A holidic medium for *Drosophila melanogaster*

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A critical requirement for research using model organisms is a well-defined and consistent diet. There is currently no complete chemically defined (holidic) diet available for *Drosophila melanogaster*. We describe a holidic medium that is equal in performance to an oligidic diet optimized for adult fecundity and lifespan. This holidic diet supports development over multiple generations but at a reduced rate. Over 7 years of experiments, the holidic diet yielded more consistent experimental outcomes than did oligidic food for egg laying by females. Nutrients and drugs were more available to flies in holidic medium and, similar to dietary restriction on oligidic food, amino acid dilution increased fly lifespan. We used this holidic medium to investigate amino acid–specific effects on food-choice behavior and report that folic acid from the microbiota is sufficient for *Drosophila* development.

In nature, *Drosophila melanogaster* feeds on fermenting fruit[1]. Fruit flies gain the majority of their nutrition from ingested microbes and experience relatively high concentrations of ethanol and organic acids[2]. Thus, a simple diet of sucrose and lyophilized yeast, with a weak organic acid as preservative, is sufficient to support *Drosophila* in the laboratory[3,4]. However, *D. melanogaster* is raised on a variety of food recipes, which contain some combination of molasses, treacle, beet syrup, rolled oats, banana, potato starch, cornmeal, corn syrup, malt, soy, one of several monosaccharides or disaccharides and one of a variety of processed dead or fresh live yeasts[5]. At worst, these are reported simply as 'standard' medium. More often, a recipe is given but without the method of preparation or the nature and source of its components. Such nutritional variations could explain many inconsistent experimental outcomes between laboratories.

Early work to specify the nutritional requirements of *Drosophila*[4,6–8] has been more recently extended to include semi-defined (meridic) media for longevity studies[9–12]. However, the nonpurified ingredients in these media can vary substantially from batch to batch. We presently lack a defined synthetic medium for *Drosophila* made entirely from purified ingredients (holidic) that is adequate to support development, egg laying in adults and normal lifespan.

Here we describe such a medium and demonstrate that it is sufficient for development of fruit flies, albeit at a reduced rate. When adult flies are maintained on the diet, they are phenotypically similar to those kept on a natural yeast-based (oligidic) diet. We show that holidic medium greatly improved oral drug delivery to flies and also yielded more stable egg-laying data between trials compared to oligidic media, which could potentially improve interlaboratory comparability. Finally, this medium offers the opportunity to investigate the effects of subtle nutrient manipulations that could not be achieved otherwise. In particular, we found that egg-associated microbes inherited from the mother could overcome a deficiency in dietary folic acid during development. We propose that this holidic medium should serve as the starting point for studies requiring a precisely controlled nutritional context.

RESULTS

A holidic medium for adult *Drosophila*

During the last 7 years of experimental work on *Drosophila* lifespan in our laboratory using the same experimental conditions, we observed that lifespan has varied over a more than 25-d range (**Fig. 1a**). To minimize the nutritional variability arising from changes to natural ingredients, such as yeast, we developed a holidic medium suitable for *Drosophila* development, egg laying in adults, lifespan and behavior.

The key features of our holidic medium that differentiate it from previously reported diets[4,6–12] are: first, the medium has an acetate buffer base, which we adjusted to pH 4–4.5, rather than neutral, to match the pH of natural food sources[13] and of our laboratory sugar, yeast medium (SY)[14], and second, it contains only purified ingredients (**Table 1**) to substitute for the nondefined components casein, lecithin and RNA extracts. Initially, we adopted proportions of nutrients identified as optimal in refs. 7,15 (referred to as HUNTaa). In later experiments, we found that the diet was improved by modifying the amino acid mixture to

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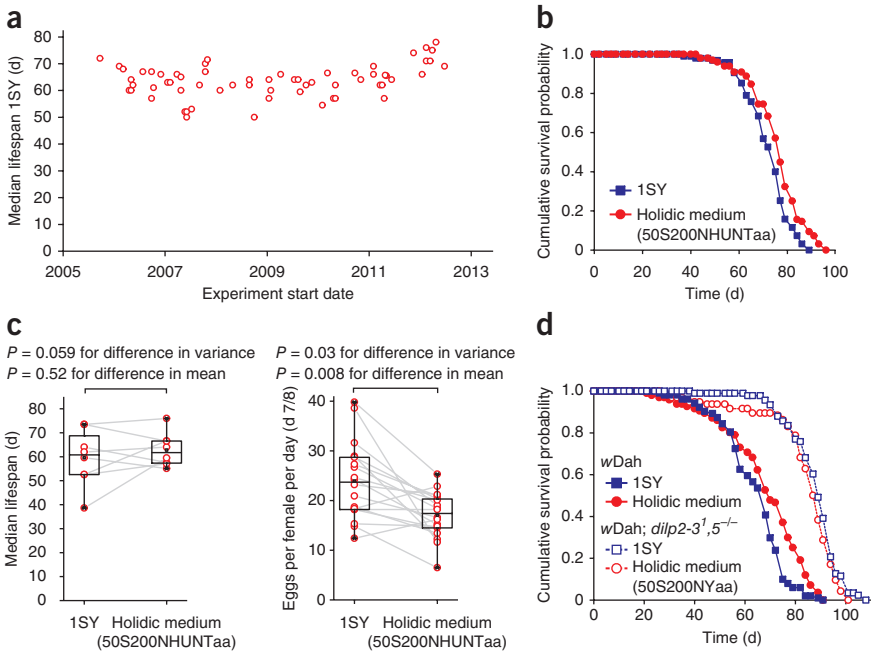

