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Body size, insulin/IGF signaling and aging in the nematode *Caenorhabditis elegans*

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Abstract

A number of recent studies of aging in *Drosophila*, mice and dogs have shown an association between reduced body size and increased lifespan. It is unclear (a) whether such an association is a general feature of animal species; and (b) whether the association reflects an effect of body size on aging, or pleiotropic effects of common determinants of growth and aging. To address these issues, we have studied the relationship between size and lifespan in the nematode *Caenorhabditis elegans*, and surveyed related findings in *Drosophila*. In *C. elegans*, we compared 12 wild isolates with varying body size and lifespan, but saw no correspondence between these traits. We also examined aging in giant and dwarf mutants, but observed only reduced lifespan in all cases. In a comparison of 15 long-lived *daf-2* insulin/IGF receptor mutants, we saw a positive correlation between body length and lifespan, and up to a 28% increase in *daf-2* mutant body volume. Thus, in *C. elegans*, insulin/IGF signaling may limit growth rather than promote it. Studies of *Drosophila* show no consistent correlation between body size and lifespan. These results indicate that the negative correlation between body size and lifespan seen in some mammals is not typical of invertebrates, but support the view that co-variation of size and longevity may occur via effects on insulin/IGF signaling.

Keywords: *Caenorhabditis elegans*; Aging; Body size; Insulin/IGF signaling; *Drosophila*

1. Introduction

Interventions such as dietary restriction (Masoro, 1995, 2000), gene mutation (Kenyon, 2001) and removal of the germline (Hsin and Kenyon, 1999) reduce the rate of aging in some model organisms, but how they do so remains unclear. Clues to the mechanisms of aging may be obtained by looking for correlated responses to such treatments. One such correlated trait identified in a number of recent studies is reduced body size. Lifespan increases have been observed in mouse lines selected for reductions in body size (Roberts, 1961; Eklund and Bradford, 1977; Miller et al., 2000). Moreover, a number of dwarf mouse mutants have recently been shown to have increased lifespan (Brown-Borg et al., 1996; Coschigano et al., 2000; Flurkey et al., 2001). An inverse relationship between body size and lifespan has also been reported between breeds of domestic dog (Michell, 1999). In *Drosophila melanogaster*, two long-lived dwarf mutants have also been identified (Clancy et al., 2001; Tatar et al., 2001). Such an association between size and longevity *within* species is the opposite of that seen *between* species (mammals in particular), where larger animal species tend to be longer lived (with notable exceptions) (Promislow, 1993).

Why might smaller individuals live longer? Either a smaller body size results in greater longevity, or growth and longevity share common determinants. One candidate for a determinant of body size and longevity is the growth hormone/insulin-like growth factor I (IGF-I) axis (or somatotropic axis) in mammals, and the homologous insulin/IGF signaling (IIS) pathway in invertebrates (reviewed in Bartke, 2000; Gems and Partridge, 2001).

We have examined the relationship between body size, IIS and lifespan in the nematode *Caenorhabditis elegans*, and reviewed related findings in *Drosophila*. Our aims were (1) to establish whether reduced body size is generally associated with increased lifespan, and (2) whether IIS promotes increased body size in *C. elegans* as it does in mammals and in *Drosophila* (Weinkove and Leevers, 2000). To this end, we measured body size and lifespan in (a) 12 wild isolates of *C. elegans* which exhibit differences

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in body size and lifespan; (b) giant and dwarf *C. elegans* mutants; and (c) 15 *daf-2* insulin/IGF receptor mutants exhibiting varying degrees of extended lifespan. Our results show that a) in *C. elegans*, as in *Drosophila*, there is no clear general effect of body size on lifespan; and b), as in *Drosophila*, IIS has a role in the determination of adult body size. Surprisingly, though, in *C. elegans* IIS retards rather than promotes growth.

