Fat Metabolism Links Germline Stem Cells and Longevity in *C. elegans*

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Summary

Fat metabolism, reproduction, and aging are intertwined regulatory axes; however, the mechanism by which they are coupled remains poorly understood. We found that germline stem cells (GSCs) actively modulate lipid hydrolysis in *Caenorhabditis elegans*, which in turn regulates longevity. GSC arrest promotes systemic lipolysis via induction of a specific fat lipase. Subsequently, fat mobilization is promoted and life span is prolonged. Constitutive expression of this lipase in fat storage tissue generates lean and long-lived animals. This lipase is a key factor in the lipid hydrolysis and increased longevity that are induced by decreased insulin signaling. These results suggest a link between *C. elegans* fat metabolism and longevity.

Abalance of fat storage and mobilization is a universal feature of animal physiology (1). Reproduction is an energy-intensive process, which is modulated by the availability of nutrients and in turn influences lipid metabolism (2). Reproductive ability declines with age, and many organisms undergo reproductive senescence (3). Obesity increases with age and is also associated with the transition to menopause in women (4). Genetic studies have suggested endocrine roles of adipose tissue and the reproductive system in regulation of life span (5–8). Thus, understanding the mechanisms by which fat metabolism is coupled to reproductive cues may reveal systemic regulation of fat metabolism and provide insights into the control of aging.

In *C. elegans*, the energetic demands of progeny production are profound. The gonad undergoes many more mitoses than does somatic tissue, and the biomass of the oocytes produced is approximately equal to the biomass increase from egg to adult. Thus, in the absence of reproduction, a surfeit of available energy could lead to an increase in fat storage. To test this idea, we ablated the precursor cells of the germ line in *C. elegans* with the use of a laser microbeam. The vital dye Nile Red was used to visualize fat storage droplets in living animals (9). Opposite to the expected increase in fat storage, germ line–ablated animals stored 50% as much fat as untreated animals (Fig. 1, A to C). This finding suggested a regulatory mechanism coupling reproduction and fat metabolism.

Fat storage is also aberrant in the sterile mutants *glp-1(e2141ts)* and *glp-4(bn2ts)*, which are defective in germline proliferation (10,11). The *glp* mutants showed a 50% decrease in fat storage at the nonpermissive temperature relative to the wild type (*N2*) (Fig. 1, D to G). A similar decrease was observed by staining with a BODIPY-labeled fatty acid analog (fig. S1)

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(12) or SudanBlack, a fat-specific dye (fig. S2) (13). At the permissive temperature, the *glp* mutants reproduced normally and their fat storage was similar to that of the wild type (fig. S3).

Fat storage can be altered by changes in either energy input or expenditure. Food intake and retention in the gut are unchanged in *glp-1* (Fig. 1, H and J); the food absorption rate is also normal (Fig. 1I). Normal locomotion in *glp-1* suggests that less fat storage is not due to an increase in physical activity (Fig. 1K). Therefore, decreased fat storage in the germ line–defective mutants is unlikely to be the result of alterations in energy intake and/or physical activity, and more likely reveals an altered endocrine signaling axis.

Production of vitellogenin-rich oocytes is the most energy-intensive reproductive function. We used *fem-3* sterile mutants to examine whether gametogenesis influences fat storage. The gain-of-function allele *fem-3(q20ts)* produces only sperm, whereas the loss-of-function allele *fem-3 (e2006ts)* produces only oocytes at the nonpermissive temperature (14,15). Neither *fem-3* mutant exhibited abnormal lipid accumulation (Fig. 2, A to D). This result excludes the possibility that gametogenesis regulates fat storage, and it also suggests that sterility per se does not cause a change in lipid accumulation.

