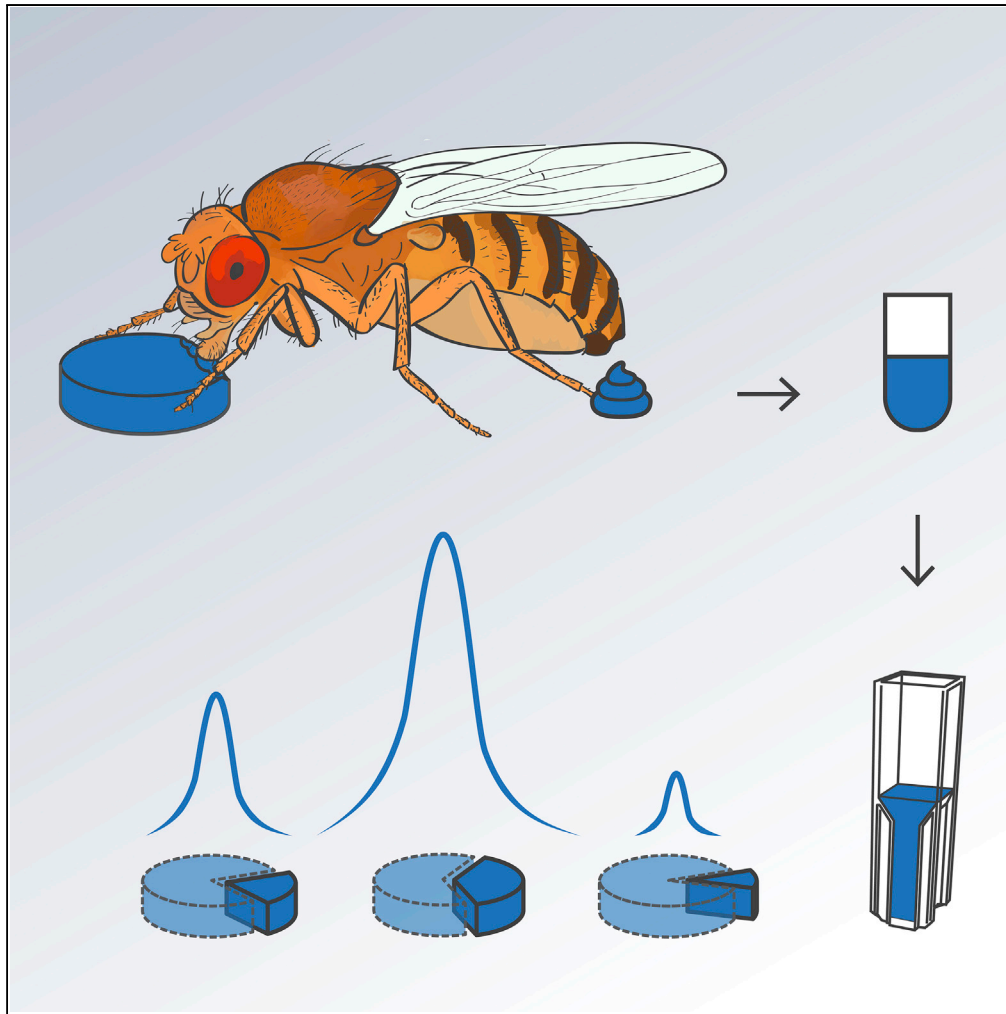


Article

Excreta Quantification (EX-Q) for Longitudinal Measurements of Food Intake in *Drosophila*

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HIGHLIGHTS

Drosophila food intake can be accurately measured by excreta quantification (EX-Q).

A smaller food surface area maximizes excreta recovery and improves EX-Q accuracy

EX-Q enables longitudinal studies since flies are not sacrificed for measurements

Females show greater age- and diet-specific effects on food intake than males

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Article

Excreta Quantification (EX-Q) for Longitudinal Measurements of Food Intake in *Drosophila*

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SUMMARY

Longitudinal measurements of food intake remain a challenge in *Drosophila* studies of nutrition and behavior. Here, we report an improved method for measuring fly food intake using dye-labeled food and excreta quantification (EX-Q). Reducing the surface area of the medium maximized excreta recovery and the accuracy in estimating total consumption. The EX-Q method is compatible with agar-based medium and makes it possible to measure consumption over an extended period and at multiple time points without sacrificing flies. Using EX-Q, we revealed nutrient- and age-specific features of *Drosophila* feeding behavior. Daily consumption of a chemically defined diet was relatively consistent over the first 25 days of adulthood. Omitting amino acids or vitamins from the diet reduced consumption in both sexes, whereas omitting sugars or cholesterol primarily affected female food intake. Our results demonstrate EX-Q as a simple, reliable, and nondestructive method for longitudinal studies of solid food intake in *Drosophila*.

INTRODUCTION

Food intake is a critical consideration in diverse studies. Despite the prevalent use of *D. melanogaster* as a research model for nutritional physiology (Piper, 2017; Tatar et al., 2014), behavior (Hollis and Koppik, 2019; Murphy et al., 2016; Thimman et al., 2010), and drug administration (Bjedov et al., 2010; Castillo-Quan et al., 2016; Slack et al., 2015), accurate, longitudinal measurements of food intake remain challenging. Several methods have been developed to assay fly feeding behavior, including the capillary feeder (CAFE) assay (Ja et al., 2007; Murphy et al., 2017), use of radioactive tracers (Carvalho et al., 2005; Ja et al., 2009; Yamada et al., 2015) or dyes (Edgecomb et al., 1994; Wood et al., 2004) to label food, and monitoring proboscis extension or food contact (Itskov et al., 2014; Ro et al., 2014; Wong et al., 2009). Although the CAFE assay allows accurate measurement of food intake over long periods by quantifying liquid food consumed from a micro capillary, it cannot be used to quantify feeding behavior in the more commonly used agar-based diets. Labeling the food and quantifying tracer accumulation in the fly facilitates the use of any medium. However, food labeling is dependent on the efficiency of label accumulation over time. Ingested dyes are excreted rapidly, limiting accurate food intake measurements using dye labeling to no more than 30 min. Radioisotope labeling has been successfully used to accurately measure food intake for at least 24 h (Carvalho et al., 2005; Deshpande et al., 2014; Ja et al., 2009; Yamada et al., 2015), but the efficiency and stability of radiotracer accumulation is likely dependent on multiple factors, including sex, genotype, and diet (Deshpande et al., 2014). Additionally, labeling techniques require sacrificing the animals to quantify tracer accumulation, eliminating the possibility of longitudinal studies. Other methods assess feeding behavior by monitoring feeding frequency, based on either manual observations of fly proboscis extension onto the food surface (Wong et al., 2009) or capacitive or circuit-based measurements to detect the physical interaction of flies with food (Itskov et al., 2014; Ro et al., 2014). Although these methods do not require sacrificing flies, accurate quantification of the volume or mass of food consumption remains challenging using these tools.

