Omega-3 and -6 fatty acids allocate somatic and germline lipids to ensure fitness during nutrient and oxidative stress in *Caenorhabditis elegans*

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Animals in nature are continually challenged by periods of feast and famine as resources inevitably fluctuate, and must allocate somatic reserves for reproduction to abate evolutionary pressures. We identify an age-dependent lipid homeostasis pathway in *Caenorhabditis elegans* that regulates the mobilization of lipids from the soma to the germline, which supports fecundity but at the cost of survival in nutrient-poor and oxidative stress environments. This trade-off is responsive to the levels of dietary carbohydrates and organismal oleic acid and is coupled to activation of the cytoprotective transcription factor SKN-1 in both laboratory-derived and natural isolates of *C. elegans*. The homeostatic balance of lipid stores between the somatic and germ cells is mediated by arachidonic acid (omega-6) and eicosapentaenoic acid (omega-3) precursors of eicosanoid signaling molecules. Our results describe a mechanism for resource reallocation within intact animals that influences reproductive fitness at the cost of somatic resilience.

soma | germline | trade-off | lipids | survival

Trade-offs between fecundity and viability fitness components are thought to drive life-history traits when resources are limited (1). In *Caenorhabditis elegans*, previous studies that removed proliferating germ cells led to an increase in somatic fat (2) and a ∼60% increase in lifespan (3), which is hypothesized to result from the reallocation of germline resources to the soma, promoting survival through enhanced proteostasis (4) and attuned metabolism (5).

Although these previous studies are compelling, the use of reproduction-deficient animals confounds the interpretation of their results with regard to trade-off models, and raises the question of how altered reallocation may affect intact animals. During reproduction, somatic resources are deposited to the germline by the actions of vitellogenins (6), which assemble and transport lipids in the form of yolk from the intestine to developing oocytes. The increased survival of germline-defective animals and their accumulation of somatic lipids suggest that the levels of somatic and germline lipids may influence the age-related decline of somatic cell function in postreproductive life (5). The mechanisms that regulate the distribution of energy resources remain elusive, however.

SKN-1 is the worm homolog of mammalian Nrf2, a cytoprotective transcription factor that impacts multiple aspects of animal physiology (7). Early work on SKN-1 defined its essential roles in development (8) and oxidative stress responses (9), whereas more recent work has identified a role mediating changes in diet availability and composition (10, 11). In the present study, we examined the SKN-1–mediated dietary adaptation pathways (10–12) of *C. elegans* and uncovered a sophisticated mechanism for mobilizing somatic lipids to the germline when animals sense stressful environments. This altruistic act by the soma impacts organismal viability to promote fecundity during oxidative and nutrient stress conditions. The universality of oxidative stress responses among aerobic organisms is a tantalizing source of energetic "cost" to maintain homeostasis that can compete with resources for reproduction. As such, an understanding of how oxidative stress responses impact reproduction, and vice versa, will likely yield insights into how the complex regulation of survival and reproduction trade-offs depend on resource reallocation (13). Here we report a SKN-1–dependent axis of regulating the distribution of somatic and germ cell resources.

Results

Age-Dependent Somatic Depletion of Fat Is Induced by Activated SKN-1. Over the course of an individual's lifespan, lipids are continually mobilized to afford organismal energy demands for growth, cellular maintenance and repair, and reproduction (14). We first examined total fat stores by Oil-red-O (15) (*SI Appendix*, Fig. S1 *A–E*) and fixed Nile red (*SI Appendix*, Fig. S2 *A–D*) in the standard wild type (WT) laboratory *C. elegans* strain N2-Bristol throughout reproduction, from early adulthood (72 h postfeeding) through reproductive senescence (144 h postfeeding). (Herein, hours postfeeding refers to the amount of time that animals have been provided with food following synchronization at larval stage 1 via starvation from hatching.) In these animals, similar to most metazoans, somatic lipid stores increased throughout this time period (Fig. 1 *A* and *B* and *SI Appendix*, Figs. S1 *A–E* and S2 *A–D*).

Significance

Food availability in nature changes continually over an organism's lifetime. As such, animals must diligently assess resource availability and appropriately allocate reserves that have been stored during times of feast for reproduction, to abate evolutionary pressures during times of famine. Our findings functionally link the availability of somatic (survival-promoting) and germline (reproduction-promoting) lipids to SKN-1 responses to oxidative and nutrient stress. We have defined this physiological response at the molecular, genetic, and organismal levels and identified a specific signaling system for regulating this process within intact animals. These findings will inform not only laboratory-based studies, but also ecological studies that have long sought to functionally integrate oxidative stress responses (like the SKN-1 pathway) into life-history traits.