To test whether germline proliferation regulates fat storage, we shifted *glp-1* mutants to the restrictive temperature at different evelopmental stages to arrest germline proliferation at distinct points. Adults that are generated from L2 (early) temperature shifts carry few mitotic germ cells, whereas adults from L4 (late) temperature shifts form the germ line with essentially wild type– sized mitotic and meiotic germ cells and differentiated sperm. Despite a very different composition of the germ line, adult fat storage was decreased to a similar extent under all conditions (Fig. 2E). One process shared by all temperature shifts is germline stem cell (GSC) arrest (16), which could induce the decrease in fat storage. We therefore shifted temperature at 1 day of adulthood, after animals started to reproduce; this should affect adult GSCs but not the already proliferated germ line. By 30 hours at the restrictive temperature, fat storage in *glp-1* started to decrease (Fig. 2F). Within 48 hours, lipid accumulation in *glp-1* was reduced to an extent comparable to that seen with the developmental temperature shifts (Fig. 2F). This result suggests that GSCs regulate fat storage during adulthood.

The somatic distal tip cell forms the niche of GSCs. The Notch ligand LAG-2 expressed in the distal tip cell is required to maintain GSC identity (17). Like *glp-1* mutants, *lag-2(q420ts)* mutants (18) showed a 50% decrease in fat storage (Fig. 2G). *glp-1(ar202gf)* mutants with a hyperactive GLP-1, in which entry into meiosis is prevented and GSCs overproliferate (19), showed a factor of 1.7 fat increase (Fig. 2, H, I, and K), which suggests that a deficit of GSCs signals low fat storage and that GSC overproliferation signals high fat storage. No change in fat content was detected in *gld-1(q485)* mutants, in which early-phase meiotic germ cells reenter into the mitotic cell cycle and overproliferate (20) (Fig. 2, H, J, and K). Thus, once germ cells undergo differentiation, they lose the ability to modulate fat storage.

To understand the mechanisms by which GSCs regulate fat storage, we reduced the activities of 163 metabolic genes by RNA interference (RNAi) and screened for gene inactivations that increase fat storage in *glp-1* (table S1). Among 16 potential candidate genes identified, *K04A8.5* encoded a triglyceride lipase, which most strongly affected fat storage. Inactivation of *K04A8.5* partially restored fat storage in *glp-1* but had marginal effect on the wild type (Fig. 3A). GSC arrest caused a marked increase in the transcriptional levels of *K04A8.5* (Fig. 3B), and a promoter–green fluorescent protein (GFP) reporter that was not detected under normal conditions became detectable in the *glp-1* gut at the restrictive temperature (fig. S4). High gene dosage of *K04A8.5* decreased fat storage in the wild type, and genetic mosaic animals showed that intestinal cells that constitutively express *K04A8.5* had fewer lipid droplets than did neighboring nontransgenic cells (Fig. 3, C to E). These results imply that this lipase acts in fat

storage tissue rather than in endocrine cells or GSCs. Thus, the decrease in fat storage upon GSC arrest is induced by increased lipid hydrolysis via up-regulation of *K04A8.5*.

GSC arrest caused by *glp-1* loss of function resulted in extended life span (Fig. 3F and table S2) (8); *K04A8.5* RNAi suppressed this increased longevity but did not reduce wild-type life span (Fig. 3F and table S2). Therefore, up-regulation of this lipase gene mediates both lipid hydrolysis and longevity in GSC-arrested animals. Constitutive expression of *K04A8.5* specifically in the intestine led to life spans that were 24% longer than in control siblings (Fig. 3G and table S3). Thus, lipid hydrolysis in fat storage tissue prolongs life span, which connects the metabolic functions of adipose tissue to life-span control.

We investigated the signaling pathways regulating *K04A8.5* expression in the intestine. The forkhead transcription factor DAF-16 is translocated into nuclei in the intestine upon GSC arrest (21). To test whether *daf-16* is involved in regulation of fat storage by GSC proliferation, we inactivated *daf-16* by RNAi in wild-type and *glp-1* mutants and assayed fat storage. *daf-16* inactivation restored fat storage in *glp-1* but did not affect wild-type fat storage (Fig. 4A and fig. S5). *K04A8.5* up-regulation in *glp-1* was abolished in the absence of *daf-16* but was not altered in wild-type animals subjected to *daf-16* RNAi (Fig. 4B). Thus, upon GSC arrest, DAF-16 is activated in the intestine to promote lipid hydrolysis through induction of *K04A8.5* expression. External stresses such as heat shock and oxidative stress activate *daf-16* (22,23). After heat shock and paraquat treatment, the DAF-16 targets *hsp-16.1* and *ctl-2* were up-regulated but *K04A8.5* was not (fig. S6). These results suggest a specific regulation of *K04A8.5* by the signal from the germ line.

