

Two neurons mediate diet-restriction-induced longevity in *C. elegans*

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Dietary restriction extends lifespan and retards age-related disease in many species and profoundly alters endocrine function in mammals. However, no causal role of any hormonal signal in diet-restricted longevity has been demonstrated. Here we show that increased longevity of diet-restricted *Caenorhabditis elegans* requires the transcription factor gene *skn-1* acting in the ASIs, a pair of neurons in the head. Dietary restriction activates *skn-1* in these two neurons, which signals peripheral tissues to increase metabolic activity. These findings demonstrate that increased lifespan in a diet-restricted metazoan depends on cell non-autonomous signalling from central neuronal cells to non-neuronal body tissues, and suggest that the ASI neurons mediate diet-restriction-induced longevity by an endocrine mechanism.

Dietary restriction extends lifespan in many organisms and reduces incidence and progression of age-related disease¹. In mammals, dietary restriction dramatically alters central and secondary hormone production and target-tissue responsiveness, suggesting a mechanism by which this regimen coordinately slows ageing across a variety of tissues². A possible hormonal link of dietary restriction to longevity is suggested by the recent demonstration that serum from diet-restricted mice contains factors sufficient to induce many of the cellular phenotypes of dietary restriction *in vitro*³. However, no hormone has been shown to mediate diet-restriction-induced longevity *in vivo*, and indeed mice that almost completely lack several pituitary hormones respond normally to dietary restriction⁴, so the question of whether dietary restriction induces longevity by an endocrine mechanism remains unresolved.

A method of dietary restriction in *C. elegans*

Dietary restriction has been shown to extend lifespan in the nematode worm *C. elegans*⁵. We developed a dietary restriction protocol that reliably increases longevity in worms (see Methods; Fig. 1a). Worms cultured on standard plates seeded with the bacterial food source throughout larval development were then transferred to a small volume of liquid medium in which bacterial density was controlled at a range of different levels (see Methods). Worms maintained at high bacterial density (our *ad libitum* condition) had a slightly longer lifespan than worms cultured on agar plates seeded with a bacterial lawn (Fig. 1a). Mean lifespan increased as bacterial density was reduced until a maximum lifespan was reached at an optimal dilution of 1:10 (our dietary restriction condition), although further dilutions caused reduced mean lifespan, presumably due to starvation (Fig. 1b). Diet-restricted animals also exhibited reduced fat in the intestine and small body size (Supplementary Fig. 1a, b). Animals in the *ad libitum* condition exhibited an optimal brood size, whereas diet-restricted animals had a reduced brood size and extended reproductive period (Supplementary Fig. 1c). As reported for other methods of dietary restriction^{6,7}, this protocol extended the lifespan of both *daf-16* and *daf-2* mutants (Fig. 1c) disrupted in genes that function in the well-characterized insulin-like signalling pathway⁸.

SKN-1 mediates diet-restricted longevity

SKN-1 is a transcription factor that has previously been shown to be critical in endodermal development⁹ and control of the oxidative

stress response¹⁰, functions that are shared by the most similar mammalian proteins, the NFE2-related factors. Because oxidative damage is thought to be a key cause of ageing¹¹, it has been proposed that SKN-1 may be a determinant of ageing rate¹⁰. Notably, some isoforms of SKN-1 are expressed from an operon downstream of *bec-1*, the *C. elegans* homologue of mammalian beclin 1 (Fig. 2a). Beclin 1 mediates autophagy induced by nutrient deprivation¹², indicating that *skn-1* might be regulated in response to nutritional stress. We therefore tested whether *skn-1* functions in the diet-restriction-induced longevity response. Four loss-of-function alleles of *skn-1* all significantly impaired the response to dietary restriction (Fig. 2b; Supplementary Figs 2a and 3). *skn-1* mutant lifespan was unresponsive to diet over a wide range of food concentrations (Fig. 1b). *skn-1* mutations seemed to specifically interfere with the dietary restriction longevity response, because they did not impair the longevity increase caused by mutation of *daf-2* (Supplementary Fig. 4) or by treatment with a plant polyphenol extract¹³. We focused our subsequent analysis on two alleles, *skn-1(zu135)* and *skn-1(zu169)*, because they completely prevented the longevity increase on dietary restriction, yet had little or no effect on lifespan of worms fed *ad libitum* (Fig. 2b). These two alleles introduce premature stop codons before the sequence encoding the DNA-binding domain of all SKN-1 isoforms (Supplementary Fig. 2a; also see below). The failure of both *skn-1* mutants to respond to dietary restriction was rescued by *Is007*, an integrated transgene expressing an *skn-1-gfp* (green fluorescent protein) fusion from its native promoter¹⁰ (Fig. 2a, c; Supplementary Fig. 2a), confirming that these *skn-1* mutations caused the dietary restriction longevity defect.

SKN-1 mediates dietary restriction in two neurons

The rescuing *Is007* transgene is expressed in only two tissues: in the two ASI neurons, and in the intestine¹⁰. This is an intriguing expression pattern in the context of dietary restriction because the intestine is the food absorption and storage organ, and the ASIs are sensory neurons in the head that are critical in translating information about food availability into endocrine signals that influence the dauer decision during development¹⁴. We asked in which tissue *skn-1* acts to mediate dietary restriction longevity. The *skn-1* gene encodes three protein isoforms—SKN-1A, SKN-1B and SKN-1C—which have different amino termini but a common carboxy terminus (Fig. 2a).