2. Materials and methods

2.1. Stocks and culture methods

The *C. elegans* wild isolates studied (Table 1) are described by Hodgkin and Doniach (1997). The body size mutants used were as follows. Giants: CB185 *lon-1 (e185) III*, CB678 *lon-2(e678) X* and MT3847 *lon-2(n1630) X*. Dwarves: CB30 *sma-1(e30) V*, CB491 *sma-3 (e491) III*, DR1369 *sma-4(e729) III*, CB88 *dpy-7(e88) X*, CB128 *dpy-10(e128) II* and CB184 *dpy-13(e184) IV*. The *daf-2* alleles employed were *e979*, *e1365*, *e1368*, *e1369*, *e1370*, *e1371*, *e1391*, *m41*, *m120*, *m212*, *m577*, *m579*, *m596*, *sa193* and *sa223*. For further description of these alleles see Gems et al. (1998). The N2 wild type used was the *Caenorhabditis* Genetics Center male stock (CGCM). All *daf-2* mutant strains were back-crossed at least 3 times to N2 CGCM, to remove second site mutations and to reduce possible confounding effects due to variation in the genetic background of the N2 wild type (Gems and Riddle, 2000).

Nematodes were maintained on 60 mm Petri dishes on NGM agar, with *E. coli* OP50 as a food source (Brenner, 1974), except in the study of wild isolates, where worms were maintained in liquid culture. Liquid culture medium consisted of *E. coli* in S medium (Sulston and Hodgkin, 1988). Worms were kept singly in 96-well microtitre plates (U wells) under 50 μl of culture medium.

Table 1
Body lengths and lifespan in 12 wild *C. elegans* isolates

Strain	Median lifespan (days)[a]	Maximum lifespan (days)	N^b	Peak body length (mm) ± SE	N
N2	19.0 (17.0, 22.0)	25.5	45 (2)	1.44 ± 0.02	10
AB1	28.0 (25.5, 29.0)	33	19 (1)	1.36 ± 0.02	10
AB2	25.0 (24.0, 27.0)	31	26 (1)	1.33 ± 0.03	10
CB4555	24.0 (22.0, 27.0)	30.0	31 (1)	1.45 ± 0.02	10
CB4853	17.5 (15.5, 21.5)	32.5	48 (2)	1.27 ± 0.04	9
CB4854	28.0 (25.0, 31.0)	27.0	19 (1)	1.23 ± 0.01	10
CB4856	22.5 (19.75, 25.0)	30.5	48 (2)	1.32 ± 0.02	10
CB4857	25.0 (23.0, 26.0)	31.0	28 (1)	1.16 ± 0.02	10
CB4858	21.5 (20.0, 23.0)	28.0	48 (2)	1.32 ± 0.03	10
RC301	21.0 (19.0, 23.25)	28.0	49 (2)	1.48 ± 0.03	10
TR389	23.0 (20.0, 26.0)	29.0	16 (1)	1.33 ± 0.02	10
TR403	26.5 (22.0, 29.5)	34.5	42 (2)	1.41 ± 0.03	10

Measurements are of hermaphrodites maintained at 22.5 °C.
[a] Median lifespan (lower, upper 95% confidence limit).
[b] Number of senescent deaths scored (number of trials).

2.2. Body size measurements

To record body lengths and widths, worms were placed on agar plates and killed by heating the nearby agar with a hot scalpel. To facilitate measurement, animals were laid out straight using a platinum wire. Measurements were made using a dissecting microscope, with an eyepiece graticule calibrated with a stage micrometer. To calculate body volume, worms were treated as cylinders ($v = \pi r^2 l$). After moulting to the adult, *C. elegans* hermaphrodites continue to grow for several days (Byerly et al., 1976). In the case of wild isolates, giant and dwarf mutants, and selected *daf-2* mutants, body size was measured during the first 3 days of adulthood and maximum size recorded. In the comparison of 15 *daf-2* alleles, one day-old adults were measured.

