Title:

Dietary zinc restriction mimics protein restriction and extends lifespan in *Drosophila*

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Abstract:

Dietary restriction extends lifespan in model organisms, mainly through dietary amino acids. Compared to macronutrients, the effect of dietary micronutrients on organismal lifespan has not been intensively investigated. Here, using a synthetic diet, we test whether restriction of each micronutrient, including vitamins and minerals, affects lifespan and fecundity in adult *Drosophila*. While restriction of many of these micronutrients have either negative or no impact on lifespan, zinc (Zn) restriction alone can increase it. Dietary Zn restriction (ZnR) decreases fecundity, increases starvation resistance, and promotes preference for feeding amino acids, in adult females, phenocopying dietary amino acid restriction. Our study demonstrates that dietary intake of trace elements has profound impacts on physiology and lifespan, and that limiting dietary zinc may be a strategy to improve the healthspan of animals.

Introduction:

Nutrition is actively involved in homeostasis and determines healthspan of animals. Dietary restriction is known to increase lifespan in various organisms and its mechanism has been intensively studied[1–3]. How dietary factors involves lifespan extension has been analysed and essential amino acids are shown to be the key nutrients determining lifespan[4,5]. However, compared to macronutrients, how dietary micronutrients, such as minerals and vitamins, influence lifespan and healthspan is not studied well.

A synthetic diet, or holidic medium, is readily available in *Drosophila* and is useful for manipulating individual nutrients[5,6]. It is used to perform *loss-of-function* experiments to elucidate the effect of a single nutrient on metabolism, tissue homeostasis, behaviour, fecundity, and lifespan. The synthetic diet contains approximately forty pure ingredients, including a sugar, lipids, amino acids, vitamins, and minerals. Among the micronutrients, some trace elements, as well as vitamins, are nutritional requirements for most animals. The synthetic diet contains four trace (heavy) metals: zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn). Depletion of all metal ions can slightly increase lifespan and decrease fecundity[5]. To date, the effect of restriction of each metal on metabolism, fecundity, and lifespan have not been examined well.

In this study, we addressed how dietary restriction of each micronutrient such as vitamins or metals can influence *Drosophila* lifespan by utilising the synthetic diet. We found that restriction of Zn, but not Cu, Fe, Mn or vitamins, can extend lifespan. Zinc restriction (ZnR) decreases fecundity, increases starvation resistance, and promotes preference to amino acid feeding, suggesting that it phenocopies typical dietary amino acid restriction.

53 **Results:**

54

55 **Dietary Zinc restriction extends lifespan**

56 For dietary manipulation of micronutrients, we prepared synthetic diets (please see Methods for
57 detail). To test the effect of trace metals and vitamins, we fed female Canton-S flies with the diet
58 which lacks each one of vitamins or heavy metals (Fig. 1a). Of note, the flies were raised using a
59 standard yeast-based diet, which contained all these micronutrients, to avoid developmental
60 lethality, then flip the adult female flies to the synthetic diets at day 2 post eclosion (Fig. 1a,
61 Table 1). We assumed that restriction of an essential nutrient throughout adult life would lead to
62 early mortality. As we expected, dietary restriction of many vitamins shortened organismal
63 lifespan (Fig. 1b). In contrast, complete drop out of each metal ion did not strongly shorten
64 lifespan, while dietary restriction of Fe slightly increased lifespan (Fig. 1c). Notably, dietary Zn
65 restriction (ZnR) significantly extended lifespan (Fig. 1c). Since it was intriguing to see the
66 specific and striking effect of Zn on lifespan, we decided to focus on this micronutrient for the
67 rest of the study.

68 Substantial evidence indicated that ISC hyperproliferation is a hallmark of *Drosophila*
69 ageing, at least of the gut, that number of which inversely correlates with lifespan and
70 healthspan[7–10]. We found that ZnR suppressed the increased ISC division during ageing,
71 suggesting that gut homeostasis is maintained by ZnR (Fig. 1d). Together, these data suggested
72 that ZnR extends organismal lifespan and increases intestinal healthspan.

73

74 **Dietary Zinc restriction decreases internal Zinc levels**

75 The profound positive impact of Zn, but not other metals, restriction on female lifespan implied
76 that, in physiological conditions, internal Zn levels might be specifically elevated with age,
77 promoting pathology of organismal ageing. We measured the level of four metals in the whole
78 body of young (day5) and aged (day42) flies reared on the standard yeast-based diet, by using
79 inductively coupled plasma-mass spectrometry (ICP-MS). The analysis measured both of free
80 and protein-bound metal ions. The level of Zn was indeed increased in the aged flies (1.7-fold
81 increase) (Fig. 2a). However, this tendency was also the case for other metals, suggesting
82 specificity of lifespan extension by ZnR is not due to the specific accumulation of internal metal
83 levels (Fig. 2b-d). We then quantified how strongly Zn levels are decreased in female flies fed
84 with ZnR diet. The average Zn levels in the whole body of female flies after one-week of ZnR
85 was 13.8 ng/mg body weight, compared to the control 112 ng/mg body weight, indicating that
86 whole body Zn levels decreased to 12% of the control within a week (Fig. 2e).