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Using 5' rapid amplification of cloned/cDNA ends (RACE), we obtained evidence that each of these isoforms is expressed from an independent promoter (Supplementary Fig. 2b–d). Because most of the unique 5' coding region of *skn-1a* is not included in the rescuing *Is007* transgene (Fig. 2a), we reasoned that *skn-1b* and/or *skn-1c* must be sufficient to rescue dietary restriction defects in the *skn-1* mutants. We used RNA interference (RNAi) feeding clones corresponding to the unique 5' end of either *skn-1b* or *skn-1c* to knock down each isoform separately in GFP reporter strains, and determined that *skn-1b* is expressed in the ASI neurons but not detectably in the gut, and *skn-1c* is expressed in the gut but not detectably in the ASI neurons (Fig. 2d–f). An extrachromosomal transgene that drives *skn-1b-gfp* expression from the ASI-specific *gpa-4* promoter¹⁵ showed expression in the ASI neurons, but not the gut, and rescued the dietary restriction longevity defects of *skn-1(zu135)* animals completely (Fig. 2g). Similar results were obtained with the same transgene

in a *skn-1(zu169)* background, and also with a chromosomally integrated version of the transgene (Supplementary Table 1). In contrast, extrachromosomal and integrated transgenes driving *skn-1c-gfp* from the *ges-1* promoter¹⁶ showed expression only in the gut, and had no effect on the dietary restriction defect of *skn-1(zu135)* (Fig. 2h). Furthermore, ectopic expression of *skn-1c-gfp* in the ASI neurons using the *gpa-4* promoter failed to rescue the dietary restriction longevity defect of *skn-1(zu135)* (Supplementary Table 1). Taken together, these data indicate that *skn-1b* functions in the ASI neurons to mediate dietary restriction longevity.

To confirm that the ASI neurons are required for diet-restriction-induced longevity, we used a laser microbeam to specifically kill these two cells and tested the effect in subsequent dietary restriction. Ablation of the ASI neurons completely suppressed the response to dietary restriction (Fig. 3a), but also caused a small increase in basal longevity, consistent with a previous report¹⁷. The ASIs can apparently affect lifespan by two independent pathways because the increase in basal longevity was previously reported to be dependent on *daf-16* (ref. 17), and dietary restriction longevity is not (Fig. 1c). We reasoned that the use of a *daf-16* mutant would separate the two effects of ablating the ASI neurons and allow us to determine their role in dietary restriction longevity specifically. As expected, when we repeated the ASI ablation in *daf-16* animals, we found that basal longevity was unaffected by the ablation (Fig. 3b). Importantly, dietary restriction longevity was still suppressed (Fig. 3b). These results support the model that *skn-1* functions in the ASI neurons to extend lifespan in response to dietary restriction.

We next investigated how *skn-1* acts in the ASI to mediate dietary restriction longevity. To rule out the possibility that *skn-1* mutation disrupts development of the ASI neurons, we used a panel of ASI-specific GFP reporters to confirm that the ASI neurons exhibit normal morphology and cell fate in a *skn-1* mutant background (Supplementary Fig. 5). We then tested whether SKN-1–GFP is induced by dietary restriction in *Is007* animals. Although we saw no specific induction in the intestine (data not shown), dietary restriction significantly increased SKN-1–GFP fluorescence in the ASI neurons (Fig. 4a). The fluorescence increase was specific to SKN-1–GFP expressed from its native promoter, because if the protein was expressed from the ASI-specific *gpa-4* promoter, we observed instead a slight decrease in expression during dietary restriction (Fig. 4a). This dependence of *skn-1* induction on the native promoter indicates that *skn-1* may be transcriptionally regulated during dietary restriction.

The ASI neurons activate respiration

In yeast, dietary restriction induces an increase in respiratory rate¹⁸, and there is evidence that this is also the case in worms^{19,20}. We measured the whole-body oxygen consumption rate of large populations of wild-type worms on day 3 of *ad libitum* feeding or dietary restriction (see Methods), and found that diet-restricted worms exhibited elevated respiration (Fig. 4b). The respiration rate increase on dietary restriction was absent in a *skn-1(zu135)* mutant, but could be rescued, and somewhat enhanced, by the *Is007* transgene (Fig. 4b), demonstrating that *skn-1* is necessary for the increased respiration. The increase in respiration is likely to be necessary for the dietary restriction longevity effect because two different specific inhibitors of the mitochondrial electron transport chain complex III, myxothiazol and antimycin, suppressed dietary restriction longevity without shortening the lifespan of worms fed *ad libitum* (Fig. 4c, d). Furthermore, myxothiazol completely suppressed the increase in respiration under dietary restriction (data not shown). The effect of inhibiting electron transport was specific to dietary restriction longevity because the long life of a *daf-2* mutant was not affected by antimycin (Fig. 4e). Finally, to determine whether *skn-1* in the ASI neurons alone is sufficient to rescue the absence of increased respiration in the *skn-1(zu135)* mutant during dietary restriction, we constructed two stably integrated transgenes: *gels9*, which drives