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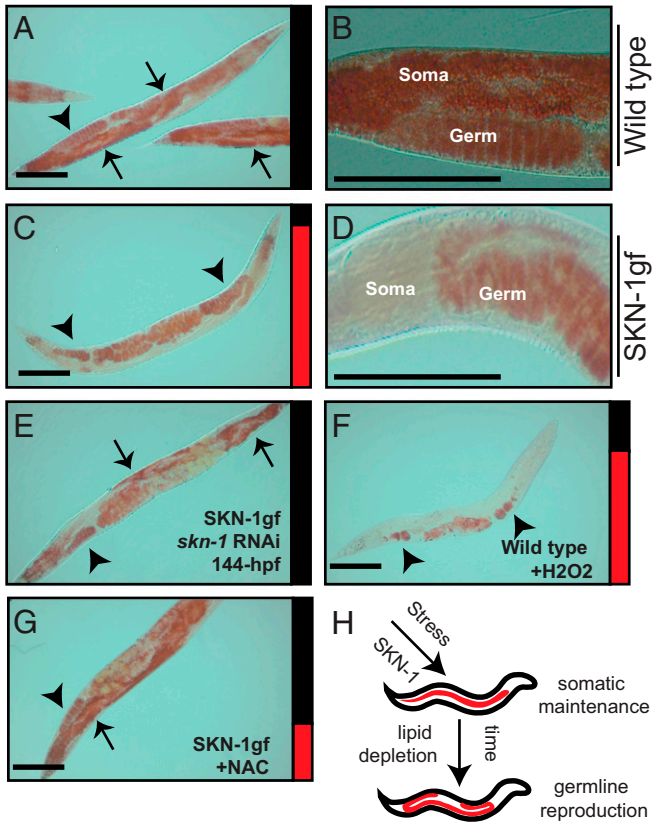

Fig. 1. SKN-1 activation mobilizes somatic fat to the germline. (*A–D*) Oil-red-O staining of somatic and germline lipids in WT animals, but only germline lipids in SKN-1gf mutants, at 144 h postfeeding. (*E*) *skn-1* RNAi suppresses Asdf in SKN-1gf animals. (*F*) Asdf is induced in WT animals by acute exposure to H_2O_2. (*G*) NAC treatment suppresses Asdf in SKN-1gf animals. (*H*) Cartoon of the Asdf phenotype. Arrows indicate soma, and arrowheads indicate germ. Bar graphs accompanying each panel indicate the percent of population scored with the Asdf phenotype (red) vs. normal lipid distribution (black) from a minimum of two biological replicates for each genotype and condition (*SI Appendix*, Fig. S3). (Scale bars: 100 μm.)

Based on the recent discovery that SKN-1 can potently influence the ability of organisms to metabolically adapt to changes in the environment (10, 11), we next looked at total fat stores during reproduction in SKN-1 gain-of-function (gf) mutant animals (Fig. 1 *C* and *D* and *SI Appendix*, Figs. S1 *A* and *F–M* and S2 *E–L*) and observed the *skn-1*–dependent rapid depletion of somatic, but not germline, lipid stores near the end of the reproductive period (Fig. 1 *C* and *D* and *SI Appendix*, Fig. S1 *I* and *M*, Fig. S2 *H* and *L*, and Table S1), a phenotype that, based on its characteristics, we call the age-dependent somatic depletion of fat (Asdf) phenotype. We assessed the Asdf phenotype in each cohort by quantifying the number of animals that displayed Asdf with those that did not. *SI Appendix*, Fig. S3 provides all % Asdf measurements. The Asdf phenotype was similar in all SKN-1–activating mutants tested, which includes strains harboring mutations in *alh-6* (10, 11) (*SI Appendix*, Fig. S4 *A* and *B*) or *wdr-23* (16) (*SI Appendix*, Fig. S4 *C* and *D*), whereas *skn-1* RNAi suppressed Asdf in SKN-1gf mutant animals (Fig. 1*E*). These data indicate that activated SKN-1 is sufficient to induce Asdf.

SKN-1 activation is correlated with increased levels of reactive oxygen species from endogenous sources or environmental exposure to oxidizing agents (17). Following acute exposure to H_2O_2, which can activate SKN-1, WT animals rapidly (within 12 h) deplete most somatic lipids (Fig. 1*F*). The Asdf response is not a generalized stress response and is specific to oxidative stress; WT animals exposed to heat (*SI Appendix*, Fig. S4*G*) or osmotic (*SI Appendix*, Fig. S4*H*) stress environments did not induce the lipid depletion phenotype. Further supporting the need for *skn-1* in the Asdf response, *skn-1*(−/−) null mutants did not deplete somatic fat following H_2O_2 exposure, and heterozygous *skn-1*(+/−) animals showed an intermediate response (*SI Appendix*, Fig. S4 *I* and *J*). Asdf was suppressed when animals with activated SKN-1 were treated with the antioxidant *N*-acetylcysteine (NAC) (Fig. 1*G* and *SI Appendix*, Fig. 4 *K–N*). Intriguingly, treatment of WT animals with NAC or *skn-1* RNAi led to excessive accumulation of somatic lipids (*SI Appendix*, Fig. S4 *O–R*), similar to the increased fat observed in *skn-1*(−/−) animals (*SI Appendix*, Fig. S4 *S* and *T*) and consistent with previous reports of lipid phenotypes in animals with reduced *skn-1* (18). This finding supports previous predictions in the life-history theory proposing that the energetic costs to maintain organismal oxidative stress capacity over the animal's lifetime represent a major trade-off variable (19). Taken together, our data indicate that the somatic depletion phenotype is sensitive to oxidative stress and requires SKN-1 (Fig. 1*H*).

