## Supplementary Figure legends

**Figure S1. Model illustrating the effects of nutrition on lifespan and fecundity in *Drosophila*.** DR flies are long-lived with low fecundity, which is reversed with yeast or EAA addition. Addition of methionine alone to DR increased fecundity without shortening lifespan. Further addition of the remaining nine EAAs to DR + methionine caused lifespan shortening without any further increase in fecundity. Thus fecundity and lifespan are altered by different nutrients: the former by methionine alone and the latter through a combination of methionine and other EAAs.

**Figure S2. The life shortening effect of amino acids is not caused by differences in pH, osmolarity or salt stress.** Addition of all 20 amino acids used for protein synthesis (All AA) to DR food significantly shortened lifespan ($P$<0.0001). This effect could not be accounted for by changes in pH or osmolarity as these differed only slightly from DR. Additional osmolarity data from amino acid additions presented in Fig. 4b (DR+EAA, DR+EAA-met and DR+EAA-trp) further demonstrate there is no correlation between osmolarity and lifespan. Furthermore, increased availability of free water did not rescue the life-shortening effect of amino acids, even when added at twice the concentration indicated in Table S1 (DR+2xAll AA v DR+2xAll AA+water, $P$=0.37). In contrast, additional water rescued the life-shortening effect of 0.8% salt addition to the food (DR v DR+salt+water, $P$=0.16), demonstrating the efficacy of this method of water supply. Survivorship curves were compared using the log rank test.

**Figure S3. Essential amino acids limit egg laying in DR food.** Increasing the concentration of EAAs added to DR caused a dose-dependent increase in fecundity (DR v DR+22mM EAA, $P$=0.000043; DR+22mM EAA v DR+43mM EAA, $P$=0.015), indicating they are the only nutrients limiting for egg laying in yeast. Furthermore, under DR conditions, all other nutrients are in sufficient excess that additional EAAs can increase egg laying beyond the level found with full feeding. Accompanying the fecundity increase was a progressive decreased in lifespan (DR v DR+22mM EAA, $P$=0.014; DR+22mM EAA v DR+43mM EAA, $P$<0.0001). Error bars represent ± s.e.m.

Egg laying data were compared using the non-parametric Wilcoxon rank sum test and survivorship using the log rank test.

**Figure S4. Effect on lifespan and fecundity of individually adding the essential amino acids to DR food.** Supplementation of the DR diet with each essential amino acid (at twice the concentration of additions made in Table S1) had no effect on fecundity with the exception of methionine that caused an increase ($P$=0.00001) to the same level as the fully fed condition ($P$=0.28). This effect was specifically due to methionine limitation and could not be accounted for by a general increase in biologically available nitrogen caused by amino acid addition (Table S1). No single amino acid addition had an effect on DR lifespan ($P$>0.05 for all single amino acid additions v DR). Error bars represent ± s.e.m. Egg laying data were compared using the non-parametric Wilcoxon rank sum test and survivorship using the log rank test.

**Figure S5. No difference in hatchability of eggs laid on different add-back media.** After 48 h habituation to the media indicated, mated 4-day-old adult females were allowed to lay eggs for a period of up to 5 hours. The unhatched eggs in these vials were counted immediately at the end of the laying period and then again after 30h incubation at 25C. There were no significant differences amongst groups ($P$=0.70, chi-squared test). The data show the total proportion from 10 replicate vials for each condition; the total number of eggs scored is indicated above each bar. Error bars represent ± s.e.m.

**Figure S6. Adding amino acids to DR food does not alter fly feeding behaviour or rate of food uptake.** A behavioural assay [2] was employed to measure possible differences in feeding when flies were fed altered foods. This assay has two parts: 1) a calibration step that measures the relationship between observed feeding behaviour and the quantity of food eaten, and; 2) steady-state feeding observations of flies. (a) Calibration: we found a strong positive linear correlation between the volume of dyed food consumed and the proportion of feeding events observed ($P$<0.001, linear mixed effects model) that was unchanged by addition of methionine to the food ($P$=0.855, linear mixed effects model). Thus, the actual food consumption by these flies is accurately reflected by observations of feeding behaviour. (b & c) During observations of steady-state feeding behaviour, no differences were observed between flies feeding on DR, DR+met, DR+EAA or fully-fed at any time point measured ($P$>0.32 in all comparisons, generalised linear model). Panels (b) and (c) show independent replicate experiments carried out by different observers. Consequently under these conditions, flies spent the same time feeding on each of the food types and thus ingested similar amounts of food. Error bars represent ± s.e.m.