KRI-1, the human KRIT 1 homolog, and DAF-12, the nuclear hormone receptor, are both required for the intestinal nuclear localization of DAF-16 in GSC-arrested animals (21). These factors could act upstream of DAF-16 to sense signals from GSC and, in response, regulate lipid accumulation. Like *daf-16* RNAi, *kri-1* RNAi significantly reduced *K04A8.5* expression and increased the fat content in *glp-1* (Fig. 4, A and B, and fig. S5). In contrast, reducing *daf-12* function did not affect *K04A8.5* levels and caused a slight decrease in lipid accumulation in both wild-type and *glp-1* mutants (fig. S7). Therefore, GSC arrest promotes lipid hydrolysis in the intestine through activation of the *kri-1/daf-16* signaling pathway, but independently of *daf-12* lipophilic hormone signaling.

We examined *K04A8.5* expression in other long-lived animals, such as worms with reducing function in insulin receptor/*daf-2*. *daf-2* is crucial in regulation of fat metabolism during larval development (24). Therefore, we reduced *daf-2* function only at adulthood by RNAi feeding. Reducing *daf-2* activity at adulthood caused up-regulation of *K04A8.5* and decreased fat storage (Fig. 4, C and D, and fig. S8). Loss of the germ line and reduced *daf-2* signaling synergistically induced *K04A8.5* and decreased fat storage (Fig. 4, C and D, and fig. S8). We also found that *K04A8.5* RNAi partially suppressed the longevity of *daf-2* mutants (Fig. 4E and table S4). These results suggest that lipid hydrolysis is also connected to life-span control in the *daf-2* long-lived animals.

Our findings reveal an endocrine signaling axis from GSCs to fat storage tissue, with feedback from the fat storage to the longevity of the animal. Somatic stem cells are thought to mediate tissue regeneration after wounding, and such regeneration is also known to decline with aging. How the proliferation of adult stem cells is coupled to the requirement for replacement cells during normal and pathological aging may be related to the metabolic pathways we have discovered between germline stem cells and the longevity of *C. elegans*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and Notes