Asdf Mobilizes Somatic Lipids During Nutrient Stress. Our observation that animals with Asdf retained lipids in the germline suggests that Asdf might result from mobilization of stored somatic lipids to the reproductive system. Members of the vitellogenin family of proteins facilitate transport of stored lipids from the intestine to developing oocytes (20) (Fig. 2*A*). RNAi of all *vit* genes tested resulted in suppression of Asdf (i.e., restoration of somatic lipids), indicating that vitellogenesis is required for Asdf in the SKN-1gf mutants (Fig. 2*B* and *SI Appendix*, Fig. S5 *A–D*). The presence of somatic lipids in SKN-1gf animals was restored

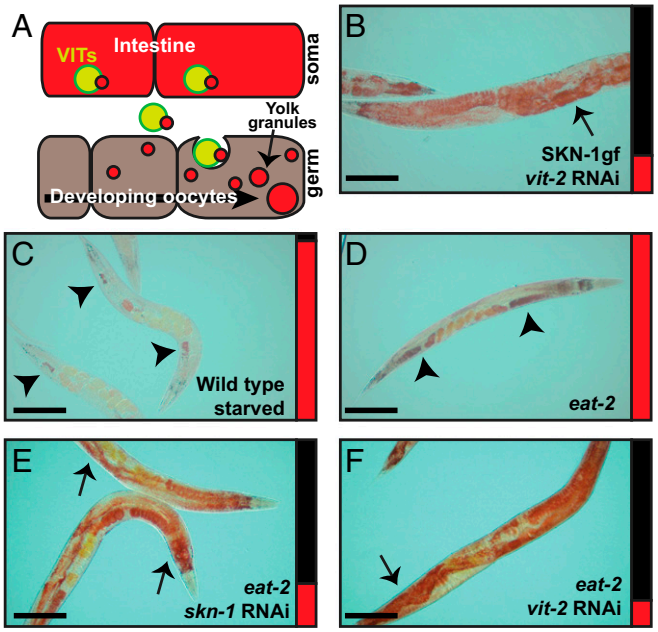

Fig. 2. Asdf is a starvation response dependent on vitellogenesis. (*A*) Cartoon representation of the vitellogenin lipid transport system from the intestine to the germline. (*B*) *vit-2* RNAi suppresses Asdf in SKN-1gf animals. (*C*) WT animals starved for 24 h deplete somatic lipids but retain a lipid pool in the germline. (*D*) *eat-2(ad456)* mutants display Asdf at 144 h postfeeding. (*E* and *F*) *skn-1* (*E*) and *vit-2* (*F*) RNAi suppresses Asdf in *eat-2* mutant animals. Arrows indicate soma, and arrowheads indicate germ. Bar graphs accompanying each panel indicate the percent of population scored with the Asdf phenotype (red) vs. normal lipid distribution (black) from a minimum of two biological replicates for each genotype and condition (*SI Appendix*, Fig. S3). (Scale bars: 100 μm.)

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when *vit-2*, *-3*, or *-5* was targeted by RNAi, or was even increased with reduced expression of *vit-4*. As such, the age-dependent loss of lipids in the soma in SKN-1gf animals is not simply the result of somatic utilization, but rather is a consequence of the unidirectional mobilization of stored lipids by the vitellogenins.

SKN-1 activity is essential for the longevity response to dietary deficiencies (21), and starvation itself can induce oxidative stress (22). Indeed, the depletion of stored lipids in WT animals after 24 h of starvation, albeit more extreme, resembled the Asdf observed in well-fed animals with activated SKN-1 (Fig. 2*C*). Consistent with the idea that the Asdf phenotype in SKN-1gf is a response to a perceived nutritional deficiency, *eat-2* mutants, which eat significantly less food than WT animals (23), also displayed Asdf at the same time point in their reproductive span, whereas WT animals failed to display Asdf (Fig. 2*D* and *SI Appendix*, Fig. S6 *A–D*). Asdf was not observed in *daf-2*/insulin-IGF1 receptor (*SI Appendix*, Fig. S6 *E* and *F*) and *isp-1*/mitochondrial iron sulfur protein (*SI Appendix*, Fig. S6 *G–J*) mutants, and thus is not universal to all longevity-promoting mutations. The Asdf phenotype observed in *eat-2* mutants was suppressed by *skn-1* (Fig. 2*E*) and *vit-2* (Fig. 2*F*) RNAi-treated animals. Note that Asdf is suppressed by the HT115 diet and glucose; thus, all RNAi experiments reported herein were performed in an OP50-background RNAi strain (*SI Appendix*, Fig. S7 and Tables S2 and S3). Our findings support an intriguing model of resource reallocation between the *C. elegans* soma and germline, where activation of the cytoprotective transcription factor SKN-1 under limited food and oxidative stress leads to the mobilization of stored lipid pools to the germline, presumably to ensure fitness.