Another dye-based method, consumption-excretion (Con-Ex), was recently described that quantifies solid food intake in *Drosophila* (Shell et al., 2018). With the Con-Ex method, the volume of food consumed is reflected by the sum of the dye inside the fly body and that excreted by flies. This method still has two obvious disadvantages: (1) because most of the excreted dye accumulates on the surface of the medium rather than on the wall of fly vials, it is impossible to collect all of the fly excreta; and (2) flies are also sacrificed in this method. Therefore, we currently lack a method for accurate, longitudinal measurements of food intake with repeated observations from the same animals.

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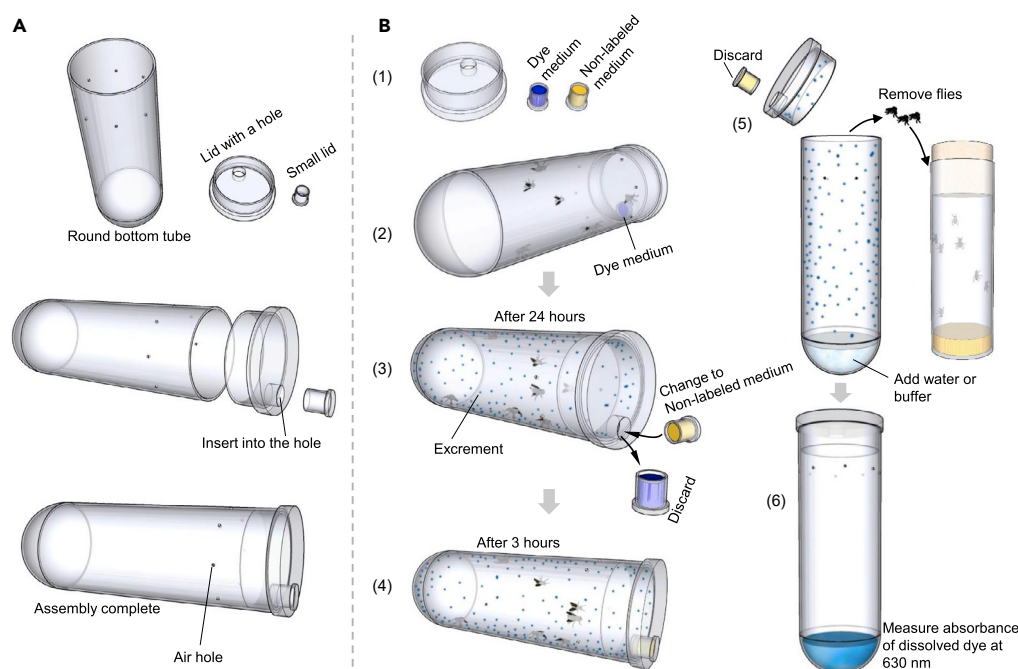


Figure 1. Schematic Diagram of Excretion Quantification (EX-Q) Method to Quantify Food Intake in *Drosophila*

(A) The EX-Q tube consists of three parts: a round-bottom centrifuge tube with several air holes in the side wall, a large lid with a hole and a small lid with the same diameter as the hole in the big lid. The small lid can be inserted into the hole of the large lid to form a complete device with the round bottom centrifuge tube.

(B) Typical protocol for quantifying food intake using the EX-Q tube. (1) Prepare dye- and non-labeled food in small lids. (2) Insert the dye medium into the hole of the big lid, transfer flies into the round bottom tube, and cover with the big lid. (3) Flies (typically 10 age-matched adults) feed on dye medium for 24 h in the EX-Q tube. The small lid with dye food is subsequently replaced with non-labeled food. (4) Flies are maintained on non-labeled medium for another 3 h to allow time for the remaining dye to be excreted from their bodies. (5) Live flies are recovered from the EX-Q tube, and the small lid containing food is discarded. Water or buffer is then added to the tube (typically 2–5 mL). (6) To recover excreta, air holes and the hole in the lid are sealed with tape, the tube is shaken to dissolve the dye completely, contents are collected by centrifugation, and the absorbance of the dye solution is measured at 630 nm. Absorbance measurements are converted to volumes according to a standard curve generated from dye solutions or aliquots of dye food (see [Transparent Methods](#)).

See also [Figures S1 and S2](#).

To address this problem, we developed a method to measure food intake of *Drosophila* based on excreta quantification (EX-Q), which does not require sacrificing flies and can quantify food intake over a long period of time and at multiple time points. Using EX-Q, we have uncovered a substantial sexual dimorphism in food intake response when specific nutrients are omitted from the diet.

RESULTS

The EX-Q Assay for *Drosophila* Food Intake

To recover fly excreta efficiently, we designed an EX-Q tube that consists of three parts: a round-bottom centrifuge tube with several air holes in the side wall, a large lid with a hole, and a small lid containing food with the same diameter as the hole in the big lid. The small lid with the food plug can be inserted into the hole of the large lid to form a complete device with the round bottom centrifuge tube ([Figures 1A and S1A–S1D](#)). To measure food intake with EX-Q, flies (typically 10 per tube) are transferred to the EX-Q chamber and provided with food containing 1% erioglaucine dye in a small lid. After the experimental feeding period (typically 24 h), the dye medium is replaced with non-labeled medium and flies are left in the chamber for another 3 h, which is sufficient for the flies to excrete almost all residual dye in the body ([Figure S2](#)). Flies are subsequently removed from the EX-Q tube and the small lid containing unlabeled food is discarded. Holes on the EX-Q tube are covered with tape, and the excreta are recovered from

the tube walls and cap with distilled water or buffer (typically 2–5 mL). Food intake is determined by quantifying absorbance at 630 nm and converting the values to volume according to a standard curve (Figures 1B and S1E–S1H).

Food Surface Area Determines the Accuracy of Food Intake Quantification

We hypothesized that using a small food surface area would reduce the amount of excreted dye lost to the food surface and maximize recovery of excreta without affecting total consumption. To test this hypothesis, we first compared food intake between small (5.5-mm diameter) and big (23.5-mm diameter) food plugs (Figure 2A). Indeed, estimated consumption on the small-diameter food surface was significantly greater than that on the big food surface (Figure 2B). When we further tested food plugs ranging in diameter from 2 mm to 23.5 mm, we found that as the food surface area decreased, estimated consumption increased and was maximized on the 3.5 mm diameter food surface (Figure 2C). We speculate that this increased estimated consumption is due to better excreta recovery, not due to actual changes in feeding. However, the estimated consumption was significantly reduced by further decreases in food surface area, possibly due to limited access and competition between flies on 2-mm diameter food (Figure 2C).

