Correspondence

Pitfalls of measuring feeding rate in the fruit fly *Drosophila melanogaster*

Richard Wong, Matthew D W Piper, Eric Blanc & Linda Partridge

Supplementary figures and text:

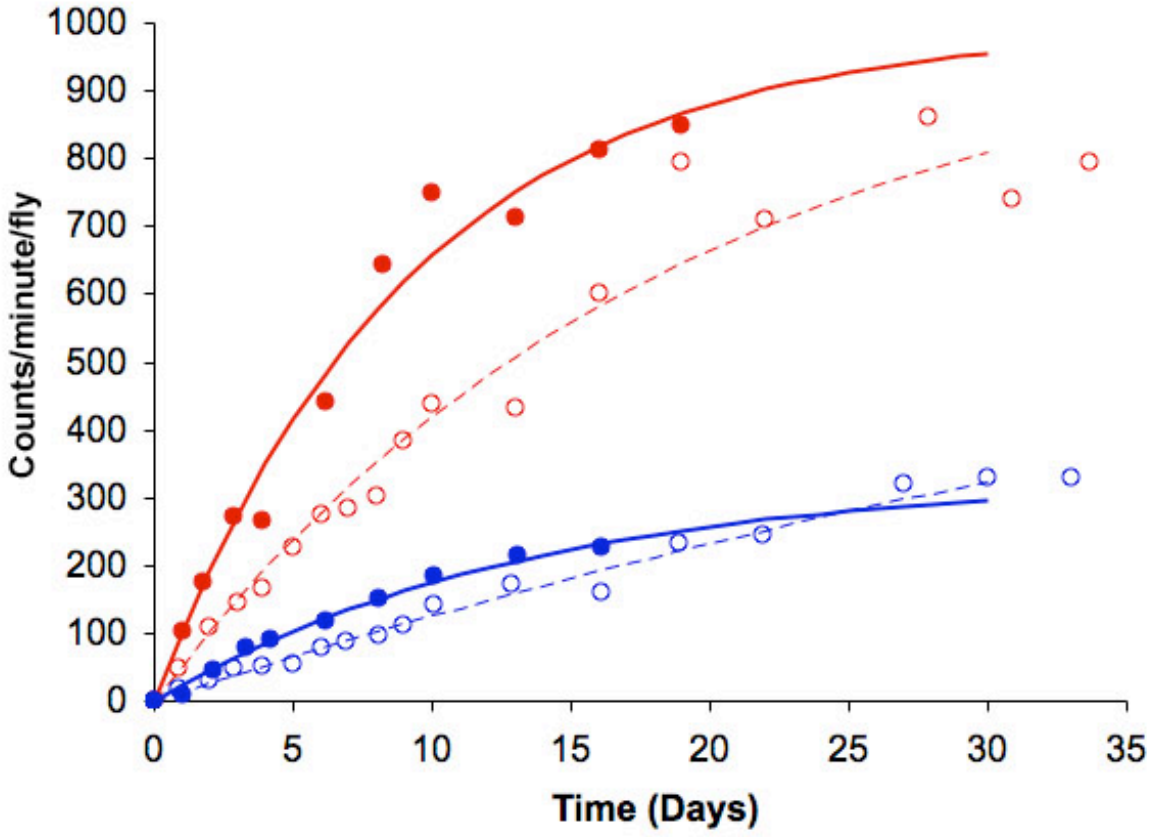
Supplementary Figure 1 ^{14}C-choline labeled food accumulation in *Drosophila*

Supplementary Methods

Supplementary Files

Supplementary Figure 1

	Parameters	
Fly group	Feeding rate (counts/min/fly)	Fraction of label removal rate (s)
Non-reproducing females	53.689	-0.053
Reproducing females	108.237	-0.109
Non-reproducing males	13.344	-0.016
Reproducing males	24.825	-0.075



Supplementary Figure 1 Legend

To parameterise the model, we used ^{14}C-choline accumulation data from Geer et al. (1970) [1], who transferred reproducing and non-reproducing flies of both sexes onto labelled food at time 0 and measured label accumulated by the flies at the various time points. The parameters were obtained by least-squares fit. The lines in the graph show the fit of the model to the data (circles) and the table gives the parameter estimates from the model. Reproducing females solid red line and circles, non-reproducing females dashed red line and open circles, reproducing males solid blue line and circles, non-reproducing males dashed blue line and open circles.