Oleic Acid Deficiency Is Sufficient to Induce Asdf. To understand the mechanisms underlying Asdf, we identified the specific lipid molecules altered in the SKN-1gf mutants by HPLC/GCMS (*SI Appendix*, Fig. S8 *A* and *B*). We noted a significant reduction in C17-branched fatty acids and the monounsaturated fatty acid (MUFA) oleic acid (C18:1 n-9) in the triglyceride fraction of the SKN-1gf mutants compared with WT animals. Oleic acid was the sole lipid species restored to WT levels in SKN-1gf animals when the Asdf phenotype was suppressed by dietary glucose (*SI Appendix*, Figs. S7 *R–U* and S8*C*). *C. elegans* can synthesize oleic acid and all polyunsaturated fatty acid (PUFA) species from dietary or de novo synthesized C16:0 (24) (*SI Appendix*, Figs. S8*D* and S9*A*).

fat-6 and *fat-7* encode the major isoforms of the Δ9 desaturases that convert stearic acid to oleic acid (25). We subsequently tested for a direct relationship between oleic acid and Asdf. First, we decreased *fat-6*/*-7* by RNAi in WT animals, which phenocopied the Asdf phenotype at the same 144-h postfeeding time point observed in the SKN-1gf mutants (Fig. 3*A*). We measured *fat-6* and *fat-7* mRNA in SKN-1gf and WT animals and found similar levels of expression. Thus, the Asdf phenotype in SKN-1gf mutant animals is not due simply to reduced expression of the transcripts (*SI Appendix*, Table S4). Next, we supplemented the OP50 diet fed to SKN-1gf mutants with 160 μM and 320 μM oleic acid and observed a concentration-dependent reversal of Asdf, with 60.7% and 81.6% suppression of the Asdf phenotype, respectively, in this population (Fig. 3*B* and *SI Appendix*, Fig. S9 *B–G*).

To test the hypothesis that the suppression of Asdf by oleic acid is related to a general increase in total lipids, we assessed the ability of additional lipid supplements to suppress Asdf. We tested lipid species that are biosynthetic precursors to oleic acid, including C18:0 stearic acid (Fig. 3*C* and *SI Appendix*, Fig. S9*H*) and C12:0 lauric acid (*SI Appendix*, Fig. S9 *I* and *J*), as well lipids that are further desaturated products of oleic acid, including C18:2 n-6 linoleic acid, C18:3 n-3 α-linolenic acid, and C18:3 n-6 γ-linolenic acid. Similar to supplementation with stearic and lauric acid, each of these supplements dramatically increased total fat in WT animals; however, they could not suppress Asdf in SKN-1gf mutants (*SI Appendix*, Fig. S9 *K–P*). We also tested

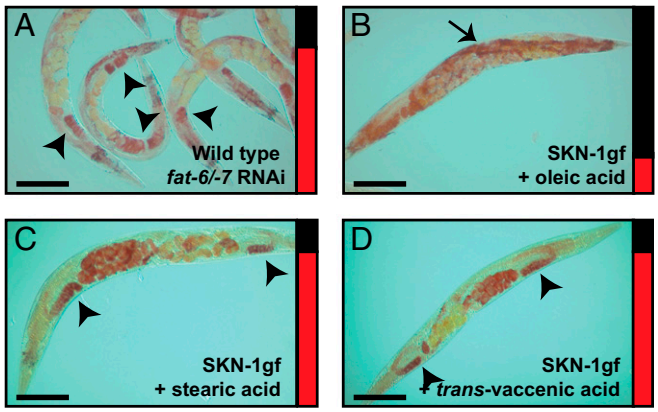

Fig. 3. Oleic acid deficiency is causal for Asdf. (*A*) RNAi inactivation of *fat-6*/*-7* in WT animals is sufficient to induce Asdf. (*B*) Dietary supplementation of oleic acid suppresses Asdf in SKN-1gf mutant animals. (*C* and *D*) Dietary supplementation of stearic acid (*C*) and *trans*-vaccenic acid (*D*) do not suppress Asdf in SKN-1gf mutant animals. Arrows indicate soma, and arrowheads indicate germ. Bar graphs accompanying each panel indicate the percent of population scored with the Asdf phenotype (red) vs. normal lipid distribution (black) from a minimum of two biological replicates for each genotype and condition (*SI Appendix*, Fig. S3). (Scale bars: 100 μm.)

trans-vaccenic acid (C18:1 *trans*-11), a MUFA that can be desaturated by FAT-6 and FAT-7 (26), but found that, unlike oleic acid, it was incapable of any observable suppression of Asdf in the SKN-1gf mutants (Fig. 3*D* and *SI Appendix*, Fig. S9*Q*). Taken together, these findings suggest that a lipid deficiency, specifically in oleic acid (C18:1), is causal for the Asdf phenotype in SKN-1gf animals as animal reproduction declines.