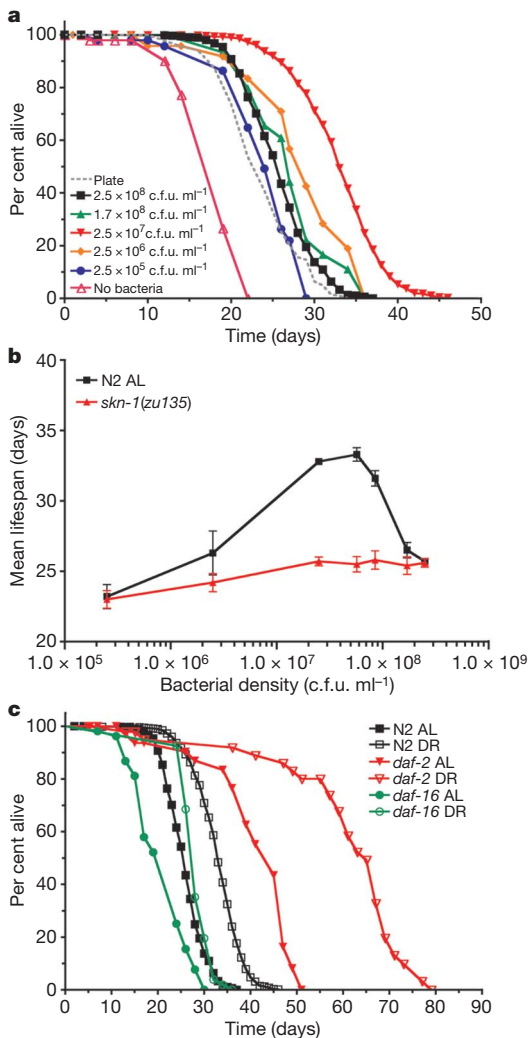


Figure 1 | Lifespan extension by dietary restriction. Lifespan curves represent combined data from independent experiments as indicated in Supplementary Table 1. Complete lifespan data are presented in Supplementary Table 1. **a**, Survival curves of wild-type N2 animals fed various concentrations of bacteria in liquid medium, or on agar plates of otherwise identical composition seeded with a bacterial lawn. **b**, Local maximum in N2 mean lifespan at an optimal level of bacterial dilution. In contrast, the lifespan of *skn-1(zu135)* is not altered by food level. Error bars, s.e.m. **c**, The dietary restriction (DR) protocol extends lifespan of the insulin pathway mutants *daf-16(mgDf50)* and *daf-2(e1370)*. *Ad libitum* (AL) bacterial concentration is 2.5×10^8 c.f.u. ml⁻¹; dietary restriction is 2.5×10^7 c.f.u. ml⁻¹.