Figure 1 | A holidic diet for adult *Drosophila*. (**a**) *Drosophila* median lifespans on an oligidic diet (n = 65 biological replicates). (**b**) Adult lifespan of flies on holidic and oligidic diets. (**c**) Fitness traits of flies on holidic and oligidic diets, for data collected May 2006 to November 2011. Average median lifespan (left, n = 7 biological replicates) and egg laying (right, n = 17 biological replicates) are shown. Lines connect cohorts in the same experiment. F test and Wilcoxon rank-sum test were used to assess differences in variance and mean, respectively, between median lifespans. (**d**) Lifespan of insulin-mutant white Dahomey (*w*Dah); *Df(3L)ilp2-3*^{1}, *ilp5* (*w*Dah; *dilp2-3*^{1},*5*$^{-/-}$) and control (*w*Dah) flies on holidic and oligidic diets (n = 100 flies per condition). Data are pooled from one experiment (**a**,**d**), representative of seven trials (**b**), and pooled from seven trials (left, **c**) and 17 trials (right, **c**).

make it more similar to that found in yeast (referred to as Yaa).

We found that holidic medium containing 50 mM sucrose and 200 mM biologically available nitrogen (50S200N HUNTaa; Online Methods and **Supplementary Table 1**) was adequate to support lifespan to a similar extent as our longevity-promoting oligidic diet (**Fig. 1b**). Median lifespan between trials showed a trend toward greater stability on holidic medium than on SY food, but this did not reach statistical significance (**Fig. 1c**). Egg-laying showed significantly less variation between replicate experiments (P = 0.03, n = 17 biological replicates, F test) but at ~25% lower level than on 1SY (**Fig. 1c**). After we adjusted the proportion of amino acids to match that in yeast (Yaa), both lifespan and egg laying matched those on 1SY (**Supplementary Fig. 1**). We also assayed the lifespan of long-lived flies lacking three of the seven insulin-like peptides (encoded by *dilp2* (*Ilp2*), *dilp3* (*Ilp3*) and *dilp5* (*Ilp5*))[16] on both SY and holidic diets. Mutant flies were ~30% longer-lived than controls on both media (P < 0.0001, n = 100 flies per condition, Cox proportional hazard; **Fig. 1d**).

Compared with flies fed medium containing all nutrients, those fed medium without cholesterol, vitamins, amino acids or only the single essential amino acid arginine or isoleucine had lifespan shortened by 30–70% and egg laying decreased by at least 30% (**Fig. 2a**). Omission of sugar did not affect egg laying but reduced lifespan (**Fig. 2a**), similar to the effect of removing sugar from yeast-based foods[3], whereas a medium lacking all nonessential amino acids except for glutamate, which we provided to replace the nitrogen lost by omitting nonessential amino acids, had no detrimental effect on lifespan or egg laying (**Fig. 2a**). Finally, we found that a medium containing only the ions and salts considered essential in ref. 7 (K, P, Mg and Na) resulted in flies that were no shorter-lived than on a complete medium, but with a 50% reduction in lifetime egg laying (**Fig. 2a**). Addition of Ca, Cu, Fe, Mn and Zn was required to establish normal egg laying after the first week of adult life (**Supplementary Fig. 2**). Thus, each of the major nutrient groups in the holidic diet, except for the nonessential amino acids, was required for full egg laying and lifespan.

To ensure that the flies were acquiring their nutrition directly from the holidic medium rather than from microbes growing on it, we tested the effect of replacing folic acid with equimolar *para*-aminobenzoic acid (PABA), which microbes, but not flies, can readily convert to folate. Irrespective of the presence of PABA, absence of folic acid reduced both lifespan (P < 0.016 in both comparisons, n = 100 flies per condition, log-rank test) and lifetime egg laying (P < 0.002, n = 10 technical replicates, Wilcoxon rank-sum test) of the flies (**Fig. 2b**).

Dietary amino acids determine egg laying and lifespan

Fly lifespan and fecundity are both altered by changes in the balance of sugar and yeast in SY food, where yeast is the flies' only source of protein[17–19]. The holidic medium allowed us to dissect this by testing the effect of a concentration range of both sugar and amino acids (**Supplementary Fig. 3**). Increasing the sugar:amino acid ratio caused both lifespan and egg laying to decline, whereas decreasing this ratio caused egg laying to rise and lifespan to decline (**Supplementary Fig. 3**). We standardized sucrose at 50 mM (50S) because this led to significantly longer lifespan than did 0 mM sucrose (P < 0.0001, n = 100 flies per condition, log-rank test), and any additional increase in the amount of sucrose reduced egg laying (P < 0.001 for all comparisons of 50 mM sucrose to higher doses, n = 10 technical replicates, Wilcoxon rank-sum test). Adding amino acids at 200 mM biologically available nitrogen (200N HUNTaa; Online Methods) produced both long lifespan and relatively high egg laying. Further amino acid addition caused egg laying to increase and lifespan to decrease. This inverse relationship between lifespan and egg laying with dietary restriction is also observed by changing amounts of either yeast[17] (**Supplementary Fig. 3**) or amino acids[14] in SY food.