Omega-3 and -6 C20 PUFAs Oppose Asdf. We were surprised to find that lipid defects in the SKN-1gf mutant animals were specific to a single MUFA, oleic acid, and that this defect did not propagate to longer and more unsaturated species (*SI Appendix*, Fig. S8*A*). However, in our assessment of the lipid biosynthesis pathways, we uncovered a role for specific C20 omega-3 and omega-6 PUFAs in the regulation of Asdf. Like mammals, *C. elegans* synthesize a variety of lipid signaling molecules that are epoxy and hydroxyl derivatives of dihomo-γ-linolenic acid (DGLA), arachidonic acid (ARA), and eicosapentaenoic acid (EPA) PUFAs, which influence complex physiological processes that maintain homeostasis (27, 28) (Fig. 4*A*). DGLA and eicosatetraenoic acid (ETA) are biosynthetic precursors for ARA and EPA, respectively; however, ARA can be further desaturated to make EPA, and thus DGLA is a precursor for both ARA and EPA. *fat-4(wa14); fat-1(wa9)* double-mutant animals, which cannot generate ARA or EPA (29), prominently displayed Asdf at the same 144-h postfeeding time point, but not early in reproduction at 72 h postfeeding, as was observed in SKN-1gf mutant animals (Fig. 4*B* and *SI Appendix*, Fig. S10*A*). The levels of *fat-1* and *fat-4* were similar in SKN-1gf and WT animals, indicating that the Asdf phenotype is not due to a reduction in gene expression in SKN-1gf mutant animals (*SI Appendix*, Table S4).

The foregoing data suggest that one function of C20 omega-3 and omega-6 PUFAs is to help maintain the distribution of somatic and germline lipids, and that reduced levels of these lipid species promote Asdf. Treatment of SKN-1gf mutants with 160 μM or 320 μM ARA resulted in potent suppression of Asdf, by 82% and 91%, respectively (Fig. 4*C* and *SI Appendix*, Fig. S10 *B–E*). Similarly, EPA supplementation suppressed Asdf to 40% and 54% of animals at the same concentrations (Fig. 4*D* and *SI Appendix*, Fig. S10 *F* and *G*). The suppression of Asdf was specific to ARA and EPA; SKN-1gf mutants fed OP50 supplemented with DGLA or ETA, even at high concentrations, still

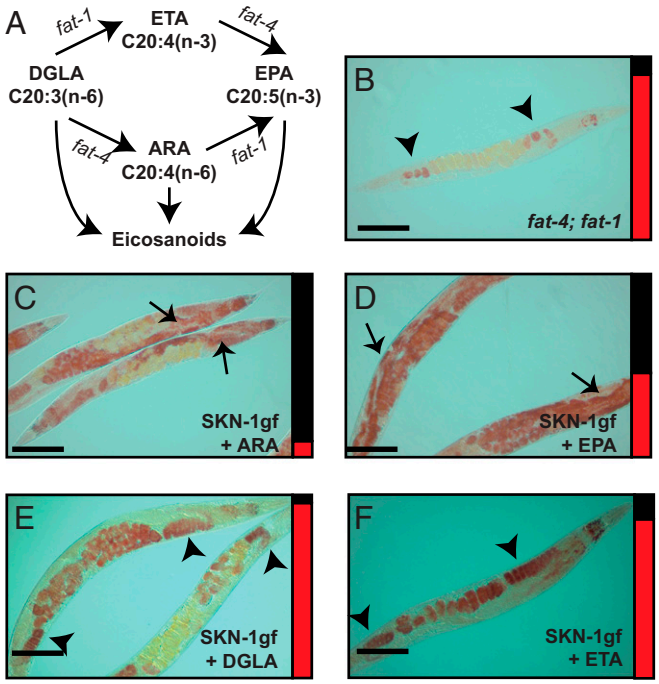

Fig. 4. ARA (omega-6) and EPA (omega-3) fatty acids regulate Asdf. (*A*) Schematic of eicosanoid biosynthesis pathways in *C. elegans*. (*B*) ARA and EPA deficient *fat-4(wa14); fat-1(wa9)* animals induce Asdf at 144 h postfeeding. (*C–F*) Dietary supplementation of ARA (*C*) or EPA (*D*), but not of DGLA (*E*) or ETA (*F*), can suppress Asdf in SKN-1gf mutant animals. Arrows indicate soma, and arrowheads indicate germ. Bar graphs accompanying each panel indicate the percent of population scored with the Asdf phenotype (red) vs. normal lipid distribution (black) from a minimum of two biological replicates for each genotype and condition (*SI Appendix*, Fig. S3). (Scale bars: 100 μm.)

displayed Asdf (Fig. 4 *E* and *F* and *SI Appendix*, Fig. S10 *H–J*). Taken together, these findings further support a dose-dependent role for specific omega-6 and omega-3 PUFAs in the homeostatic balance of somatic and germline lipid reserves.

