

# Caenorhabditis elegans integrates food and reproductive signals in lifespan determination

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## Summary

**Dietary restriction extends lifespan and inhibits reproduction in many species. In *Caenorhabditis elegans*, inhibiting reproduction by germline removal extends lifespan. Therefore, we asked whether the effect of dietary restriction on lifespan might proceed via changes in the activity of the germline. We found that dietary restriction could increase the lifespan of animals lacking the entire reproductive system. Thus, dietary restriction can extend lifespan independently of any reproductive input. However, dietary restriction produced little or no increase in the long lifespan of animals that lack germ cells. Thus, germline removal and dietary restriction may potentially activate lifespan-extending pathways that ultimately converge on the same downstream longevity mechanisms. In well-fed animals, the somatic reproductive tissues are generally completely required for germline removal to extend lifespan. We found that this was not the case in animals subjected to dietary restriction. In addition, in these animals, loss of the germline could either further lengthen lifespan or shorten lifespan, depending on the genetic background. Thus, nutrient levels play an important role in determining how the reproductive system influences longevity.**

**Key words:** *C. elegans*; caloric restriction.

## Introduction

Food scarcity jeopardizes an animal's ability to survive and reproduce. In response to dietary restriction, the lifespans of many species are extended and their reproduction is inhibited and delayed (Partridge & Harvey, 1985; Audesirk & Audesirk, 1996; Guarente, 2005). This response is likely to have selective value in nature. For example, if food is restored to rodents subjected to dietary restriction when age-matched well-fed animals are postreproductive, they are able to produce progeny

(Merry & Holehan, 1979). Thus the response to dietary restriction can allow an animal to postpone reproduction until conditions improve and its progeny are more likely to survive.

In *Caenorhabditis elegans*, genetic and microsurgical approaches have yielded valuable insights into the aging process. The lifespan of *C. elegans*, like that of other organisms, is increased by dietary restriction (Klass, 1977; Lakowski & Hekimi, 1998; Houthoofd *et al.*, 2005). In this organism, there is an interesting link between the reproductive system and aging, as removing the germ cells increases lifespan (Hsin & Kenyon, 1999; Tatar, 2002; Mukhopadhyay & Tissenbaum, 2007). This lifespan extension is not a consequence of sterility, because removing the somatic reproductive tissues as well as the germline also sterilizes the animal but does not extend lifespan. Instead, the germline and somatic gonad appear to regulate counterbalancing signaling pathways that influence lifespan. These pathways regulate longevity, at least in part, by controlling the activity of the lifespan-extending forkhead box O (FOXO)-family transcription factor DAF-16 (Hsin & Kenyon, 1999; Libina *et al.*, 2003; Berman & Kenyon, 2006; Gerisch *et al.*, 2007). In nature, this pathway may allow an animal to coordinate its rate of aging with its timing of reproduction. For example, if something happens to retard germline development, changes in the activities of germline-dependent signaling pathways may slow the rate of aging, ensuring that the animal remains youthful enough to reproduce successfully. Transplanting reproductive tissues from young into old female mice lengthens lifespan (Cargill *et al.*, 2003; J. Carey, pers. comm.). Thus, reproductive signals affect lifespan in mammals as well as in *C. elegans*, although whether the underlying mechanisms are related is not known.

Nutrient intake, reproduction and aging are central aspects of an animal's life history. Because germline removal increases *C. elegans*' lifespan and dietary restriction inhibits reproduction, it seemed possible that food limitation could potentially extend lifespan by exerting an effect on the germline that mimics the effect of germline ablation. This idea is not new. For example, studies with mice and medflies have suggested the hypothesis that the reduced mortality of calorically restricted animals is caused by their reduced reproduction (Merry & Holehan, 1979; Carey *et al.*, 1998). Calorically-restricted animals shifted to *ad libitum* conditions acquire the same rates of reproduction and mortality as animals fed *ad libitum* their entire lives, and their reproductive behaviors are accurate predictors of subsequent mortality rates. Similarly, the timing of reproduction and subsequent death are correlated in *Drosophila* (Sgro & Partridge, 1999).