Holidic medium to study behavior

We assessed the activity and sleep levels of flies kept on holidic medium. During a 72-h period, there was no difference in the activity patterns of flies between the two diets, whether assessed in real time or as the cumulative time spent active or sleeping (**Fig. 3a**,**b** and **Supplementary Fig. 4**). These data establish a baseline for using the medium to examine the effects of specific nutrients on

Table 1 | Composition of holidic medium

	Ingredient	Stock	Amount per liter	Notes	Manufacturer, example order number
Gelling agent	Agar		20 g		Difco, 214530
Base	Buffer	10×: 30 ml/l glacial acetic acid 30 g/l KH_2PO_4 10 g/l $NaHCO_3$[a]	100 ml		Fisher, A/0400/PB15 Sigma, P9791 Sigma, S8875
Sugar	Sucrose		17.12 g		Sigma, S1888
Amino acids	L-isoleucine L-leucine L-tyrosine		1.82 g 1.21 g 0.42 g	Amounts for HUNTaa	Sigma, I2752 Sigma, L8912 Sigma, T3754
Metal ions	$CaCl_2 \cdot 6H_2O$ $CuSO_4 \cdot 5H_2O$ $FeSO_4 \cdot 7H_2O$ $MgSO_4$ (anhydrous) $MnCl_2 \cdot 4H_2O$ $ZnSO_4 \cdot 7H_2O$	1,000×: 250 g/l 1,000×: 2.5g/l 1,000×: 25 g/l 1,000×: 250 g/l 1,000×: 1 g/l 1,000×: 25 g/l	1 ml 1 ml 1 ml 1 ml 1 ml 1 ml		Sigma, C7902 Sigma, C7631 Sigma, F7002 Sigma, M7506 Sigma, M3634 Sigma, Z0251
Cholesterol	Cholesterol Water (milliQ)	20 mg/ml in EtOH	5 ml or 15 ml[b] 1 l minus combined volume to be added after autoclaving		Sigma, C8667
Autoclave 15 min at 120 °C along with glassware (vials) for experiment and tubing for pump. All additions below should be performed using sterile technique.					
Amino acids	Essential amino acid stock solution	8 g/l L-arginine 10 g/l L-histidine 19 g/l L-lysine(HCl) 8 g/l L-methionine 13 g/l L-phenylalanine 20 g/l L-threonine 5 g/l L-tryptophan 28 g/l L-valine	60.51 ml[c]	Amounts for HUNTaa	Sigma, A5131 Sigma, H8000 Sigma, L5626 Sigma, M9625 Sigma, P2126 Sigma, T8625 Sigma, T0254 Sigma, V0500
	Nonessential amino acid stock solution	35 g/l L-alanine 17 g/l L-asparagine 17 g/l L-aspartic acid 1 g/l L-cysteine HCl 25 g/l L-glutamine 32 g/l glycine 15 g/l L-proline 19 g/l L-serine	60.51 ml	Amounts for HUNTaa	Sigma, A7627 Sigma, A0884 Sigma, A6683 Sigma, C1276 Sigma, G3126 Sigma, G7126 Sigma, P0380 Sigma, S4500
	Sodium glutamate stock solution	100 g/l sodium glutamate	15.13 ml	Amount for HUNTaa	Sigma, G5889
Vitamins	Vitamin solution	125×: 0.1 g/l thiamine (aneurin) 0.05 g/l riboflavin 0.6 g/l nicotinic acid 0.775 g/l Ca pantothenate 0.125 g/l pyridoxine (HCl) 0.01 g/l biotin	14 ml[d]		Sigma, T4625 Sigma, R4500 Sigma, N4126 Sigma, P2250 Sigma, P9755 Sigma, B4501
	Sodium folate[e]	1,000×: 0.5 g/l	1 ml		Sigma, F7876
Other nutrients		125×: 6.25 g/l choline chloride 0.63 g/l myo-inositol 8.13 g/l inosine 7.5 g/l uridine	8 ml		Sigma, C1879 Sigma, I7508 Sigma, I4125 Sigma, U3750
Preservatives	Propionic acid Nipagin	 100 g/l methyl 4-hydroxybenzoate in 95% EtOH	6 ml 15 ml		Sigma, P5561 Clariant Nipagin M

[a]During preparation of the buffer stock, add $NaHCO_3$ slowly as escaping CO_2 will cause solution to foam vigorously. Despite this loss, its addition is necessary as omitting $NaHCO_3$ causes flies to be short-lived. [b]5 ml was used in all experiments except those in **Figures 1d**, **3a**,**b**, **4** and **5**, and **Supplementary Figures 1** and **5**. [c]The volumes shown for glutamate, essential and non-essential amino acid solutions deliver 200 mM biologically available nitrogen. [d]50% more vitamin solution (to 21 ml/l improved the proportion of flies surviving from egg to adult; see **Fig. 5b**). [e]Folic acid brought into solution by drop-wise addition of 2 N NaOH solution.

