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Quantitative Lipid Analysis and Life Span of the *fat-3* Mutant of *Caenorhabditis elegans*

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The long-chain polyunsaturated fatty acids (LC PUFAs) docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3) are important for health and development of organisms, but the precise biological function of these molecules is not known. It has been suggested that they may play a part in aging, as they are highly susceptible to oxidation. A genetic mutant of *Caenorhabditis elegans* (*fat-3*), which lacks a functional δ-6 desaturase, and thus LC PUFAs including EPA, allows dietary manipulation of long-chain n3 fatty acids in this nematode. The life span of *C. elegans* strains N2 (wild-type) and BX30 [*fat-3*(wa22)] with and without supplemental EPA and DHA was analyzed. In addition, quantitative analysis was performed on total lipids, phospholipids, and triglycerides, as it is important to understand where fatty acids are being partitioned among the various lipid classes. The results show a beneficial effect of these molecules on the life span of *C. elegans* and will aid in the elucidation of the underlying causes of PUFA deficiency in the simple animal *C. elegans* as well as in humans.

KEYWORDS: ***Caenorhabditis elegans*; docosahexaenoic acid; eicosapentaenoic acid; lipid analysis; aging; life span**

INTRODUCTION

With the vast number of papers on the n3 fatty acids docosahexaenoic acid (DHA, 22:6n3) and eicosapentaenoic acid (EPA, 20:5n3)—a Pubmed search yielded over 10000 papers for EPA and DHA combined—it is surprising that the biological function of these molecules remains largely a mystery. It is well-known that they are essential for proper brain and nerve development and function (*1*); however, it is not clear what role(s) they serve in these processes. The level of unsaturation in bacterial membranes, most of which do not contain long-chain polyunsaturated fatty acids (LC PUFAs), is positively correlated with the rate of certain membrane-associated processes, such as oxygen uptake (*2*). Along this same line, membranes have been proposed to act as "pacemakers" for metabolism and possibly for aging (*3*). This theory is based largely on a correlation between metabolic rate and level of membrane fatty acid unsaturation in mammals and birds (*3*); that is, the greater the amount of unsaturated fatty acids in the membrane, the higher the metabolic rate of the animal tends to be. In mammals, high metabolic rates are generally associated with smaller species and shorter life spans, and lower metabolic rates are generally associated with larger species and longer life spans (*3*, *4*). Taken together, these findings indicate that unsaturated fatty acids (UFAs) may play a role in determining the metabolic rate of membrane-associated processes, yet direct evidence in support of this idea has not been produced in any organism beyond bacteria.

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It remains unclear what role, if any, oxidation plays in aging, but it seems likely that if there is a link between the two processes, then highly oxidizable molecules, such as EPA and DHA, would be implicated as key molecules involved in this process. This may be especially true in the mitochondrial inner membrane. Cardiolipin, a phospholipid present only in the mitochondrial inner membrane, was shown to have a high specificity for accumulating DHA (*5*). When cardiolipin contains large amounts of DHA, up to 50% of esterified fatty acids in some circumstances, free radical production increases drastically (*5*). The combination of highly oxidizable DHA in close proximity to reactive free radicals could increase the oxidative load of the membrane significantly. Unfortunately, there is no way to determine, in a controlled experiment, how altering the LC PUFA content of human tissues would affect the aging process and, thus, the longevity of a person as a function of their fatty acid profile. It is possible, however, to control the fatty acid content of a simple organism such as *Caenorhabditis elegans* through a combination of genetic manipulation and dietary control and then to measure its life span.