87 It is worth noting that the Zn concentrations of the flies fed with the complete synthetic
88 diet were higher than those in the standard yeast-based diet (Fig. 2a,e). However, these diets
89 contained approximately the same amount of total Zn levels (Table 1). This discrepancy is
90 probably due to the bioavailability as Zn in the standard diet is likely chelated, while that in the
91 synthetic diet is free ions. Difference in the Zn levels in these two dietary conditions would
92 impact the physiology and lifespan of flies, however it is very difficult to compare them as
93 synthetic diet contains minimal number of nutrients. Empirically, however, higher Zn levels in
94 the flies fed with the control synthetic diet do not show the shorter lifespan compared to that with
95 standard yeast diet.

96

97 **Small amount of dietary Zinc is necessary for lifespan extension**

98 Given that complete Zn restriction does not enhance mortality, we suspected that Zn was
99 contaminated in some components of the synthetic diet and provided minimal Zn requirement for
100 flies to maintain homeostasis. Especially, an agar can contain various trace elements. To check this,
101 we quantified heavy metals in agar or agarose obtained from several manufacturers. Indeed, the
102 agar we used for the synthetic diet has relatively higher amount of Zn as well as other trace elements
103 (1,523 ng/g for Zn, 164 ng/g for Cu, 68,994 ng/g for Fe, 8,261 ng/g for Mn) (Fig. 3a-d). Among
104 tested, we found that an agarose from Nippon Gene Inc. has minimum amount of contaminated Zn
105 (15 ng/g for Zn, 350 ng/g for Cu, 808 ng/g for Fe, 46 ng/g for Mn) (Fig. 3a-d). Therefore, we
106 analysed lifespan of female flies under synthetic diets made with this agarose. ZnR using the
107 agarose failed to increase lifespan and rather slightly decrease it (Fig. 3e), suggesting that the
108 lifespan extension by ZnR demands a small amount of contaminated Zn in the agar and that harsher
109 ZnR has thus negative impact on longevity.

110

111 **Zinc restriction phenocopies dietary yeast restriction**

112 In general, dietary restriction induces the trade-off between fecundity and lifespan. As we
113 expected, one-week ZnR strongly decreased fecundity, which was not the case for the restriction
114 of other metals (Fig. 4a). ZnR with the synthetic diet using Nippon gene agarose (less Zn
115 contamination) induced a greater effect, suggesting that egg production is sensitive to the dose of
116 dietary Zn (Fig. 4b). The female flies fed with the ZnR diet for a week had smaller ovaries (Fig.
117 4c,d). This observation indicated that the decreased fecundity is owing to the defective egg
118 production/maturation, but not to the oviposition behaviour.

119 Importantly, lifespan extension by ZnR was not observed in a heterozygous *ovo*^{D1}
120 mutant, in which egg production is compromised, suggesting that reduction of fecundity is
121 required for ZnR-longevity (Fig. 4e). We also tested other physiological traits induced by the
122 typical dietary restriction, such as starvation resistance and lipid accumulation. The flies fed with
123 ZnR diet for a week showed increased starvation resistance and lipid storage (Fig. 4f-h).

124

125 **Zinc restriction increases feeding preference to yeast**

126 Restriction of dietary yeast increases preference for feeding yeast over sugar[11]. To test whether
127 ZnR changes feeding behaviour, we performed two-choice assay using yeast and sugar. The
128 female flies fed with the ZnR diet for one week showed the preference for yeast (Fig. 5a).
129 Intriguingly, this effect is not due to the increased preference for Zn contained in yeast, as they
130 did not show any augmented preference for Zn-contained sugar over sugar only diet (Fig. 5b). In
131 contrast, they showed increased preference index compared to control when choices were given
132 for the diet contained sucrose but not amino acids versus one contained amino acids but not
133 sucrose, suggesting an increased amino acid preference (Fig. 5c). These data further confirmed
134 that ZnR mimics the dietary yeast restriction phenotype.

135 We noticed that female flies preferred Zn-contained sugar regardless of their dietary
136 environment prior to the choice assay, when the Zn concentration was 87 μM which is equal to
137 the one in the synthetic diet (Fig. 5b). The data suggest that flies can sense Zn and show
138 preference to it. Previous studies revealed that higher concentration of Zn (above 3.3 mM)
139 provokes avoidance as it stimulates some taste neurons such as bitter neurons[12,13]. Thus, we

140 assumed that physiological concentration of Zn (such as in the synthetic diet) can have ability to

141 stimulate sweet or other taste neurons to trigger the attractive behaviour.