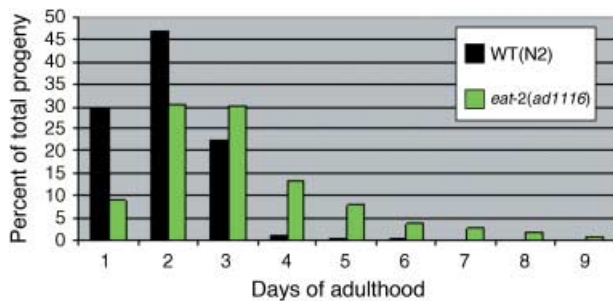
In addition to the possibility that food influences lifespan via effects on the reproductive system, it also seemed possible that, conversely, food limitation might alter the influence that

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**Fig. 1** *eat-2(ad1116)* mutations slow the rate of reproduction and decrease brood size. The histogram shows the fraction of total progeny (defined as the mean brood size) produced on each day of adulthood. The mean brood size of wild-type (N2) was  $298 \pm 11.6$  (SEM); 95% confidence interval, 272–323;  $n = 12$  (these 12, of 24, animals survived the fertile period and were included in the data set. Animals that crawled off the plates or ruptured before the fertile period ended were discarded; the mean number of progeny produced each day by these animals was similar to that of those that survived the entire fertile period; data not shown). The mean brood size of *eat-2(ad1116)* mutants was  $145 \pm 5.1$  (SEM); 95% confidence interval, 135–156;  $n = 21$  (three of 24 total animals crawled off the plate or ruptured during the fertile period. As with N2, these animals produced numbers of progeny each day that were similar to the numbers produced by the animals that survived the entire fertile period; data not shown).  $P < 0.0001$  for the mean brood size of *eat-2* vs. N2 animals.

the reproductive system has on lifespan. To investigate these relationships in *C. elegans*, we have used microsurgical approaches to ask how the reproductive system influences the response to dietary restriction, and how dietary restriction influences the effect of the reproductive system on lifespan.

## Results and discussion

The simplest way to ask whether the response to dietary restriction proceeds via changes in the reproductive system is to remove the reproductive system altogether and ask whether food limitation still increases lifespan. To subject *C. elegans* to dietary restriction, we used the *eat-2(ad1116)* mutation (Avery, 1993; Lakowski & Hekimi, 1998; McKay *et al.*, 2004). *eat-2* mutants lack a pharyngeal-specific nicotinic acetylcholine receptor subunit that is required for pharyngeal pumping (feeding) (McKay *et al.*, 2004). Like worms subjected to direct dietary restriction in liquid media, *eat-2* mutants live ~20–30% longer than well-fed wild-type animals (Lakowski & Hekimi, 1998) in a *pha-4* dependent fashion (Panowski *et al.*, 2007) and have a pale and thin appearance (Avery, 1993). In addition, we found that the brood size of *eat-2* mutants (~145) was smaller than that of well-fed wild-type animals (~298), and that their reproductive period was extended (Fig. 1), as expected for animals subjected to dietary restriction.