Whereas humans cannot synthesize n3 fatty acids de novo, the nematode *C. elegans* can synthesize all of its necessary fatty acids up to and including EPA, making it an attractive model in which to further investigate the function of PUFAs (*6*, *7*). Using nematodes that produce EPA, but not eicosanoids, it is possible to study DHA and EPA in the absence of one of the known functions of LC n3 fatty acids, so that their role as structural components of the phospholipid membrane bilayer can be independent of other factors. The *fat-3* mutant of *C.*

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elegans is lacking a functional δ-6 desaturase, and, as such, cannot synthesize sufficient amounts of any fatty acid beyond linoleic acid (18:2n6) and linolenic acid (18:3n3) (*7*). Unsurprisingly, this strain of nematode exhibits pleiotropic affects from the lack of LC PUFAs, nearly all of which can be rescued by the addition of various fatty acids that contain the n12 unsaturation (the product of δ-6 desaturase activity on an 18-carbon fatty acid). Subsequent studies on the *fat-3* mutant have focused on neurotransmission and sensory signaling as the underlying mechanisms for the pleiotropic effects of LC PUFA deficiency in this strain. Lesa et al. (*8*) showed that the *fat-3* mutant has lower levels of synaptic vesicles and neurotransmitters at certain neuromuscular junctions, and they suggested that this may be the underlying cause of clinical symptoms associated with LC PUFA deficiency. Kahn-Kirby et al. (*9*) demonstrated that LC PUFAs are needed for TRPV-dependent sensory signaling in the *fat-3* mutant and hypothesize that this dependency may play a role in LC PUFA function in human processes such as vasodilation.

Although it was shown that removing nearly all LC PUFAs from *C. elegans* did not alter their life span, only a single trial was performed, and no statistical data were provided (*10*). Additionally, it is unknown whether exogenously fed DHA can affect the overall life span of both wild-type (WT) and *fat-3* mutant worms. Lesa et al. (*11*) reported that both WT and *fat-3* mutant worms convert exogenously fed DHA to EPA. This indicates that it may be detrimental for *C. elegans* to have DHA in their membranes, and retroconversion of DHA to EPA may be a mechanism that evolved to cope with DHA. The added oxidative stress of DHA may be too great for the repair mechanisms of the worm to overcome, which may cause a shortened life span. It is also possible that there are benefits from these molecules, such as an increase in proton gradient in the mitochondria (*5*).

To understand the function of fatty acid molecules, it is important to determine where the LC PUFAs partition among the lipid classes in a quantitative, as opposed to relative, manner. Quantitative data can reveal changes in lipid metabolism that semiquantitative, or mole percent, data cannot (*12*). Furthermore, because fatty acids are building blocks for more complex lipid species such as triglycerides and phospholipids, in addition to determining the quantitative concentrations of total fatty acids, that is, fatty acids from all lipid classes, separating lipids into different classes provides a more holistic measurement of metabolism. It is well-known in biology that function follows structure; as such, EPA esterified to a phospholipid will have metabolic and physiological consequences drastically different from those of EPA esterified to a triglyceride molecule.

Using a quantitative lipid separation and analysis technique described previously (*13*), the concentrations of fatty acids in total lipid (all classes), phospholipids (all phospholipid classes combined), and triglyceride were determined in both WT worms and *fat-3* mutants. Life span analysis was also performed on WT and *fat-3* cultures. The analyses presented here will further aid in the elucidation of the underlying causes of PUFA deficiency in the simple animal *C. elegans* and, by extension, in humans.

MATERIALS AND METHODS

C. elegans strains N2 (WT) and BX30 [*fat-3*(wa22)] and *Escherichia coli* OP50 were obtained from the *Caenorhabditis* Genetics Center. *E. coli fadE*$^-$ was provided by Dr. John Cronan (University of Illinois, Urbana, IL). Worms were fed *E. coli fadE*$^-$ instead of *E. coli* OP50—which is typically used—because the *fad*E$^-$ mutant lacks the ability to catabolize fatty acids (*14*) and did not affect *C. elegans* life span as compared with worms fed *E. coli* OP50 (data not shown). Free fatty acids were purchased from Nu-Chek Prep (Elysian, MN).

