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Extension of Life-Span by Loss of CHICO, a *Drosophila* Insulin Receptor Substrate Protein

David J. Clancy,¹ David Gems,^{1*} Lawrence G. Harshman,² Sean Oldham,³ Hugo Stocker,³ Ernst Hafen,³ Sally J. Leever,^{4,5} Linda Partridge¹

The *Drosophila melanogaster* gene *chico* encodes an insulin receptor substrate that functions in an insulin/insulin-like growth factor (IGF) signaling pathway. In the nematode *Caenorhabditis elegans*, insulin/IGF signaling regulates adult longevity. We found that mutation of *chico* extends fruit fly median life-span by up to 48% in homozygotes and 36% in heterozygotes. Extension of life-span was not a result of impaired oogenesis in *chico* females, nor was it consistently correlated with increased stress resistance. The dwarf phenotype of *chico* homozygotes was also unnecessary for extension of life-span. The role of insulin/IGF signaling in regulating animal aging is therefore evolutionarily conserved.

Mutations that extend life-span illuminate the molecular mechanisms underlying aging and longevity. In *Caenorhabditis elegans*, mutation of the genes *daf-2* and *age-1*, which encode components of an insulin/IGF signaling (IIS) pathway, enhances stress resistance and increases adult life-span by up to 200% (1). This pathway also controls the formation of dauer larvae, which are developmentally arrested, stress resistant, long-lived, and produced in response to crowding and reduced food (2). Potentially, insulin/IGF mutants could be long-lived by virtue of expression of dauer longevity in the adult, in which case the extension of adult life-span by these mutations could be a peculiarity of *C. elegans*. We examined whether the role of IIS in aging has been evolutionarily conserved and therefore might also operate in humans.

In the fruit fly *Drosophila melanogaster*, the insulin/IGF receptor INR, the insulin re-

ceptor substrate CHICO, the phosphatidylinositol 3-kinase (PI3K) Dp110/p60, and the PI3K target protein kinase B (PKB, also known as DAK1) form a signaling pathway that regulates growth and size (3–7). We examined the effects on aging of hypomorphic mutations in *Inr* (equivalent to *daf-2*) and *PKB*, and null mutations in *chico* and the catalytic (*Dp110*, equivalent to *age-1*) and adapter (*p60*) PI3K subunits (8). All mutants were tested as heterozygotes. We also tested *chico*¹ (3) and *PKB*³ (9) homozygotes and *Inr*^{GC25}/*Inr*^{E19} transheterozygotes, which form viable dwarf adults. The remaining mutations were homozygous lethal.

Most mutants tested had normal or significantly decreased life-span (10). For example, *PKB*³ homozygotes and *Inr*^{GC25}/*Inr*^{E19} flies were short-lived. By contrast, *chico*¹ extended life-span (Fig. 1). Homozygous *chico*¹ females exhibited an increase of median and maximum life-span of up to 48 and 41%, respectively. *chico*¹ heterozygotes also exhibited increases in median life-span of up to 36 and 13% in females and males, respectively. Homozygous males, however, were slightly short-lived.

To confirm that *chico*¹ itself extended life-span, we tested the effect on life-span of pCSR4-*chico*, a P element containing *chico*(+). This construct fully rescues the dwarf phenotype of *chico*¹ (3). *chico*¹ was crossed to two stocks containing independent pCSR4-*chico* in-

sertions (pCSR4-*chico* 1.1 and 2.3). As a control, *chico*¹ was also crossed to the base stock in which the P element insertions were made. Progeny with either two copies (*chico*¹ heterozygotes with one *chico* transgene) or one copy (*chico*¹ heterozygotes alone) of *chico*(+) were compared (11). The rescue construct significantly reduced life-span relative to the +/*chico*¹ control. The median female life-span of 54 days in +/*chico*¹ was reduced to 46 days in +/*chico*¹, +/*pCSR4-chico* 1.1 flies and 52 days in +/*chico*¹, +/*pCSR4-chico* 2.3 flies ($P = 0.0002$ and 0.0243 , respectively). Similar effects were observed in males (10). Thus, mutation of *chico* itself increases life-span. Because *chico*¹ is a null allele, its effect on life-span indicates that the wild-type *chico* gene acts to accelerate aging.