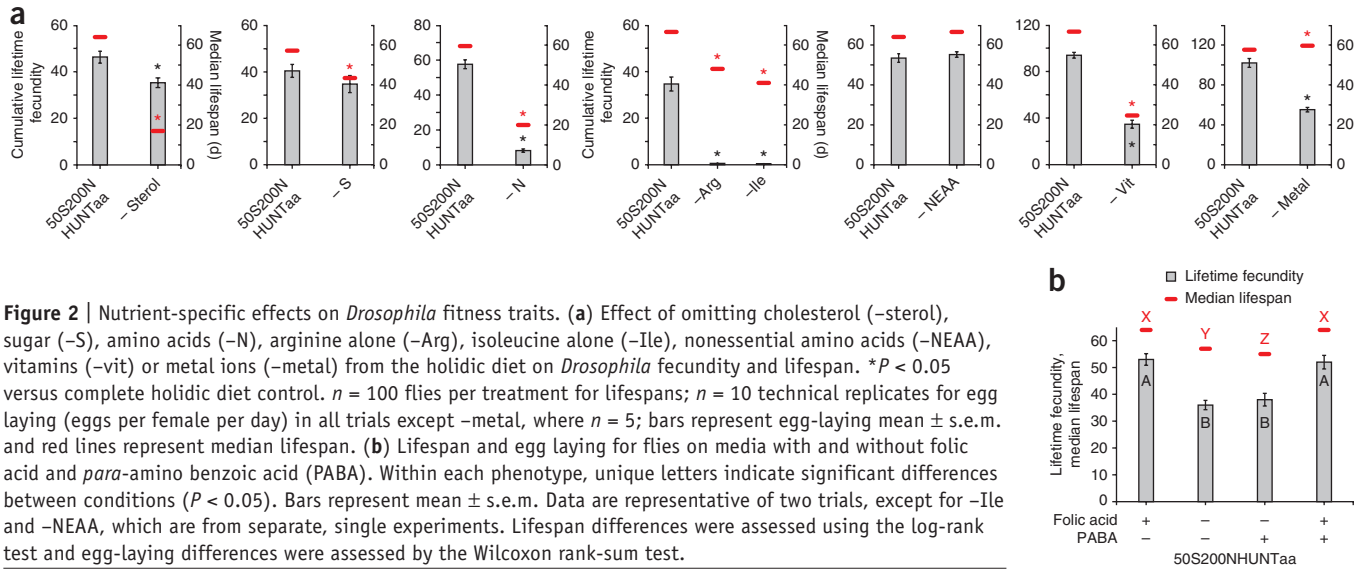

Figure 2 | Nutrient-specific effects on *Drosophila* fitness traits. (**a**) Effect of omitting cholesterol (–sterol), sugar (–S), amino acids (–N), arginine alone (–Arg), isoleucine alone (–Ile), nonessential amino acids (–NEAA), vitamins (–vit) or metal ions (–metal) from the holidic diet on *Drosophila* fecundity and lifespan. *$P < 0.05$ versus complete holidic diet control. $n = 100$ flies per treatment for lifespans; $n = 10$ technical replicates for egg laying (eggs per female per day) in all trials except –metal, where $n = 5$; bars represent egg-laying mean ± s.e.m. and red lines represent median lifespan. (**b**) Lifespan and egg laying for flies on media with and without folic acid and *para*-amino benzoic acid (PABA). Within each phenotype, unique letters indicate significant differences between conditions ($P < 0.05$). Bars represent mean ± s.e.m. Data are representative of two trials, except for –Ile and –NEAA, which are from separate, single experiments. Lifespan differences were assessed using the log-rank test and egg-laying differences were assessed by the Wilcoxon rank-sum test.

activity. After a period of yeast deprivation, flies choose yeast over sugar[20,21], which is interpreted as compensation for protein deprivation. However, yeast provides many nutrients for flies as well as possessing hedonic properties that confound the interpretation of how and why flies choose it as a food source. To test if the preference for yeast is induced by a lack of amino acids, we maintained flies on holidic medium lacking either sugar or amino acids and then gave them a choice of either sugar alone or yeast alone. After 3 d or 6 d of nutrient-specific deprivation on holidic diets, flies deprived of amino acids preferentially ingested yeast instead of sugar, whereas those deprived of sugar preferred sucrose over yeast (**Fig. 3c**). These data indicate that flies sense an internal deficiency in amino acid, leading to an enhanced preference for proteinaceous food and highlight the value of the holidic diet in the dissection of feeding behavior.