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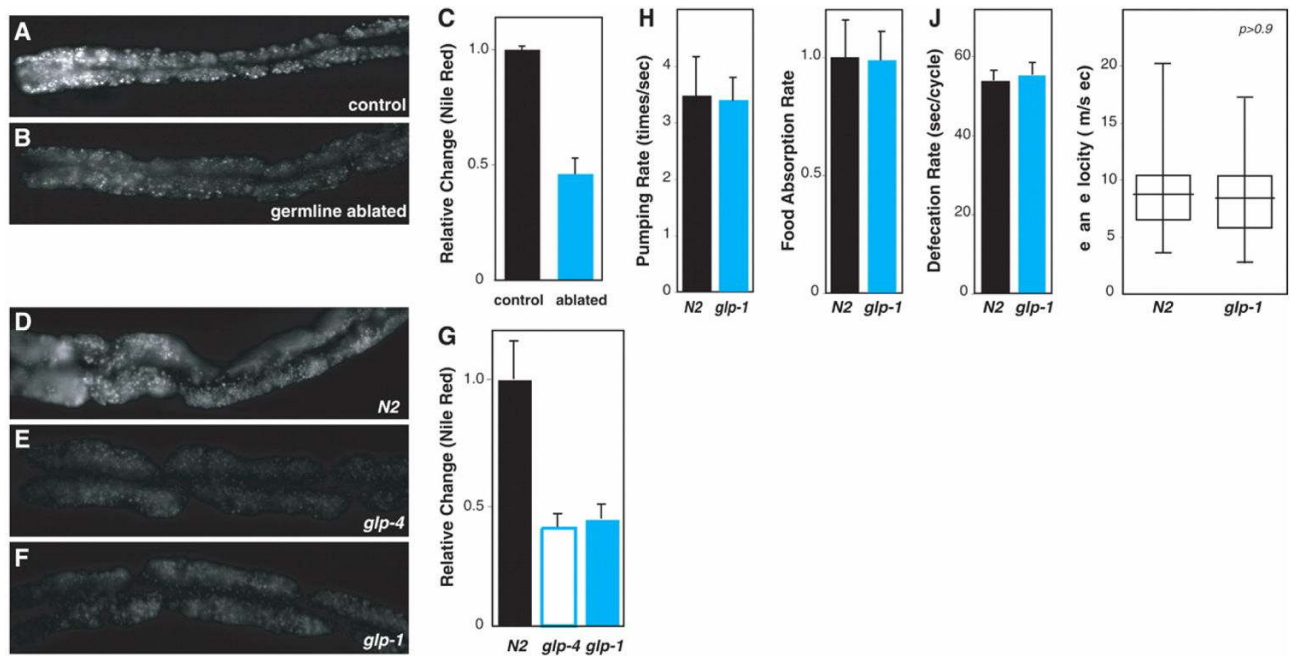
Fig. 1. Influence of reproductive activity on fat metabolism
(**A** to **C**) Ablation of germline precursor cells resulted in a 50% reduction in adult fat storage
($P < 0.0001$) ($n = 15$). (**D** to **G**) The same degree of reduction was observed in *glp-4(bn2)* and
glp-1(e2141) mutants defective in germline proliferation ($P < 0.0001$) (*N2* and *glp-4, n = 15*;
glp-1, n = 18). (**H** to **J**) Decreased fat in *glp-1* was not due to less food intake (measured as
pharyngeal pumping and food absorption rates) or food retention time (measured as defecation
rate) ($P > 0.5$ for each) (pumping rate, *N2, n = 12*; *glp-1, n = 18*; food absorption rate, *N2* and
glp-1, n = 5; defecation rate, *N2* and *glp-1, n = 7*). (**K**) Comparison of locomotory behavior
showed that physical activity does not change in *glp-1*. Experiments were performed at the
restrictive temperature (25°C) for both wild-type (*N2*) and *glp* mutants ($n = 21$ for each).
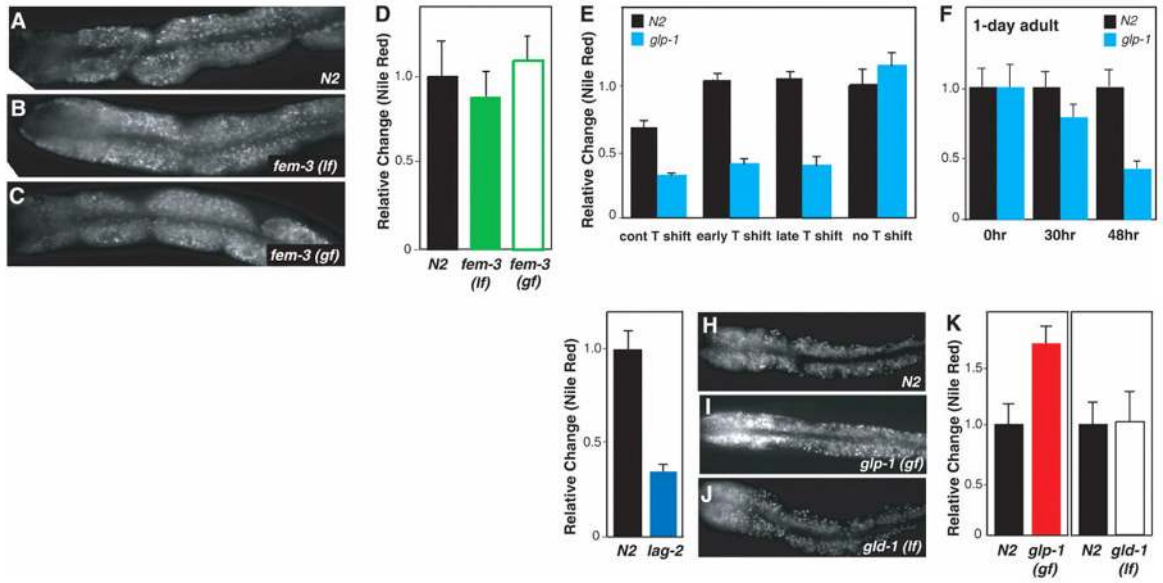
Fig. 2. Regulation of fat metabolism by GSC proliferation