To further investigate whether the differences in food intake measured by EX-Q on food surfaces of different diameters are due to the different proportions of excreta accumulated on the surface of the medium, we transferred flies that had been fed dye food for 12 h into EX-Q tubes with non-labeled medium of different sizes and collected excreta after 3 h (Figure 2D). Our results showed that more excreta accumulates on the larger food surfaces (Figure S3), leading to reduced recovery of excreta from the tube wall and lids (Figure 2E). Recovery of excreted dye on the 3.5- and 5.5-mm food surfaces was equivalent to that collected by direct homogenization of the flies (Figure 2E), showing that most or all of the ingested dye was recovered.

Our results suggest that the smaller, 3.5- and 5.5-mm diameter food surfaces may be optimal for maximizing excreta recovery and estimating total consumption. To test for the possibility that food cup diameter affects actual food intake, we measured total consumption using radioisotope food labeling in the EX-Q tubes (Figure 2F). Accumulation of the ^{32}P label in the fly body accurately reflects total consumption (Deshpande et al., 2014), and we maximized tracer absorption efficiency by using a sucrose/tryptone diet that lacks nucleotides that might compete with the label for gut absorption. Based on ^{32}P accumulation in the fly, we found that food consumption on the 23.5-mm diameter surface did not differ from that on the 5.5-mm diameter surface but was 11% greater than consumption on the smallest (3.5 mm) surface diameter (Figure 2G). Recovery of excreted radiotracer was maximized on the 3.5- and 5.5-mm diameter surfaces, with significantly less radiotracer recovered from the 23.5-mm diameter surface, consistent with reduced excreta collection on the larger food surface (Figure 2G). Based on the total excreted radiolabel, the efficiency of ^{32}P absorption in the fly body was >98%. These results are consistent with the idea that quantification of food intake with EX-Q using 3.5- and 5.5-mm diameter food cups accurately reflects total consumption, but there may be an effect of food surface area on feeding behavior on the smallest sizes.

To test how suitable the EX-Q assay is for longitudinal measurements of food consumption, we performed timecourse experiments in the EX-Q tube using a 3.5-mm diameter food surface. Estimated food consumption increased proportionally over 48 h (Figure 2H), demonstrating that EX-Q is suitable for the measurement of food intake over a relative long period of time without losing sensitivity. To determine the optimal density of flies in the EX-Q tube, we compared food intake of flies in different housing densities ranging from 1 to 20 animals per enclosure. We found that the average food intake per fly is not significantly affected by the number of flies per tube, but data are less variable as the fly density increases (Figure 2I). These results suggest that competition on smaller food surfaces is not greatly impacting total consumption.

EX-Q Can Resolve Small Differences in Food Intake

Previous studies have shown that reducing food concentration can increase consumption in *Drosophila* (Carvalho et al., 2005; Deshpande et al., 2014). To test whether the EX-Q assay can resolve compensatory feeding, we measured intake on different food concentrations (0.25 \times , 0.5 \times , 1 \times , 2 \times , and 4 \times). We found that food intake measured by EX-Q decreased with increasing food concentration in both sexes (Figure 3A). To investigate if the EX-Q assay can detect more subtle compensatory changes in feeding (Deshpande et al., 2014), we further measured intake on 1 \times , 1.1 \times , 1.2 \times , 2 \times , 2.2 \times and 2.4 \times food concentrations