**Figure S7. Methionine addition to DR food rescues fecundity to the level of full feeding but has no effect on lifespan.** (a) Methionine addition to DR food up to 13mM did not increase fecundity more than the fully-fed condition, indicating another nutrient became limiting for egg laying at 0.7mM methionine addition (Fig. 3c). (b & c) Lifespan was not altered by any level of methionine addition to DR food ($P$>0.18 in all comparisons, log-rank test). Lifespan data in panel (c) is for flies from Fig. 3c. Error bars represent ± s.e.m.

**Figure S8. Addition of essential amino acids to DR food can raise fecundity beyond that in fully-fed conditions given sufficient methionine in the diet.** Egg laying in DR food was limited by methionine until 0.7mM addition, beyond which there was no further increase in egg laying. To test if this is because another amino acid became limiting, we added 6.5mM methionine to DR food (excess) and increased the concentration of the remaining 9 essential amino acids. Egg laying progressively increased with additions of 43mM and 82mM ($P$=0.00288 and $P$=0.00105, from DR+6.5mM met to DR+6.5mM met+43mM EAA to DR+6.5mM met+86mM EAA) but no further increase to 168 mM ($P$=0.1903). (b) Lifespan decreased with each increasing concentration of EAA to DR+6.5mm met ($P$<0.0005 between each increasing in EAA addition). Error bars represent ± s.e.m. Egg laying data were compared using the non-parametric Wilcoxon rank sum test and survivorship using the log rank test.

**Figure S9. Methionine increases fecundity with no effect on lifespan when alternative DR diet is used.** To test if the effect of methionine addition to DR was a curiosity of our DR protocol, we made the food using another yeast that has been extensively used in the literature (described in [1]). Addition of 0.7mM methionine to DR food caused fecundity to increase (*P*=0.0147) and match that of the fully fed condition (*P*=0.436). Moreover, addition of methionine did not alter lifespan from DR (*P*=0.279) while the fully-fed condition was shorter lived (*P*<0.0002 in both comparisons). Error bars represent ± s.e.m. Egg laying data were compared using the non-parametric Wilcoxon rank sum test and survivorship using the log rank test.

**Figure S10. Insulin receptor mutants have extended lifespan and are insensitive to the fecundity and lifespan effects of amino acid additions.** (a) Survivorship data for Fig. 4c. (b) Median lifespan (connected points) and lifetime reproductive success (bars) of each genotype on different foods. Ubiquitous overexpression of a dominant negative form of the *Drosophila* insulin receptor (daGal4->UAS InRDN) extended lifespan under DR conditions (*P*<0.0001 compared with either control). Lifespan was unchanged by methionine addition, but unlike controls, was only marginally reduced with addition of EAA to DR (*P*<0.0001; 9% reduction in median v > 41% reduction for controls). Similarly, the effect of amino acid addition on fecundity was reduced by the InRDN mutation. Methionine addition had no effect on fecundity in the mutant (*P*=0.4813), whereas it did in controls (*P*<0.0001) and EAA addition caused only a 19% increase in egg laying in the mutant (*P*=0.0029), whereas the increase was 62% or greater for controls (*P*<0.0001). (c) Survivorship curves for medians shown in panel (b). Panels (b) and (c) show an independent biological replicate of Fig. 4c. Error bars represent ± s.e.m. Egg laying data were compared using the non-parametric Wilcoxon rank sum test and survivorship using the log rank test.

**Table S1.** Constituents and concentrations of add-back solutions

| Add-back group | Nutrients used for add-back solution | Concentration in add-back media ($g.l^{-1}$) | Biologically available nitrogen (mM)[1] |
|---|---|---|---|
| Essential amino-acids (EAA)[2] | L-arginine | 0.43 | 4.1 |
| | L-histidine | 0.21 | 2.7 |
| | L-isoleucine | 0.34 | 2.6 |
| | L-leucine | 0.48 | 3.7 |
| | L-lysine | 0.52 | 2.8 |
| | L-methionine | 0.1 | 0.7 |
| | L-phenylalanine | 0.26 | 1.6 |
| | L-threonine | 0.37 | 3.1 |
| | L-tryptophan | 0.09 | 0.4 |
| | L-valine | 0.4 | 3.4 |
| | | | Total = 25.1mM |
| Non-essential amino acids (N-EAA) | L-alanine | 0.4 | 4.5 |
| | L-asparagine | 0.27 | 4.1 |
| | L-aspartate | 0.27 | 1.7 |
| | L-cysteine[3] | 0.01 | 0.06 |
| | L-glutamate | 0.42 | 2.9 |
| | L-glutamine | 0.42 | 5.8 |
| | glycine | 0.34 | 3.2 |
| | L-proline | 0.2 | 1.7 |
| | L-serine | 0.29 | 2.8 |
| | L-tyrosine | 0.12 | 0.7 |
| | | | Total = 27.3mM |
| Carbohydrates[4] | Lactose | 3.9 | |
| | Sucrose | 0.5 | |
| | Glycogen | 4.7 | |
| | Trehalose | 3.1 | |
| Vitamins[5] | Biotin | 0.00004 | |
| | Pantothenate | 0.004 | |
| | Nicotinic acid | 0.01 | |
| | Pyridoxine | 0.002 | |
| | Riboflavin | 0.002 | |
| | Thiamine | 0.005 | |
| | Folate | 0.0005 | |
| Lipids[6] | Phosphatidyl-choline | 0.1 | |