142

143

144 **Discussion:**

145 In this study, we identified dietary Zn as a regulator of fecundity, starvation resistance, feeding
146 preference, and lifespan. ZnR can phenocopy dietary restriction of protein/amino acids, even
147 under the condition where they have full set of nutrients including twenty amino acids. Thus, Zn
148 is a key micronutrient to determine lifespan-fecundity trade-off, which is consistent with the
149 recent work[14]. Considering the fact that the trade-off is necessary for lifespan extension by ZnR,
150 the decreased fecundity is a first event to trigger lifespan extension. The decreased fecundity in
151 flies with ZnR can be attributed to both the indirect (through IIS) and the direct impact of Zn on
152 oocyte maturation[15]. During oogenesis in *Drosophila*, Zn contents are increased and enriched to
153 form granules in the mature oocyte. Zn is then decreased and distributed uniformly upon the egg
154 activation[15]. The requirement of Zn for oocyte maturation and egg activation is also reported in
155 mammals, including humans[16–18]. The regulation of Zn levels in oocytes, as well as in other
156 tissues, requires Zn transporters, thus manipulation of Zn levels specifically in cells or tissues in
157 the body would be necessary to narrow down the responsible cells for ZnR longevity.

158

159 Lifespan extension by the dietary Zn depletion observed in the adult fly was unexpected, given
160 that Zn is an essential nutrient required for biological processes, such as immune, metabolic, or
161 tissue homeostasis. Indeed, in humans, even a mild Zn deficiency (intake of 3.0-5.0 mg/day Zn,
162 instead of 12 mg/day for a control) leads to adverse effects on immunological and biochemical
163 health parameters[19]. Although we did not test all the physiological traits in *Drosophila*, we did
164 not observe striking negative impact on fly health by ZnR, except decreased fecundity. We found
165 that contamination of Zn, such as in agar of the synthetic diet, was sufficient for maintaining a

166 minimum requirement for maintaining health, since animals demand a tiny amount for trace
167 elements, compared to macronutrients such as amino acids. We show that the internal Zn levels
168 during ZnR is approximately 12% of the control. We could not tell whether the zinc level is
169 further decreased during prolonged ZnR or its level is maintained throughout the entire life.
170 However, this level of reduction of internal Zn might not be as severe as it can negatively impact
171 lifespan, at least in *Drosophila*. We assume that adult flies have a way to store Zn obtained
172 during the larval stage and utilise it efficiently throughout the adult life. Of note, the Zinc is
173 reported to be enriched and stored in the Malpighian tubules[20]. There must be mechanisms to
174 augment the absorption of Zn and to block the excretion of it to maintain its level, probably by
175 sensing the decreased level of internal or dietary Zn. It is known that Zn transporters Zip44C.1
176 and Zip44C.2 in the gut are responsible for Zn absorption, the gene expression of which are
177 regulated by dietary Zn[21]. In the Malpighian tubules, a Zn transporter Zip71B regulates Zn
178 excretion and its expression is also known to be responsive to the dietary Zn levels[22]. Regulatory
179 mechanisms of these Zn transporters by internal Zn levels and the effect of their manipulation on
180 lifespan are yet to be elucidated.

181

182 The mechanism of how ZnR extends lifespan is not entirely understood. More than 2800
183 proteins, reaching 10% of the human genome, are potential Zn proteins[23]. Decrease in cellular Zn
184 levels can therefore influence various processes. One of such mechanisms would be a Zn-gated
185 chloride channel pHCl-2 (also known as Hodor), which regulates IIS through Ilps secretion
186 triggered by TOR signalling in the gut enterocytes[24]. Alternatively, insulin stability and secretion
187 from the mammalian pancreatic β cells are Zn-dependent[25], although the requirement of Zn for
188 *Drosophila* Ilps might not be conserved. Beyond IIS, there are 302 putative Zinc finger

189 transcription factors in *Drosophila* genome (flybase.org), such as GATA transcription factors
190 which are known to be involved in DR longevity[26]. It is known that age-related activation of
191 innate immunity is one of the hallmarks of ageing, which is related to organismal lifespan[9,27].
192 Since Zn is tightly linked with immunity[28], suppression of immune signalling by ZnR would
193 contribute to the lifespan extension.

194

195 As far as we know, this study provides the first evidence that limiting dietary Zinc availability by
196 utilising a synthetic diet can extend organismal lifespan. However, it is noteworthy that reducing
197 Zn levels achieved by a zinc-specific chelator TPEN can increase lifespan in *C. elegans*[29]. This
198 manipulation led to the activation of Daf-16, the FoxO ortholog in *C. elegans*, although the
199 lifespan extension was not completely blocked by the loss of *daf16* function alone[29]. It is pivotal
200 to test whether Zinc restriction can activate FoxO to extend lifespan in Drosophila and in
201 mammals.