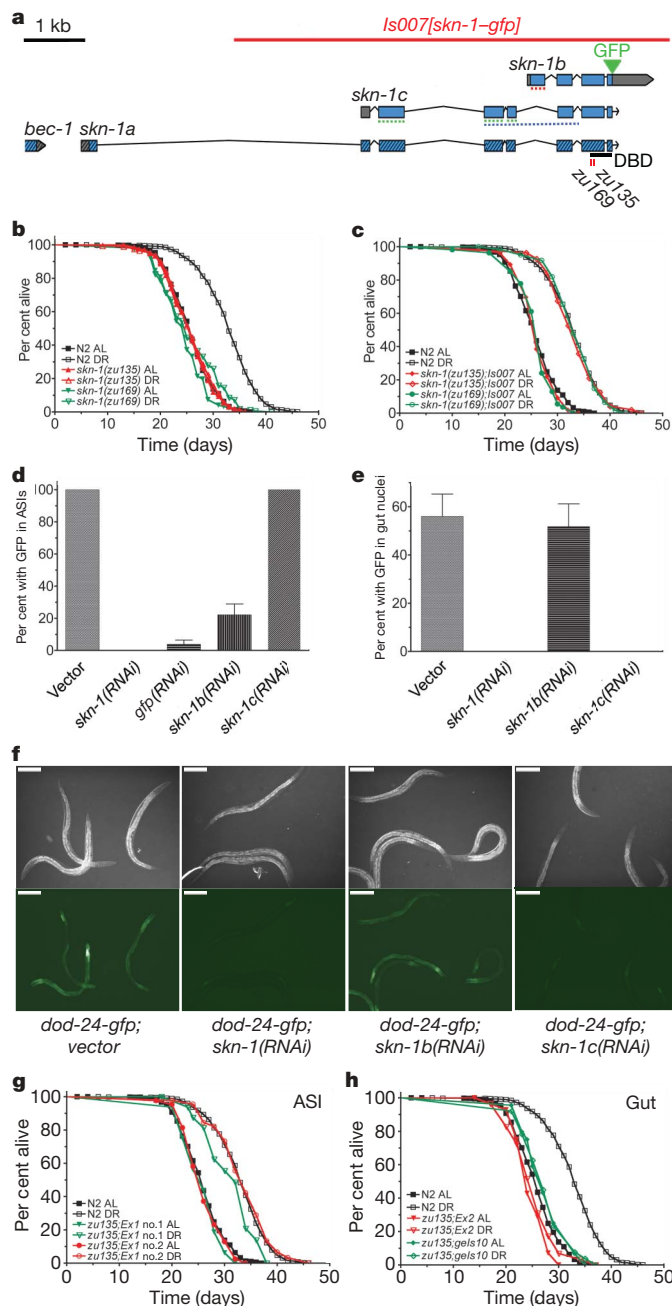


Figure 2 | *skn-1* functions in the ASI neurons to mediate dietary restriction longevity. **a**, The *skn-1* gene encodes three protein isoforms, with different N termini but a common C terminus. Predicted transcripts (Wormbase) and their genomic organization are depicted, with exons shown in blue and untranslated regions in grey. Striped boxes indicate an operonic transcript including the upstream gene—the beclin-1 homologue *bec-1*—and downstream *skn-1a*. The extent of the *Is007[skn-1-gfp]* integrated transgene¹⁰ and the insertion site of GFP in the transgene are also indicated. The purple and red dotted lines indicate the DNA segments used in the *skn-1(RNAi)* and *skn-1b(RNAi)* constructs, respectively (see below); the *skn-1c(RNAi)* construct was made using a fusion of the exons indicated by the green dotted line. The sites of the *zu169* and *zu135* nonsense mutations are shown. DBD, DNA binding domain. See Supplementary Fig. 2a for more detail. **b**, Two strains carrying *skn-1* mutations *zu135* and *zu169* have normal basal lifespans, but fail to respond to dietary restriction. **c**, Both *skn-1* mutants are rescued by the integrated transgene *Is007*, which drives *skn-1-gfp* from its native promoter and expresses *skn-1-gfp* in the ASI neurons and the gut. **d–f**, The *skn-1b* isoform is expressed primarily in the ASI neuron, and *skn-1c* is expressed primarily in the intestine. RNAi constructs

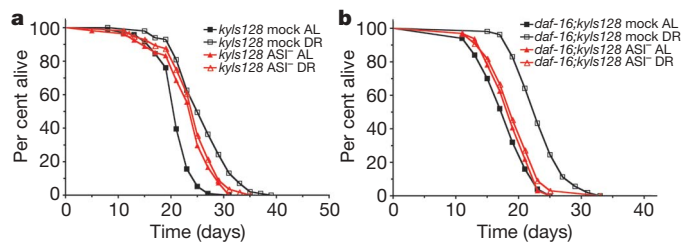
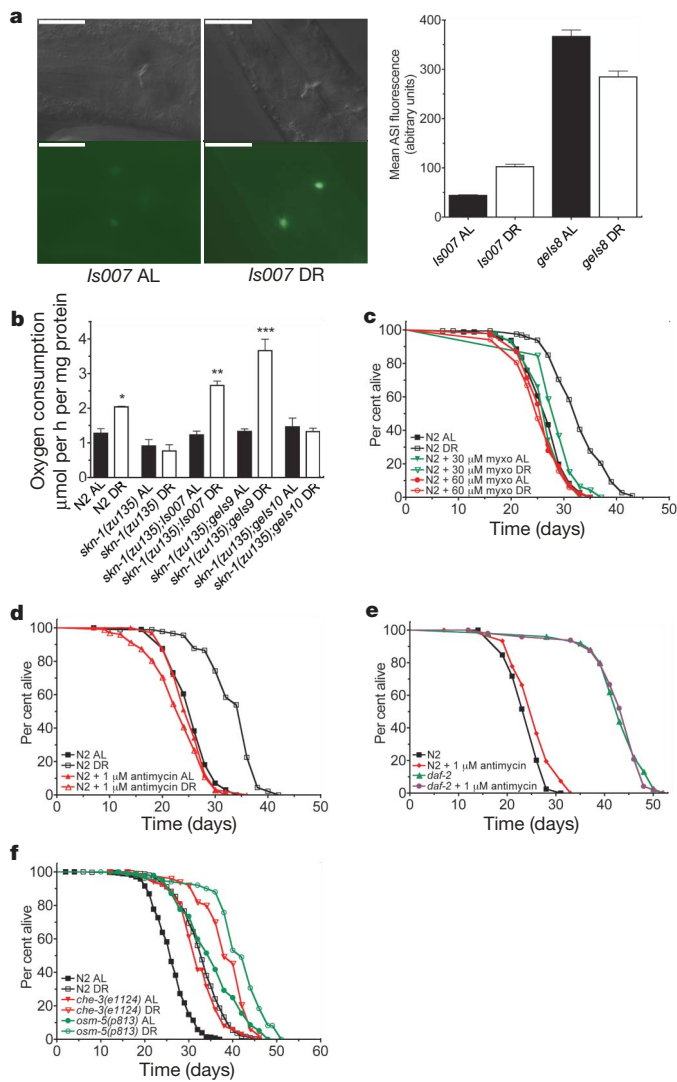


Figure 3 | The ASI neurons are necessary for dietary restriction longevity. ASI neurons were laser-ablated in strains carrying the integrated array *kyIs128*, which expresses GFP from the ASI-specific *str-3* promoter²⁷. ‘Mock’ strains represent non-operated controls (see Methods). **a**, Animals with killed ASI neurons (ASI⁻) do not respond to dietary restriction. These animals have a longer basal lifespan, as previously reported¹⁷ (for *kyIs128* ASI⁻ AL versus *kyIs128* mock AL, Mantel–Cox logrank $P < 0.0001$). **b**, *daf-16* mutation suppresses the longer basal lifespan caused by ASI ablation (for *daf-16(mgDf50);kyIs128* ASI⁻ AL versus *daf-16(mgDf50);kyIs128* mock AL, $P = 0.3902$), but ASI-ablated animals remain refractory to dietary restriction.