2.3. Lifespan measurements

These were performed as previously described (Gems et al., 1998), except that in some cases monoxenic liquid culture was used (see above). Lifespan and body length measurements were made at the same time and temperature within each trial, apart from the IIS mutants, where growth curves for selected strains were performed after the lifespan measurements (see Section 3).

2.4. Statistical methods

Median rather than mean lifespans were calculated since typical age-specific death frequencies do not show a normal distribution. For wild isolate and body size mutant trials, survival data were re-sampled 50,000 times using R software (www.r-project.org) to obtain a mean of median values across replicates, and the 95% confidence limits around them. Survival analyses were performed using the Kaplan-Meier method. Differences in mean body size within trials were analysed using analysis of variance, and statistical comparisons for pairs of means performed using a Tukey-Kramer honestly significant difference test. Correlation analyses were carried out for lifespan against body length, width or volume. Statistical software used was JMP v.3 (SAS Institute Inc.).

3. Results

3.1. Body size and lifespan in wild isolates

To ask whether body size per se determines lifespan we examined the relationship between naturally occurring variation in body size and longevity in 12 *C. elegans* wild isolates from around the world (Hodgkin and Doniach,

1997) (Table 1). Although we saw significant variation in body length ($P < 0.001$) and lifespan ($P < 0.001$), there was no significant correlation between lifespan and body length (Fig. 1(a) and (b)).

3.2. Aging in giant and dwarf mutants

We also asked whether mutations that increase or decrease body size result in correlated effects on aging. A major regulatory pathway for body size in *C. elegans* involves the TGFβ-like ligand DBL-1, signaling via the DAF-4 and SMA-6 TGFβ receptors and the SMA-2, SMA-3 and SMA-4 SMAD proteins (reviewed in Savage-Dunn (2001). Mutations in these components result in reduced body size, apparently due to a reduction in cell size rather than in cell number (Suzuki et al., 1999; Flemming et al., 2000). Mutations in *lon* (*long*) genes result in giant adults that exhibit an increased body length. LON-1 is a protein of unknown biochemical function whose expression is regulated by DBL-1 (Morita et al., 2002). We examined mutants affected in *sma-1*, *sma-3*, *lon-1* and *lon-2*. Another class of genes affecting body size and morphology are the *dpy* (dumpy) genes. Dpy mutants are short and fat, and in many cases the genes involved encode cuticle collagens, including those examined here: *dpy-7*, *dpy-10* and *dpy-13* (von Mende et al., 1988; Johnstone et al., 1992; Levy et al., 1993).

If there were a general correlation between small size and longevity, then we would expect giant Lon mutants to be short lived, and dwarf Sma and Dpy mutants to be long lived. All strains had body lengths significantly different from N2. In both the large and the small mutants the median lifespan was significantly reduced relative to N2. Maximum lifespan was also decreased in all mutants but *lon-1(e678)*, and *sma-3(e491)* where it was increased. (Table 2). However, these mutants displayed a range of deleterious effects to various extents, including high incidences of death due to internal hatching of eggs, and extrusion of the gonad through the vulva. Moreover, in many cases, in mid-life and beyond animals appeared pale and/or shrivelled relative to wild type. It is therefore possible that the short lifespans of these strains is due to deleterious pleiotropic effects of the mutations they bear, masking any potential effect of body size on lifespan.

3.3. Body size and lifespan in IIS mutants

In *C. elegans*, mutations affecting a number of genes in an insulin/IGF signaling (IIS) pathway increase lifespan.