Asdf Occurs in Natural Isolates of *C. elegans*. *C. elegans* represent a species of particularly low genetic diversity at the molecular level (30), and recent work to isolate and document the phenotypes of the ever-expanding library of wild *C. elegans* strains has revealed interesting phenotypic variation among them when cultured under laboratory conditions (31). A dearth of ecological data has hindered a better understanding of the relevance of this variation in the natural context, however (32). We analyzed a small collection of wild isolates of *C. elegans* and examined the abundance of somatic and germline lipids and their propensity for Asdf (*SI Appendix*, Fig. S11 *A–H* and Table S5). None of the wild isolates displayed Asdf at early time points in their reproductive span; however, four of the wild isolate strains tested displayed Asdf at the same 144-h postfeeding time point as animals with activated SKN-1, albeit with varying penetrance. NL7000 and ED3040 had the strongest Asdf phenotype, ED3021 displayed an intermediary phenotype, and ED3049 had a weak Asdf response in this population. RW7000, TR403, CB4856, and CB4869 were most similar to N2-Bristol in that they did not display Asdf at any time point.

Strains NL7000 and RW7000 are isolates of the same strain of *Bergerac* that recently diverged in the laboratory setting. Although derived from the same parental isolate, NL7000 displays Asdf at 144 h postfeeding, whereas RW7000 does not. Moreover, and consistent with the idea that Asdf promotes reproductive fitness, NL7000 animals have more progeny and remain reproductive longer than RW7000 animals (*SI Appendix*, Fig. S11*I*). Taken together, our data suggest that the Asdf phenotype is present in some, but not all, wild *C. elegans* strains, and that the propensity for Asdf may be correlated with reproductive success.

Asdf Promotes Reproduction at the Cost of Survival. We next assessed the role of Asdf in animal physiology and the resulting impact of deregulating Asdf capacity. During periods of scarce resources, fertile *C. elegans* hermaphrodites exhibit matricide, an altruistic behavior in which fertilized eggs are held in the uterus and hatch internally, and the resulting larvae feed on the hermaphrodite mother as a nutrient source (33). We observed an intriguing matricide phenomenon that correlated with Asdf in SKN-1gf mutants. When day 3 (120 h postfeeding) adult SKN-1gf mutants with early signs of Asdf were starved for 24 h, they became filled with newly hatched larvae, phenotypically defined as bags of worms (Bag) (Fig. 5*A* and *SI Appendix*, Fig. S12 *A–D*). This is in contrast to day 1 adult (72 h postfeeding) SKN-1gf mutant animals and day 1 or 3 adult WT animals, which have only one, if any, internally hatched larvae after 24 h of starvation. During the 48 h separating these two periods in reproduction, WT *C. elegans* accumulate lipids in their somatic tissues (*SI Appendix*, Figs. S1 *B–E* and S2 *A–D*), whereas SKN-1gf mutants mobilize somatic fat to the germline (*SI Appendix*, Figs. S1 *F–M* and S2 *E–L*). The Bag phenotype observed in day 3 adult SKN-1gf mutants with Asdf could be a consequence of the Asdf-mediated increase in germline lipids.

A primary function of somatic cells is to protect the germline, but this comes at the cost of depleting somatic resources. ARA

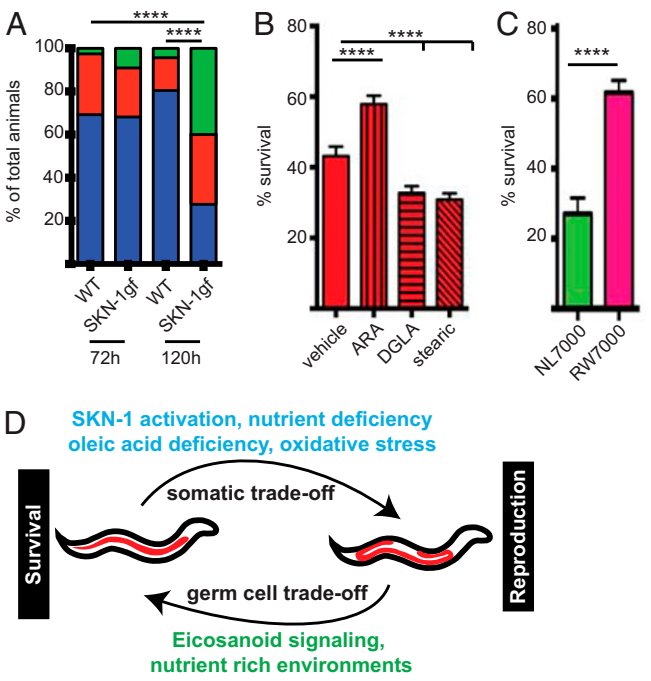

Fig. 5. Asdf fuels germ cell maturation to ensure fitness. (*A*) Following 24 h of starvation, SKN-1gf mutants display an age-dependent increase in the incidence of matricide (Bag phenotype) that coincides with Asdf and is not induced in WT animals when starved for 24 h. Blue indicates zero to one internal progeny; red, two to four internal progeny; green, five or more internal progeny. ****$P <$ 0.0001, ANOVA. (*B*) OP50 diet supplemented with ARA, but not with DGLA or stearic acid, can increase somatic resistance to acute H_2O_2 exposure in SKN-1gf mutant animals at 144 h postfeeding. ****$P < 0.0001$, ANOVA. (*C*) Somatic resistance to H_2O_2 in NL7000 and RW7000 *Bergerac* strains correlates with Asdf competency. Data are mean ± SEM for at least 40 animals, with a minimum of two biological replicates for each genotype and condition. ****$P < 0.0001$, two-tailed *t* test. (*D*) Model for the mechanisms underlying somatic survival and germline reproduction trade-offs of lipid reallocation within intact animals.

