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.

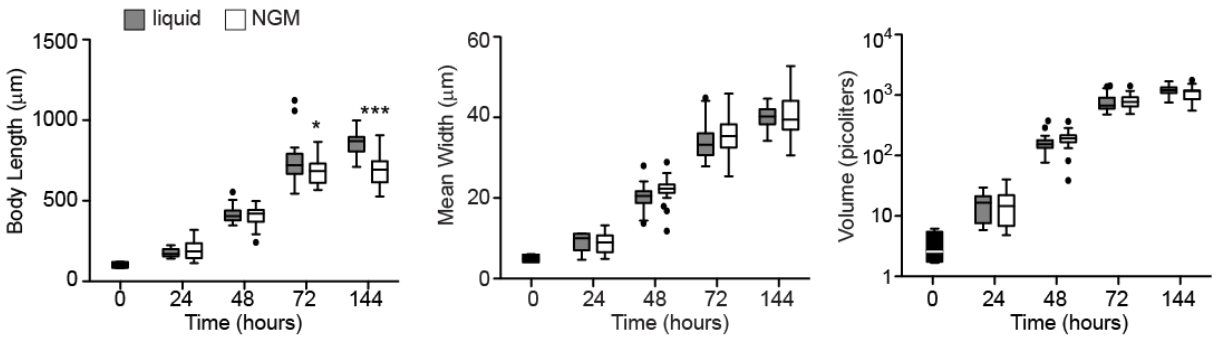

Figure S1 Comparison of Body Size and Growth Rate of Worms Grown in Liquid Versus Solid Media. Data are representative of three independent experiments. $N_{worms} > 20$ for each condition and all time points except at 0 hrs. For data at 0 hrs starved synchronized worms ($N_{worms} = 5$) were measured prior to plating in liquid or NGM. *** $P < 0.001$, * $P < 0.05$, Two-way ANOVA with Bonferroni post-hoc test.

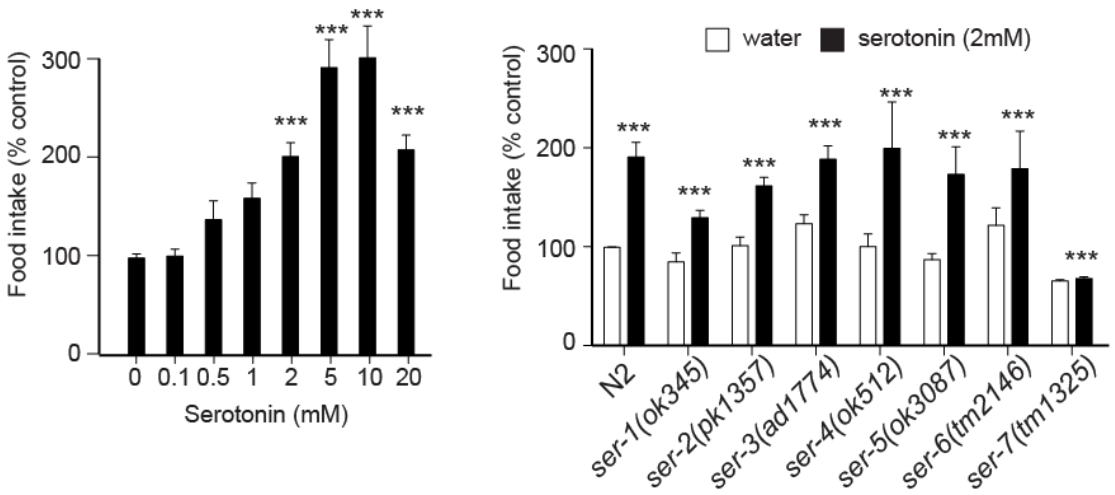

Figure S2 Same experiments as shown in Figure 3A and C using bar plots of the mean and standard error of the mean (S.E.M) instead of the Tukey plots. Some error bars are too small to see.

Table S1 List of proteins induced by serotonin in *C. elegans* that were identified by hierarchical clustering of fraction labeled values. Relationships of proteins within these groups were explored by KEGG pathways analysis. (A) Proteins in which the increase in fraction labeled were highest. (B) Proteins in which the increase in fraction labeled were lower. Genes in bold extend lifespan when suppressed by RNAi and thus are likely to be age-promoting when highly expressed.