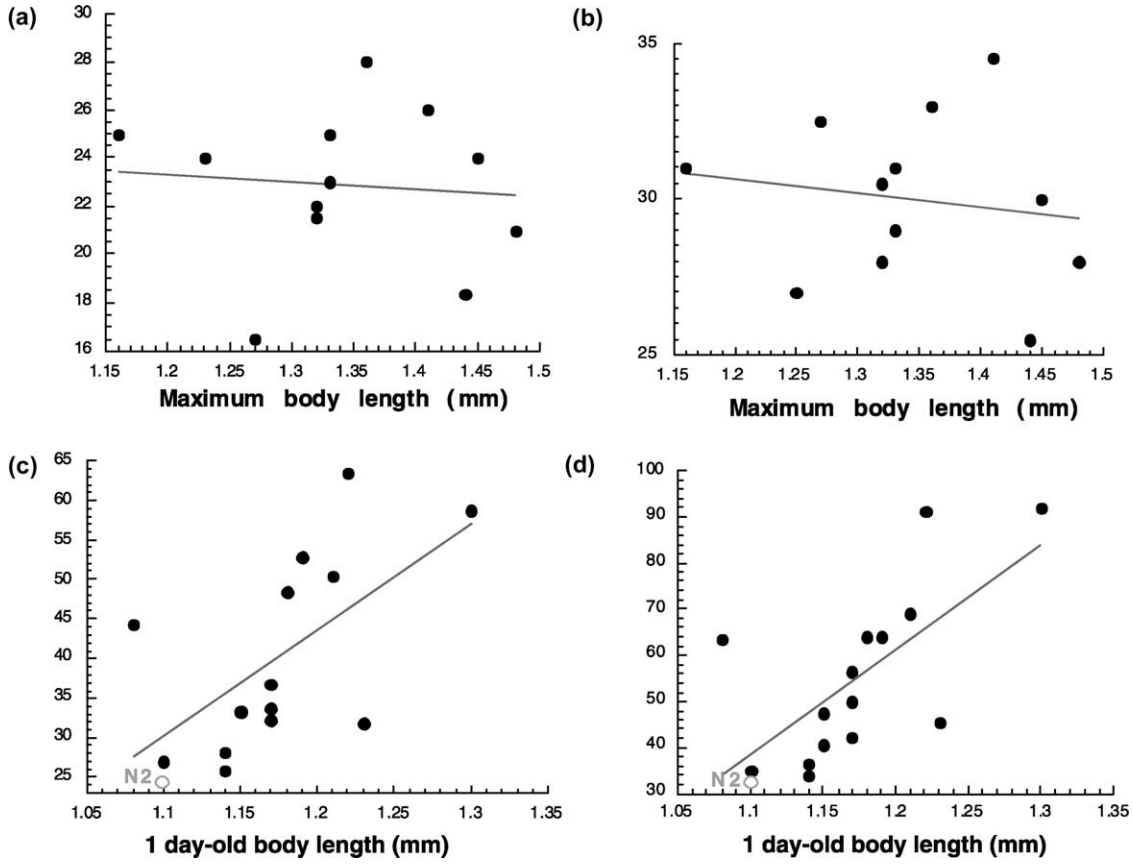

Fig. 1. (a), (b) Comparison of body size and lifespan in hermaphrodites of 12 *C. elegans* wild isolate strains. (a) Median lifespan and body length (correlation: −0.093 $P = 0.773$). (b) Maximum lifespan and body length (correlation: −0.154 $P = 0.633$). (c), (d) Comparison of body size and lifespan in 15 *daf-2* mutants, 15 °C. (c) Median lifespan and body length (correlation: 0.599, $P = 0.018$). (d) Maximum lifespan and body length (correlation: 0.651, $P = 0.0086$).