(**A** to **D**) *fem-3(e2006)* loss-of-function mutants (*lf*) producing only oocytes or *fem-3(q20)* gain-of-function mutants (*gf*) generating only sperm showed the same fat storage as in the wild type (*P* > 0.1) (*n* = 15 for each). (**E**) Shift to 25°C during early or late larval development did not affect fat storage in the wild type (*P* > 0.1) but caused a 50% decrease in *glp-1(e2141)* mutants (*P* < 0.0001) (*n* = 15 for each genotype and treatment). (**F**) GSC arrest, caused by temperature shifting of 1-day-old *glp-1* adults, caused a decrease in fat storage (0 hours, *P* > 0.5; 30 hours, *P* < 0.005; 48 hours, *P* < 0.0001)(*n* = 17 for each genotype and treatment). (**G**) *lag-2(q420)* showed reduced fat (*P* < 0.0001) (*N2, n* = 12; *lag-2, n* = 15). (**H** to **K**) GSC overproliferation in the *glp-1(ar202)* gain-of-function mutant causes increased fat (*P* < 0.0001). In contrast, the loss-of-function mutant of *gld-1(q485)*, in which early-phase meiotic germ cells overproliferate, did not change fat storage (*P* > 0.1) (*N2, n* = 12; *glp-1, n* = 15; *gld-1, n* = 13).
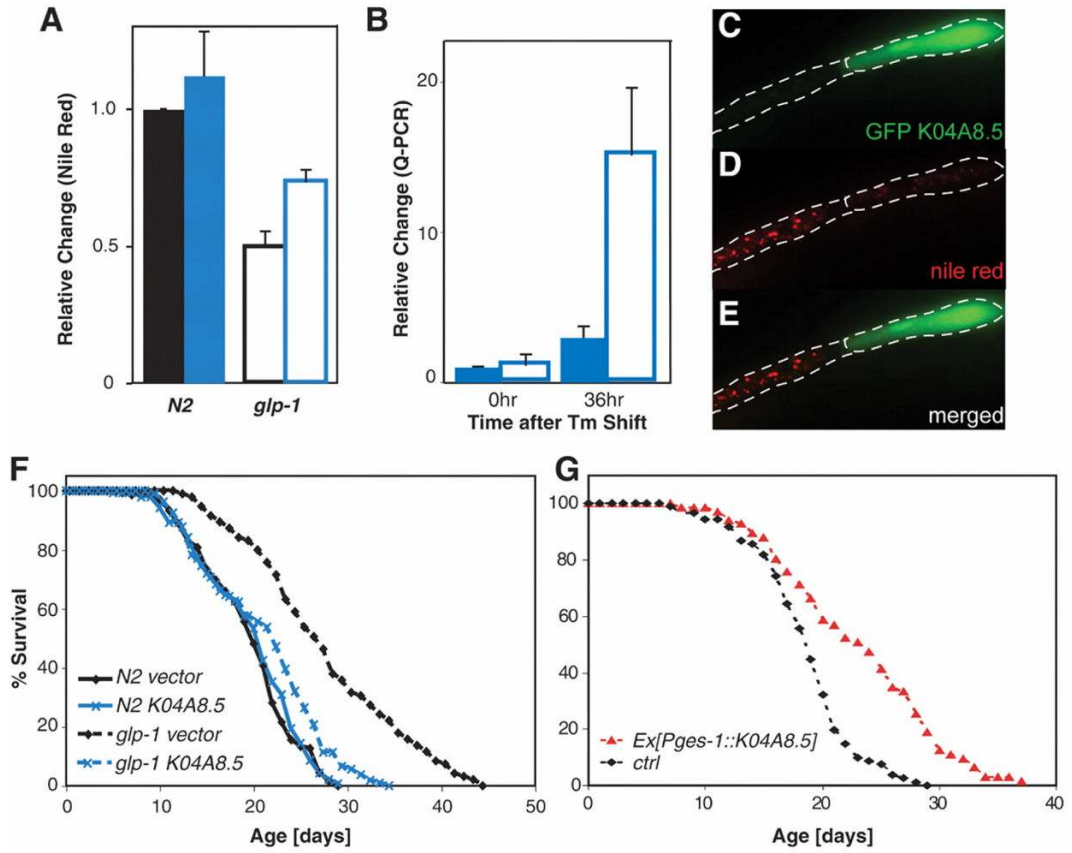
