File S1

Supplementary Discussion

In this supplementary discussion, we will discuss some technical aspects of our assays. In the manuscript, we assert that nutrient absorption is proportional to the food intake of *C. elegans*. We based this assertion on data present in the manuscript and the fact that amino acids cannot be stored unless in the proteome.

I) First, we compare the results of our bacterial clearance assay for serotonin-induced food intake in wild-type animals (see Figure 3B, D5:D8) to the results described with the nutrient absorption assay (see Figure 5B, D5:D7). In the bacterial clearance assay, we observed an approximately 2-fold increase in food intake when comparing basal and serotonin-induced feeding during the D5:D8 interval. Similarly, in the nutrient absorption assay we observed a 1.8-fold increase in N15 labeling in wild-type N2 animals treated with serotonin as compared to water-treated animals. Both assays indicate that food intake, or a proxy of food intake, has increased by approximately 2-fold upon exposure to serotonin.

II) Comparing published results of pharyngeal pumping (HUANG *et al.* 2004) with both our bacterial clearance and nutrient absorption data show the highest feeding rate to be for worms transitioning from the L4 larval stage to adulthood (L4 to D2, Figure 1K). We observed significantly higher rates of N15 labeling over a shorter period of time with the nutrient absorption assay in young adult animals (L4 to D1) as compared to animals at later stages of adulthood (see Figure 5B, 5C, comparing fraction labeled).

While at first food intake seems simple, it is a rather complex issue. Food intake is not a single process but involves an entire chain of events that involves the feeling of hunger, food-searching behavior, eating of food, ingesting of food and finally metabolizing it. Thus, different assays, measuring different aspects of this chain, may occasionally result in opposing outcomes without any real biological disagreement. Consider an animal with a gut defect that impairs nutrient uptake. As a consequence, it is likely that the animal will try to compensate for the defect by over-eating. Thus, in such a case, the pharyngeal pumping assay as well as the bacterial clearing assay would detect an increase in food intake while the pulse-feeding assay would detect a decrease in food intake. While these results would look contradictory, in fact they correctly represent the underlying biology.

With the development of the bacterial clearing assay and the pulse-feeding assay, we intended to add to the toolbox necessary to investigate the *C. elegans* food-intake chain. With the bacterial clearance assay, we attempted to generate an assay equivalent to those used in other model organisms, where food intake is measured by calculating the difference of food initially given to an animal minus food not consumed. The bacterial clearance assay is the *C. elegans* equivalent of those assays. Similarly, we generated the pulse-feeding assay to ensure that our estimates of food intake reflect the actual absorption and utilization of ingested nutrients. In our view, these two assays are to be used in combination with current assays to dissect food searching, detection, ingestion, and usage (protein synthesis).

HUANG, C., C. XIONG and K. KORNFELD, 2004 Measurements of age-related changes of physiological processes that predict lifespan of Caenorhabditis elegans. Proc Natl Acad Sci U S A **101:** 8084-8089.