202

203 # Methods

204

205 ***Drosophila* stocks and husbandry**

206 Flies were reared on a standard yeast-based diet containing 4.5% cornmeal (NIPPN
207 CORPORATION), 6% brewer's yeast (ASAHI BREWERIES, HB-P02), 6% glucose (Nihon
208 Shokuhin Kako), and 0.8% agar (Ina Food Industry S-6) with 0.4% propionic acid (Wako 163-
209 04726) and 0.15% butyl p-hydroxybenzoate (Wako 028-03685). For dietary manipulations, we
210 used the exome-matched version of a synthetic diet, or holidic medium, with some
211 modifications[30,31]. Adult flies for experiments were maintained under 25°C, 65% humidity with
212 12h/12h light/dark cycles. To allow synchronised development and constant density, embryos were
213 collected up to over night using agar plates (2.3% agar, 1% sucrose, and 0.35% acetic acid) with
214 live yeast paste. The equal volume of collected embryos were spread onto plastic bottles. The fly
215 lines used in this study were Canton-S and ovo^{D1} (Bloomington Drosophila Stock Center (BDSC)
216 1309).

217

218 **Lifespan analysis and starvation resistance assay**

219 Adult flies eclosed within one day were collected and maintained for an additional two days, in
220 order for female flies to mature and mate with males, on the standard yeast-based diet. Flies were
221 then sorted by sex and maintained at a fixed density (30 flies/vial). For lifespan analysis, the
222 number of dead flies was counted every three to four days when flies were transferred to fresh vials.
223 For each lifespan curve, at least six vials were used in parallel to minimise inter-vial variation. For

224 starvation resistance assay, female flies were transferred to vials with 1% agar, and the number of
225 dead flies was counted 2-3 times per day until all flies were dead.
226

227 **Inductively coupled plasma-mass spectrometry**

228 Metal contents in flies or in agar (FUJIFILM 010-15815) or agarose (SIGMA A9539-10G, Fast
229 Gene NE-AG01, or Nippon Gene 316-01191) were analysed by ICP-MS (Agilent 7700x). Frozen
230 samples were weighed and placed in a quartz glass test tube. Then 1 mL of 65% HNO_3 was added
231 and boiled in a water bath at 100°C for eight minutes. After cooling, the volume was fixed at 10
232 mL with ultrapure water. These solutions were diluted and introduced into an ICP-MS to determine
233 the concentration of metal elements in the eluate.
234

235 **Quantification of intestinal stem cell proliferation**

236 Whole guts from female flies were dissected in PBS and fixed in 4% PFA for 1 hr. After washing
237 with PBST (0.1% Triton-X100), the guts were incubated with blocking buffer (PBST (PBS with
238 0.1% Triton-X100) with 5% normal donkey serum) for 30 minutes. The guts were incubated with
239 anti-Histone H3 (phosphor S28) (1:1000, rat, Abcam, ab10543) antibody diluted in blocking buffer
240 overnight at 4 °C. After washing with PBST, guts were incubated with anti-rat IgG 488 (1:500,
241 Thermo Fisher Scientific, A32766) diluted in blocking buffer for two hours at room temperature.
242 After washing, the guts were mounted using SlowFade Gold (Thermo Fisher Scientific, S36936).
243 Hoechst 33342 (0.4 mM for 1:100, Thermo Fisher Scientific, H3570) was used for nuclear
244 visualisation. The number of phospho-Histone H3 positive cells in the whole gut was counted
245 manually.
246

247 **Fecundity analysis**

248 Flies reared on the standard yeast diet until day two post eclosion were maintained for one week
249 in vials with the synthetic diet with 17 females and 17 males. During this period, adult flies were
250 transferred to fresh vials every three days. Flies were then quickly anaesthetised and separated
251 into groups of three males with three females. After 24 hours, the egg number in each vial was
252 counted manually.

253

254 **Measurement of triacylglycerol**

255 Four female or male flies were homogenised in PBST (PBS with 5% TritonX-100), then incubated
256 for 5 min at 70°C. Using triglyceride reagent (Wako 632-50991), free glycerol amount derived
257 from triacylglycerol was measured according to the manufacturer's instruction, based on A_{600}. The
258 absorbance was determined by Infinite M Plex (Tecan). Total protein concentration in the same
259 sample was quantified using BCA reagent mix (Wako, 164-25935) for the normalisation.

260

261 **Food preference assay**

262 A two-choice test on a Petri dish was modified from the previously described one[32]. Four pieces
263 of square filter paper (2 cm × 2 cm, ADVANTEC No.50) were placed on the bottom of a Petri
264 dish (90 mm diameter). Two pieces in diagonal position were soaked with 150 µl distilled water
265 to humidify the dish. Then, 150 µl of one taste solution (coloured with the red dye, 0.25 mg/mL
266 acid red 52, Wako 018-10012) was applied to one of the remaining pieces of paper, and the other
267 taste solution (coloured with the blue dye, 0.125 mg/mL brilliant blue, Wako 027-12842) was
268 applied to the other. For the two-choice assay using yeast and sugar, 1% yeast extract and 5 mM
269 sucrose were coloured blue and red, respectively. To assess Zn preference, 5 mM sucrose with

270 0.87 μM (1/100×), 8.7 μM (1/10×), 87 μM (1×), or 870 μM (10×) $ZnSO_4$ solution and 5 mM
271 sucrose were coloured blue and red, respectively. To examine AA preference, agar, cholesterol,
272 propionic acid, and nipagin were excluded from the holidic medium solution. Holidic medium
273 solution from which 20 AAs were omitted and holidic medium solution from which sucrose was
274 omitted were coloured red and blue, respectively.

