Supporting Information

Ja et al. 10.1073/pnas.0908016106

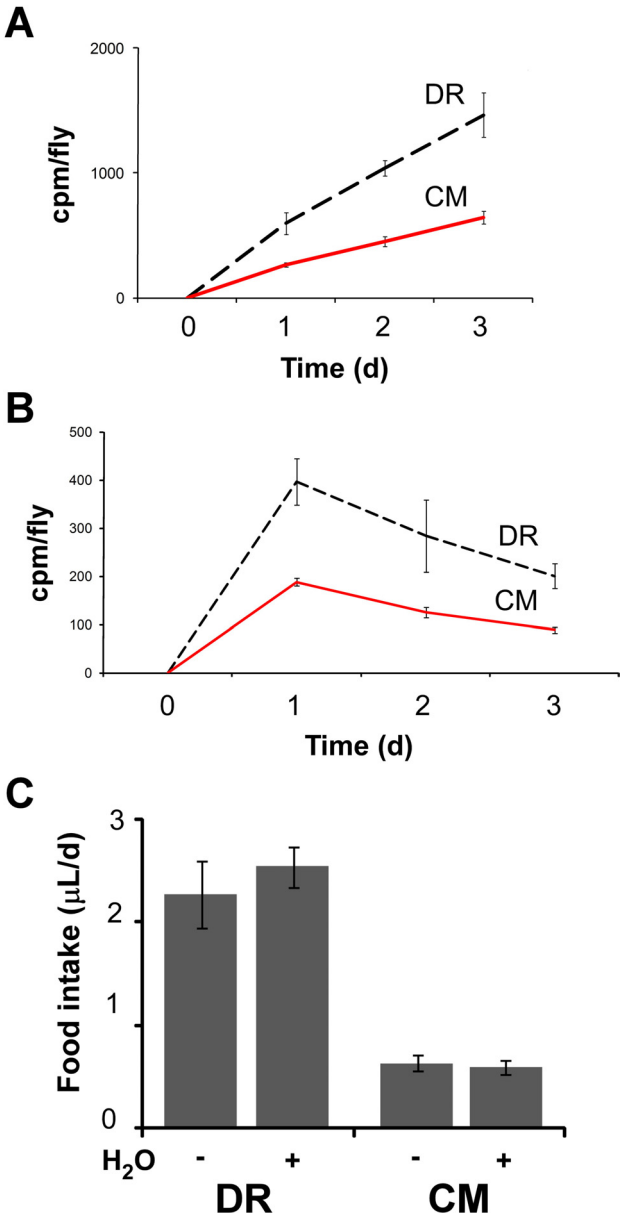

Fig. S1. Food dilution elicits compensatory feeding as measured by radiolabeling the medium (1–3) or by using the Capillary Feeder (CAFE) assay (4). (*A*) Time-course of isotope accumulation for flies fed yeast extract/sucrose (YE/S: CM = 5% YE + 5% S; DR = 1% YE + 1% S) show near-linear accumulation rates of differing slope, with neither curve reaching saturation over the trial period (72 h). (*B*) Radioactive pulse-chase of flies fed isotope-labeled food for 24 h and then transferred to nonlabeled medium of identical composition. Isotope levels were assayed at three time points: 24 h (immediately before transfer), 48 h, and 72 h. Isotope levels in flies fed diluted food show a faster rate of decline, ruling out that impaired excretion or metabolization of the label underlies the observed differences. Results in *A* and *B* are expressed as an average (± SD) of 3–4 trials, each containing 15 Canton-S males. (*C*) Food consumption measured in the CAFE (4) using YE/S (CM = 10% YE + 10% S; DR = 2.5% YE + 2.5% S) is consistent with radiolabeling results (Fig. 1*B*). The presence of an ad libitum water source in the CAFE chamber did not affect feeding ($P > 0.05$, Student's *t* test). Each time point is expressed as an average (± SD) of four trials, each containing three Canton-S males.

1. Ayaki T, Oshima K, Yoshikawa I (1985) Linear relationship between lethal mutation yield and intake of ethyl methanesulfonate in *Drosophila melanogaster. Environ Mutagen* 7:147–153.
2. Carvalho GB, Kapahi P, Anderson DJ, Benzer S (2006) Allocrine modulation of feeding behavior by the sex peptide of *Drosophila. Curr Biol* 16:692–696.
3. Thompson ED, Reeder BA, Bruce RD (1991) Characterization of a method for quantitating food consumption for mutation assays in *Drosophila. Environ Mol Mutagen* 18:14–21.
4. Ja WW, et al. (2007) Prandiology of *Drosophila* and the CAFE assay. *Proc Natl Acad Sci USA* 104:8253–8256.