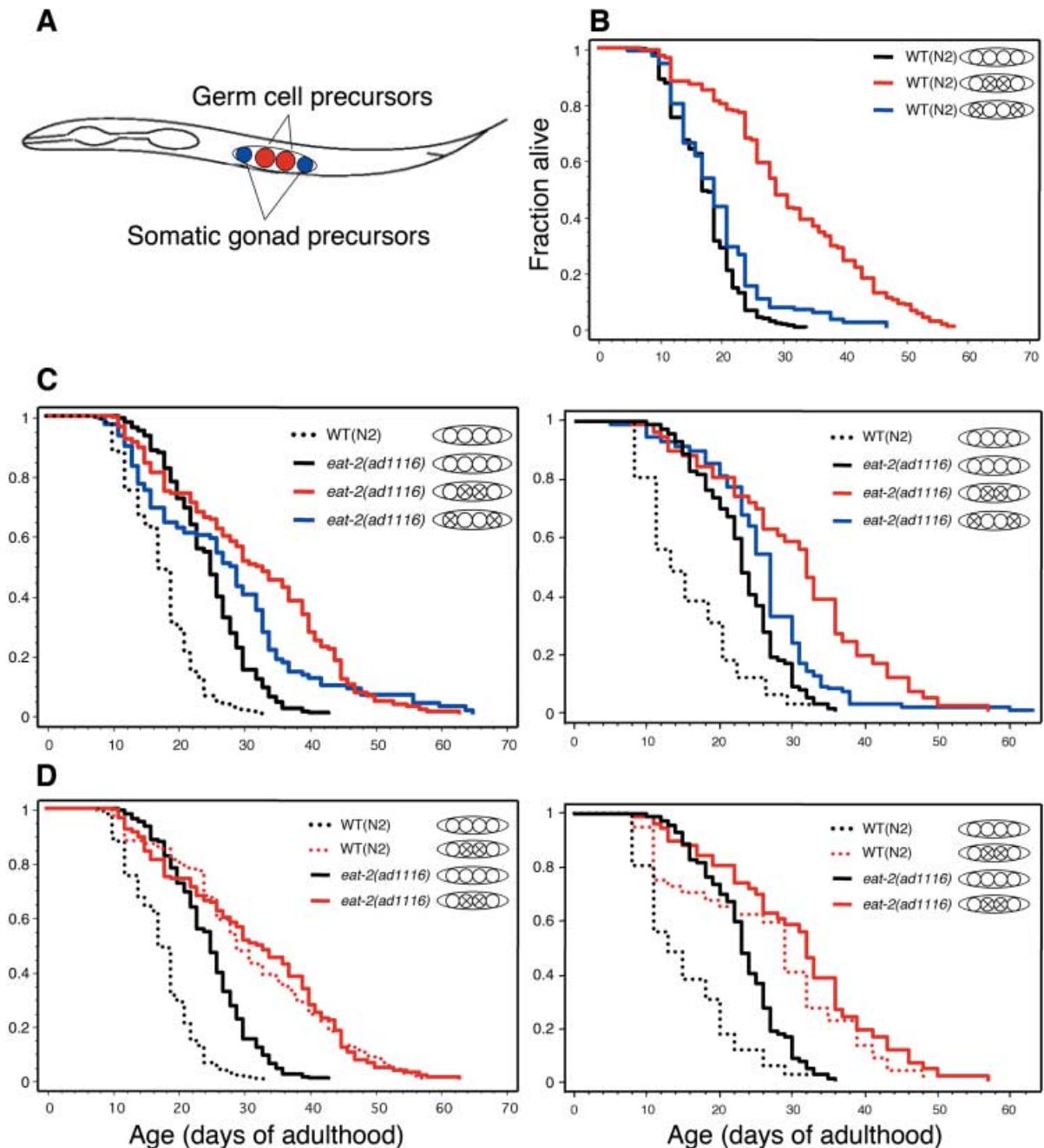
When *C. elegans* hatches from the egg, its reproductive system consists of four cells, Z1–Z4 (Fig. 2A). Z1 and Z4 give rise to the somatic reproductive tissues (uterus, spermatheca, etc.) and Z2 and Z3 give rise to the germline (Kimble & White, 1981). When Z2 and Z3 are killed with a laser microbeam, the resulting adults have somatic reproductive tissues but no germ cells. Killing Z1 and Z4 with a laser microbeam removes the entire

reproductive system, including the germline, because the germline cannot develop without the somatic reproductive tissues. To test the hypothesis that the longevity response to dietary restriction is mediated through a change in the reproductive system, we removed the entire reproductive system in both wild-type and *eat-2(ad1116)* animals by killing Z1 and Z4 at hatching, and asked whether the *eat-2* mutation could still extend lifespan. We found that it did (compare Fig. 2B with 2C; Table 1). Thus, the ability of food limitation to extend lifespan does not require the intervention of the reproductive system. Instead, the mechanism that triggers lifespan extension in response to food limitation can bypass the reproductive system completely. This finding suggests that dietary restriction initiates processes that act in parallel to reduce reproduction and to extend lifespan.