supplementation has been linked to the survival of somatic tissues during starvation and can increase the lifespan of ad libitum-fed WT animals (34). SKN-1gf mutants display significant resilience to H_2O_2 exposure in early reproductive life compared with WT animals (*SI Appendix*, Fig. S13 *A* and *B*); however, the afforded resistance to exogenous oxidative stress in SKN-1gf mutants declines at 144 h postfeeding (*SI Appendix*, Fig. S13*B*). We hypothesized that the reduction in somatic energy reserves as lipids are mobilized to the germline during Asdf is causal for the diminished oxidative stress resistance capacity. To test this, we inhibited Asdf by ARA supplementation to the OP50 diet, which resulted in a marked increase in resilience to acute H_2O_2 exposure in 144 h postfeeding, but not 80 h postfeeding, SKN-1gf animals (Fig. 5*B* and *SI Appendix*, Fig. S13 *B* and *C*). The restoration of somatic resistance to oxidative stress was specific, because supplementation with DGLA and stearic acid did not increase survival at either time point (Fig. 5*B* and *SI Appendix*, Fig. S13*C*). Intriguingly, postreproductive WT animals, which no longer need to devote as many resources to reproduction, exhibited a significantly increased survival response to acute H_2O_2 exposure (*SI Appendix*, Fig. S13*A*).

Finally, we examined somatic stress resistance to H_2O_2 in the NL7000 ($Asdf^+$) and RW7000 ($Asdf^-$) *Bergerac* strains. Although both strains had enhanced resistance at 72 h postfeeding (*SI Appendix*, Fig. S13*D*), NL7000 displayed a significant loss of resilience at 144 h postfeeding, whereas RW7000 was more apt to survive acute exposure to H_2O_2 (Fig. 5*C*). These findings are consistent with an increased capacity for stress resistance that is fueled by additional somatic resources, and regulated by specific omega PUFAs.

Taken together, our results describe a pathway for the reallocation of resources between the soma and germ cells of an intact organism (Fig. 5*D*). Our findings link the availability of somatic and germline lipids to SKN-1 responses to oxidative stress and nutrient limitation. This reallocation impacts somatic survival during stress and reproductive output, which may have universal implications for organisms with specialized soma and germ cells.

Discussion

In the present study, we examined organismal age-related levels of lipids during the *C. elegans* reproductive span and found a remarkable lipid reallocation phenotype between somatic and germ cells that impacts survival and reproduction trade-offs. We used Oil-red-O and Nile red staining of fixed animals, because the former allows for qualitative assessment of tissue distribution and the latter affords more quantitative measurements, albeit with reduced spatial resolution. We observed similar patterns of lipid distribution with either dye, but each could have unique specificity for different lipid species (35, 36), and differences in the intensity and size of the lipid droplets might reflect a change in the composition of lipid molecules affected.

Our discovery was facilitated by a collection of SKN-1gf mutants that we previously characterized as having reduced lipid levels on fat-inducing diets (10, 11), perhaps owing in part to their starvation-like behaviors, despite being fed ad libitum (12). Although resistant to acute exposure to oxidative stress, none of the constitutively activated SKN-1 mutants have proven to be long-lived. This finding is surprising, given that SKN-1 is a cytoprotective transcription factor essential for mounting an appropriate stress response. The near-complete depletion of somatic lipid reserves from the soma in the animals could explain this lack of longevity in the SKN-1gf mutants. The eventual depletion of somatic lipids was apparent at 144 h postfeeding, but clear differences in lipid abundance between the somatic and germline cells were obvious by 120 h postfeeding. Our data suggest that following the peak of reproduction, somatic resources are mobilized to the germline, but these resources are effectively "wasted" as animals enter reproductive senescence, because postreproductive animals no longer need to devote as many resources to reproduction. Intriguingly, SKN-1gf mutants do indeed have an extended self-reproductive period that does require Asdf, and thus an intriguing model for the function of Asdf is to promote late reproductive output. Although recent reports have shown that mated *C. elegans* hermaphrodites lose fat after mating, future assessment of the impact of Asdf on the fertility of mated animals will be of great interest, considering that maximal reproductive capacity is limited by sperm production in hermaphrodites (37, 38).