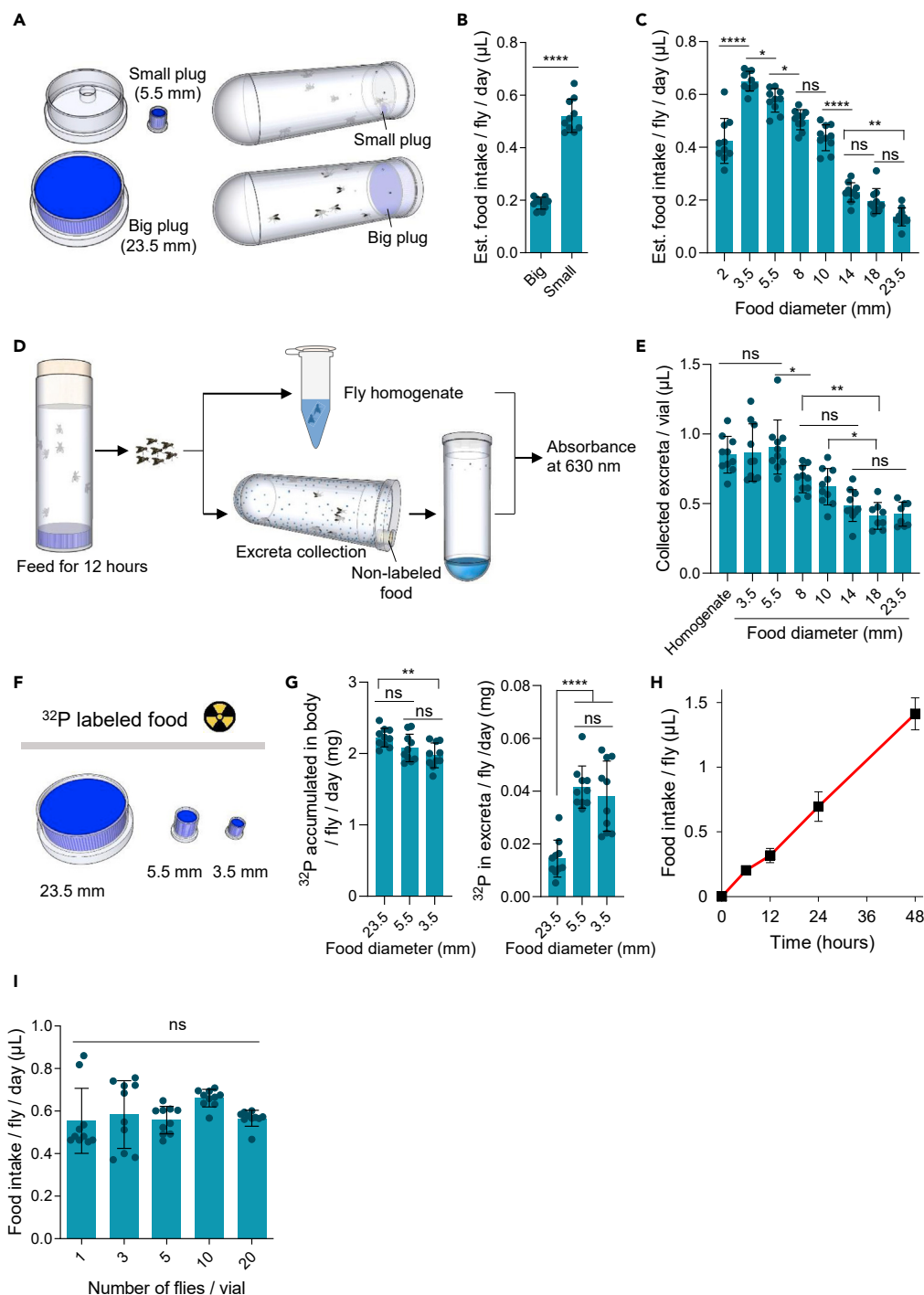


Figure 2. Effect of Food Surface Area on EX-Q Food Intake Measurements

(A–C) Relationship between estimated consumption and food surface diameter in EX-Q tubes. (A) Schematic of big (23.5-mm diameter) and small (5.5-mm diameter) food plugs for the EX-Q tubes. (B) Estimated consumption is greater on the small food plug compared with that on the plug. (C) Estimated food intake on food surfaces of diameters ranging from 2 to 23.5 mm.

(D and E) Relationship between excreta recovery and food surface diameter. (D) Flies were fed on dye medium for 12 h. Flies were then either transferred to Eppendorf tubes and homogenized, or transferred to EX-Q tubes containing non-labeled medium. After 3 h, excreta were collected. Dye was quantified from filtered fly homogenates or excreta solutions by measuring absorbance at 630 nm. (E) The amount of excreta collected was negatively correlated with the diameter of

Figure 2. Continued

the medium. Using the smaller 3.5- and 5.5-mm diameter food allowed recovery of most of the excreta. On larger-diameter food, excreta are likely lost to the food surface.

(F and G) Food intake of flies in EX-Q tubes with different medium size measured by radioisotope labeling. (F) Flies fed on radiolabeled medium for 24 h. The medium was also dyed to facilitate recovery of excreta. (G) Quantified radiolabel in fly bodies and recovered excreta reveal efficient absorption of the radioisotope (>98%). Although there is minimal radiolabel in the excreta, results are consistent with greater recovery of excreta on the smaller (5.5 and 3.5 mm)-diameter food surfaces, compared with the 23.5-mm surface.

(H) Timecourse of measured food intake by EX-Q over 48 h. Each time point is a subsample of 10 replicates of 10 flies each.

(I) Housing density does not affect average food intake over 24 h.

For panels B, C, E, H, and I, 5- to 6-day-old Dahomey females were fed on yeast-sugar medium (10% yeast, 5% sucrose). In panel G, 6- to 7-day-old Dahomey females were fed on tryptone-sugar medium (3.5% tryptone +5% sucrose). In all experiments, $n = 10$ biological replicates. Ten flies were used per replicate except as indicated in panel I. p values were determined by unpaired t test or one-way ANOVA followed by Tukey's multiple comparisons: * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data are shown as mean \pm SD. See also Figure S3.

(Figure 3B). Relative intake on diets from 1 \times to 2.4 \times was 1: 0.91: 0.77: 0.49: 0.43: 0.44 and 1: 0.95: 0.83: 0.57: 0.53: 0.48 in males and females, respectively, with statistically significant differences suggesting that the EX-Q assay is capable of distinguishing 10%–20% differences in food intake using 10 replicates per condition.

Previous studies have shown that the CAFE assay is a relatively accurate method for the measurement of *Drosophila* food intake (Deshpande et al., 2014). We compared the resolving power of EX-Q and CAFE (Figure S4) by quantifying consumption of different food concentrations. Compensatory feeding was observed between the 1 \times , 2 \times , and 4 \times diets in both the CAFE and EX-Q assays (Figure 3C). We further compared the consumption of diets of different amino acid levels (Piper et al., 2014) measured by CAFE and EX-Q. Both assays resolved the 15%–20% difference in consumption between 100N (100 mM total amino acids) food and the 200N or 300N diets (Figure 3D).

To validate the resolving power of the EX-Q assay, we measured 24-h consumption in a large trial comprised of 60 EX-Q chambers for each sex with 10 flies per chamber. Data for each sex are consistent with a Gaussian distribution (Shapiro-Wilk normality test: $p = 0.32$, males; $p = 0.55$, females). Based on the variance of the data, a sample size of 10–15 chambers is appropriate for resolving a 10% difference in feeding (80% power, $\alpha = 0.05$), whereas 40–50 chambers per condition would be required to resolve a 5% effect size. Thus, the EX-Q method is comparable with the CAFE assay for resolving small differences in food intake, and both techniques are less powerful than radioisotope labeling (Deshpande et al., 2014).

Using EX-Q to Study Age-Related Changes to Food Intake in Response to Nutrition

Changes to food composition could have immediate or gradual effects on feeding behavior. Using EX-Q, we measured the age-related food consumption of flies maintained on food in which specific nutrients were omitted. To investigate the food intake on these diets, four-day-old flies were transferred from yeast-sugar (SY) medium to a chemically defined medium (Yaa) (Piper et al., 2014), and 24-h food intake was measured by EX-Q every five days (Figure 4A). When amino acids were omitted from the medium, female food consumption was significantly decreased at all ages, including immediately after the food was changed (Figure 4B). In contrast, a significant reduction in feeding was not observed in males until day 10, after five days of amino acid deprivation (Figure 4C). Omission of sugar did not affect female food intake on days 5, 10, and 25 but significantly increased consumption on days 15 and 20 (Figure 4D). No obvious change in food consumption was found in males on sugar-free medium except on day 5, when flies were switched to the novel diet (Figure 4E). Lack of cholesterol did not generally affect female food intake, except at day 25 when reduced consumption was observed (Figure 4F). Although the interaction between diet and age was significant in males, post hoc analyses showed no significant differences between diets at any age, suggesting that the effect of cholesterol—age dependent or not—is small (Figure 4G). Similar to the results on amino acid-free food, both sexes showed decreased feeding of a medium without vitamins, with this reduction observed earlier in females (starting on day 10) than males (starting on day 20) (Figures 4H and 4I). In conclusion, female food intake generally appears more sensitive than that of males in response to the exclusion of particular nutrients.