Of the mutations tested, only *chico*¹ increased life-span. This may be because the effect of reduced IIS on life-span depends on the degree to which signaling is reduced. Unlike the other null mutations in IIS genes tested, *chico*¹ is not homozygous lethal, presumably because the INR receptor can signal to PI3K directly, as well as indirectly via CHICO (3). Thus, *chico*¹ mutants may be long-lived because of the relatively mild reduction in pathway activity that they bring about. Notably, severe IIS mutations in *C. elegans* can cause premature mortality in some adults, although the maximum life-span of populations is invariably increased (1). This is probably why *Inr*^{GC25}/*Inr*^{E19} flies are short-lived: Demographic analysis indicates that a reduction in the age-specific mortality rate acceleration occurs, whose effect on survival is masked by an elevated rate of age-independent mortality (12). Furthermore, a different heteroallelic *Drosophila Inr* mutant to that tested here exhibits an 85% increase in female life-span (13). By contrast, in short-lived *PKB*³ populations, no reduction in mortality rate acceleration is seen (12). This raises the possibility that a second pathway downstream of *chico* might regulate aging in *Drosophila*. Interestingly, CHICO contains potential binding sites for the Drk/Grb2 docking protein, consistent with signaling via Ras/mitogen-activated protein kinase.

We next investigated whether extension of life-span by *chico*¹ was mediated by processes previously shown to affect aging. A reduction in fecundity extends life-span in *Drosophila* females (14, 15); *chico*¹ heterozygous females have reduced fecundity, and the homozygotes are almost sterile (3, 12). To test whether the

¹Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK. ²School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588, USA. ³Zoologisches Institut, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland. ⁴Ludwig Institute for Cancer Research, 91 Riding House Street, London W1W 7BS, UK. ⁵Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.

*To whom correspondence should be addressed. E-mail: david.gems@ucl.ac.uk

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increased life-span of *chico*¹ females was due to reduced fecundity, we examined the interactions between *chico*¹ and the dominant, female-sterile mutant *ovo*^{D1}. This mutation blocks oogenesis at stage 4, before vitellogenesis commences (16), and extends female life-span (15). If *chico*¹ extends female life-span by the exact same mechanism as *ovo*^{D1}, then the three sterile genotypes (*chico*¹, *+/ovo*^{D1}, and *+/ovo*^{D1} *+/chico*¹) should have similar life-spans and live longer than the subfertile *chico*¹ heterozygotes. In fact, the *chico* homozygotes lived significantly longer than all other genotypes (Fig. 2). In addition, the partially fertile *chico*¹ heterozygotes lived as long as the sterile flies that were heterozygous for both *ovo*^{D1} and *chico*¹ and lived significantly longer than the sterile *ovo*^{D1} heterozygotes. The effect of *chico*¹ on female life-span is therefore not a consequence of the same mechanism of reduced fecundity as is produced by *ovo*^{D1}. If *chico*¹ does extend female life-span through an effect on reproductive effort, the interaction must occur through some process other than oogenesis (for instance yolk protein synthesis) or before stage 4 in oogenesis because *ovo*^{D1} flies are blocked at that stage.