Drug bioavailability is enhanced in the holidic medium

The *Drosophila* GeneSwitch[22] system can be used to direct the expression of cloned genes in response to a drug (RU486) administered in the food. We have used this system to generate an adult-onset fly model of Alzheimer's disease by ectopically expressing the toxic amyloid beta peptide species Aβ42 (ref. 23). Using the holidic diet, we found that expression of Aβ42 was induced at lower doses of RU486, compared with drug additions to SY food. In a dose-response analysis, 25 μM RU486 in the holidic medium reproduced both Aβ42 expression levels ($P = 0.05$, $n = 3$ biological replicates, Student's *t*-test) and a similar defect in the flies' ability to perform negative geotaxis (climbing against gravity) at older ages when receiving 200 μM RU486 in SY food ($P = 0.47$ for diet by age interaction, $n = 3$ biological replicates, two-way analysis of variance (ANOVA); **Fig. 4a** and **Supplementary Fig. 5**). Similarly, the minimum dose of rapamycin required to completely suppress egg laying[24] in 1-week-old females after 5 d of drug exposure was 10 μM in holidic diet, whereas on SY food it was 50–200 μM (**Fig. 4b**).

Development on the holidic diet

We observed that the average time for development on holidic diet from egg to the appearance of the first pupa was 9.9 d ± 0.3 d (±s.e.m.), which is considerably longer than on SY medium (**Fig. 5a**). Development time was not shortened by addition of extra amino acids, carbohydrates (as sucrose, glycogen or trehalose), carnitine, folic acid, nucleosides, choline or metal ions to the medium. The addition of extra vitamins in the context of higher amino acid amounts improved the proportion of flies surviving from egg to adult (**Fig. 5b**), but this did not improve development time. Adding water-soluble yeast extract to the defined medium, which did not change the texture, reduced development time to 6.3 d ± 0.3 d (**Fig. 5a**), which is only 1 d slower than on SY food. We do not know the active ingredient(s) for this improvement, but it is not

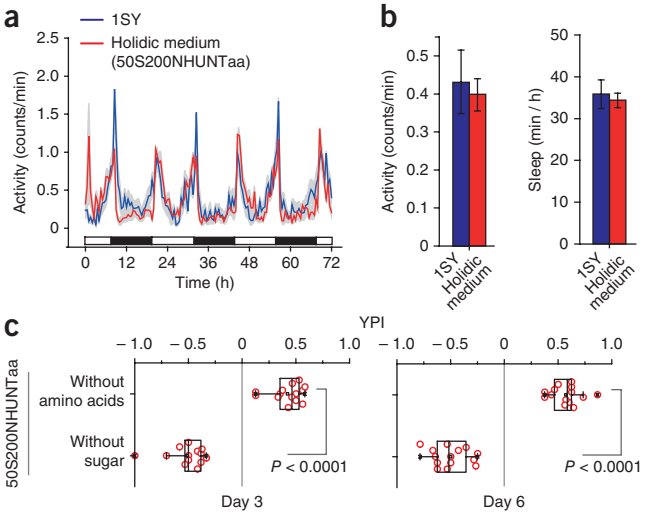

Figure 3 | Holidic diet as a tool to study behavior. (**a**,**b**) Activity and sleep profiles of flies on holidic and oligidic diets (**a**; summed into 30-min bins) for female and male flies recorded for 72 h. White and black boxes represent light and dark periods, respectively. (**b**) Average sleep amount and total waking activity, summed during the 72-h recording. Plotted data represent mean ± s.e.m., $n = 16$ technical replicates. Data are representative of six trials (**a**,**b**). (**c**) Diet preference test. After maintenance on holidic diets lacking either sucrose or amino acids for a period of 3 d or 6 d, flies were then assayed for their choice of sugar alone or yeast alone. YPI, yeast preference index. $n = 14$ biological replicates per pretreatment, except day 3 sugar-deprived, $n = 13$ biological replicates.

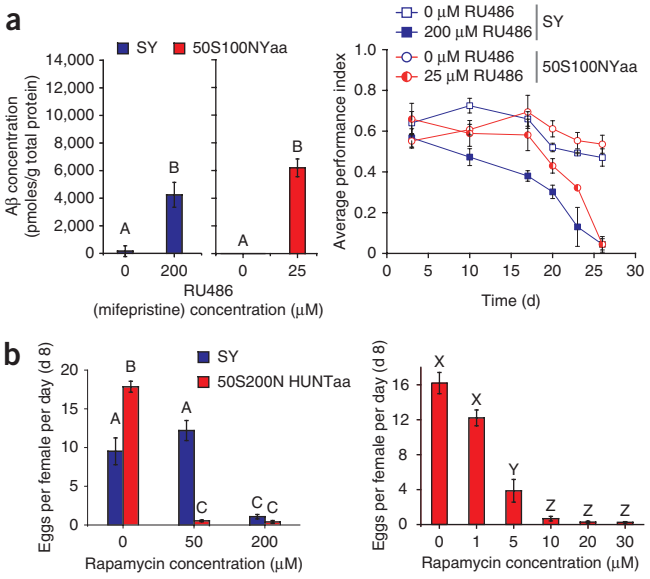

Figure 4 | Drug bioavailability is increased on holidic medium. (**a**) RU486 induction of transgenic Aβ42 (Aβ) expression on holidic and oligidic diets (left, $n = 3$ biological replicates). Climbing ability (right) of flies on both media when Aβ42 was induced to the same level ($n = 3$ biological replicates). Bars represent mean ± s.e.m. Unique letters indicate significant differences in Aβ42 expression ($P < 0.05$), assessed using Student's t-test. (**b**) Effect of rapamycin on egg laying for holidic and oligidic diets. For each diet, unique letters indicate significant differences in egg laying between rapamycin doses ($P < 0.05$, Wilcoxon rank-sum test). $n = 5$, technical replicates. Bars represent mean ± s.e.m. Data are pooled from two experiments.

an essential element because, at the time of writing, we have been able to rear *Drosophila* on the holidic medium without yeast extract for 12 successive generations with a stable developmental duration. We could not extract the limiting nutrient(s) with chloroform, and the limiting nutrient(s) is heat-stable (95 °C for 135 min) and is smaller than 10 kDa; this indicates that the critical element is not a lipid, large protein or a heat-labile vitamin.