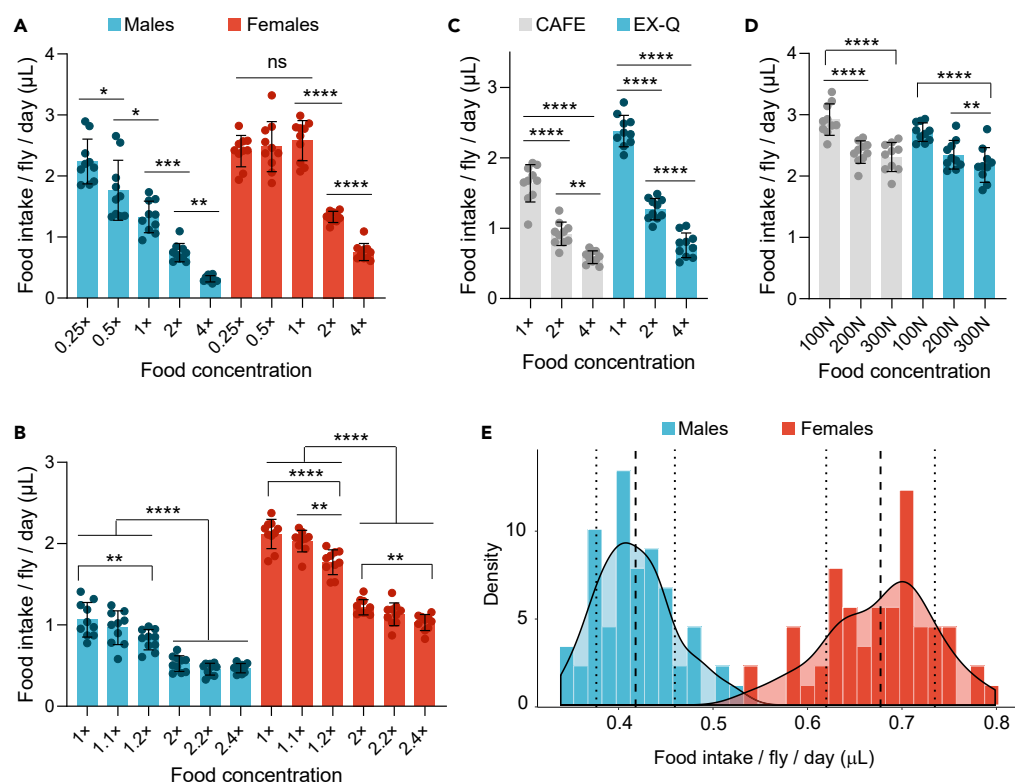


Figure 3. Assessment of Compensatory Feeding Using the EX-Q Method

(A and B) 24-h food intake of males and females on yeast-sugar medium of different concentrations (1 × medium = 2.5% yeast + 2.5% sucrose).

(C and D) Comparison of 24-h food intake of females measured by CAFE and EX-Q on (C) yeast extract-sucrose medium of different concentrations (1 × medium = 2.5% yeast extract + 2.5% sucrose) or (D) cholesterol-free Yaa holidic medium of different amino acid levels.

(E) 24-h food intake of males and females on a yeast-sugar medium (10% yeast, 5% sucrose). Data for 60 biological replicates for each sex (10 flies per replicate) are shown as a histogram with dashed and dotted lines showing mean ± 1 SD for each sex. Solid curves show the distribution of daily food intake. For all panels, 5- to 7-day-old Dahomey flies were used. Except in panel E, all experiments used 10 biological replicates with 10 flies per replicate. p values were determined by one-way ANOVA followed by Tukey's multiple comparisons: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Data are shown as mean ± SD. See also Figure S4.

DISCUSSION

We have characterized the EX-Q method for measuring fly food intake. The key advantage of EX-Q is that it can be used for repeated measurements of food intake of the same individual or group of animals at multiple time points. If the small lids containing food plugs in the EX-Q tubes are periodically replaced, it should also be feasible to measure food intake for long periods of time without sacrificing accuracy.

Accuracy in quantifying food intake using EX-Q relies on maximizing recovery of excreted dye. Our results are consistent with the idea that increased food surface area leads to greater excretion on the food itself, such that more of the dye is irrecoverable. However, it is also possible that food presentation rather than surface area has the greater impact on excreta recovery. To decouple these possibilities, future studies might compare food intake using multiple small-diameter food cups per chamber such that food surface area is matched to a larger diameter medium.

The impact of changes in nutritional composition of food and some drug interventions on the physiological health of animals tend to occur gradually over time. Therefore, brief measurements of food intake at a single time point may not reflect chronic changes in feeding behavior. We observed varying