skn-1b-gfp expression in the ASI neurons, and *gels10*, which drives *skn-1c-gfp* expression in the intestine. *gels9* expressed strongly in the ASIs, and also rescued and enhanced the respiration response to dietary restriction in the *skn-1(zu135)* mutant (Fig. 4b). The *gels9* line sometimes showed weak expression in a few cells apart from the ASIs, although these are unlikely to contribute to the observed rescue (see Methods). *gels10*, in contrast, failed to rescue the dietary restriction respiration defect of *skn-1(zu135)*, despite expressing efficiently in the intestine (Supplementary Fig. 6). These findings suggest that diet-restriction-induced activation of *skn-1* in the ASIs causes release of a signal that promotes metabolism in peripheral tissues and leads to long life. Interestingly, it seems that *skn-1* has two functions in the adult that are separable by isoform and tissue of expression: mediation of dietary restriction longevity by *skn-1b* in the ASI neurons,

specific to the unique 5' end of either *skn-1b* or *skn-1c* (see panel a) were used to examine *skn-1* isoform expression. Note that the *skn-1c* RNAi construct will also knock down endogenous *skn-1a* owing to sequence identity in this region. The *skn-1(RNAi)* construct targets the 3' region common to all *skn-1* isoforms (see panel a). **d**, *Is007* expresses SKN-1B–GFP, but not SKN-1C–GFP, in the ASI. The indicated RNAi constructs were fed to RNAi-hypersensitive³⁰ *lin-35(n745);Is007[skn-1-gfp]* animals. *skn-1b(RNAi)*, but not *skn-1c(RNAi)*, reduced GFP expression in the ASI. Data shown are from two independent experiments; errors are s.e.m. Total *n* of animals observed in trial 1 and trial 2, respectively: vector, 24, 29; *skn-1(RNAi)*, 20, 24; *gfp(RNAi)*, 22, 26; *skn-1b(RNAi)*, 36, 24; *skn-1c(RNAi)*, 23, 20. **e**, *Is007* expresses SKN-1C–GFP, but not SKN-1B–GFP, in the intestine. Following RNAi exposure, *Is007* worms were heat-shocked to induce nuclear accumulation of SKN-1–GFP in the intestine. *skn-1c(RNAi)* reduced SKN-1–GFP, whereas *skn-1b(RNAi)* had no effect. Data shown are from two independent experiments; errors are s.e.m. Total *n* of animals examined in trial 1 and trial 2, respectively: vector, 24, 25; *skn-1(RNAi)*, 25, 23; *skn-1b(RNAi)*, 28, 24; *skn-1c(RNAi)*, 28, 21. **f**, *skn-1a* and/or *skn-1c* account for the majority of basal *skn-1* transcriptional activity in the intestine. The gene *dod-24* has several *skn-1* consensus binding sites in its promoter region and is expressed in a *skn-1*-dependent manner (data not shown). An integrated *dod-24-gfp* transgenic strain (a gift of D. Kim and F. Ausubel) was therefore used as a reporter for intestinal *skn-1* activity. *skn-1c(RNAi)* reduced reporter expression, whereas *skn-1b(RNAi)* did not. Scale bars, 100 μ m. **g**, *Ex1*, an extrachromosomal array that drives *skn-1b-gfp* expression from the *gpa-4* promoter exclusively in the ASI neurons of adult animals, rescues the *skn-1(zu135)* dietary restriction longevity defect. Two independently transformed lines, labelled no. 1 and no. 2, are shown. **h**, *Ex2*, an extrachromosomal array driving *skn-1c-gfp* expression from the *ges-1* promoter exclusively in the gut, does not rescue *skn-1(zu135)* dietary restriction longevity. *gels10*, an integrated version of the same array, also fails to rescue.



and resistance to oxidative stress by *skn-1c* in the intestine (Supplementary Fig. 6).

Discussion

How might dietary restriction activate *skn-1* in the ASIs? Although the ASI is a sensory neuron²¹, sensation of the environment by the ASI is unlikely to be necessary for dietary restriction to increase longevity because mutants lacking functional sensory cilia still expressed SKN-1-GFP in the ASI neurons (not shown) and responded normally to dietary restriction (Fig. 4f). It has recently been shown that, within particular cells of the mammalian hypothalamus, levels of key intracellular metabolites indicative of energy availability control organismal food intake and energy metabolism²². We propose that the ASI neurons similarly detect their own cellular energy state and adjust the animal's overall metabolism in response to nutrient availability. Because increased expression of *skn-1-gfp* in the ASI is not sufficient by itself to extend the lifespan of worms fed *ad libitum* or to enhance the response to dietary restriction (Supplementary Table 1), other pathways or neurons must act in parallel to *skn-1* to orchestrate dietary restriction longevity.