Table 2
Body size and lifespan in body size mutants

Mutation	Encoded protein	Median lifespan (days)[a]	Maximum lifespan (days)	N^b	Mean peak body length (mm) ± SE	Mean peak body width (mm) ± SE	N	Mean peak body volume (mm^3)
+		19.5 (17.7, 20.7)	27.3	166 (3)	1.34 ± 0.02	0.078 ± 0.001	10	0.0064
lon-1(e185)	Novel protein	13.0 (11.5, 14.5)	21.0	68 (2)	1.84 ± 0.02	0.086 ± 0.002	10	0.0107
lon-1(e678)		11.0 (9.0, 13.0)	27.0	59 (1)	1.55 ± 0.04	0.069 ± 0.002	10	0.0058
lon-2(n1630)	Novel protein	10.5 (9.0, 11.5)	21.0	98 (2)	1.59 ± 0.01	0.063 ± 0.001	10	0.0050
lma-1 (e30)	Spectrin	11.5 (10.5, 12.5)	19.5	87 (2)	1.14 ± 0.04	0.086 ± 0.002	10	0.0066
sma-3(e491)	SMAD	14.0 (12.0, 20.0)	34.0	19 (1)	0.70 ± 0.01	0.061 ± 0.002	10	0.0021
dpy-10(e128)	Collagen	13.5 (12.5, 13.5)	18.5	153 (2)	0.80 ± 0.02	0.083 ± 0.002	8	0.0043
dpy-7(e88)	Collagen	10.5(10.0, 11.0)	16.5	155 (2)	0.88 ± 0.02	0.085 ± 0.002	10	0.0050
dpy-13(e184)	Collagen	11.5 (10.5, 13.5)	9.5	206 (2)	0.77 ± 0.01	0.096 ± 0.002	10	0.0056

Measurements are of hermaphrodites maintained at 22.5 °C.
[a] Mean of median lifespans (lower, upper 95% confidence limit).
[b] Number of senescent deaths scored (number of trials).

This pathway is regulated via *daf-2*, which encodes a receptor tyrosine kinase similar to the mammalian insulin and IGF-1 receptors (Kenyon et al., 1993; Kimura et al., 1997). In contrast to the case of long-lived mice with mutations affecting the somatotropic axis (Brown-Borg et al., 1996; Coschigano et al., 2000; Flurkey et al., 2001), and IIS mutant *Drosophila* (Clancy et al., 2001; Tatar et al., 2001), no alteration of adult body size has been reported in IIS mutant *C. elegans*. However, IIS mutations can affect larval body size by causing constitutive formation of the thin-bodied, diapausal dauer larva form (Riddle et al., 1997).

Table 3
Body size of *daf-2* mutants

Allele	Mean 1-day adult body length (mm)	Mean 1-day adult body width (mm)	Mean 1-day adult body vol. (mm^3)
+	1.10 ± 0.018	0.068 ± 0.004	0.0040
daf-2(e1365)	1.14 ± 0.024	0.067 ± 0.005	0.0040
daf-2(m577)	1.14 ± 0.036	0.071 ± 0.005	0.0045
daf-2(sa193)	1.15 ± 0.056	0.068 ± 0.004	0.0042
daf-2(e1371)	1.23 ± 0.041	0.072 ± 0.004	0.0050
daf-2(e1368)	1.15 ± 0.046	0.063 ± 0.006	0.0036
daf-2(sa223)[a]	1.30 ± 0.062	0.063 ± 0.007	0.0041
daf-2(m120)	1.17 ± 0.038	0.071 ± 0.003	0.0046
daf-2(e1370)	1.17 ± 0.026	0.064 ± 0.006	0.0038
daf-2(m579)	1.08 ± 0.029	0.065 ± 0.005	0.0036
daf-2(m596)	1.17 ± 0.038	0.062 ± 0.006	0.0035
daf-2(m41)	1.10 ± 0.044	0.065 ± 0.005	0.0037
daf-2(e1391)	1.22 ± 0.036	0.062 ± 0.006	0.0037
daf-2(m212)	1.18 ± 0.047	0.067 ± 0.006	0.0042
daf-2(e979)	1.21 ± 0.083	0.071 ± 0.006	0.0048
daf-2(e1369)	1.19 ± 0.024	0.063 ± 0.007	0.0037

Measurements are of 1 day old adult hermaphrodites (15 °C). Values show ± SE. Body dimension measurements were taken at same time as lifespan determination (Gems et al., 1998). Twelve animals were measured for each allele. Alleles are in order of increasing severity of the dauer constitutive phenotype.
[a] Progeny of *daf-2(sa223)/qC1* [*dpy-19(e1259ts) glp-1(q339)*] hermaphrodites.