In *C. elegans*, long-lived IIS mutants are stress resistant and overexpress the antioxidant enzyme superoxide dismutase (SOD) (1). We examined the resistance of *chico*¹ flies to three stressors, but only one showed any correspondence with life-span (17) (Fig. 3). No resistance to heat stress (37°C) was seen (Fig. 3A). Slight resistance to oxidative stress (methyl viologen) was observed in *chico*¹ heterozygotes but not in homozygotes (Fig. 3B). However, some correspondence between starvation resistance and life-span was seen (Fig. 3C). Increased SOD levels were seen in *chico*¹ homozygotes but not in heterozygotes (17) (Fig. 4). Thus, modulation by IIS of longevity, and of SOD levels, has evidently been conserved between *C. elegans* and *Drosophila*. Furthermore, effects of this pathway on fertility are widespread (1, 3, 18). However, effects on stress resistance are not well conserved, nor do any of the above associated affects appear to be causal in extending life-span.

Our results raise the question of whether IIS regulates aging in mammals. Whereas both the *C. elegans* and *Drosophila* genomes contain a single insulin/IGF receptor, mammals possess distinct receptors for insulin and IGF-I, plus a third insulin receptor–like receptor of unknown function. Potentially, any or all of these receptors may play a role in regulating aging. Caloric restriction (CR), which increases life-span in rodents (19), and possibly primates (20), reduces circulating levels of both insulin and IGF-I (21, 22). In the case of IGF-I, there is further evidence for a role in the control of longevity (22, 23). Growth hormone (GH) acts via IGF-I to control mammalian body size, and circulating

IGF-I levels correlate with body size in mice, dogs, and humans (23, 24). Furthermore, CR can reduce body size (22). In mice and dogs (and possibly humans), there is a marked negative correlation between body size and longevity (24–26). In addition, long-lived Ames hypopituitary mouse dwarves are deficient in GH and other pituitary hormones and have reduced circulating IGF-I (27). Mutation of the human equivalent of the Ames dwarf gene, *Prop-1*, also causes dwarfism and, possibly, delayed aging (28). The Laron dwarf mouse, which has no GH receptor and very low IGF-I levels, exhibits life-span increases of up to 55% (29).

Whereas the effects of *chico*¹ on development that result in reduced body size are recessive, its effects on life-span are semidominant. This may reflect the noncatalytic and dosage-dependent nature of the function of CHICO as a docking protein. It has been proposed that reduced body size per se increases life-span in mammals (23). Alternatively, the same genes may independently regulate growth during the preadult period and regulate survival during the adult period. Our data support the latter interpretation because *chico*¹ heterozygotes are long-lived, yet of normal size. Likewise, the effect of CR on aging may be observed in the absence of its effects on body size.