Here we demonstrate that cell non-autonomous signals from central neuroendocrine cells to the periphery can activate respiration and mediate dietary restriction longevity in a metazoan (Fig. 5). The ASIs have well-established endocrine functions, and express at least 15 known or potential hormones, including members of the insulin, TGF- β , and neuropeptide classes^{23,24}. We propose that, on sensing

Figure 4 | Dietary restriction activates *skn-1* in the ASI neurons and increases whole-body respiration. **a**, Five days of dietary restriction increases GFP expression in the ASI neurons of *Is007[skn-1-gfp]* worms. Left panel, bright field and fluorescence Nomarski images; scale bars, 10 μ m. Right panel, quantification of fluorescence in *Is007[skn-1-gfp]* and in *gels8*, a specificity control strain that drives *skn-1-gfp* expression from the ASI-specific *gpa-4* promoter. Each value represents pooled data from two independent trials; errors are s.e.m.; *n* for trial 1 and trial 2, respectively: *Is007* AL, 42, 42; *Is007* DR, 58, 21 (unpaired two-tailed *t*-test $P < 0.0001$); *gels8* AL, 19, 31; *gels8* DR, 19, 39 ($P < 0.0001$). **b**, Respiration rate is increased in N2 animals assayed on day 3 of dietary restriction. The *skn-1(zu135)* mutation prevents the respiration increase on dietary restriction. Integrated transgenes driving *skn-1-gfp* expression, either from the native promoter (*Is007*), or from the ASI-specific *gpa-4* promoter (*gels9*), rescue the dietary restriction respiration defect of *skn-1(zu135)*, elevating respiration rates above the wild-type level. The integrated transgene *gels10*, which drives *skn-1-gfp* expression in the gut using the *ges-1* promoter, fails to rescue the respiration defect of *skn-1(zu135)* on dietary restriction. Unpaired two tailed *t*-test *P* values (AL versus DR for each strain): N2, * $P = 0.0221$; *skn-1(zu135);Is007*, ** $P = 0.0011$; *skn-1(zu135);gels9*, *** $P = 0.0005$. No other values differ significantly from N2 AL. N2 data represent pooled triplicate measurements from each of four independent experiments. Other strains were measured in triplicate in two experiments each; errors are s.e.m. **c, d**, Electron-transport-chain-inhibiting drugs prevent the dietary restriction longevity response without shortening AL lifespan. The electron transport chain complex III inhibitor myxothiazol (myxo) partially blocks dietary restriction longevity at 30 μ M, and completely blocks dietary restriction longevity at 60 μ M (**c**). Another complex III inhibitor, antimycin, also blocks dietary restriction longevity (**d, e**). Antimycin does not affect the longevity induced by *daf-2(e1370)* mutation. This assay was performed on agar plates with otherwise identical compositions to the liquid medium used for dietary restriction experiments. **f**, The dietary restriction longevity response does not require environmental chemosensation. *che-3(e1124)* and *osm-5(p813)* mutants lack functional sensory neuron cilia and are long-lived under AL conditions³¹, but dietary restriction still increases lifespan in these mutants by a similar percentage as it does in N2.

dietary restriction, the ASI neurons release, or cause other neurons to release, a hormonal signal that promotes metabolic activity in the periphery and leads to increased respiration and long life. Mutations in genes encoding two hormones produced by the ASI neurons, an insulin (*daf-28*) and a TGF- β (*daf-7*), do not affect the dietary restriction longevity response, suggesting that these genes do not have an essential role in dietary restriction (N.B and L.G., unpublished results). We anticipate that future work will dissect the critical central hormones mediating diet-restriction-induced longevity of worms and mammals.

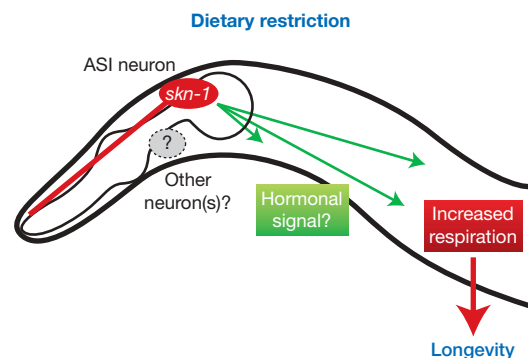


Figure 5 | Model of *skn-1* function during dietary restriction. Dietary restriction activates *skn-1* specifically in the ASI neurons. This promotes cell non-autonomous signalling to the peripheral tissues that increases respiration and extends lifespan. Other neurons or genetic pathways may be required in parallel to *skn-1* to mediate dietary restriction longevity (see text).

METHODS SUMMARY

Dietary restriction protocol. Lifespan assays were performed in 6-well tissue culture plates, with each well containing 2.5 ml of dietary restriction bottom medium and 2.5 ml of dietary restriction top medium. Dietary restriction bottom medium is composed of standard NGM medium²⁵, supplemented with 1 mg ml⁻¹ erythromycin to prevent bacterial division, 12.5 µg ml⁻¹ fluorodeoxyuridine to inhibit progeny hatching, 50 µg ml⁻¹ ampicillin, and 1 mM isopropyl-β-D-thiogalactoside; dietary restriction top medium is identical to dietary restriction bottom medium, excluding the agar. Ampicillin-resistant bacteria were added from a concentrated stock to the desired concentration, and concentration was monitored using a spectrophotometer. Worms were grown to the L4/young adult stage on NGM plates seeded with OP50 bacteria, then transferred individually into the wells and maintained at 20 °C with gentle gyratory shaking.