Hermaphrodite lifespan and body length was measured in 15 mutants bearing reduction-of-function alleles of *daf-2*. The effect of many of these mutations on lifespan is temperature sensitive, and the study was carried out at 15 °C, where median lifespan increases relative to wild type ranged from 8 to 120% (Gems et al., 1998). We observed a significant positive correlation between *daf-2* mutant body length and both median and maximum lifespan (Table 3) (Fig. 1(c) and (d)). However, no significant correlations were found between body width and median or maximum lifespan (correlation: -0.425, $P = 0.114$ and -0.412, $P = 0.127$, respectively), or between mean body volume and either median or maximum lifespan (correlation: -0.198, $P = 0.480$, and -0.141, $P = 0.617$, respectively). Body lengths in all *daf-2* mutants were significantly greater than wild type, except for *e1365*, *e1368*, *m41*, *m577*, *m579* and *sa193* (Tukey–Kramer test).

C. elegans adults continue to grow for the first few days of adulthood. As body size was measured in 1-day-old adults, it is possible that any potential positive correlation between body length and lifespan resulted from an accelerated growth rate in *daf-2* mutants, rather than increased final body size. We therefore measured body size in *daf-2(e979)*, *daf-2(e1370)* and *daf-2(m41)*, during the first 3 days of adulthood at 15 °C. At this temperature only the *e979* and *e1370* alleles cause marked increases in lifespan. *m41*, *e979* and *e1370* all resulted in a significant increase in adult body length over N2, and *e1370* in a significant increase of 28% in adult body volume (Fig. 2). The fact that *daf-2(e979)* was not larger in volume than wild type despite being longer was due to its reduced body width: the width of 3-day old *e979* adults was 6.0% of their length, compared to 6.6% in N2 adults of the same age. Taken together, these results indicate that wild-type insulin/IGF signaling acts to reduce adult body size.

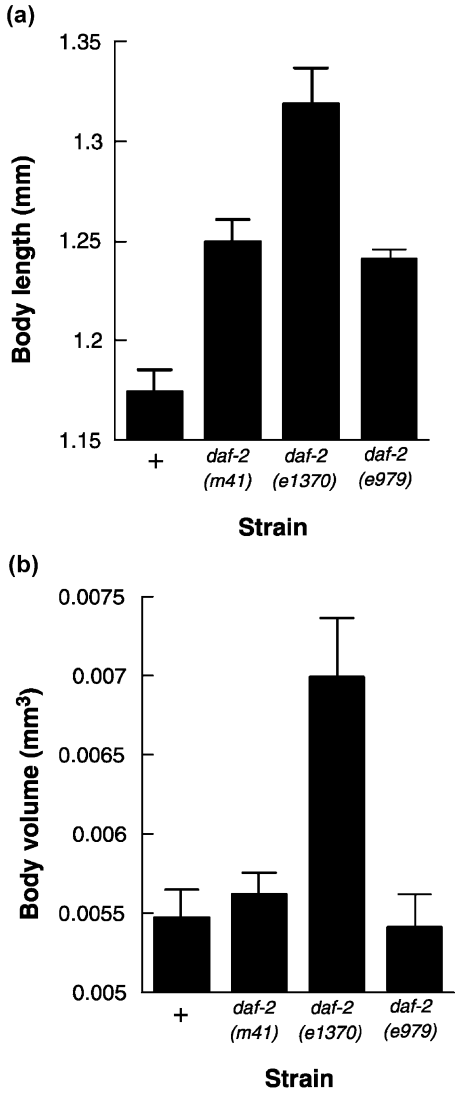

Fig. 2. Effects of three *daf-2* alleles on body size. (a) Body length. (b) Body volume. Error bars represent standard error.