275 Adult female flies were allocated to vials containing a synthetic diet at day two post
276 eclosion. Six vials, each containing 30 individuals, were prepared for each condition. After one
277 week, flies were transferred to vials containing 1% agar for starvation for 28 hours. The starved
278 flies were then quickly anesthetised by exposure to a temperature of 4°C for 10 minutes and
279 subsequently transferred to the assay plate. The plate was covered by aluminium foil and
280 positioned in the incubator at 25°C with a humidity level of 60% for two hours. Subsequently, the
281 plate was retrieved and subjected to freezing at -30°C for at least two hours. The coloration of the
282 abdomen was manually categorised into blue, purple, and red. The preference index (PI) towards
283 yeast was calculated as follows: PI[blue]={N(blue)-N(red)}/{N(blue)+N(purple)+N(red)}. Vials
284 with more than 30% colorless flies were excluded from the analysis.

285

286 **Quantification and statistical analysis**

287 Statistical analysis was performed using Graphpad Prism 9 and 10. A two-tailed Student's *t*-test
288 was used to test between samples. One-way ANOVA with Holm-Šídák's multiple comparison test
289 was used to test among groups. One-way ANOVA with Dunnett's multiple comparison test was
290 used to test between control and multiple samples. Bar graphs were drawn as mean and SEM with

291 all the data point shown by dots to allow readers to see the number of samples and each raw data.

292 OASIS 2 was used to perform log-rank test for lifespan analysis[33].

293

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Author Contributions:

F.O. and H.K. conceived the project. H.K., H.A., R.O., performed most of the experiments and analysed the data. S.K. quantified ISC proliferation and performed food choice assay. C.S. established the method for food choice assay. H.K. and F.O. wrote the initial manuscript. M.M. and F.O. supervised the study. All authors edited and approved the final manuscript.