Laser ablation. Synchronized L1 animals were ablated, or mock-ablated in parallel, using a laser microbeam as described²⁶. Worms were recovered to plates and grown to the L4/young adult stage before lifespan analysis. The *kyIs128* strain²⁷ proved to have a somewhat shorter lifespan than N2, but this was attributable to intrinsic strain differences and not to the mounting procedure (Supplementary Fig. 7).

Respiration assay. Mass plate cultures of synchronized worms were grown on OP50 plates to the L4 stage, then transferred to Erlenmeyer flasks containing *ad libitum* or dietary restriction levels of bacteria. After three days of culture, animals were collected by centrifugation and washed free of bacteria in S buffer. Respiration rate was measured at 25 °C and normalized to protein content as described²⁸.

Fluorescence intensity quantification. Fluorescent images were collected at 1,000× magnification (Zeiss) from worms subjected to 5 days of *ad libitum* feeding or dietary restriction, as described above. Fluorescence brightness in the ASI was quantified using NIH ImageJ software.

RNAi feeding. RNAi feeding assays were done as described previously²⁹.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Strains. We used the following strains: the wild-type N2, EU1 *skn-1(zu67)/nT1[unc-?(n754);let-?]*, EU31 *skn-1(zu135)/nT1[unc-?(n754);let-?]*, EU35 *skn-1(zu169)/nT1[unc-?(n754);let-?]*, EU40 *skn-1(zu129)/nT1[unc-?(n754);let-?]*, LG335 *skn-1(zu135)/nT1[qIs51]*, LG336 *skn-1(zu169)/nT1[qIs51]*, LG333 *skn-1(zu135);Is007[skn-1-gfp]*, LG326 *skn-1(zu169);Is007*, LG340 *skn-1(zu135)/nT1[qIs51];Ex(gpa-4-sk-1b-gfp)* no. 1, LG341 *skn-1(zu169)/nT1[qIs51];Ex(gpa-4-sk-1b-gfp)* no. 2, LG343 *skn-1(zu135)/nT1[qIs51];Ex(ges-1-sk-1c-gfp)*, LG344 *geIs8[gpa-4-sk-1b-gfp]*, LG345 *geIs9[gpa-4-sk-1b-gfp]*, LG348 *skn-1(zu135)/nT1[qIs51];geIs9*, LG349 *geIs10[ges-1-sk-1c-gfp]*, LG357 *skn-1(zu135)/nT1[qIs51];geIs10*, CB1370 *daf-2(e1370)*, GR1307 *daf-16(mgDf50)*, CX3596 *kyIs128[scr-3-gfp]*, LG347 *daf-16(mgDf50);kyIs128*, CB1124 *che-3(e1124)*, PR813 *osm-5(p813)*, LG331 *lin-35(n745);Is007*, LG360 *skn-1(zu135)/nT1[qIs51];kyIs128*, FK181 *kIs2[daf-7-gfp]*, LG359 *skn-1(zu135)/nT1[qIs51];kIs2*, CX3594 *kyIs87[srd-1-gfp]*, LG363 *skn-1(zu135)/nT1[qIs51];kyIs87*, CX3465 *kyIs39[sra-6-gfp]*, LG367 *skn-1(zu135)/nT1[qIs51];kyIs39*, LG313 *kyIs140[scr-2-gfp]*, LG361 *skn-1(zu135)/nT1[qIs51];kyIs140*, AU10 *agIs1[dod-24-gfp]* (a gift of D. Kim and F. Ausubel), and the *Is007[skn-1-gfp]* and *Ex(gcs-1A2-gfp)* strains¹⁰.

Transgenic strain construction. Extrachromosomal-array-carrying transgenic strains were generated using standard microinjection methods³², injecting 50 ng μl^{-1} each of the transgene plasmid and the co-injection marker plasmid *pRF4*. Details of transgene construction are available on request.

The *gpa-4-sk-1b-gfp* transgene included 2.9 kb of *gpa-4* promoter fused to the full-length *skn-1b* complementary DNA and GFP. Adult animals carrying this transgene exhibited GFP fluorescence in the nuclei of the ASI neurons, and nowhere else. In larvae, weak expression was observed in one other pair of head neuron nuclei, which may be the AWAs³³. Integrants of the *Ex(gpa-4-sk-1b-gfp)* transgenes, called *geIs8* and *geIs9*, were isolated independently from a standard γ -ray integration screen³⁴, and were backcrossed three times to N2 before analysis. Following integration, non-specific low-level GFP expression accumulated in the extreme posterior few gut nuclei of aged adult animals. It is improbable that this weak intestinal expression accounts for the observed effects on dietary restriction respiration in *geIs9* for several reasons: (1) the intestinal expression is very low-level, detectable only in aged adult animals on normal plates and not in our *ad libitum* or dietary restriction conditions, and limited to only a few posterior cells; (2) *skn-1b* is not normally present in the intestine (Fig. 2e, f) and lacks the transactivation domain of the *skn-1* isoforms that are normally present (Fig. 2a), and therefore is not necessarily capable of performing any normal intestinal function of *skn-1*; and (3) the low level of intestinal *skn-1b* that is present in *geIs9* animals is definitely not capable of rescuing the only intestine-specific function of *skn-1* that we are aware of, that is, arsenate resistance (Supplementary Fig. 6).