After rearing flies on a holidic diet, we measured their egg laying and wing size, which is a proxy for body size. Flies that developed in SY food were ~18% bigger and had ~2.5-fold higher egg laying than those that developed as larvae in holidic medium (effect of larval diet, **Fig. 5c**). Irrespective of the developmental conditions, the holidic medium was equally good a source of nutrients for adult egg laying as SY food (effect of adult diet $P = 0.31$, larval diet by adult diet interaction $P = 0.85$, $n = 8$ technical replicates, linear model).

Microbes can adhere to *Drosophila* eggs and promote larval growth under nutrient-limiting conditions[25]. These microbes can be removed by bleach-treating eggs to produce germ-free animals[7]. We reared larvae from both untreated and bleach-treated eggs on sterile holidic medium, with or without folic acid. Absence of folic acid from the medium caused a developmental delay of ~2 d for untreated eggs ($P < 0.0001$, $n = 40$ technical replicates, Wilcoxon rank-sum test), indicating that dietary folates are not required for larval development (**Fig. 5d**). Bleach treatment, in contrast, led to a complete failure to produce pupae on medium without folic acid (**Fig. 5d**). This was not simply a detrimental effect of bleach treatment because adding bleached eggs to defined medium that had previously been exposed to male flies and that had therefore presumably acquired their associated microbes, rescued development time back to the level of untreated eggs (**Fig. 5d**). Bleaching also delayed development on folic acid–containing food by ~0.5 d ($P = 0.015$, $n = 40$ technical replicates, Wilcoxon rank-sum test). Taken together, these data indicate that *Drosophila* larvae require exogenous folic acid for development, and this demand can be met either from dietary sources or from egg-associated microbes.

DISCUSSION

We report a medium for *Drosophila* for which the chemical composition is entirely defined. To our knowledge, it is the only holidic diet reported to support adult lifespan and egg laying to the same extent as oligidic food that we had optimized for these traits[3]. Although a meridic medium that supported egg laying to nearly the same extent as yeast-based food has been reported[8], to our knowledge there is no record of the effect on lifespan. Lifespan has been measured on several other meridic media[9,12,26–29], and one[9] supported lifespan to the same extent as yeast-based

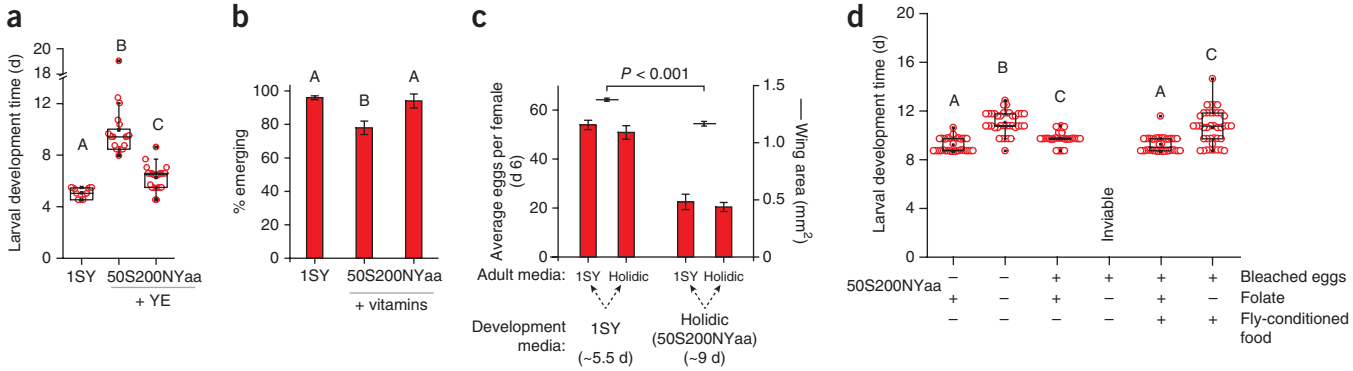

Figure 5 | Development on holidic media. (**a**) Time from egg to appearance of first pupa for larvae developing in oligidic and holidic diets. YE indicates addition of water-soluble yeast extract. $n = 13$ biological replicates for oligidic diet and 20 for holidic diet. (**b**) Percentage of flies surviving from egg to adult when developing on oligidic or holidic diets. Where indicated, the vitamin concentration was enhanced by 50%. $n = 3$ biological replicates. (**c**) Wing area and egg laying for adult flies assigned to oligidic or holidic diets, measured after larval development on holidic or oligidic diets, as indicated. Wing measurements: $n = 22$ technical replicates for oligidic diet and 16 for holidic; egg laying: $n = 8$ technical replicates. (**d**) Development time for untreated or bleach-treated eggs after transfer to sterile holidic media either with or without folic acid. $n = 40$ technical replicates. Within graphs, unique letters indicate significant differences at a threshold $P < 0.05$ using the Wilcoxon rank-sum test. Data are means ± s.e.m. Data are from separate, single experiments (**b**–**d**) and representative of 13 independent experiments (**a**).

food, but egg-laying data are not available for this diet and the exact concentration of some components of the medium remains unclear (Harlan Teklad diet TD.04310)[10].