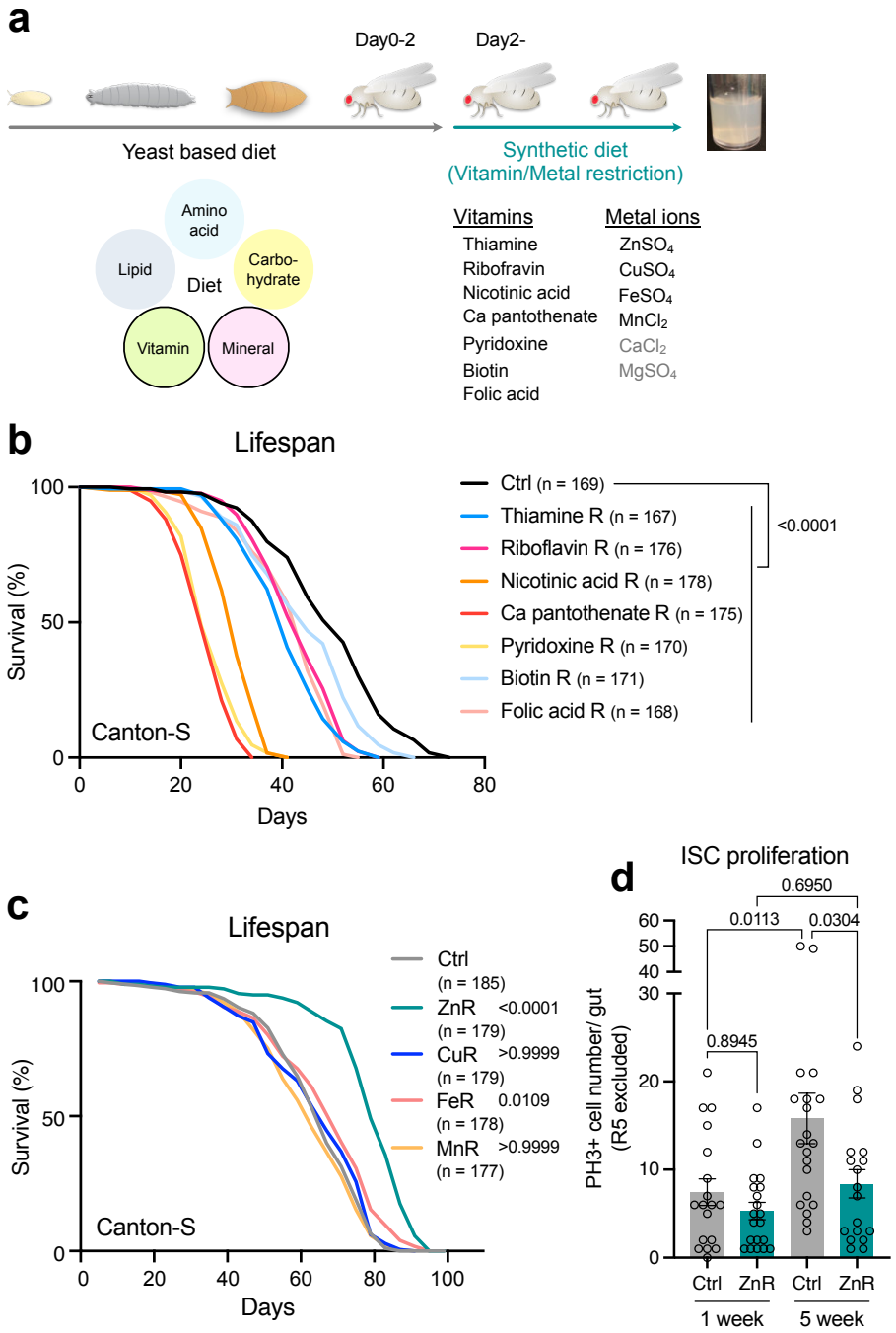
Competing interests

The authors declare no competing interests.

Materials & Correspondence

All the materials generated in this study are available upon request to F.O.

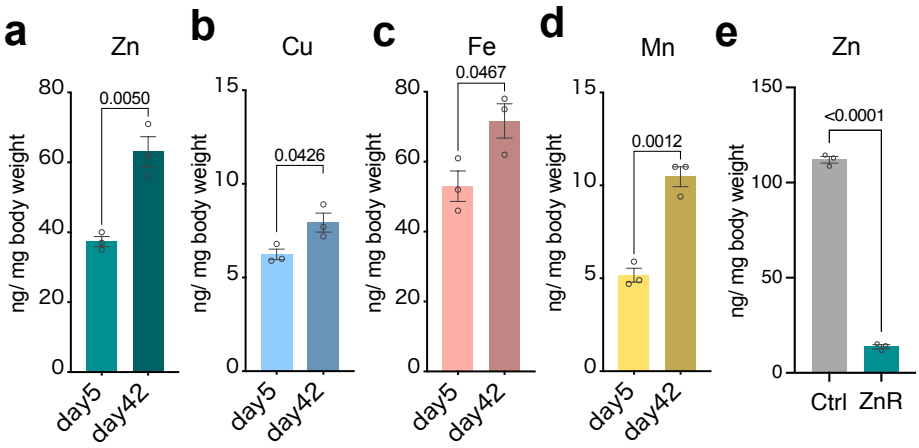
389 **Figures:**



390 **Fig. 1 Zinc restriction extends female lifespan and gut healthspan.**

391 **a,** The scheme of dietary manipulation. Each of seven vitamins and four trace metals was dietary
392 restricted from day 2. **b,c,** Lifespans of female Canton-S flies fed with or without vitamin- (**b**) or
393 metal- (**c**) restricted diets. Sample sizes (n) are shown in the figure. For the statistics, a log-rank
394 test was used. **d,** Intestinal stem cell proliferation indicated by phospho-histone H3 positive cells

in the guts of female Canton-S flies. The flies were fed the synthetic diet with or without zinc for one or five weeks. n = 18 (1-week Ctrl and 5-week ZnR) or 20 (1-week ZnR and 5-week Ctrl). For the statistics, one-way ANOVA with Holm-Šídák's multiple comparison test was used. For the graph, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.



401 **Fig. 2 Internal levels of metal ions during ageing and dietary restriction.**

402 **a-d,** Internal levels of zinc (**a**), copper (**b**), iron (**c**), and manganese (**d**) in female Canton-S flies at
403 day 5 and 42 reared on a standard yeast-based diet. n = 3. For the statistics, a two-tailed Student's
404 *t* test was used. **e,** Internal levels of zinc in female Canton-S flies fed the synthetic diet with or
405 without zinc. n = 3. For the statistics, a two-tailed Student's *t* test was used.