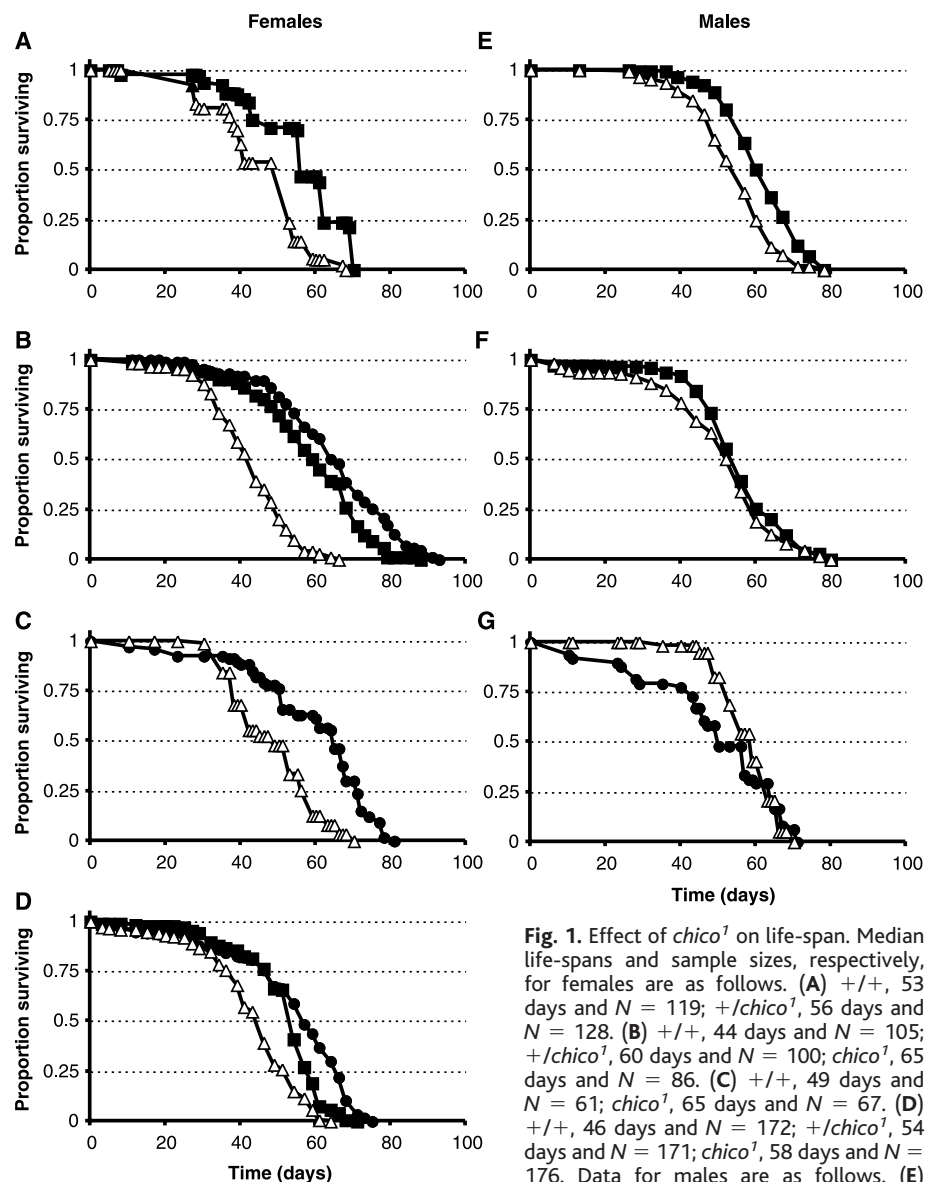


Fig. 1. Effect of *chico*¹ on life-span. Median life-spans and sample sizes, respectively, for females are as follows. (A) *+/+*, 53 days and *N* = 119; *+/chico*¹, 56 days and *N* = 128. (B) *+/+*, 44 days and *N* = 105; *+/chico*¹, 60 days and *N* = 100; *chico*¹, 65 days and *N* = 86. (C) *+/+*, 49 days and *N* = 61; *chico*¹, 65 days and *N* = 67. (D) *+/+*, 46 days and *N* = 172; *+/chico*¹, 54 days and *N* = 171; *chico*¹, 58 days and *N* = 176. Data for males are as follows. (E) *+/+*, 54 days and *N* = 136; *+/chico*¹, 56 days and *N* = 135. (F) *+/+*, 57 days and *N* = 104; *+/chico*¹, 62 days and *N* = 140. (G) *+/+*, 59 days and *N* = 54; *chico*¹, 50 days and *N* = 48. Statistical comparisons (log rank test) are as follows for females: *+/chico*¹ versus *+/+*, *P* < 0.0001 (A, B, and D); *chico*¹ versus *+/chico*¹, *P* = 0.0008 and < 0.0001 (B and D, respectively); and *chico*¹ versus *+/+*, *P* < 0.0001 (B, C, and D). Statistical comparisons (log rank test) are as follows for males: *+/chico*¹ versus *+/+*, *P* < 0.0001 and < 0.0866 (not significant) (E and F, respectively). In all panels, circles represent *chico*¹/*chico*¹; squares represent *+/chico*¹, and triangles represent *+/+*.