Table 1. Average Life Span of *C. elegans* WT and *fat-3* at 20 °C with and without EPA and DHA Supplementation

strain	treatment	av life span (days)	SEM	*N*
WT	control	14.5	1.0	17
WT	EPA	14.0	0.9	21
WT	DHA	15.8	1.2	14
fat-3	control	10.9[a]	1.4	9
fat-3	EPA	12.8	0.5	22
fat-3	DHA	12.6	1.1	9

[a] significantly different from WT control at $P < 0.05$.

Briefly, life span analysis was performed at 20 °C by transferring adult worms from a synchronized culture to a fresh nematode growth media plate. Then, live worms were transferred every other day using a platinum pick to fresh plates. Worms were determined to be dead if they did not move after being prodded with the platinum pick. The life span analysis procedure followed has been previously described by Larsen et al. (*15*), with the following exceptions. Fatty acids and bacteria were spread on the surface of the nematode growth medium plates immediately before worms were transferred to the plates in order to reduce the oxidation of the PUFAs prior to life span analysis. Free fatty acids were added to the plates as an ethanol solution at a concentration of 2.3 μg of EPA/cm^2 of plate surface or 2.5 μg of DHA/cm^2 of plate surface.

Cultures for lipid analysis were incubated on 15 cm plates with nematode growth medium, spread with either *E. coli fadE*$^-$ alone (control) or *E. coli fadE*$^-$ in addition to either EPA or DHA as described above. Cultures were allowed to grow at 16 and 20 °C to maximum density, and then they were washed from the plates with 10 mL of deionized water, placed in 15 mL conical tubes, and centrifuged at approximately 100*g* for 2 min. Bacteria and dead nematodes were removed from the cultures by flotation on sucrose (*16*). All samples were then briefly rinsed in a small amount (∼1 mL) of 100% ethanol and centrifuged to remove any nonesterified EPA or DHA on the outside of the nematodes. The pellets were finally rinsed with 1 mL of water and recentrifuged, and excess water was removed by aspiration. All samples were frozen by the addition of a small amount of liquid nitrogen and stored at −80 °C until lipid analysis was performed. Lipid analysis was performed as described by Watkins et al. (*13*). Each sample for lipid analysis was the combination of cultures from three separate plates of the same treatment (strain, fatty acid supplementation, and growth temperature) grown at the same time. Fatty acid concentrations given are the average of different analyses performed on three different samples of each treatment.

Statistical analysis was performed using Statistical Package for Social Science Inc., version 12.0 (Chicago, IL). Independent *t* tests were used to assess differences in life span between all stains and treatments as compared to WT control and for each fatty acid in a given lipid class (total lipids, phospholipids, triglyceride) relative to the WT control. Statistical significance is reported at the $P < 0.05$ or $P < 0.1$ level, as specified.

RESULTS AND DISCUSSION

Life span analysis at 20 °C revealed that there was a significant difference ($P < 0.05$) between the average life span of WT and *fat-3* mutant worms (**Table 1**). The average life span of WT worms grown without fatty acid supplementation was 3.6 days longer than that of *fat-3* mutant worms grown without fatty acid supplementation. Supplementing either strain of *C. elegans* with exogenous EPA or DHA had no significant effect on the average life span. DHA supplementation of WT and *fat-3* mutant worms increased the average life span by 1.3 and 1.7 days, respectively, over that of the controls of the same strain grown without fatty acid supplementation (**Table 1**). **Figure 1** shows that, in addition to a decrease in average life span of the