Supplementary Methods

DR vs. fully fed flies on blue labelled food

The flies used in **Figure 1a** were from our outbred lab stock Dahomey and reared as for lifespan experiments [2]. 7-day old once-mated females were housed 5 per vial during the assay. One hour after lights-on (t=0) flies were transferred without CO_2 anaesthesia, to either DR blue-labelled food (1xSY) or fully fed blue-labelled food (2xSY; diet recipes can be found in Bass et al., 2007) [2]. The blue dye was FD&C Blue no. 1 previously described in Edgecomb et al., 1994 [3] used at a concentration of 2.5% (w/v). At the indicated times, flies were transferred without anaesthesia to eppendorf tubes and snap-frozen in liquid nitrogen. Flies were maintained on their appropriate non-dyed food medium throughout their lifetime at 25ºC, 65% humidity, on a 12:12 light: dark cycle.

Spectroscopy

Sampled flies were homogenised in a 1.5ml eppendorf with 1ml of distilled water using a plastic pestle and passed through a 0.22μm Millipore filter to remove debris and lipids. The absorbance of the liquid sample was then measured by spectrophotometry [Hitachi U-2001 Spectrophotometer (Lambda Advanced Technology Ltd., UK); 629nm]. The reading was recorded and the amount of food was determined from a standard curve. Corrections of background absorbance were achieved by using readings from control flies that were not exposed to the dyed food source.

Modelling the dye-labelled data

We fitted the model to the blue dye data for DR and fully fed flies using a least-squares regression analysis in R v2.2.1 (R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2005). Using the model, we estimate feeding rates for DR fed and fully fed flies to be 3.96 +/- 0.246 and 3.87 +/- 0.841 μg of food per hour, respectively, and a proportional rate of label removal at 0.137 +/- 0.00706 and 0.317 +/- 0.0674 of the gut volume per hour, respectively.

Measurement of gut passage time

Females (n = 60) were reared as for lifespan assays and maintained as adults on either DR or fully-fed food [2] and were transferred to either DR or fully-fed labelled food for 15, 30 and 60 minutes and allowed to feed. Flies were then transferred back to their respective non-labelled food condition and observed. The timing of appearance and quantity of blue-labelled faeces was then recorded for each condition. From the 15-minute and 30-minute trials, it took an average total time of 48 ± 1.5 minutes (s.d.), from first exposure to the label to the first appearance of blue-labelled faeces. This appeared to be a true indicator of gut passage time as a third to a half of total blue label consumed was egested in the subsequent 45 minutes of the assay.

Crop measurements

Females (n = 50) from each food condition were dissected in phosphate buffer solution and placed onto a dimple slide. Crops were then photographed using a camera (Marlin F-145C2, Allied Vision Technologies) attached to a dissection microscope (Nikon C-DSD230, Japan) at 60x magnification. The surface area of the crops was measured using Object-Image v2.10 (imaging software by Norbert Vischer, University of Amsterdam) and the size calculation converted from an image of a 1mm^2 reticule. To avoid bias, another laboratory member coded and de-coded the image labels so their identity was unknown to the observer while measurements were performed.

References

1. Geer, B. W., Olander, R. M. & Sharp, P. L. Quantification of dietary choline utilization in adult Drosophila melanogaster by radioisotope methods. *Journal of Insect Physiology* **16**, 33-43 (1970).
2. Bass, T. M. et al. Optimization of dietary restriction protocols in Drosophila. *J Gerontol A Biol Sci Med Sci* **62**, 1071-81 (2007).
3. Edgecomb, R. S., Harth, C. E. & Schneiderman, A. M. Regulation of feeding behavior in adult Drosophila melanogaster varies with feeding regime and nutritional state. *J Exp Biol* **197**, 215-35 (1994).