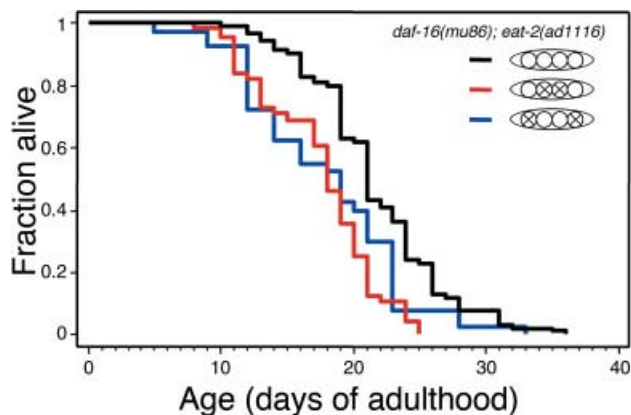
The finding that the reproductive system is not required for the longevity response to dietary restriction does not rule out the possibility that signals from the reproductive system modulate the longevity response to dietary restriction. In fact, this seemed to be the case. In two out of three experiments, dietary restriction produced an even greater lifespan extension in the absence of the reproductive system than it did in normal, intact animals (Table 1). Thus, the reproductive system is not simply a neutral bystander in the response to dietary restriction, instead it prevents dietary restriction from producing an even greater extension of lifespan.

In normal, well-fed animals, removing the entire reproductive system has little or no effect on lifespan (Hsin & Kenyon, 1999). As removing the entire reproductive system consistently produced a further increase in the lifespan of *eat-2* mutants, dietary restriction appears to alter an aspect of this longevity system. In principle, either the germ cells or the somatic gonad, or both, could limit the lifespan of dietary-restricted animals. To investigate this, we killed the germline precursors (Z2 and Z3) in *eat-2(ad1116)* mutants, but left the somatic gonad precursors intact. We found that killing only the germline precursors further extended the lifespan of *eat-2* mutants. In fact, these animals lived even longer than did *eat-2* mutants lacking the entire reproductive system (Fig. 2, Table 1). This finding indicates that it is the loss of the germ cells rather than the loss of the somatic gonad that extends lifespan when the reproductive system is removed in *eat-2* mutants. The somatic gonad, in contrast, promotes lifespan extension in these animals. As killing the germ cells also extends lifespan in well-fed, wild-type animals, this finding suggests that somatic gonad signaling, rather than germline signaling, changes in response to dietary restriction. Apparently, when the germline is removed under conditions of dietary restriction, a longevity factor that must normally be provided by the somatic gonad can either be provided by other tissues, or is no longer completely required for lifespan extension. For this reason, removing the entire reproductive system further extends lifespan.

Upon closer inspection, it appears that not only does the effect of the somatic gonad change in response to dietary restriction, but the effect of germline removal changes as well



