

Supplemental Data

Role of CYP-eicosanoids in the regulation of pharyngeal pumping and food uptake in *C. elegans*

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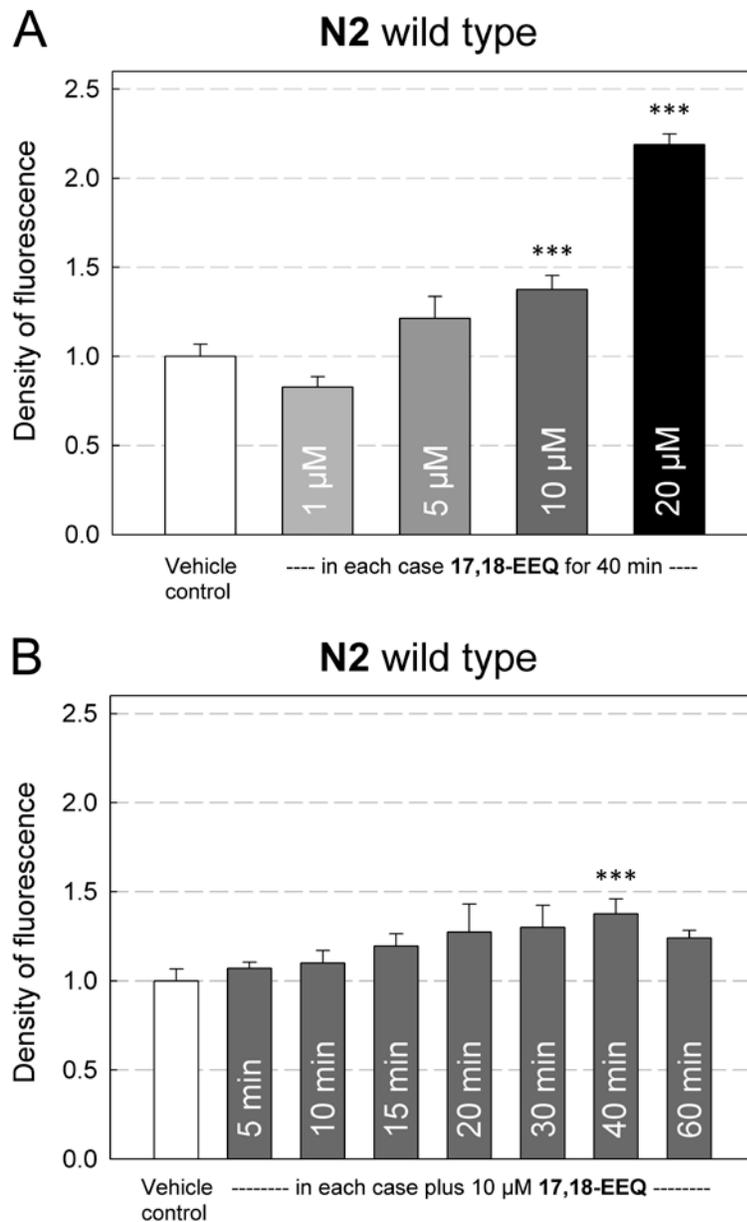
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Supplemental Figure 1

Concentration- and time-dependent effects of 17,18-EEQ treatment on the food uptake.

Shown is the relative effect of 17,18-EEQ on the accumulation of 0.5 μm fluorescent beads. The accumulation was assayed in well-fed worms pre-incubated for 40 min with different concentrations of 17,18-EEQ (A) or pre-incubated with 10 μM 17,18-EEQ for different time periods (B). Data are means + SEM; n=15. *P*-values were obtained from one way ANOVA, ****P* < 0.001; vehicle: 0.3% DMSO.



Supplemental Table 1: Total fatty acid composition of wild type and mutant strains

Fatty acid composition (mol %)					
Fatty acid	N2 (wild type)	<i>emb-8</i> (<i>hc69</i>)	<i>fat-1</i> (<i>wa9</i>)	<i>fat-2</i> (<i>wa17</i>)	<i>fat-3</i> (<i>wa22</i>)
	<i>n</i> =5	<i>n</i> =9	<i>n</i> =3	<i>n</i> =4	<i>n</i> =3
C16:0 ¹	16.3±2.0	6.2±1.0 ^{***}	10.0±0.9 [*]	17.7±3.3	14.6±1.2
C18:0	9.3±2.3	6.3±1.0	8.2±0.2	8.7±0.6	8.1±0.1
C18:1 ²	29.0±4.8	23.0±2.6	28.4±2.8	66.1±2.3 ^{***}	31.2±1.6
C18:2 n-6	7.8±1.4	17.1±1.1 ^{***}	5.5±1.0	3.7±0.1	25.6±1.1 ^{***}
C18:3 n-6	5.3±0.6	6.0±1.2	13.1±1.0 ^{***}	n.d.	1.1±0.1 ^{***}
C18:3 n-3	0.6±0.1	1.1±0.1 [*]	1.0±0.12 [*]	n.d.	14.8±0.1 ^{***}
C20:1 n-9	0.1±0.1	0.2±0.1	n.d.	n.d.	n.d.
C20:3 n-6	5.7±0.9	6.7±0.5	15.2±1.4 ^{**}	n.d.	2.0±0.2 [*]
C20:4 n-6	1.4±0.3	1.4±0.3	17.0±1.1 ^{***}	0.4±0.1 [*]	0.5±0.0 [*]
C20:4 n-3	3.3±0.5	3.6±0.6	n.d.	n.d.	n.d.
C20:5 n-3	21.2±3.0	28.4±1.1 [*]	1.6±0.1 ^{**}	2.6±0.1 ^{***}	0.9 ^{**} ±0.1