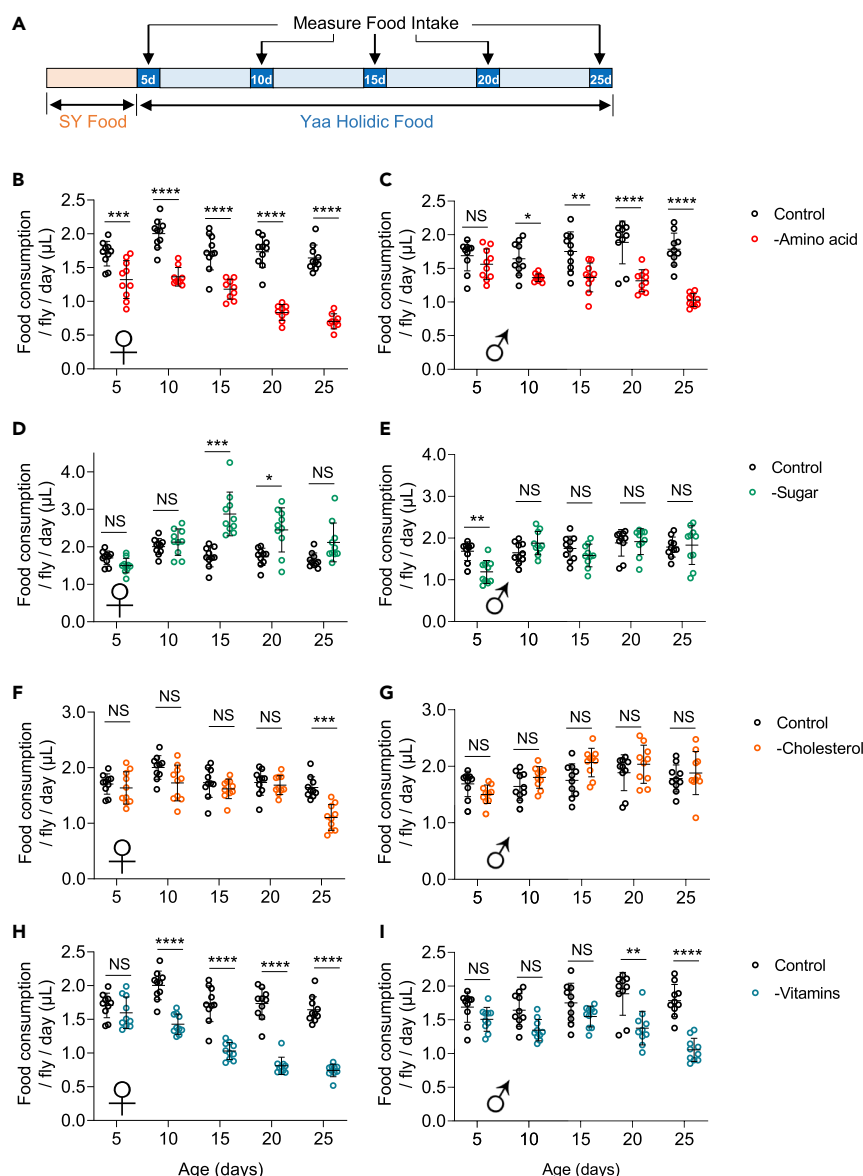


Figure 4. Omission of Different Nutrients Can Have Immediate or Gradual Effects on Food Intake

(A) Diagram of the experimental scheme.

(B–I) Effect of omitting (B and C) amino acids, (D and E) sugar, (F and G) cholesterol, or (H and I) B-group vitamins from the 100N50S Yaa holidic diet on fly food intake. Dahomey flies were used in a longitudinal study. $n = 10$ biological replicates of 10 flies each per condition. The small lid with a 5.5-mm food surface diameter was used for EX-Q assays. The interaction between age and diet was significant in each panel ($p < 0.001$, panels B, C, D, E, H, I; $p < 0.01$, panel F; $p < 0.05$, panel G). p values were determined by two-way ANOVA followed by Sidak's multiple comparisons test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Data are shown as mean \pm SD.

degrees of hysteresis on total consumption when food excluded specific nutrients, and such hysteresis also varied between sexes. These differences might reflect the sexually dimorphic effects of specific nutrient deficiencies on aging. Although omission of nutrients generally reduced or had no effect on feeding, sugarless food resulted in increased female food intake at two intermediate ages. We speculate that the change in protein:carbohydrate balance in a sugarless diet might have led to differences in egg laying. Because eggs laid on the food surface are also recoverable from the EX-Q chamber, future longitudinal studies might examine how the kinetics of egg laying correlates with individual fly feeding behavior.

Repeated observations or continuous monitoring of food intake will be valuable in studies on nutritional physiology, behavior, and drug administration. The EX-Q method should shed light on progressive changes in fly physiology and metabolism through monitoring of long-term feeding behavior. Furthermore, the method should be amenable to choice or preference studies because different food plugs labeled with dyes with non-overlapping absorbance spectra could be provided simultaneously. Finally, the EX-Q tube could also be used to maximize the collection of deposited hydrocarbons or fecal microorganisms, facilitating diverse studies.

Limitations of the Study

The EX-Q method for quantifying food intake offers a number of advantages over existing techniques, including high accuracy, ease-of-use, compatibility with typical lab diets, and the potential for longitudinal studies because flies are not sacrificed for the measurements. Nonetheless, there are potential limitations. First, the EX-Q assay might be less accurate in estimating food intake of older flies because intestinal barrier dysfunction increases with age (Rera et al., 2012), which may result in consumed dye that cannot be recovered from the fly body. Second, the EX-Q assay still relies on dye labeling, which may affect food properties. Although our previous studies showed that 1% erio-glucaine dye does not affect total consumption (Deshpande et al., 2014), commonly used dyes at high concentrations can be tasted by flies, resulting in preference for one dye over another in food choice experiments (Bantel and Tessier, 2016; Chen et al., 2019). This caveat could be mitigated by using a lower dye concentration and adjusting the volume used to dissolve excreta such that absorbance measurements are still in an optimal range.

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2019.100776>.

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AUTHOR CONTRIBUTIONS

Conceptualization, Q.W., W.W.J., and M.Y.; Investigation, Q.W., G.Y., S.J.P., Y.G., and W.W.J.; Formal Analysis, Q.W., G.Y., S.J.P., Y.G., W.W.J., and M.Y.; Writing - Original Draft, Q.W., W.W.J., and M.Y.; Writing - Review & Editing, Q.W., G.Y., S.J.P., Y.G., W.W.J., and M.Y.; Funding Acquisition, W.W.J. and M.Y.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Excreta Quantification (EX-Q) for Longitudinal Measurements of Food Intake in *Drosophila*

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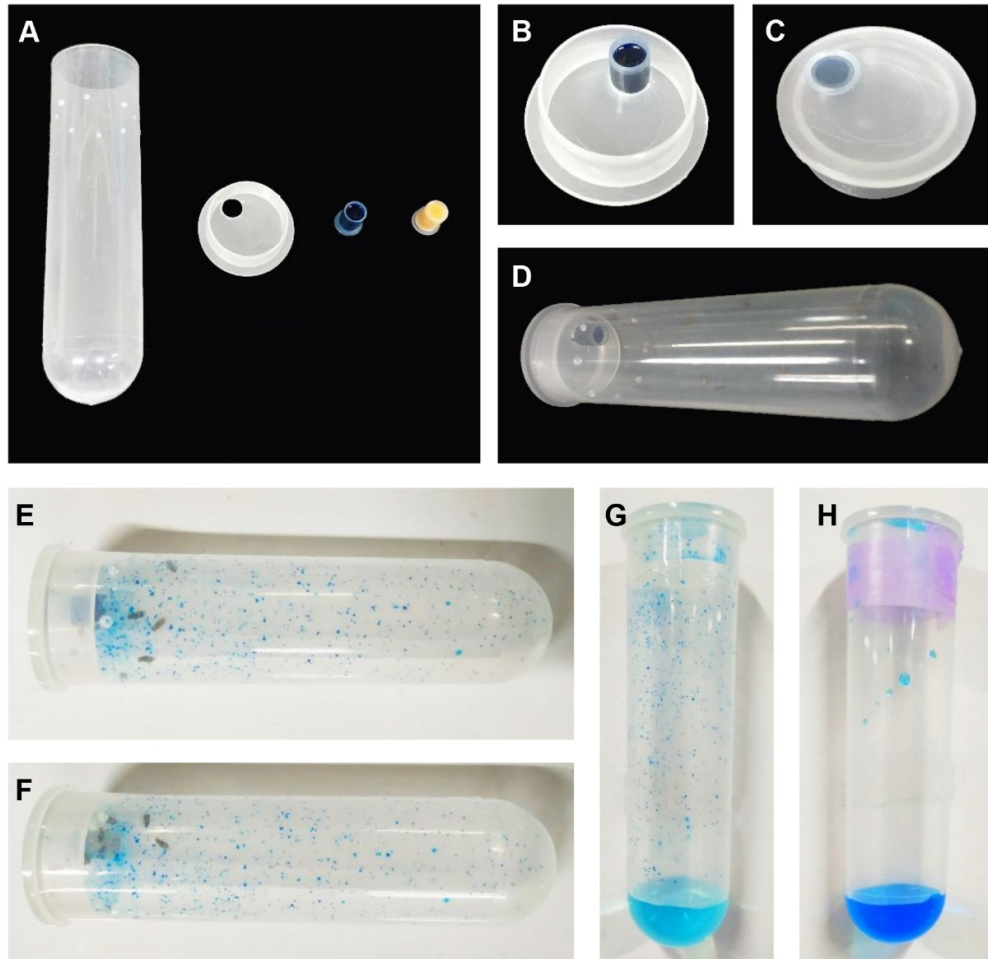