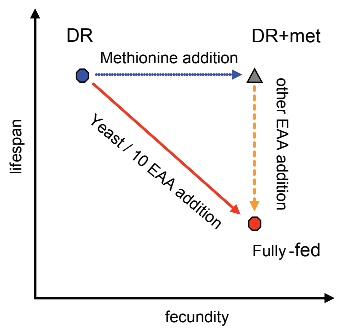
[1] refers to nitrogen available upon complete catabolism. Arg, His, Asn and Gln can each donate 2 moles of N for each mole of amino acid catabolised.
[2] amino acids added from 10x stock made in water
[3] scaled concentration range estimate 0.01 – 0.1 $g.l^{-1}$
[4] added from 5x stock made in water
[5] added from 100x stock, except folate which was added from a separate 1000x stock
[6] added from 100x stock made in ethanol

Reference List

[1] T. M. Bass, *et al.*, "Optimization of dietary restriction protocols in *Drosophila*," J. Gerontol. A Biol. Sci. Med. Sci. **6**, 1071 (2007).

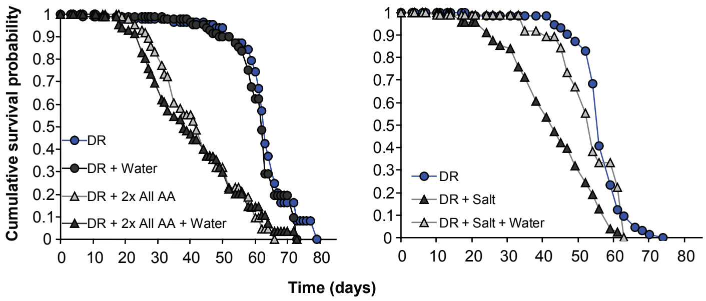
[2] R. Wong, *et al.*, "Quantification of food intake in Drosophila," PLoS One. **4**(6), e6063 (2009).

# Figure S1_Grandison et al



# Figure S2_Grandison et al

| Food | mOsM | pH |
|---|---|---|
| DR | 446 | 4.6 |
| DR+ALL AA | 495 | 4.65 |
| Fully Fed | 1081 | 4.9 |
| DR+EAA | 472 | |
| DR+EAA-met | 471 | |
| DR+EAA-trp | 472 | |



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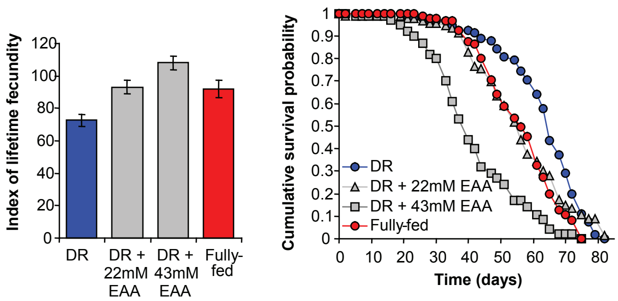

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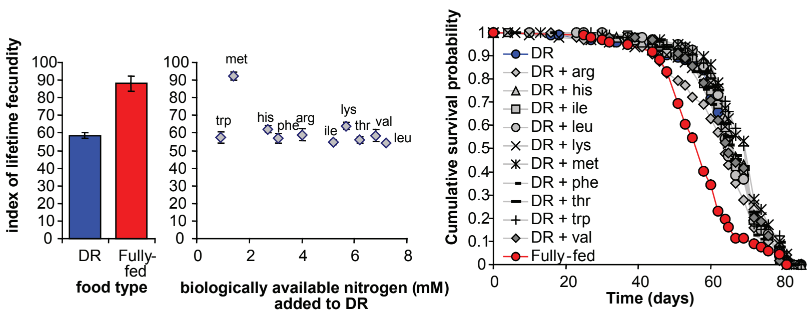
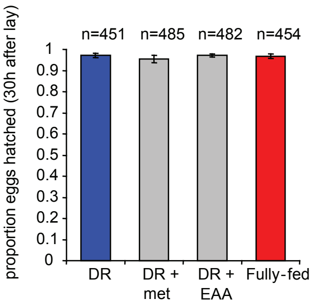