Collectively, our data support a genetic role for *skn-1* in the Asdf phenotype. Further refinement of the role SKN-1 plays in the distribution of somatic and germline lipids will be of particular interest. This work expands the known impact of SKN-1 on organismal physiology beyond its role as a mediator of cellular and organismal stress responses (7). One interpretation of this study is that SKN-1 activity is restricted to the soma, which leads to loss of lipids in this compartment; however, the fact that both SKN-1gf and *eat*-2 mutant animals no longer deplete somatic lipids when vitellogenesis is impaired suggests that mobilization of lipids to the germline is at least partially causal for the loss of somatic lipids. In addition, the supplementation of all lipid species resulted in an increase of somatic fat in WT animals and in SKN-1gf mutants early in reproduction, but only oleic acid, ARA, and EPA could suppress Asdf. The fact that most fatty acid supplements did not impact Asdf but also did not increase somatic stress resistance in the SKN-1gf mutants suggests that the depletion of somatic lipids is not simply a result of increased utilization in the soma.

This lipid reallocation has consequences for both somatic and germline tissues. The enhanced resistance to oxidative stress afforded in the SKN-1gf mutant animals is progressively impaired as animals proceed through reproduction, which correlates with the temporal progression of Asdf. Furthermore, if Asdf is suppressed, then the decline in stress resistance is attenuated. Thus, the reallocation of lipids between the soma and the germline is physiologically relevant, because the ultimate location where the lipids reside impacts the function of that compartment. Although body mass index (BMI) has proven to be an imperfect predictor of human metabolic disease risk (39), recent work has suggested that moderate increases in BMI above "normal" can be protective (40). Perhaps the reduction in mortality resulting from increased somatic reserves is the result of enhanced utilization of those stores for adaptation.

We have identified a role for C20 PUFAs in the mobilization of somatic resources to the germline in the SKN-1gf mutant animals. Dietary supplementation with the omega-6 PUFA ARA and the omega-3 PUFA EPA effectively suppressed Asdf, whereas that with the omega-6 PUFA DGLA did not. ARA, EPA, and DGLA are precursors of specific classes of eicosanoid signaling molecules (41), which play multiple and complex roles in animal physiology. Our finding that only ARA and EPA can suppress Asdf suggests that specific species of eicosanoids could be responsible for the physiological responses that we observed. C20 PUFAs also play a critical role in maintaining membrane fluidity (42), and thus the addition of these C20 PUFAs could alter membrane function and signaling capacity; however, the opposing responses to DGLA compared with ARA and EPA suggest that this is not simply a general disorganization of the lipid bilayer (43). Nevertheless, future assessment of the phospholipid composition of membranes, the signaling pathways that influence Asdf, and the functional consequences of perturbing these components on Asdf capacity and resulting phenotypes will be of great interest.

Although we examined reproductive-stage adults, previous studies of germline starvation responses in developing larvae have documented the scavenging of material from the germline to fuel reproduction (44) and even reproductive diapause (45). The increased germline lipid stores in $Asdf^+$ animals could promote two

non-mutually exclusive outcomes: (*i*) provide additional fuel for the rapid maturation of progeny and (*ii*) provide adequate nutrients to escape diapause initiation and/or maintenance. Alternatively, because SKN-1gf mutants Bag only when starved at the end of the reproductive period, this phenotype could represent a time-dependent failure to decrease ovulation in response to nutrient limitation when SKN-1 is constitutively activated. Nevertheless, because progeny's success is subject to the deposition of maternal factors, and their life-history parameters are sensitive to the experiences of the parental and grandparental generations (46–48), future studies to assess the cumulative effects of Asdf capacity on fitness in successive generations are needed.

We analyzed a collection of natural *C. elegans* isolates from diverse climates that revealed that Asdf capacity is variable in the wild (*SI Appendix*, Table S5). The RW7000 *Bergerac* isolate does not display Asdf and has a diminished reproductive period and brood compared with the recently diverged NL7000 strain, which displays Asdf at 144 h postfeeding and has a much larger brood size and a longer self-reproductive period. The number of SNPs between these strains is unknown, and these strains quite possibly could be significantly divergent from each other because they are classical mutator lines, originally used for the active transposons in their genomes. Nonetheless, in light of our finding that single gene mutations are sufficient to induce Asdf, future assessment of the genomic differences between these two strains and all of the wild isolates tested will be of particular interest.

Our results identify a SKN-1 and eicosanoid signaling pathway that balances somatic lipid mobilization to developing germ cells at the cost of survival. Our study provides insight into the trade-offs resulting from the reallocation of lipid stores within intact animals, which are critically important during nutrient and oxidative stress (Fig. 5*D*). The fundamental similarities of the *C. elegans* and mammalian lipid metabolism and eicosanoid biosynthesis and signaling pathways (41) suggests that the resource reallocation pathways and resulting trade-offs may be conserved.

Methods

C. elegans was cultured by standard techniques at 20 °C unless noted otherwise. Statistical analyses were performed with GraphPad Prism 6 software. Data are presented as mean ± SEM. Data were analyzed with the unpaired Student *t* test and two-way ANOVA. All of the methods used in this study are described in detail in *SI Appendix*.

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