Fig. 3. A role for triglyceride lipase in lipid hydrolysis and longevity
(**A**) *K04A8.5* RNAi partially restored fat storage in *glp-1(e2141)* (open bars, *P* < 0.001) but had a marginal effect in the wild type (solid bars, 10% increase in fat storage, *P* > 0.1)(*n* = 20 for each genotype and RNAi feeding). Blue, *K04A8.5* RNAi; black, vector control. (**B**) *K04A8.5* expression was up-regulated in *glp-1* (*P* < 0.0001; solid bars, *N2*; open bars, *glp-1*). (**C** to **E**) Genetic mosaic analysis shows that the number and intensity of lipid droplets both decreased in the cell constitutively expressing *K04A8.5*, marked by GFP, relative to its sister cell. (**F**) *K04A8.5* RNAi had no effect on the life span of wild-type animals (*P* > 0.01) but suppressed the increased longevity of *glp-1* (*P* < 0.0001; 24% reduction in mean life span). (**G**) Constitutive expression of *K04A8.5* in the intestine extended life span (*P* < 0.0001; 24% mean life-span extension).
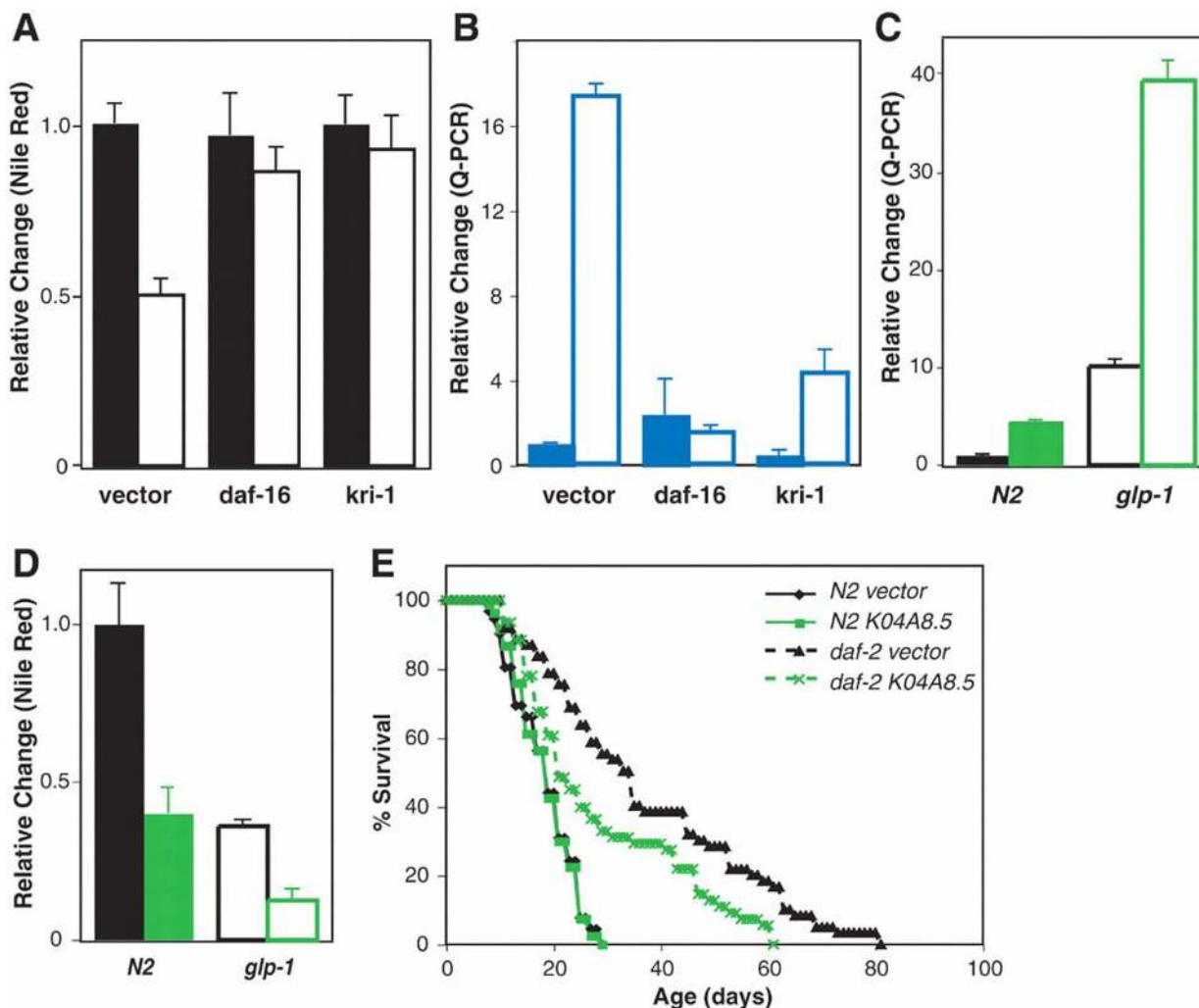
Fig. 4. Synergistic regulation of fat metabolism by GSC proliferation and insulin signaling