**Figure S5_Grandison et al**



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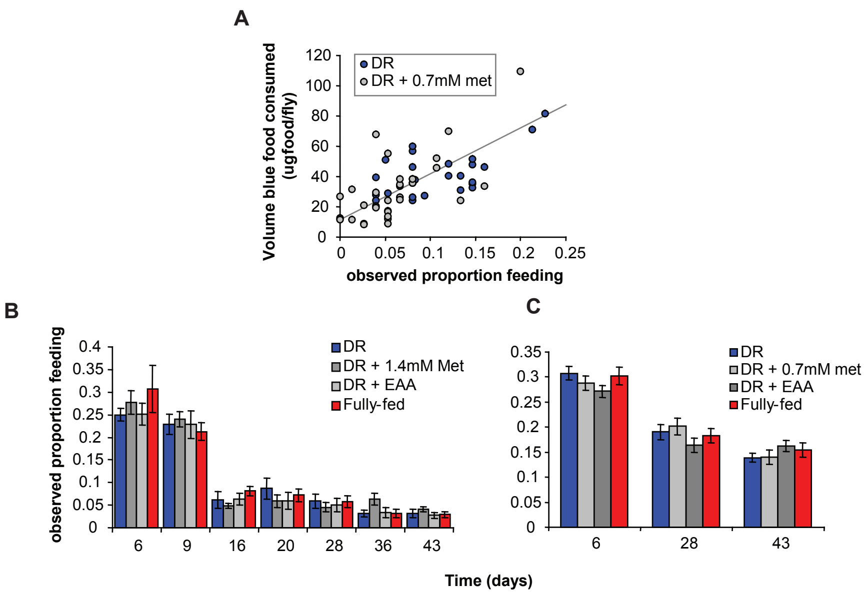

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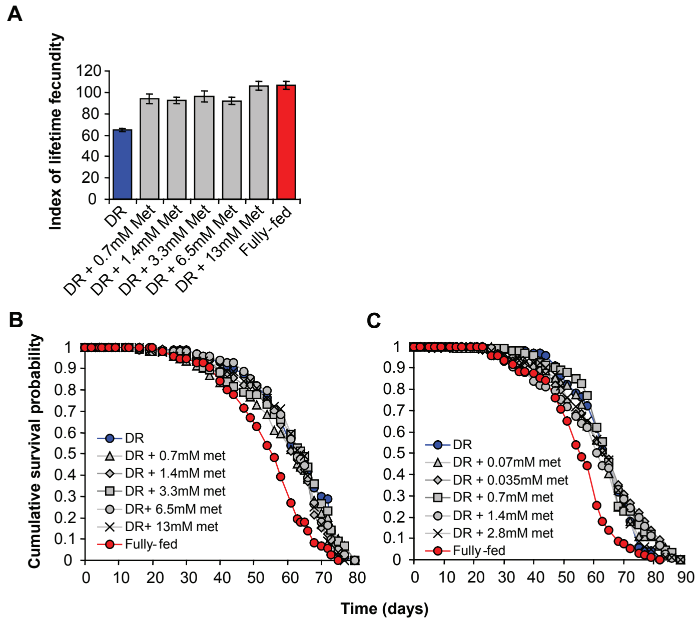

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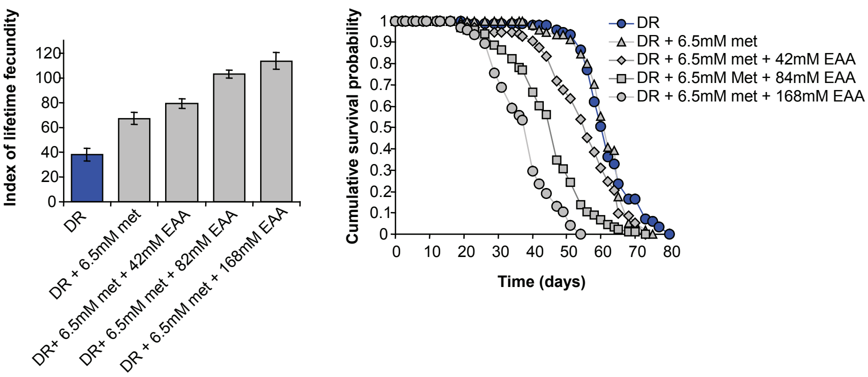

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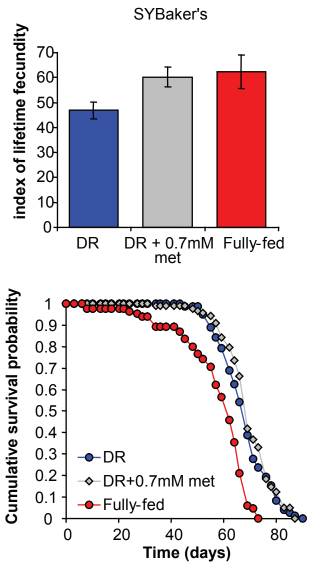

# Figure S10_Grandison et al