4. Discussion

4.1. Body size and lifespan in C. elegans

We have examined the relationship between body size and lifespan in a range of *C. elegans* strains. Comparing variation in naturally occurring *C. elegans* isolates we found that there is no effect of body size on longevity. Moreover, dwarf Sma and Dpy mutants as well as giant Lon mutants were short lived. Previously, extended lifespan was not seen in a *daf-4* dwarf mutant (Larsen et al., 1995). However, it is possible that the effects of retarded aging on lifespan in Dpy and Sma mutants were masked by deleterious pleiotropic effects. The increase in *sma-3* mutant maximum lifespan is consistent with this.

We also examined body size in 15 *daf-2* IIS mutants, exhibiting a range of severities of the extended lifespan trait. There was a significant positive correlation between body length and lifespan, and of three alleles tested further, one, *daf-2(e1370)*, resulted in increased body volume. Thus, in *C. elegans*, wild type IIS can act to limit body size. However, a more severe loss-of-function allele, *e979*, resulted in increased body length but reduced body diameter. This suggests a possible non-linear effect of IIS levels on body size. Thus, moderate reductions in IIS (as in *e1370*) may result in proportional increases in both length and diameter, while severe reductions in IIS (as in *e979*) may result in increases in body length, but reduction in body width. Possibly *e979* mutants are thin as the result of partial expression of dauer larva-like morphology. The mechanism of action of IIS on body size remains to be investigated. DBL-1 TGFβ signaling promotes growth by stimulating endoreduplication of hypodermal nuclei (Flemming et al., 2000). Possibly there is cross talk between IIS and DBL-1 TGFβ signaling, as there is between IIS and DAF-7 TGFβ signaling in the regulation of dauer larva formation (Ogg et al., 1997).

The effect of IIS on body size in *C. elegans* described here differs from that in other organisms. For example, in *Drosophila*, mutation of *Inr*, which encodes an insulin/IGF receptor, results in retarded development, and dwarf adults (Chen et al., 1996). In mammals, disruption of somatotropic signaling, where circulating IGF-1 levels are reduced, leads to reduced body size (Sara and Hall, 1990; Jones and Clemmons, 1995). Why might the role of IIS in the regulation of body size in *C. elegans* be reversed?

One clue is that overexpression of INS-1, the *C. elegans* insulin-like peptide that best resembles mammalian insulin, appears to antagonise signaling from the DAF-2 receptor (Pierce et al., 2001). From this it was suggested that the function of the DAF-2 receptor might be reversed relative to the mammalian insulin receptor, such that ligand binding blocks receptor activation rather than potentiating it. Thus, perhaps INS-1 promotes growth by inhibiting DAF-2 action. A further possibility is that the action of IIS on body size is germline dependent. Like mutation of *daf-2*, removal of the germline by ablation of germline precursor cells using a laser microbeam results in increased body volume (up to 46%) (Patel et al., 2002), and increased longevity (Hsin and Kenyon, 1999). However, while the effects of both *daf-2* and germline signaling on lifespan require the activity of the forkhead transcription factor DAF-16 (Kenyon et al., 1993; Hsin and Kenyon, 1999), this is not true of the effect of the germline on body size (Patel et al., 2002).

Overall, these results are consistent with the view that correlations between body size and lifespan occur as a consequence of the effect of insulin/IGF signaling on these two traits. Thus, the lack of any correlation between body size and longevity seen in the study of *C. elegans* wild isolate strains may reflect the fact that neither body size or lifespan variation in these strains is the result of variation in IIS levels.

4.2. Body size and lifespan in Drosophila

The absence of a general correspondence between small body size and increased lifespan in *C. elegans* is consistent with results in *Drosophila*. Several studies have noted a positive phenotypic correlation between *Drosophila* body size and lifespan (Partridge and Farquhar, 1981, 1983; Partridge et al., 1986), while genetic correlations between these traits have been either positive (Tantawy and Rakha, 1964; Tantawy and el-Helw, 1966; Partridge and Fowler, 1992), or have not been detected (Zwaan et al., 1995).