Table S1A. Highest Fraction Labeled

UniProt ID	WB Gene Name
ARF12_CAEEL	arf-1
BAF1_CAEEL	baf-1
BTF3_CAEEL	icd-1
CGL2_CAEEL	cth-2
CH60_CAEEL	hsp-60
CHIT_CAEEL	cht-1
CLC87_CAEEL	clec-87
CLC91_CAEEL	clec-91
CPG2_CAEEL	cpg-2
CPG3_CAEEL	cpg-3
CPR6_CAEEL	cpr-6
CYP1_CAEEL	cyn-1
CYP2_CAEEL	cyn-2
CYP3_CAEEL	cyn-3
CYP5_CAEEL	cyn-5
CYP6_CAEEL	cyn-6
CYP7_CAEEL	cyn-7
DPY30_CAEEL	dpy-30
EF1A_CAEEL	eft-3
EF1B1_CAEEL	**eef-1B.1**
EF1B2_CAEEL	eef-1B.2
EF2_CAEEL	**eef-2**
ETFA_CAEEL	F27D4.1
FABP2_CAEEL	lbp-2
FABP9_CAEEL	lbp-9
G3P1_CAEEL	gpd-1
GBLP_CAEEL	rack-1
GLC7A_CAEEL	gsp-1
GLYC_CAEEL	mel-32
GST7_CAEEL	gst-7
H2B1_CAEEL	his-11
H4_CAEEL	his-1
HSP7A_CAEEL	hsp-1

HSP7D_CAEEL	hsp-4
HSP7F_CAEEL	hsp-6
KARG2_CAEEL	ZC434.8
NACA_CAEEL	icd-2
PCNA_CAEEL	pcn-1
PFD2_CAEEL	pdf-2
PLBL1_CAEEL	Y37D8A.2
PLBL2_CAEEL	F09B12.3
PSA1_CAEEL	pas-6
PSA2_CAEEL	pas-2
PSA3_CAEEL	pas-7
PSA5_CAEEL	pas-5
PSA6_CAEEL	pas-1
PSA7_CAEEL	pas-4
PSB1_CAEEL	pbs-6
PURA_CAEEL	C37H5.6
RAN_CAEEL	ran-1
RIR2_CAEEL	rnr-2
RL10_CAEEL	rpl-10
RL10A_CAEEL	rpl-1
RL11_CAEEL	rpl-11.1
RL12_CAEEL	rpl-12
RL13_CAEEL	rpl-13
RL13A_CAEEL	rpl-16
RL15_CAEEL	rpl-15
RL17_CAEEL	rpl-17
RL18_CAEEL	rpl-18
RL18A_CAEEL	rpl-20
RL22_CAEEL	rpl-22
RL23_CAEEL	rpl-23
RL24_CAEEL	rpl-24.1
RL27_CAEEL	rpl-27
RL28_CAEEL	rpl-28
RL3_CAEEL	rpl-3
RL4_CAEEL	**rpl-4**
RL5_CAEEL	rpl-5
RL6_CAEEL	**rpl-6**
RL7_CAEEL	rpl-7
RL7A_CAEEL	rpl-7A
RL8_CAEEL	rpl-2
RL9_CAEEL	rpl-9
RLA1_CAEEL	rla-1
RLA2_CAEEL	rpa-2

RS12_CAEEL	rps-12
RS13_CAEEL	rps-13
RS14_CAEEL	rps-14
RS15_CAEEL	**rps-15**
RS17_CAEEL	rps-17
RS19_CAEEL	rps-19
RS2_CAEEL	rps-2
RS21_CAEEL	rps-21
RS23_CAEEL	**rps-23**
RS26_CAEEL	**rps-26**
RS27A_CAEEL	ubl-1
RS28_CAEEL	rps-28
RS3_CAEEL	rps-3
RS3A_CAEEL	rps-1
RS4_CAEEL	rps-4
RS5_CAEEL	**rps-5**
RS6_CAEEL	**rps-6**
RS7_CAEEL	rps-7
RS8_CAEEL	**rps-8**
RS9_CAEEL	rps-9
RSSA_CAEEL	rps-0
SMD1_CAEEL	snr-3
SODM1_CAEEL	**sod-2**
SUMO_CAEEL	smo-1
SYSC_CAEEL	sars-2
TCTP_CAEEL	tct-1
TDX1_CAEEL	prdx-3
TIMPL_CAEEL	tag-225
U375A_CAEEL	C08F11.11
UBIQ_UBIQ1_CAEEL	ubq-1
UBIQ_RL40_CAEEL	ubq-2
VIT1_CAEEL	vit-1
VIT2_CAEEL	vit-2
VIT3_CAEEL	**vit-3**
VIT4_CAEEL	**vit-4**
VIT5_CAEEL	**vit-5**
VIT6_CAEEL	vit-6
YOCA_CAEEL	ZC395.10