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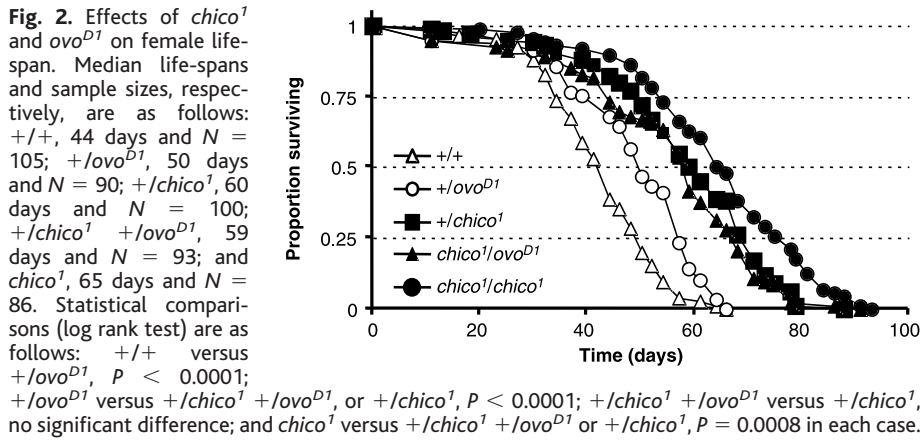


Fig. 2. Effects of *chico*¹ and *ovo*^{D1} on female life-span. Median life-spans and sample sizes, respectively, are as follows: +/+, 44 days and N = 105; +/*ovo*^{D1}, 50 days and N = 90; +/*chico*¹, 60 days and N = 100; +/*chico*¹ +/*ovo*^{D1}, 59 days and N = 93; and *chico*¹, 65 days and N = 86. Statistical comparisons (log rank test) are as follows: +/+ versus +/*ovo*^{D1}, *P* < 0.0001; +/*ovo*^{D1} versus +/*chico*¹ +/*ovo*^{D1}, or +/*chico*¹, *P* < 0.0001; +/*chico*¹ +/*ovo*^{D1} versus +/*chico*¹, no significant difference; and *chico*¹ versus +/*chico*¹ +/*ovo*^{D1} or +/*chico*¹, *P* = 0.0008 in each case.

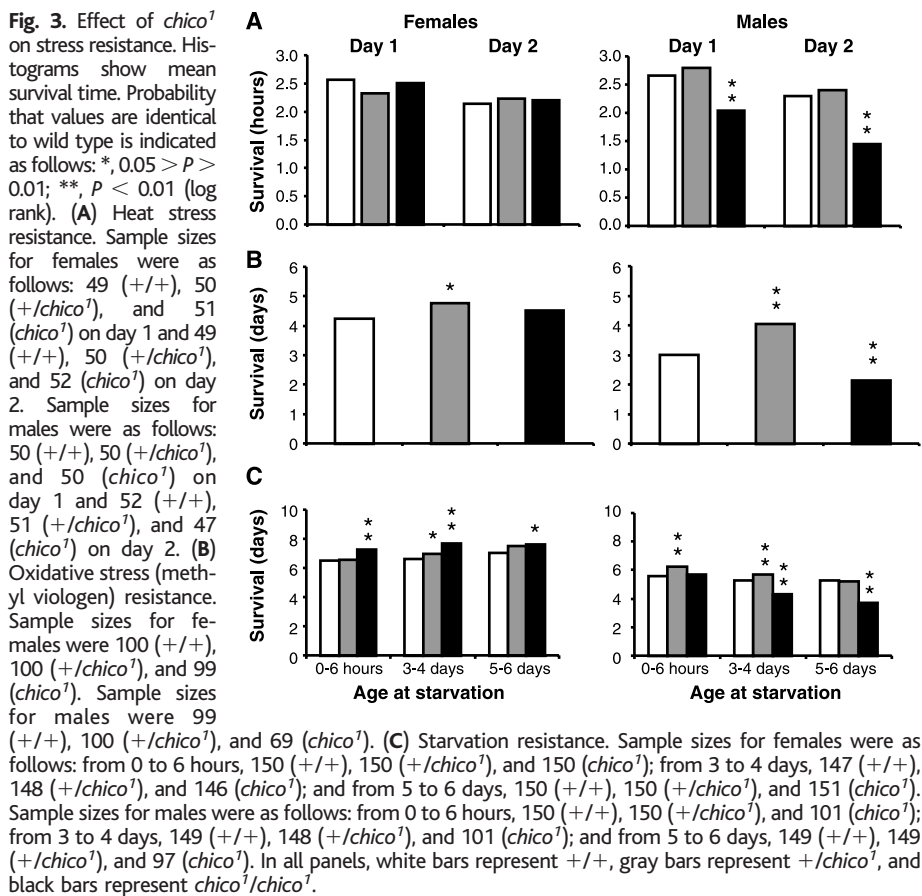
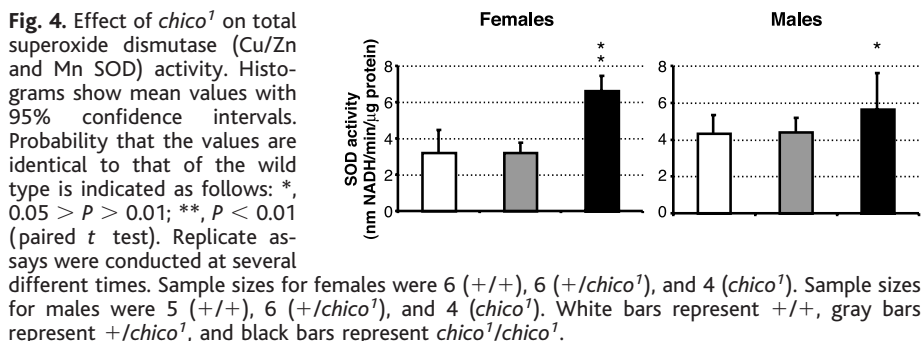