Nevertheless, flies reared on our holidic medium develop more slowly, are smaller and have lower egg-laying capacity than those maintained on SY food. Reduced fecundity is a known correlate of smaller body size in many animals, including *Drosophila*[30], but the reasons for this effect are unknown. We do not currently know what components in the holidic diet limit development.

Two drugs that are widely used in *Drosophila* research had eightfold to tenfold higher bioavailability in our holidic diet than in SY food. Given that these drugs, RU486 and rapamycin, cost about eight times and about four times more, respectively, than gold at its record high in September 2011, the cost advantage of enhanced bioavailability is obvious. Additionally, the requirement for lower doses of drugs with poor palatability reduces the need to prestarve flies before treatment and for drugs whose water solubility is low, enhanced bioavailability avoids the addition of toxic doses of vehicles, such as DMSO[31]. Given the effects of nutritional status on drug efficacy, we also note that this holidic diet offers a more stable platform than oligidic diets in which to undertake drug studies.

We demonstrated how the holidic medium may be used to study *Drosophila* nutritional requirements and behavioral responses. We expect that the medium will prove useful for dissection of a variety of responses in this model organism, and of their underlying molecular and neuronal mechanisms.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.D.W.P. and L.P. conceived and developed the project, and wrote the manuscript. M.D.W.P., R.L.-G., M.Y., X.H., N.J.L., M.P.H., C.H., G.A.S., C.N. and F.K. performed experiments. All authors contributed to data analysis and interpretation.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Media and flies. In all experiments except those for RU486 calibration, we used our laboratory stock of outbred wild-type *D. melanogaster*, Dahomey, which has been cured of *Wolbachia* by tetracycline treatment[32]. Flies were maintained in large population cages with overlapping generations at 25 °C with a 12 h:12 h light:dark cycle. *UAS-ArcAβ42* and *elavGS* transgenic flies were backcrossed into *w1118*, as reported in ref. 33 and *w*Dah; *Df(3L)ilp2-3*^{1}*, ilp5* deletion mutants (lacking (*ilp2, ilp3* and *ilp5*) and *Wolbachia*-positive white Dahomey (*w*Dah) controls are those reported in ref. 34. All genetic constructs were backcrossed into the genetic background of their control for at least six generations before experiments were performed.

For all experiments using adult flies, other than food choice, flies were reared on sugar, yeast food (1SYBrewer's; 1SY) as described[35] for lifespan experiments. Egg collections were used to synchronize fly age as described[36]. Flies for the food choice assay were reared in a medium containing, per liter, 80 g cane molasses, 22 g beetroot syrup, 8 g agar, 80 g corn flour, 10 g soya flour, 18 g yeast extract, 8 ml propionic acid and 12 ml nipagin (15% in ethanol).

Holidic media. An open-access editable version of this protocol is available through Nature Protocol Exchange (http://dx.doi.org/10.1038/protex.2013.082).

Components of the holidic medium as well as example supplier order numbers for all components are listed in **Table 1**. Medium is prepared in two stages. In the first, sucrose, agar, amino acids with low solubility (l-isoleucine, l-leucine and l-tyrosine) as well as stock solutions of buffer, metal ions and cholesterol are combined in a 1-l autoclavable bottle with a magnetic stirrer and milliQ water up to 1 l, minus the volume of solutions to be added after autoclaving. After autoclaving at 120 °C for 15 min, the solution is allowed to cool at room temperature with stirring to ~65 °C. Stock solutions for the amino acids (**Supplementary Table 1**), vitamins, nucleosides, choline, inositol and preservatives are then added as are the drugs mifepristone (Sigma) or rapamycin (LC Laboratories), where appropriate. With constant stirring, sterile tubing is used to dispense the solution into sterile vials. These are covered, allowed to cool for 90 min at room temperature and then stored at 4 °C until use. Feedback from other users of the medium has highlighted that this method of preparation may result in medium that does not set. This varies with the autoclave used and can be resolved by adding the sterilized buffer base after autoclaving; this indicates that it is caused by acid hydrolysis of the agar during autoclaving.

Stock solutions were prepared in milliQ water, except for the cholesterol stock, which was prepared in absolute ethanol. Cholesterol stock, buffer stock, amino acid solutions and stock containing nucleosides, choline and inositol were stored at 4 °C; $FeSO_4$, vitamin and folic acid stocks were stored at −20 °C. Before freezing the latter stocks, we typically made 1 l and aliquoted smaller volumes so that once thawed, they could be used quickly and without refreezing. Before storing aliquots, pH of amino acid stocks was adjusted to 4.5 using HCl. All aqueous solutions were filter-sterilized by passing through a 0.22 µm syringe-fitted filter. Note also, that cholesterol precipitates out of solution during storage at 4 °C, but it easily redissolves with stirring at room temperature. The amino acid ratio shown in **Table 1** refers to HUNTaa. The proportions and amounts of the amino acid stock solutions as well as their value in terms of biologically available nitrogen are available in **Supplementary Table 1**.