406

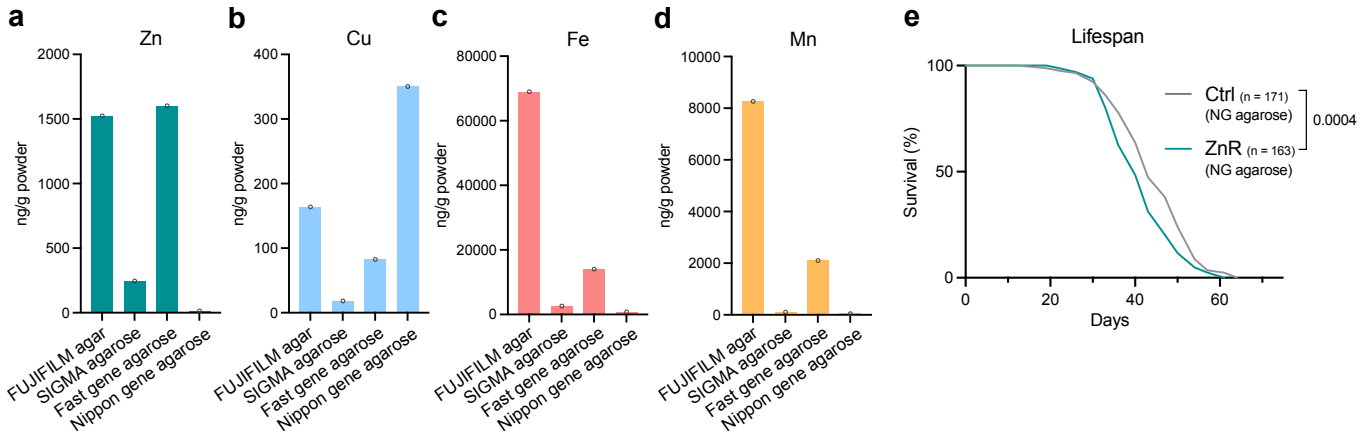


Fig. 3 Zinc restriction with a minimum zinc contamination shortens lifespan.

a-d, The content of zinc (**a**), copper (**b**), iron (**c**), and manganese (**d**) in the agar or agarose powder. n = 1. **e,** Lifespans of female Canton-S flies fed with or without a zinc-restricted diet using Nippon gene agarose instead of FUJIFILM agar. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used.

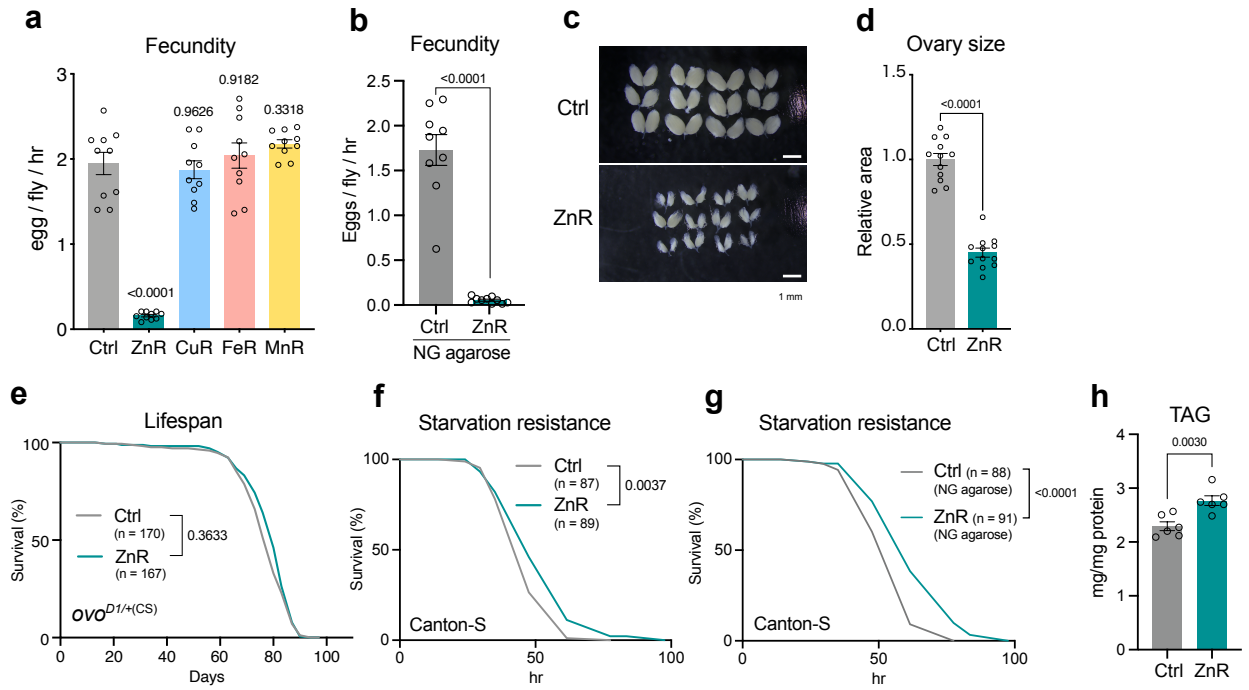


Fig. 4 Zinc restriction mimics amino acid restriction phenotypes.

a, Fecundity of Canton-S flies fed the synthetic diet with or without metal restrictions. n = 10. For the statistics, one-way ANOVA with Dunnett's multiple comparison test was used. **b,** Fecundity of Canton-S flies fed with or without a zinc-restricted diet using Nippon gene agarose instead of FUJIFILM agar. n = 9 (Ctrl) or 10 (ZnR). For the statistics, a two-tailed Student's *t* test was used. **c,d,** Representative images (**c**) and the quantification (**d**) of ovaries of Canton-S flies fed with or without a zinc-restricted diet. n = 12. For the statistics, a two-tailed Student's *t* test was used. Scale bars: 1 mm. **e,** Lifespans of female $ovo^{D1/+}$ flies fed with or without a zinc-restricted diet. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **f,** Survivability of female Canton-S flies upon complete starvation after feeding with or without a zinc-restricted diet for one week. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **g,** Survivability of female Canton-S flies upon complete starvation after feeding with or without a zinc-restricted diet using Nippon gene agarose instead of FUJIFILM agar for one week. Sample sizes (n) are shown in the figure. For the statistics, a log-rank test was used. **h,** The level of triacylglycerol in the female Canton-S flies fed with or without a zinc-restricted diet. For the statistics, a two-tailed Student's *t* test was used. For all graphs, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.