Table S1B. Lower Fraction Labeled

UniProt ID	WB Gene Name
3HAO_CAEEL	haao-1
ACBP1_CAEEL	acbp-1
ACOC_CAEEL	aco-1
ACON_CAEEL	**aco-2**
ACT1_CAEEL	act-1
ACT2_CAEEL	act-2
ACT4_CAEEL	act-4
ADH1_CAEEL	**sodh-1**
ADHX_CAEEL	H24K24.3
AL7A1_CAEEL	alh-9
ALF1_CAEEL	**aldo-1**
ALF2_CAEEL	aldo-2
AMPL_CAEEL	lap-1
ANC1_CAEEL	anc-1
ARP3_CAEEL	arx-1
ATPA_CAEEL	**H28O16.1**
ATPB_CAEEL	**atp-2**
ATPD_CAEEL	F58F12.1
BAT45_CAEEL	bath-45
CALR_CAEEL	crt-1
CISY_CAEEL	cts-1
CORO_CAEEL	cor-1
CYC21_CAEEL	cyc-2.1
DEOC_CAEEL	F09E5.3
DNPEP_CAEEL	F01F1.9
ECHM_CAEEL	ech-6
ENO_CAEEL	**enol-1**
F37C4_CAEEL	F37C4.5
FABP6_CAEEL	**lbp-6**
FAR2_CAEEL	far-2
FUMH_CAEEL	fum-1
G3P2_CAEEL	**gpd-2**
GABT_CAEEL	gta-1
GCP_CAEEL	**gei-7**
HCDH2_CAEEL	B0272.3
HPPD_CAEEL	**hpd-1**
HSP7C_CAEEL	hsp-3
IFB1_CAEEL	ifb-1
IMPA1_CAEEL	ttx-7
IPYR_CAEEL	pyp-1
KARG1_CAEEL	F46H5.3

LDH_CAEEL	ldh-1
MDHM_CAEEL	mdh-2
METK1_CAEEL	sams-1
MIF2_CAEEL	mif-2
MLE_CAEEL	mlc-3
MLR1_CAEEL	mlc-1
MLR2_CAEEL	mlc-2
MMSA_CAEEL	alh-8
MPI_CAEEL	ZK632.4
MSP10_CAEEL	msp-10
MSP49_CAEEL	msp-49
MYO4_CAEEL	unc-54
MYSP_CAEEL	unc-15
OAT_CAEEL	C16A3.10
ODO1_CAEEL	ogdh-1
ODPA_CAEEL	T05H10.6
PCCA_CAEEL	pcca-1
PDI2_CAEEL	pdi-2
PGK_CAEEL	pgk-1
PPN1_CAEEL	mig-6
PROF2_CAEEL	pfn-2
SAHH_CAEEL	ahcy-1
SCOT_CAEEL	C05C10.3
SODC_CAEEL	sod-1
TBA2_CAEEL	tba-2
TBB2_CAEEL	tbb-2
TNNI2_CAEEL	unc-27
TPIS_CAEEL	tpi-1
TPM1_CAEEL	lev-11
TPM3_CAEEL	lev-11
TTR15_CAEEL	ttr-15
UNC87_CAEEL	unc-87
VATA_CAEEL	vha-13
VATB_CAEEL	vha-12
VATF_CAEEL	vha-9
YH24_CAEEL	lap-2
YUW5_CAEEL	F41C3.5
YVRI_CAEEL	F37H8.5

File S2

Extended Materials and Methods

Part 1: Materials.

Potassium phosphate buffer, pH 6.0, 1000ml

136 g KH2PO4
Add deionized water to 900 ml
Adjust pH to 6.0 with 5M KOH
Add deionized water to 1000 ml
Autoclave

Trace metal solution

1.86 g Na2EDTA
0.69 g FeSO4·7H20
0.20 g MnCl2·4H20
0.29 g ZnSO4·7H20
0.016 g CuSO4
1000 ml deionized water
Autoclave and store in the dark

S-basal medium, 1000 ml

5.9 g NaCl
50 ml 1M potassium phosphate, pH 6.0
1000 ml deionized water
Autoclave

Potassium citrate 1M, 1000 ml

268.8 g tripotassium citrate
26.3 g citric acid monohydrate
Add 900 ml deionized water
Adjust pH to 6.0 with 5M KOH
Add deionized water to 1000 ml
Autoclave

Part 2: Preparation of S-complete medium

S-complete medium, 1000ml

977 ml S-basal medium
10 ml 1M potassium citrate, pH 6.0 (sterile)
10 ml Trace metals solution (sterile)
3 ml 1M CaCl2 (sterile)
3 ml 1M MgSO4 (sterile)
1 ml 5mg/ml Cholesterol (dissolved in EtOH)