(**A**) Either *daf-16* or *kri-1* RNAi restored lipid accumulation in *glp-1* (*P* < 0.0001); neither of them affected fat storage in the wild type (*P* > 0.05) (*n* = 17 for each genotype and RNAi feeding). Solid bars, *N2*; open bars, *glp-1*. (**B**) *daf-16* and *kri-1* were required to up-regulate *K04A8.5* upon GSC arrest. *daf-16* or *kri-1* RNAi suppressed *K04A8.5* induction in *glp-1* (*P* < 0.001) (*n* = 15 for each genotype and RNAi feeding). Solid bars, *N2*; open bars, *glp-1*. (**C**) *K04A8.5* was induced in animals subjected to *daf-2* RNAi only at adulthood (*P* < 0.0001). This induction by *daf-2* RNAi was enhanced in the *glp-1* mutant (*P* < 0.0001). Green, *daf-2* RNAi; black, vector control. Solid bars, *N2*; open bars, *glp-1*. (**D**) Adult-specific *daf-2* RNAi decreased fat storage by 50% in the wild type (*P* < 0.0001). Loss of the germ line and reduction of *daf-2* activity were synergistic in reducing fat storage (*P* < 0.0001). Green, *daf-2* RNAi; black, vector control. Solid bars, *N2*; open bars, *glp-1*. (**E**) *K04A8.5* RNAi partially suppressed the longevity of *daf-2(e1370)* mutants (*P* < 0.005; 24% decrease in mean life span) but had no effect on the life span of the wild type (*P* > 0.01).