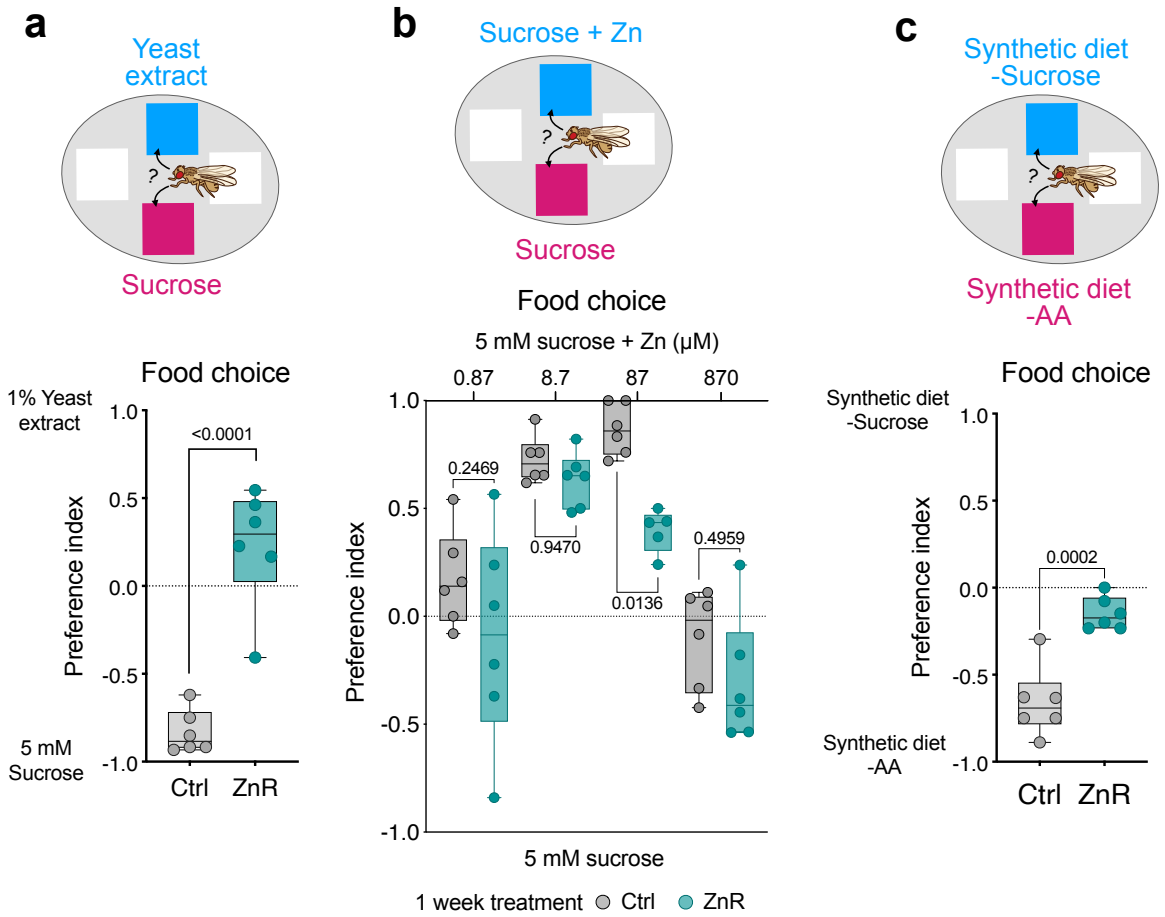


Fig. 5 Zinc restriction increases food preference towards yeast rather than sugar.

a, Food preference towards yeast extract compared to sucrose in female Canton-S flies fed with or without a zinc-restricted diet for 1 week. n = 6. For the statistics, a two-tailed Student's *t* test was used. b**,** Food preference towards zinc-supplemented sucrose compared to mere sucrose in female Canton-S flies fed with or without a zinc-restricted diet for 1 week. n = 6. For the statistics, one-way ANOVA with Holm-Šídák's multiple comparison test was used. **c,** Food preference towards sucrose depleted holidic medium compared to AA depleted holidic medium in female Canton-S flies fed with or without a zinc-restricted diet for 1 week. n = 6. For the statistics, a two-tailed Student's *t* test was used. For all graphs, the mean and SEM are shown. Data points indicate biological replicates. Source data are provided as a Source Data file.

443

444 Table 1. Metal ion content in fly diets.

Metal ion	Yeast based diet (μM)	Synthetic diet (μM)
Zn	101	86.9
Cu	2.90	10.0
Fe	41.8	89.9
Mn	8.26	5.05

445