Figure S1. Photos of the EX-Q, Related to Figure 1. (A) Components of EX-Q include the main chamber (a round-bottom centrifuge tube), and a lid for the main chamber with a hole that fits a small food cup containing dye-labeled or non-labeled medium. (B-C) The small food cup is inserted into the main chamber lid (B, inside view; C, outside view). (D-H) Overview of the EX-Q method. (D) Flies are transferred into the EX-Q chamber and allowed to feed on dye-labeled medium. (E) After 24 hours of feeding, excreta containing blue dye accumulates on the inner surface of the chamber. (F) The dye food is replaced with non-labeled medium and flies are maintained in the chamber for another 3 hours to allow remaining dye to be excreted. (G) Flies are removed from the chamber and water or buffer is added. (H) Holes are sealed with tape and excreta are dissolved by shaking the tube. Dye can then be quantified from an aliquot of the excreta solution by spectrophotometry.

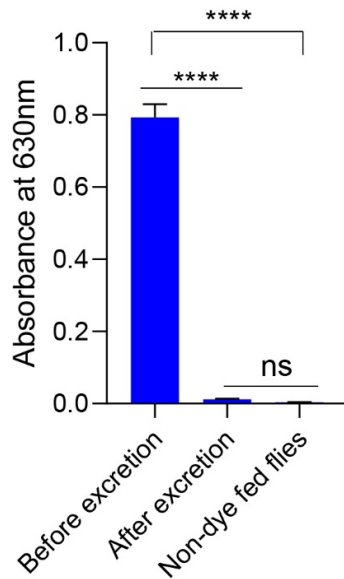


Figure S2. Three hours is sufficient for flies to excrete residual dye, Related to Figure 1. Flies were fed on medium containing 2.5% (w/v) erioglaucline disodium salt for 12 hours, and then transferred into Eppendorf tubes and homogenized in distilled water (left bar), or transferred into EX-Q tubes to excrete for 3 hours. After 3 hours of excretion, flies were transferred into Eppendorf tubes and homogenized in distilled water (middle bar). Homogenate from flies fed non-labeled food are shown as a control (right bar). Absorbance of filtered fly homogenates were measured at 630 nm. 5-day-old Dahomey females flies were fed on yeast-sugar medium (10% yeast, 5% sucrose). $n = 10$ replicates with 10 flies per replicate. P values were determined by one-way ANOVA followed by Tukey's multiple comparisons: **** $P < 0.0001$. Data are shown as mean \pm SEM.

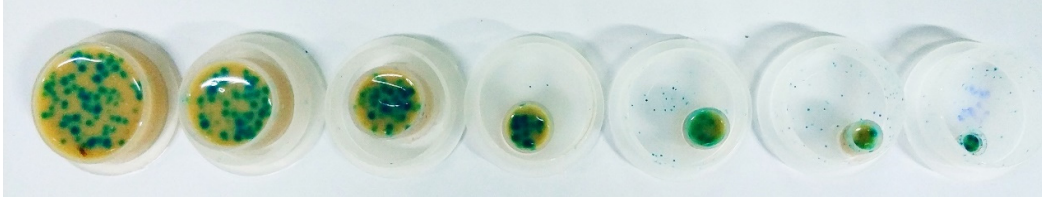


Figure S3. Excreta accumulation on the food surface is increased with greater surface area of the medium, Related to Figure 2. 5-day-old Dahomey female flies were fed on dye medium for 12 hours, and then transferred into EX-Q tubes with non-dye medium of several different sizes for 3 hours. The excreta accumulated on the food surface were then photographed. From left to right, the diameters of the food surfaces were 23.5, 18, 14, 10, 8, 5.5 and 3.5 mm.

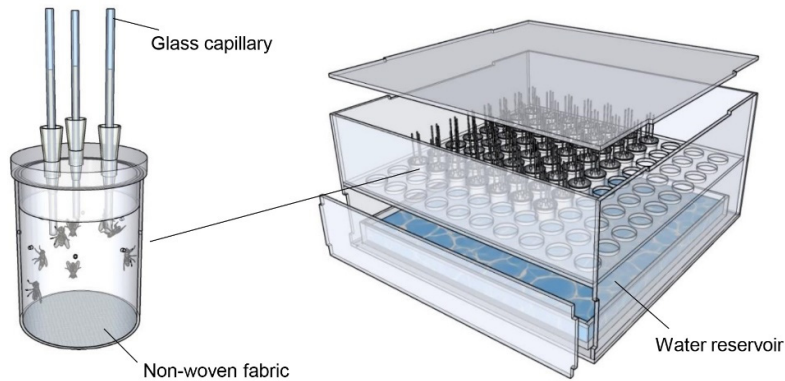


Figure S4. Schematic of the CAFE assay, Related to Figure 3. The CAFE chambers were made from the same round-bottom centrifuge tubes that were used to make EX-Q chambers, but the lower portion was cut off to keep only 3.5 cm of the upper region. CAFE chambers were sealed on the bottom with non-woven fabric to allow air circulation. Three trimmed 10- μ l pipette tips were inserted into the lid to hold glass microcapillaries. Capillaries containing 10 μ l of liquid food each were provided per chamber and were replaced every \sim 12 hours. Identical chambers without flies were maintained as controls for evaporation (evaporation was typically $<10\%$ of feeding volumes), and these measurements were subtracted from consumption data. CAFE chambers were placed in a transparent acrylic box with a water reservoir at the bottom to maintain humidity. The plate in the box has openings that are the same diameter as the CAFE tube, but slightly smaller than the diameter of the CAFE lid so that the height of the chambers can be maintained over the water reservoir.