The *ges-1-sk-1c-gfp* transgene included 2.5 kb of *ges-1* promoter fused to the *skn-1c* cDNA and GFP. Twenty-one of 30 transgenic animals from the extrachromosomal transgenic line used in this report exhibited nuclear GFP fluorescence in the intestine following heat shock for 20 h at 29 °C (this degree of nuclear localization is comparable to that observed with the *Is007* integrant; Fig. 2e). No GFP fluorescence was ever observed outside the gut with or without heat shock. *geIs10*, an integrant of the *Ex(ges-1-sk-1c-gfp)* transgene was isolated from a standard γ -ray integration screen³⁴, and was backcrossed three times to N2 before analysis.

Dietary restriction protocol. Lifespan assays were performed in 6-well tissue culture plates, with each well containing 2.5 ml dietary restriction bottom medium and 2.5 ml dietary restriction top medium. Erythromycin that was added did not affect oxygen consumption rate or the time required for larval development (data not shown). The bacterial food source used was PPD129.36 (ref. 29). Bacterial concentration in a 1 ml sample of each well was monitored using a spectrophotometer and additional bacteria were added as necessary to maintain starting concentration. Worms were grown to the L4/young adult stage on NGM plates seeded with OP50 bacteria, then transferred individually into the wells and maintained at 20 °C with gentle gyratory shaking at 80 r.p.m.

For the progeny production assays, worms were cultured as described except that FuDR was excluded to allow progeny production, and worms were transferred daily to fresh wells.

Lifespan assay. Most lifespans in this report were measured in the liquid medium described above, except where noted; in these cases lifespans either were done on plates with the same composition as dietary restriction bottom medium seeded with 50 μl of concentrated bacteria ($\sim 5 \times 10^9$ c.f.u. ml^{-1}), or on standard NGM plates seeded with OP50 bacteria. Regardless of medium, the lifespan assay was performed by prodding individual animals with a worm pick every two days to determine when they died. Animals that were lost, or exploded, or died from internal hatching of progeny were censored at the time of the event³⁵. In the assays shown in Fig. 4c–e, the dietary restriction medium contained 1% DMSO

plus 0 μM , 30 μM or 60 μM myxothiazol, or 0.1% ethanol plus 0 μM or 1 μM antimycin.

Survival curve *P* values were calculated by the Mantel–Cox logrank test using Prism statistical software (Graphpad).

Laser ablation. The strain CX3596 *kyIs128[scr-3-gfp]*, which expresses GFP specifically in the ASI neurons²⁷, was used to facilitate identification of the ASIs. Synchronized L1 animals were ablated, or mock-ablated in parallel, using a laser microbeam as described²⁶. Worms were recovered to OP50 plates and grown to the L4/young adult stage, at which time absence of GFP was taken to indicate successful ablation. (We confirmed in a separate control experiment that a cohort of ablated animals lacking GFP also all lacked the ASI itself, and we never observed a GFP-negative animal in any of the hundreds of mock-ablated controls.) CX3596 proved to have a somewhat shorter lifespan than N2, but this was attributable to intrinsic strain differences and not to the mounting procedure (Supplementary Fig. 7).

Respiration assay. Mass OP50 plate cultures of synchronized worms were grown to the L4 stage, then transferred to Erlenmeyer flasks containing *ad libitum*-fed or dietary restriction bacterial concentrations. After three days of culture, animals were collected by centrifugation and washed free of bacteria in S buffer. Respiration rate was measured at 25 °C and normalized to protein content as described²⁸, using a Clark-type oxygen electrode (Microelectrodes Inc.). At least three 1 ml samples containing approximately 500 worms each were measured for each strain and condition in each experiment.

skn-1 mutants are maternal-effect lethal and must be maintained as balanced heterozygotes. To obtain nearly pure *skn-1* populations for the oxygen assays, we first balanced *skn-1(zu135)* with the reciprocal translocation *nT1[qIs51]*, which carries a transgene expressing GFP in the pharynx. We then isolated non-GFP-pharynx, *skn-1* homozygous animals from a synchronous L4 population using the COPAS Biosorter (Union Biometrica) before transfer to the *ad libitum* or dietary restriction culture conditions. This procedure yielded greater than 95% non-GFP-pharynx populations, which we confirmed to be *skn-1* homozygotes by verifying the embryonic lethality of progeny of samples of sorted worms.

RNAi feeding. RNAi feeding assays were done as described²⁹. Worms were fed RNAi from L1 to L4. The *skn-1(RNAi)* construct has been described³⁶. The *skn-1b(RNAi)* feeding vector included the first 209 nucleotides of the coding region of a *skn-1b* cDNA. The *skn-1c(RNAi)* feeding vector included the first 455 nucleotides of the coding region of a *skn-1c* cDNA.

Rapid amplification of cDNA ends. 5' RACE (Invitrogen) was performed on total N2 RNA in accordance with the manufacturer's instructions.

Sudan Black fat staining. Day 3 adults cultured on plates, in *ad libitum* conditions, and in dietary restriction conditions were stained as described³⁷.

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