**Fig. 2** Dietary restriction changes *Caenorhabditis elegans*' response to germline and whole-gonad ablation. (A) The reproductive system of *C. elegans* at the time of hatching. Z1 and Z4 (in blue) give rise to the somatic reproductive tissues (somatic gonad) and Z2 and Z3 (in red) give rise to the germline. (B) Lifespans of wild-type animals lacking the germline (Z2 and Z3 ablated) or the entire gonad (Z1 and Z4 ablated). As reported previously (Hsin & Kenyon, 1999), germline ablation extends the lifespan of well-fed wild-type animals, and this lifespan extension requires the somatic gonad. Mean adult lifespan of intact wild-type (N2) animals, 17.5 days; mean lifespan of wild-type animals lacking the germline (Z2 and Z3-ablated), 31.1 days; mean lifespan of wild-type animals lacking the entire reproductive system (Z1 and Z4-ablated), 19.5 days.  $P = 0.065$  N2 intact vs. N2 Z1/Z4 ablated;  $P < 0.0001$  N2 intact vs. N2 Z2/Z3 ablated. (C) Two replicate lifespans of *eat-2(ad1116)* mutants lacking the germline (Z2 and Z3 ablated) or the entire gonad (Z1 and Z4 ablated). Left panel: mean lifespan of intact *eat-2(ad1116)* mutants, 23.2 days; mean lifespan of *eat-2(ad1116)* mutants lacking the germline (Z2 and Z3 ablated), 31.0 days; mean lifespan of *eat-2(ad1116)* mutants lacking the entire reproductive system (Z1 and Z4 ablated), 26.3 days; mean lifespan of intact, wild-type control (N2), 15.4 days.  $P < 0.0001$  *eat-2 Z2/Z3-ablated* vs. *eat-2 Z1/Z4*;  $P < 0.001$  *eat-2 Z1/Z4-ablated* vs. *eat-2* intact;  $P < 0.0001$  *eat-2 Z2/Z3-ablated* vs. *eat-2* intact. Right panel: mean lifespan of intact *eat-2(ad1116)* mutants, 24.7 days; mean lifespan of *eat-2(ad1116)* mutants lacking the germline (Z2 and Z3 ablated), 31.5 days; mean lifespan of *eat-2(ad1116)* mutants lacking the entire reproductive system (Z1 and Z4 ablated), 27.4 days; and mean lifespan of intact, wild-type animals, 17.5 days.  $P = 0.025$  *eat-2 Z2/Z3-ablated* vs. *eat-2 Z1/Z4-ablated*;  $P = 0.0004$  *eat-2 Z1/Z4-ablated* vs. *eat-2* intact;  $P < 0.0001$  *eat-2 Z2/Z3-ablated* vs. *eat-2* intact. (D) These two panels show the two replicate experiments in which Z2 and Z3 were ablated in N2 and *eat-2(ad1116)* animals. Some of these survival curves were also shown in Fig. 2C; the data were replotted to make it easier for the reader to compare the responses of wild-type and *eat-2(-)* animals to loss of the germline. For additional information about these experiments, see Table 1.



**Fig. 3** In *daf-16* null mutants subjected to dietary restriction, killing the germ cells shortens lifespan. *daf-16(mu86null)* single mutants live approximately 20% shorter than normal (Lin *et al.*, 1997). Killing the germline precursors of well-fed [*eat-2(+)*] *daf-16* null mutants has no effect on lifespan, whereas killing their somatic gonad precursors shortens lifespan (Hsin & Kenyon, 1999). In a *daf-16(mu86); eat-2(ad1116)* mutant background, removing either the germ cells or the entire reproductive system further shortens lifespan. Mean lifespan of intact *daf-16(mu86); eat-2(ad1116)* mutants, 21.7 days; mean lifespan of *daf-16(mu86); eat-2(ad1116)* mutants lacking the germline (Z2 and Z3 ablated), 17.5 days; and mean lifespan of *daf-16(mu86); eat-2(ad1116)* mutants lacking the entire reproductive system (Z1 and Z4 ablated), 17.9 days.  $P < 0.0001$  *daf-16; eat-2* intact vs. *daf-16; eat-2* Z2/Z3-ablated;  $P = 0.2186$  *daf-16; eat-2* Z1/Z4 vs. *daf-16; eat-2* Z2/Z3. For more information and repetitions, please see Table 1.

(Fig. 2C). When the germ cells are killed in well-fed, wild-type animals, lifespan is extended by approximately 60–70% (Hsin & Kenyon, 1999) (Fig. 2B). However, killing the germ cells in *eat-2* mutants only extended lifespan by ~30% (Fig. 2C, Table 1). Thus germline removal has a smaller effect on *eat-2* mutants than it has on wild-type. As a consequence, the lifespans of germline-ablated well-fed animals and germline-ablated *eat-2* mutants are similar to one another. In other words, dietary restriction produces little or no lifespan increase in animals lacking the germline (Fig. 2D).