Transparent Methods

Fly stocks and husbandry

The Dahomey stock was provided by Matthew D.W. Piper (Monash University). Flies were maintained at 25 °C on a 12:12-hour light:dark cycle at constant humidity using 1 SY food (10 g agar/50 g sucrose/100 g yeast). For all experiments, animals were reared at a standard larval density and adults were collected over a 12-hour period. Flies were mated for 48 hours before sorting into single-sex groups.

To obtain flies of the same age for experiments, ~1000 flies were maintained in a large enclosure containing a plate of grape juice-based medium. After 18 hours of egg laying, eggs were collected from the grape juice plate with PBS buffer into a 15-mL centrifuge tube. After allowing the eggs to settle, the supernatant was aspirated and eggs were dispensed into fly bottles (containing yeast medium) at a density of 200-300 eggs per bottle.

Preparation of EX-Q tubes

EX-Q tubes were made from round-bottom centrifuge tubes (Solarbio YA0473, length 102.8 mm, i.d. 25.5 mm, o.d. 27.9 mm). The diameter of the cap brim is 30.4 mm. The size of the EX-Q tube is nearly equivalent to a standard fly vial to facilitate fly transfers. Other equivalent tubes can be used instead (e.g., Fisher 3110-0500, with DS3111 lids). Air holes (~0.5 mm diameter) were made with a pushpin. Although air holes were made on the side of the tubes in our experiments, incorporating air holes only in the caps streamlines subsequent steps to recover excreta and improves reusability of the EX-Q tubes. For various diameter food cups, an appropriately sized hole in the big cap was cut with surgical scissors.

Food preparation for EX-Q

For yeast-sugar and yeast extract-sugar food used in EX-Q experiments, the agar (BioFroxx 1182GR500) concentration is 1% (w/v). All food used in the study were dissolved by sterilizing at 120 °C for 15 minutes. Erioglaucline (Sigma 861146, for dye food), 30 mL 10% methyl-p-hydroxybenzoate/liter and 3 mL propionic acid/liter were added to the medium after cooling to 60 °C. The holidic medium (100N50S Yaa) was prepared as described (Piper et al., 2014) except that the agar concentration was adjusted to 1.5%. The holidic medium used in Figure 3D was cholesterol-free.

The 5.5-mm diameter food container is a plastic cap for 6-mm screws (HeZhong X-M6), but can be replaced with the pipettor-end of a 200- μ L pipette tip. The 3.5-diameter food container is the pipettor-end of a 10- μ L pipette tip. When using 10- μ L pipette tips as food cups, the tips should be bent and sealed with pliers in advance. Food can then be injected into the pipettor-end. The 3.5- and 5.5-mm diameter food containers each require approximately 80 and 250 μ L of medium, respectively.

EX-Q experiment

Adult flies (~3 days post-eclosion) were acclimated on the experimental food for 48 hours before testing (except for experiments in Figure 4), and transferred into the EX-Q tubes on day

5 to measure food intake. Identical medium containing 1% (w/v) erioglaucine was used as the assay medium. See detailed methods in main text. For all EX-Q experiments, the small food cup (3.5-mm diameter) was used, unless specifically stated. EX-Q chambers were typically maintained horizontally, but estimated food consumption did not differ based on EX-Q orientation (data not shown).

Absorbance of solubilized excreta (100 μ l) was measured at 630 nm in a 96-well plate. Food intake was calculated according to a standard curve prepared from stock solutions of pure dye (Shell et al., 2018). Briefly, 100 mg of erioglaucine is dissolved in 10 ml of ddH₂O. Absorbance was then measured from multiple 2- or 3-fold dilutions from this stock solution. Accuracy of food intake measurements might also be improved by generating standard curves directly from experimental diets. An aliquot of dye food is solubilized in water or buffer by heating at 80 °C. After cooling to room temperature, a standard curve can be produced from dilutions of the initial solution.

³²P radioisotope labeling

To compare fly food intake in EX-Q tubes with different food surface sizes, adult flies were acclimated on the yeast-sugar medium (3.5% tryptone, 5% sucrose) for 48 hours before testing, and transferred into the EX-Q tubes with a medium (diameter 23.5, 5.5, or 3.5 mm) containing 1–2 μ Ci/ml [α -³²P]dCTP to measure food intake. After 24 h, flies were transferred to empty vials and killed by freezing at –80 °C. Excreta were collected from EX-Q tubes with 1 mL 1 \times PBST. Radioactivity in whole flies and excreta were quantified in 5 ml of scintillation fluid (ScintiVerse BD Cocktail, Fisher Scientific) in a multipurpose scintillation counter (LS 6500, Beckman Coulter).

Comparison between EX-Q and direct homogenization

Flies were fed on dye medium containing 2.5% (w/v) erioglaucine for 12 hours, and then transferred into Eppendorf tubes and homogenized in distilled water, or transferred into EX-Q tubes for 3 hours to collect excreta. Flies were homogenized as previously described (Wong et al., 2009). Briefly, flies were transferred to 1.5-ml Eppendorf tubes and homogenized in 200 μ l dH₂O. 800 μ l of dH₂O was then added and the suspension was filtered (0.22-mm Millex, Millipore Corporation, Bedford, MA) to remove debris and lipids. The absorbance of the liquid sample was then measured at 630 nm (Thermo Fisher Spectrophotometer 3020). Age-matched flies exposed to non-labeled food were used as the baseline. For excreta in the EX-Q tube, 1 ml ddH₂O was added into the EX-Q tube to dissolve the dye and the absorbance was then measured at 630 nm.

CAFE assay

The CAFE assay was performed essentially as described (Deshpande et al., 2014; Ja et al., 2007) with changes detailed (**Figure S4**). To be consistent with EX-Q food preparation, yeast extract medium for the CAFE assay was also sterilized at 120 °C for 15 minutes, but without dye and agar. Due to difficulty in solubilizing cholesterol, the holidic medium used in Figure 3D was cholesterol-free.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.0.2. *P* values were determined by unpaired *t*-test, one-way ANOVA followed by Tukey's multiple comparisons, or two-way ANOVA followed by Sidak's multiple comparisons test. The Shapiro-Wilk normality test was used to determine if data sets were consistent with a Gaussian distribution.