Measuring development, egg laying and lifespan. For development assays, young age-matched flies were allowed to lay eggs on grape juice plates overnight. 24 h later, first instar larvae were picked onto test media, which were kept at 25 °C. The appearance of pupae was scored twice a day.

To generate age-synchronized adult flies, larvae were allowed to develop on SY food at standard density, transferred to fresh SY food upon emerging as adults and allowed 48 h to mate. Under light CO_2 anesthesia, females were separated from males and allocated to treatment vials at a density of ten flies per vial. Flies were transferred to fresh vials three times per week at which point deaths and censors were scored. Egg-laying was scored after flies occupied vials for ~18 h, and the value was expressed as the number of eggs per vial per female.

To generate germ-free larvae, eggs were bleach-treated according to ref. 37: eggs were transferred into a sterile mesh strainer and bleached in 2.7% sodium hypochlorite for 2 min, washed twice in 70% ethanol and rinsed twice in sterile diluted phosphate-buffered saline (PBS; 1:10) for 1 min. Eggs were collected and transferred into a sterile 1.5 ml microcentrifuge tube using an autoclaved brush from which 4 µl was transferred onto the appropriate food using a pipette. In order to rescue microbe loss, food vials were exposed to five male flies for 24 h. The male flies were removed before the eggs were placed on the food.

Multiple-generation fly rearing was performed using three replicate vials of 1SY in parallel with three replicate vials of 50S200NYaa holidic medium. At each generation, flies were allowed to emerge on the food they had developed and transferred to fresh food of the same type to mate. After 3–5 d, five female and five male siblings were allocated to fresh vials for overnight egg laying, whereupon the adults were discarded, and eggs were allowed to develop. Time to emergence of adults was recorded.

Assays for drug delivery, climbing and behaviors. For administration of rapamycin and RU486, flies were reared and sorted into single-sex groups as for lifespan experiments. Flies were introduced to drug-containing food 2 d after eclosion. Negative geotaxis assays and enzyme-linked immunosorbent assay (ELISA) for Aβ42 were performed at day 14, according to the methods described[33].

The food choice assay was performed as described[38]. Groups of 1–5-day-old flies (16 females and 5 males) were reared and maintained for 72 h after eclosion in the oligidic medium described above. Flies were transferred to holidic medium from which either sucrose or amino acids were omitted and maintained for 72 h, at which point food preference was tested. Flies were given the choice between red sucrose (20 mM sucrose, 7.5 mg/ml agarose, 5 mg/ml Erytrosin B (Sigma-Aldrich) and 10% PBS) and blue yeast (10% yeast (SAF instant yeast), 7.5 mg/ml agarose, 0.25 mg/ml Indigo carmine (Sigma-Aldrich) and 10% PBS) medium. After visual inspection of the abdomen, each female fly was scored as having eaten red sucrose medium (red abdomen), blue yeast medium (blue abdomen) or both media (red and blue or purple abdomen). Dye-swap experiments were performed and this did not affect the outcome (data not shown). YPI for the

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whole female population in the assay was calculated as follows: $(n_{\text{blue yeast}} - n_{\text{red sucrose}})/(n_{\text{red sucrose}} + n_{\text{blue yeast}} + n_{\text{both}})$. Fly rearing, maintenance and behavioral testing were performed at 25 °C in a humidified and temperature-controlled chamber.

For activity and sleep monitoring, young (2–4-day-old) mated male and female Canton-S flies (n = 16 per group) were transferred to individual 5-mm polycarbonate vials containing either 1SY medium or 50S 200N HUNTaa holidic medium. Vials were transferred to a Trikinetics activity monitoring apparatus (Trikinetics) in an environmentally controlled chamber at 25 °C with a 12 h:12 h light:dark cycle. Activity counts were summed every minute, and sleep was defined as five or more minutes of continuous inactivity[39]. The average sleep and waking activity (activity counts/minutes awake) were calculated for each fly.

Statistical analyses. All statistical analyses were performed using JMP software (V9). The nonparametric Wilcoxon rank-sum test was chosen to avoid assumptions of data normality. The log-rank test, a nonparametric test for survival analysis, was used to assess survival distributions of two treatments, and Cox proportional hazard was used for survival experiments with more than one explanatory variable. Student's t-test and analysis of variance (ANOVA) were used where previous experimental data has conformed to the assumptions about normality and variance equality.

Reproducibility and experimental design. Sample sizes are at least, and in many cases beyond, what is required to give confidence in the results. These are consistent with established norms for research on *Drosophila* and aging. For lifespan and egg-laying assays, individuals that developed in the same vessels were assigned evenly and systematically between experimental treatments. None of the assays required scoring blindly.

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Corrigendum: A holidic medium for *Drosophila melanogaster*

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