If dietary restriction and germline removal extended lifespan through completely independent pathways, then one would expect the lifespan extension produced by subjecting animals to both treatments to be substantially longer than the lifespan extension produced by either treatment alone; and, in fact, many longevity mutations do substantially extend the lifespan of *eat-2* mutants (Lakowski & Hekimi, 1998; Hansen *et al.*, 2005). Thus, this unexpected finding suggests the possibility that dietary restriction and germ-cell removal activate the same longevity pathway.

The hypothesis that the longevity response to dietary restriction might be the same as the longevity response to germline removal seems problematic, because the FOXO transcription factor DAF-16 is required for the longevity response to germline removal (Hsin & Kenyon, 1999), but not for the longevity response to dietary restriction (Lakowski & Hekimi, 1998; Houtthoofd *et al.*, 2003). If this hypothesis is correct, then perhaps dietary restriction initiates a chain of events that can be initiated independently by DAF-16 in response to germline removal.

As *daf-16* is required for germline removal to extend lifespan in well-fed, wild-type animals, we asked whether *daf-16* was required for germline ablation to further extend the lifespan of *eat-2* mutants. To this end, we killed the germ cell precursors of *daf-16(mu86null); eat-2(ad1116)* double mutants, and measured lifespan (Fig. 3, Table 1). As predicted, germ-cell ablation did not further increase the lifespan of *daf-16; eat-2* double mutants. Thus, *daf-16* is required for germline ablation to extend the lifespans of both wild-type and *eat-2* animals. However, unexpectedly, we found that germline removal actually shortened lifespan. Whereas intact *daf-16; eat-2* mutants lived 21.7 days on average, germline-ablated *daf-16; eat-2* double mutants lived 17.5 days on average. Removing the entire reproductive system shortened lifespan to the same extent as removing only the germ cells. Thus, as in a *daf-16(+)* background, the somatic gonad appeared to have a smaller influence on lifespan under conditions of dietary restriction.

These findings are informative for several reasons. First, as described above, removing either the germline or the entire gonad extends lifespan in *eat-2* mutants. One could imagine that under conditions of dietary restriction, where, by definition, resources are scarce, these treatments extend lifespan because they eliminate a 'cost of reproduction' (see; Williams, 1957; Partridge & Harvey, 1985; Kirkwood & Rose, 1991; Partridge & Barton, 1993; Chapman *et al.*, 1998; Westendorp & Kirkwood, 1998; Partridge *et al.*, 2005), in which the expenditure of resources on reproduction comes at the expense of activities that would otherwise protect and repair cells and thereby extend lifespan. Our findings with *daf-16* suggest that this is unlikely to be the case, as, in dietary-restricted animals that lack *daf-16*, resources are still scarce, but now sterilizing the animal by removing the germline or the entire gonad shortens lifespan.

Second, this finding indicates that in the absence of *daf-16*, the nutritional state of the animal determines how it responds to germline removal. If a *daf-16(-)* mutant is well-fed, removing the germline has no effect on lifespan (Hsin & Kenyon, 1999). However, if the animal has been subjected to dietary restriction, its lifespan is shortened. Thus, paradoxically, the physiology of animals subjected to dietary restriction is acutely sensitive to *daf-16* activity, in spite of the fact that lifespan extension produced by dietary restriction does not require *daf-16*. Recently, Henderson *et al.* showed that *daf-16* mediates the up-regulation of *sod-3* in short-lived, starved animals and protects them from becoming extremely sensitive to oxidative damage (2006). Although this was not examined in animals subjected to dietary restriction instead of starvation, this finding, too, indicates that nutrition influences the physiological role of *daf-16*.

Finally, these results indicate for the first time that germline removal is capable of affecting lifespan independently of *daf-16* (specifically, under conditions of dietary restriction).