Fig. 3. Effect of *chico*¹ on stress resistance. Histograms show mean survival time. Probability that values are identical to wild type is indicated as follows: *, 0.05 > *P* > 0.01; **, *P* < 0.01 (log rank). (A) Heat stress resistance. Sample sizes for females were as follows: 49 (+/+), 50 (+/*chico*¹), and 51 (*chico*¹) on day 1 and 49 (+/+), 50 (+/*chico*¹), and 52 (*chico*¹) on day 2. Sample sizes for males were as follows: 50 (+/+), 50 (+/*chico*¹), and 50 (*chico*¹) on day 1 and 52 (+/+), 51 (+/*chico*¹), and 47 (*chico*¹) on day 2. (B) Oxidative stress (methyl viologen) resistance. Sample sizes for females were 100 (+/+), 100 (+/*chico*¹), and 99 (*chico*¹). Sample sizes for males were 99 (+/+), 100 (+/*chico*¹), and 69 (*chico*¹). (C) Starvation resistance. Sample sizes for females were as follows: from 0 to 6 hours, 150 (+/+), 150 (+/*chico*¹), and 150 (*chico*¹); from 3 to 4 days, 147 (+/+), 148 (+/*chico*¹), and 146 (*chico*¹); and from 5 to 6 days, 150 (+/+), 150 (+/*chico*¹), and 151 (*chico*¹). Sample sizes for males were as follows: from 0 to 6 hours, 150 (+/+), 150 (+/*chico*¹), and 101 (*chico*¹); from 3 to 4 days, 149 (+/+), 148 (+/*chico*¹), and 101 (*chico*¹); and from 5 to 6 days, 149 (+/+), 149 (+/*chico*¹), and 97 (*chico*¹). In all panels, white bars represent +/+, gray bars represent +/*chico*¹, and black bars represent *chico*¹/*chico*¹.



Together, our results with fruit flies and recent findings with nematodes and mice suggest that the role of IIS (perhaps IGF-I in mammals) in regulating longevity is evolutionarily conserved throughout the animal kingdom.

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