements were performed at 20°C, as described previously (Apfeld and Kenyon, 1999), starting with day 1 adults. In some experiments, D-glucose, L-glucose, D-sorbitol, or glycerol was added to standard NG agar plates. The chemical 5-fluoro-2′-deoxyuridine (FUDR) (75 μM, Sigma; St. Louis) was added to prefertile young adult animals to prevent their progeny from developing unless stated otherwise. For the RNAi experiments, eggs were added to plates seeded with bacteria expressing double-strand RNA of given genes.

Progeny Profiles

Single L4 stage wild-type (N2) worms grown on OP50 bacteria with or without 2% glucose were placed on individual plates at 20°C (36 worms per condition). The animals were then transferred to new plates every 24 hr for 7 days while they produced progeny. Bagged or ruptured worms were censored. All the plates containing progeny were incubated for 2–3 days after removing the parental worms, and the number of worms that developed was counted.

Dauer Assay

Animals were grown at 22.5°C or 27°C as indicated. After 3 days of incubation, dauers were scored under a dissecting microscope.

Microarray Analysis

Synchronized day 1 adult animals that were cultured at 20°C were harvested, washed three times in M9, and frozen immediately using liquid nitrogen. Total RNA extraction, mRNA purification, reverse transcription of mRNA, labeling, and hybridization were performed as described previously (Murphy et al., 2003). Six biological replicate samples of glucose-fed versus control wild-type adult animals were used to identify the glucose-responsive genes (GEO Accession GSE 18562) (Tables 1 and S3). Four biological replicate samples of *daf-2(e1370)* versus *daf-16(RNAi); daf-2(e1370)* and four biological replicate samples of *fer-15(b26); daf-2(mu150); fem-1(hc17)* versus *daf-16(RNAi); fer-15(b26); daf-2(mu150); fem-1(hc17)* animals were used for identification of genes that are regulated by DAF-16 in *daf-2* mutants (GEO Accession GSE 18561) (Tables S2 and S4).

Microarray raw data were normalized using Acuity 4.0 (Molecular Devices; Sunnyvale, CA). Significance Analysis of Microarray (SAM) (Tusher et al., 2001) was performed to identify genes that were differentially regulated in each of the comparisons. For glucose-responsive genes, 5% median false-positive discovery rate was used (Table 1). For DAF-16 regulated genes, 1% median false-positive discovery rate was used (Table S2).

DAF-16-regulated genes in *daf-2(−)* mutants were compared with previously published data (McElwee et al., 2003; Murphy et al., 2003). The overlap between DAF-16 target genes in this study and the genes from the previous reports (McElwee et al., 2003; Murphy et al., 2003) was highly significant in both comparisons (Figure S18).

Upstream Sequence Analysis

Upstream sequences for the glucose-responsive genes were extracted from WormBase (http://www.wormbase.org). Regulatory Sequence Analysis Tools (RSAT) (http://rsat.ulb.ac.be/rsat/) was used to search the sequence 2 kb upstream of the translation start site of each open reading frame for overrepresented sequences. DAF-16 and GATA transcription factor binding sites in the 5 kb upstream of glucose-responsive genes were identified using RSAT (Table 1).

Generation of ΔPTS OP50 Double Mutant *E. coli*

A ΔPTS mutation that prevents glucose uptake in *E. coli* (Deutscher et al., 2006) was introduced from the GI698 ΔPTS strain into OP50 bacteria using a standard phage-transduction method (Sambrook et al., 1989). The double mutant was selected by picking colonies on kanamycin-containing LB plates and confirmed by the formation of white colonies on McConkey agar medium plates containing 0.5% glucose (Figure S3).

Quantitative RT-PCR

RNA extraction, purification, and reverse transcription were performed as described (Taubert et al., 2006). Quantitative RT-PCR was carried out in a 7300 Real-Time PCR System (Applied Biosystems; Foster City, CA) and analyzed using the Ct method (Applied Biosystems Prism 7700 User Bulletin #2, http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf). mRNA levels of *act-1*, *nhr-23*, and/or *ama-1* were used for normalization as indicated. The average of at least two technical repeats was used for each biological data point. Primer sequences are available on request.