Several studies have examined the effect of varying body size on longevity, or vice versa. Early comparisons of long- and short-lived lines resulting from selection for late and early fecundity, respectively, saw no correlated changes in body size (Rose, 1984; Luckinbill et al., 1988). In these studies body weight was measured at least two days after eclosion. In a later study involving two sets of lines selected for early and late fecundity, body size (thorax length, and wet weight) was measured shortly after eclosion (Partridge and Fowler, 1992). Here, both sexes of longer-lived flies were larger in one set of lines (e.g. a 10% increase in weight in long-lived females). In the other set of selected lines, only males were significantly larger. Lifespan was measured in virgin flies. The long-lived flies also grew more slowly, potentially reflecting a greater resource investment into determinants of somatic durability. However, subsequent selection experiments of this type have not resulted in correlated changes in body size (Partridge et al., 1999).

Longevity has also been examined in *Drosophila* lines with increased or reduced body size. For example, in female *D. pseudoobscura*, larger females are longer-lived, and also lay more eggs than smaller females (Tantawy and Vetukhiv, 1960; Tantawy, 1961). It has also been shown that *Drosophila* evolves a larger body size in response to colder environments. These large lines are longer-lived than small and control lines, but only when survival analyses are performed at the cold temperatures at which the large lines were originally selected (McCabe and Partridge, 1997). The larger lines also display increased daily progeny production, again, only at the cold temperature. Thus, selecting for larger size in *Drosophila* by raising lines at low temperature increases female performance in terms of survival and lifetime reproduction at low temperatures.

Why larger flies should be longer-lived is still poorly understood. The authors of the temperature selection experiments point out that it is unlikely to be increased desiccation resistance, since the increased lifespan and fecundity are only seen at lower temperatures where desiccation is less of a problem. They suggest that larger flies have an improved ability to acquire, conserve and use resources.

Bigger flies that are heavier because they contain more fat are starvation resistant, and this may also affect longevity (Zwaan et al., 1991). One study of *D. melanogaster* examined longevity and fertility in large and small fly lines, raised on rich and poor food (Hillesheim and Stearns, 1992). In the case of virgin flies, no difference in longevity was seen on rich food, while on poor food, large males lived longer, but larger females were shorter lived. In the case of mated females, lifespan in the larger flies was reduced by 34% under both food regimens. However, in *Drosophila*, egg production greatly reduces lifespan (Maynard Smith, 1958; Lamb, 1964; Partridge et al., 1987), and larger females, with larger ovaries, laid more eggs early in life, and may have died sooner for this reason (Hillesheim and Stearns, 1992).

Studies involving specific mutations that increase lifespan provide little evidence for a determining effect of body size on lifespan in *Drosophila*. Mutations affecting *Inr* (equivalent to *C. elegans daf-2*) and *chico*, which encodes an insulin receptor substrate protein, can result in long-lived dwarf flies (Clancy et al., 2001; Tatar et al., 2001). However, *chico* ^{1}/ + heterozygote females are not dwarf yet show up to a 36% increase in mean lifespan. Thus, the body size reduction is not required for the increase in lifespan (Clancy et al., 2001).

In summary, these results show that in *Drosophila* there is no general negative correlation between body size and longevity. Selection for *increased* size can result in increased lifespan in *Drosophila* under specific conditions; however, selection for increased longevity does not result in increased body size. Body size mutants studied to date have shown a separation of the body size and longevity phenotypes.

5. Conclusions

The findings described here do not support the existence of a general relationship between body size and longevity in animal species, and show that in invertebrates such as *C. elegans* and *Drosophila*, correlated effects on body size and aging may only occur via IIS-associated pleiotropy. It has been suggested that reduced body size may lead to increased lifespan in mammals, for example by conserving cell proliferative capacity, or reducing the number of sites at which carcinogenesis can occur (reviewed in Bartke (2000)). In this context, it may be significant that in both *C. elegans* and *Drosophila*, aging occurs in the absence of somatic cell proliferation. Thus, in the case of mammals, it remains equally possible that body size does directly affect aging, or that it is a pleiotropic correlate of IIS activity that also affects aging, or both.

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