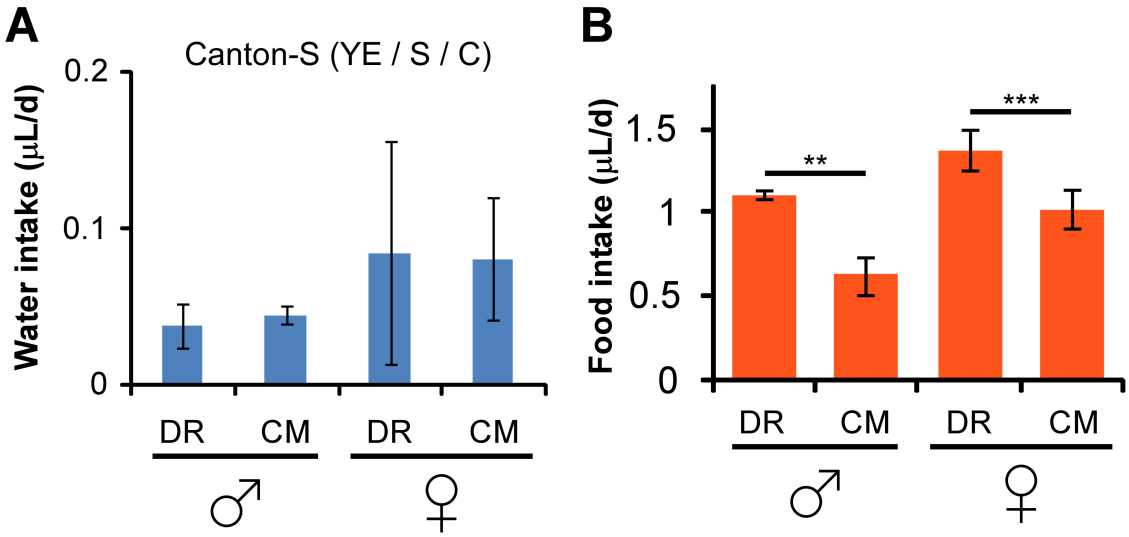

Fig. S2. Food and water intake of Canton-S males on yeast extract/sucrose/cornmeal (YE/S/C) as assayed by isotope radiolabeling (2). (*A*) Water intake per fly over 24 h. Drinking is minimal on both high (CM) and low (DR) yeast concentrations of YE/S/C. (*B*) Food intake per fly over 24 h. Results are expressed as an average (± SD) of 2–6 trials, each containing 6–16 flies. Medium composition is described in Fig. 3. Statistical significance was determined by nonpaired, two-tailed Student's *t* tests between DR and CM media: **, $P < 0.01$; ***, $P < 0.001$.

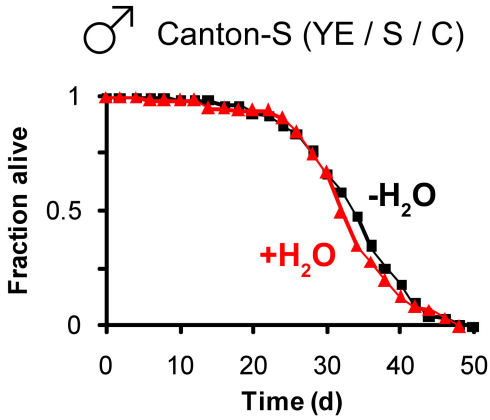

Fig. S3. Water supplementation does not affect the lifespan of Canton-S males on high-yeast (CM) YE/S/C medium. Flies were aged in vials. Medium composition is described in Fig. 3. $n = 87$ flies, -H_2O; 86 flies, +H_2O; $P = 0.40$, log-rank test.

Table S1. Statistics of Cox proportional hazards analysis for the effect of diet on survival in the presence or absence of water

	Effect	df	χ^2	p	Hazard ratio*	Lower CI	Upper CI
YE/S							
Canton-S males	DR	1	10.36	0.0013	1.728	1.244	2.401
	DR+H_2O	1	0.18	0.6694	1.059	0.815	1.376
BY/S							
Canton-S females	DR	1	16.14	0.0001	1.693	1.304	2.199
	DR+H_2O	1	2.55	0.1101	1.284	0.944	1.746
Dahomey females	DR	1	5.32	0.0211	1.450	1.059	1.986
	DR+H_2O	1	0.61	0.4269	0.879	0.640	1.208
YE/S/C							
Canton-S males	DR	1	43.65	<0.0000	3.920	2.569	5.980
	DR+H_2O	1	28.94	<0.0000	2.703	1.871	3.904
Canton-S females	DR	1	23.86	<0.0000	2.478	1.706	3.600
	DR+H_2O	1	30.65	<0.0000	3.188	2.084	4.877

*When the hazard ratio is close to 1, DR has little effect on survival.

Table S2. Dietary restriction sample sizes and lifespan statistics

Flies	Housing	Medium	Conc.	Water access	No. of flies	Mean lifespan, days	Mean DR effect, %	Max lifespan,* days	Max DR effect, %
Canton-S males	Cages	YE/S	CM		68	21.4		29	
(Fig. 2)			DR		81	25.2	18	31	7
			CM	Yes	103	27.4		35	
			DR	Yes	129	27.5	0	35	0
Canton-S males	Cages	BY/S	CM		156	32.1		39	
(Fig. 2)			DR		94	37.0	15	45	15
			CM	Yes	91	39.5		51	
			DR	Yes	79	40.2	2	53	4
Canton-S females	Cages	BY/S	CM		152	29.9		42	
			DR		153	33.6	13	47	12
			CM	Yes	163	38.3		46	
			DR	Yes	153	38.7	1	49	7
Dahomey females	Vials	BY/S	CM		78	53.3		68	
(Fig. 2)			DR		81	59.6	12	74	9
			CM	Yes	78	57.9		72	
			DR	Yes	79	57.6	−1	68	−6
Canton-S males	Cages	YE/S/C	CM		75	42.3		59	
(Fig. 3)			DR		60	56.0	32	73	24
			CM	Yes	77	46.0		63	
			DR	Yes	68	56.9	24	71	13
Canton-S females	Cages	YE/S/C	CM		86	44.7		57	
(Fig. 3)			DR		66	50.1	12	70	23
			CM	Yes	70	42.5		54	
			DR	Yes	61	51.5	21	71	31
Canton-S males	Vials	YE/S/C	CM		86	32.9		42	
(Fig. S3)			DR		87	41.7	27	54	29
			CM	Yes	85	32.0		41	
			DR	Yes	88	42.6	33	53	29

*90th percentile.