Together our findings show that the responses to diet and reproductive signals are intertwined in unexpected ways in *C. elegans*. First, one of our most significant findings is that the lifespan of an animal completely lacking the reproductive system can be extended by dietary restriction. The close correlation

**Table 1** Adult lifespans of animals lacking either the germline or the entire reproductive system

Genotype	Cells ablated	Mean lifespan (day)	Number of died/ total number of animals§ (number of trials)	Lifespan extension, percentage of increase vs. control	<i>P</i> value against control	<i>P</i> value against specified group
Experiment 1						
<i>eat-2(ad1116)</i>	None control	24.7	193/335(3)			
	Z1/Z4	27.5	112/142(1)	<b>11%</b>	0.0004	
	Z2/Z3	31.5	128/249(2)	<b>27.4%</b>	< 0.0001	0.75†
N2	None control	17.5	217/375(3)			
	Z1/Z4	19.5	107/115(1)	<b>11.7%*</b>	0.017	
	Z2/Z3	31.1	159/199(2)	<b>77.9%</b>	< 0.0001	
Experiment 2						
<i>eat-2(ad1116)</i>	None control	23.2	113/187(2)			
	Z1/Z4	26.3	121/139(2)	<b>13.3%</b>	< 0.0001	
	Z2/Z3	31.0	46/102(2)	<b>33.5%</b>	< 0.0001	0.063†
N2	None control	15.4	41/66(1)			
	Z1/Z4	16.2	31/32(1)	4.9%	0.76	
	Z2/Z3	26.5	32/77(1)	<b>72.4%</b>	< 0.0001	
Experiment 3						
<i>eat-2(ad1116)</i>	None control	24.4	28/41(1)			
	Z1/Z4	32.3	22/28(1)	<b>32.3%</b>	0.011	
N2	none control	18.1	137/196(1)			
	Z1/Z4	18.3	97/101(1)	1.1%	0.84	
Experiment 4						
<i>daf-16(mu86)</i>	None	14.6	53/101(1)			
<i>eat-2(ad1116)</i>	None	24.2	75/114(1)			
<i>daf-16(mu86); eat-2(ad1116)</i>	None control	22.0	62/98(1)			
	Z1/Z4	17.6	55/59(1)	<b>-20%</b>	< 0.0001	
	Z1/Z4	17.9	40/41(1)	<b>-18.5%</b>	< 0.0001	
	Z2/Z3	17.5	51/87(1)	<b>-20.6%</b>	< 0.0001	
Experiment 5						
<i>daf-16(mu86); eat-2(ad1116)</i>	None control‡	23.0	70/121(1)			
	Z1/Z4	17.4	72/75(1)	<b>-24.5%</b>	< 0.0001	
	Z2/Z3	19.9	29/46(1)	<b>-13.6%</b>	0.006	

All controls were treated the same as ablated animals (placed on pads of 2% agarose containing 1.5 mM NaN<sub>3</sub> as an anesthetic), unless otherwise labeled 'untreated control'.

\*Percentage of control' compared to untreated control was 0.7%.

†Compared to N2 Z2/Z3 ablated.

‡Untreated control.

§Some animals were censored. The number of independent trials is in parentheses. The percentage difference (bold-faced if significant) between mean lifespans of treated animals and those of their respective controls is indicated in the fifth column. *P* values represent the probability that the estimated survival function of the experimental group of animals is equal to that of the control group. *P* values are determined using the log-rank (Mantel–Cox) statistics. *P* values less than 0.05 are considered statistically significant, demonstrating that the two survival functions are different.

In (1)-trial experiments the 'percentage of control' and *P* values are relative to the controls assayed in parallel with the experiments. In (2) or more trial experiments 'percentage of control' and *P* values are relative to controls combined from two or more experiments. (We show cumulative statistics in this table and relevant figures because experimental animals compared to their respective controls assayed at the same time, and to cumulative controls, behaved similarly.)

between reproduction and longevity in response to changes in nutrition (Merry & Holehan, 1979; Carey *et al.*, 1998) has suggested the 'reproductive clock' hypothesis (Carey *et al.*, 1998), in which changes in reproductive activity due to changes in nutrition, which are predictive of future mortality risk, have a causal effect on lifespan. However, at least in *C. elegans*, this need not be the case, because the longevity response to dietary restriction can bypass the reproductive system entirely.