Measurement of Glucose and Glycerol

Glycerol levels were quantified as described (Lamitina et al., 2004) using Free Glycerol Reagent (Sigma). Glucose levels were determined essentially as described (Lamitina and Strange, 2005), with modifications. Briefly, neutralized perchloric acid worm extracts prepared as described (Lamitina et al., 2004) were used for quantification of glucose concentration using Amplex Red Glucose/Glucose Oxidase Kit (Molecular Probes; Carlsbad, CA). Protein content was measured with BCA Assay Kit (Pierce; Rockford, IL) and used for the normalization of glucose and glycerol levels. Approximately 10,000 synchronized day 1 adult worms were used for one data set.

Measurement of Oxygen Consumption Rates

Oxygen consumption rates were determined using the Oxytherm (Hansatech; Norfolk, UK), which is a DW1/AD Clark-type oxygen electrode, as described (Schulz et al., 2007). Approximately 5000 synchronized day 1 adult worms were collected, quickly washed three times in M9 buffer, resuspended in 1 ml of M9, and transferred into the chamber. Respiration was measured at 20°C, and the oxygen consumption rates were automatically calculated by using the "Rate Cursors" function in the Oxygraph program (Hansatech).

Fluorescence Microscopy

All pictures of transgenic animals were captured using a Retiga EXi Fast 1394 CCD digital camera (QImaging; Burnaby, BC, Canada) attached to a Zeiss Axioplan 2 compound microscope (Zeiss Corporation; Jena, Germany). The expression of *sod-3::GFP* was assayed as described previously (Libina et al., 2003). Briefly, well-fed 3-day-old adult animals grown at 20°C were mounted on 2% agarose slides (~10 per slide). The level of *sod-3::GFP* in the head muscle was scored blindly. P values were calculated using the χ^2 test as described (Tullet et al., 2008). The expression level of *ins-7::GFP* was examined as described previously (Murphy et al., 2007). Subcellular localization of DAF-16::GFP was determined as previously described (Lin et al., 2001).

ACCESSION NUMBERS

The accession number for the microarray data is GSE18563 (NCBI-GEO).

SUPPLEMENTAL DATA

Supplemental Data include Supplemental References, six tables, and 19 figures and can be found online at http://www.cell.com/cell-metabolism/supplemental/S1550-4131(09)00302-7.

ACKNOWLEDGMENTS

We thank T. Lamitina, G. Ruvkun, and the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources, for providing some strains and all Kenyon lab members for sharing outcrossed strains and for helpful comments on the experiments, data analysis, and manuscript. We also thank T. Lamitina for valuable suggestions on *osm-8* and *gpdh-1; gpdh-2* mutant experiments, M. Gaglia for initiating experiments on dauer formation, Vera Tenberg for performing some life span experiments, A.B. Hwang and J.S. Yang for technical help during revision, and B. Koo and Y.J. Seok for providing ΔPTS *E. coli*. S.-J.L. was an Ellison Medical Foundation fellow of the Life Sciences Research Foundation and was also supported by a postdoctoral fellowship from the American Heart Association, Western States Affiliate. This work was supported by NIH grant #AG11816 to C.K., who is the director of the UCSF Hillblom Center for the Biology of Aging, an American Cancer Society Professor, and a cofounder of the biotechnology company Elixir Pharmaceuticals. The data shown in Figure S7E, additional experiments requested by reviewers, and the salary of S.-J.L during revision were supported by Korean government grant World Class University Program #R31-2008-000-10100-0. The majority of the experiments were performed by S.-J.L. C.T.M. discovered that glucose affected insulin gene expression using an *ins-7::GFP* fusion strain. C.K. performed the initial glucose experiments involving wild-type and *daf-16* mutants. S.-J.L and C.K. designed experiments and wrote the paper.

Received: January 28, 2009
Revised: August 10, 2009
Accepted: October 14, 2009
Published: November 3, 2009

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