n.d. – not detectable

¹ C16:0 are the total mol % of 16:0, C16:iso, and 16:1

² C18:1 are the total mol % of C18:1 n-7 and C18:1 n-9

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, One way ANOVA

Supplemental Table 2: CYP-eicosanoid composition of wild type and mutant strains

CYP-eicosanoid pattern (ng/mg protein)					
CYP-eicosanoid	N2 (wild type)	<i>emb-8</i> (<i>hc69</i>)	<i>fat-1</i> (<i>wa9</i>)	<i>fat-2</i> (<i>wa17</i>)	<i>fat-3</i> (<i>wa22</i>)
	<i>n</i> =11	<i>n</i> =4	<i>n</i> =6	<i>n</i> =3	<i>n</i> =6
19-HETE	1.1±0.2	n.d.	8.8±0.9 ^{***}	0.3±0.0 [*]	0.2±0.0 ^{***}
20-HETE	0.8±0.1	n.d.	4.7±1.1 ^{***}	n.d.	n.d.
14,15-DHET	0.1±0.0	n.d.	0.6±0.1 ^{***}	n.d.	n.d.
14,15-EET	0.2±0.0	n.d.	1.9±0.2 ^{***}	n.d.	n.d.
11,12-DHET	n.d.	n.d.	0.2±0.0 ^{***}	n.d.	n.d.
11,12-EET	0.2±0.1	n.d.	1.5±0.1 ^{***}	n.d.	n.d.
8,9-DHET	n.d.	n.d.	n.d.	n.d.	n.d.
8,9-EET	0.2±0.1	n.d.	1.3±0.1 ^{***}	n.d.	n.d.
5,6-DHET	0.2±0.1	n.d.	0.4±0.1	0.2±0.2	n.d.
5,6-EET	0.1±0.0	n.d.	1.6±0.3 ^{***}	n.d.	n.d.
19-HEPE	1.9±0.4	0.2±0.1 ^{**}	n.d.	n.d.	n.d.
20-HEPE	1.4±0.1	0.1±0.0 [*]	n.d.	0.2±0.1 ^{***}	n.d.
17,18-DHEQ	30.3±0.3	14.5±2.6 [*]	0.4±0.1 ^{***}	1.0±0.0 ^{***}	0.6±0.19 ^{***}
17,18-EEQ	6.7±0.6	0.6±0.2 ^{**}	0.2±0.0 ^{***}	0.2±0.1 ^{***}	0.1±0.0 ^{***}
14,15-DHEQ	2.2±0.3	0.3±0.1 ^{**}	0.2±0.0 ^{***}	0.4±0.1 [*]	n.d.
14,15-EEQ	2.1±0.5	n.d.	n.d.	n.d.	n.d.
11,12-DHEQ	0.7±0.2	0.3±0.1	0.1±0.0 [*]	0.2±0.1	n.d.
11,12-EEQ	1.9±0.2	n.d.	n.d.	0.1±0.0 ^{***}	n.d.
8,9-DHEQ	0.3±0.1	0.2±0.1	n.d.	0.1±0.0	n.d.
8,9-EEQ	0.9±0.1	0.1±0.0 ^{**}	n.d.	0.1±0.0 ^{***}	n.d.
5,6-DHEQ	1.0±0.3	n.d.	n.d.	0.1±0.1 [*]	0.1±0.0 ^{***}
5,6-EEQ	1.4±0.3	n.d.	n.d.	0.3±0.1 [*]	n.d.

n.d. – not detectable, ^{*}*P* <0.05, ^{**}*P* <0.01, ^{***}*P* <0.001, One way ANOVA

Please note that all DHETs and DHEQs are hydrolysis products of previously CYP-produced corresponding EETs and EEQs, respectively.

Captures for Supplemental Videos 1 – 4

Supplemental Video 1. One minute film of the head region of a representative untreated N2 wild type worm. The worm pumps 290 times in one minute.

Supplemental Video 2. One minute film of the head region of a representative untreated *fat-3(wa22)* mutant worm. The worm pumps 207 times in one minute.

Supplemental Video 3. One minute film of the head region of a representative *fat-3(wa22)* mutant worm treated with 10 μ M 17,18-EEQ for 40 min, just before recording. The worm pumps 237 times in one minute.

Supplemental Video 4. One minute film of the head region of a representative N2 wild type worm treated with 10 μ M 20-HETE for 40 min, just before recording. The worm pumps 257 times in one minute.