Second, because dietary restriction does not further extend the lifespan of animals that lack germ cells, it seems likely that dietary restriction and germ-cell removal activate pathways that are related to one another in some way. It is possible

that dietary restriction and germ-cell removal each trigger chains of events that ultimately converge on the same downstream lifespan-extending processes, albeit in a manner that requires DAF-16 activity in the case of germline ablation but not in the case of caloric restriction.

In addition, our findings show that the animal has a means of integrating nutrient signals, the activity of the reproductive system and processes that affect aging. Dietary restriction changes the way that both the somatic gonad and the germline influence lifespan, even though dietary restriction can extend lifespan in the complete absence of both reproductive cell types. Dietary restriction appears to increase the importance of *daf-16*, as

*daf-16* now prevents defects in the germline from actually shortening lifespan. In addition, because it appears to lessen the requirement for the somatic gonad for the lifespan extension produced by germ-cell impairment, dietary restriction may broaden the range of reproductive perturbations that could potentially further extend lifespan in nature. Thus, together these effects may increase the chance that a dietary-restricted animal can outlive unfavorable environmental conditions.

Finally, we note that it is possible to subject *C. elegans* to dietary restriction in other ways (e.g. via bacterial dilution in liquid or on agar plates). We attempted to repeat our experiments using bacterial dilution in liquid media; however, we found that the somatic gonad was not required for germline ablation to extend lifespan even under 'fully fed' conditions (data not shown); that is, conditions that, by the criteria of lifespan length and reproductive profiles, were not thought to produce a classical 'dietary restriction' response. Interestingly, under similar 'fully fed' conditions, animals grown in liquid medium exhibit a spectral shift when visualized using *in vivo* spectrofluorimetry that is also seen in *eat-2* mutants (Gerstbrein *et al.*, 2005), suggesting that they express aspects of the dietary-restriction response. It is also possible to subject *C. elegans* to dietary restriction by limiting food availability on agar plates. We have not examined the response of such animals to the removal of reproductive cells. Thus it remains possible that the reproductive system will influence lifespan differently in animals subjected to a different paradigm for dietary restriction.

## Experimental procedures

### Strains

All strains were maintained as described previously (Brenner, 1974). We used the strains: N2 (wild-type), *eat-2(ad1116)* II, outcrossed four times to our laboratory N2 by Laura Mitic; and *daf-16(mu86); eat-2(ad1116)* double mutants, which were constructed from outcrossed (to N2) single *daf-16(mu86)* and *eat-2(ad1116)* mutants for this study.

### Reproductive profiling

Eggs from N2 and *eat-2(ad1116)* worms were incubated at 20 °C until the L4 stage, and then transferred to fresh plates every day until they stopped producing progeny. Worms that crawled off the plates, bagged or ruptured were removed from the data set. All progeny plates were incubated at 20 °C for about 2 days following transfer of P<sub>0</sub> worms and then shifted to 4 °C. The number of worms that developed was determined at the end of the experiment.

### Laser ablation

Laser ablations were performed on newly hatched animals using a nitrogen-pumped dye laser, which produces a wavelength of 440 nm. Successful ablation was confirmed by examining the

reproductive systems of adult animals with a dissecting microscope. Worms were anesthetized with 1.5 M NaN<sub>3</sub> during ablation.

### Lifespan analysis

Lifespan analysis was conducted at 20 °C as described previously (Libina *et al.*, 2003). Animals were cultured on standard agar plates (5–8 animals per plate). The L4 molt was used as the starting point in the lifespans. Animals were examined and transferred to fresh plates every day for the first 4 days, and then examined every 2 days and transferred once a week. Animals were considered dead when they failed to react to gentle prodding of the head. Animals that crawled off the plates, ruptured or died as a 'bag of worms' were censored at the last day they were seen alive, and were incorporated in the data set until that date. STATA software was used for statistical analysis. In all cases, *P* values were calculated using the log-rank (Mantel–Cox) method.

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