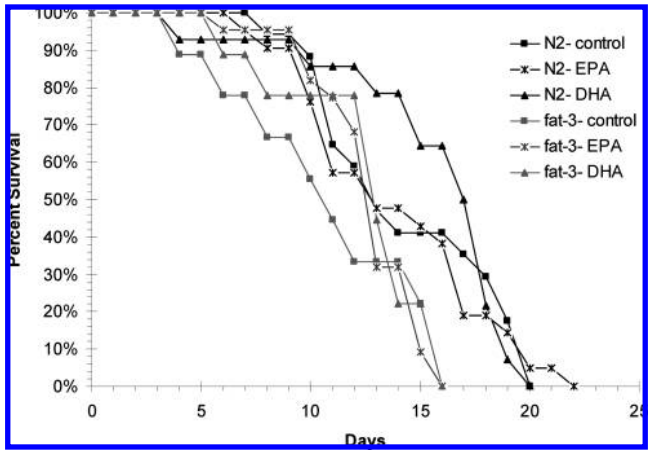

Figure 1. Survival of *C. elegans* WT and *fat-3* at 20 °C with and without EPA or DHA supplementation.

Table 2. Total Lipid Fatty Acid Concentrations in WT *C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.024 ± 0.004	0.032 ± 0.005	0.054 ± 0.029
16:0	0.057 ± 0.011	0.090 ± 0.017	0.127 ± 0.069
16:1n7	0.032 ± 0.006	0.027 ± 0.006	0.047 ± 0.024
18:0	0.206 ± 0.056	0.277 ± 0.017	0.280 ± 0.061
18:1n9	0.105 ± 0.032	0.130 ± 0.012	0.155 ± 0.057
18:1n7	0.511 ± 0.139	0.508 ± 0.064	0.673 ± 0.217
18:2n6	0.324 ± 0.130	0.416 ± 0.051	0.384 ± 0.114
18:3n6	0.023 ± 0.005	0.010 ± 0.006	0.023 ± 0.010
18:3n3	0.026 ± 0.015	0.051 ± 0.007	0.060 ± 0.019
20:0	0.026 ± 0.007	0.031 ± 0.002	0.033 ± 0.008
20:1n9	0.020 ± 0.006	0.015 ± 0.001	0.018 ± 0.002
20:2n6	0.053 ± 0.017	0.063 ± 0.005	0.046 ± 0.003
20:3n6	0.097 ± 0.027	0.072 ± 0.009	0.077 ± 0.021
20:4n6	0.043 ± 0.014	0.019 ± 0.005	0.018 ± 0.005
20:3n3	0.007 ± 0.007	0.018 ± 0.001	0.014 ± 0.003
20:5n3	0.511 ± 0.178	0.754 ± 0.067	0.517 ± 0.097
22:0	0.032 ± 0.009	0.033 ± 0.002	0.035 ± 0.006
22:6n3	nd	nd	0.411 ± 0.178 b
SFA	0.345 ± 0.087	0.464 ± 0.038	0.529 ± 0.171
MUFA	0.669 ± 0.177	0.680 ± 0.081	0.893 ± 0.299
PUFA	1.084 ± 0.391	1.402 ± 0.133	1.550 ± 0.442
n3	0.543 ± 0.200	0.823 ± 0.074	0.591 ± 0.118
n6	0.541 ± 0.192	0.579 ± 0.063	0.548 ± 0.149
n7	0.543 ± 0.144	0.535 ± 0.069	0.720 ± 0.241
n9	0.125 ± 0.038	0.145 ± 0.012	0.173 ± 0.058

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

fat-3 mutant relative to that of WT worms, there was also a decrease in the maximum life span. The *fat-3* mutant lived a maximum of 16 days regardless of treatment at 20 °C; whereas, WT worms grown either without exogenous fatty acids or with supplemental DHA had maximum life spans of 20 days, and those grown with EPA lived for a maximum of 22 days.

Lipid class speciation of WT *C. elegans* control cultures grown at 20 °C showed that supplementing DHA significantly increased ($P < 0.1$) DHA concentrations in all three lipid classes measured (**Tables 2−4**). Approximately two-thirds of the EPA in the worms was portioned into phospholipids (**Table 3**), and very little was incorporated into triglycerides. The same was true of all the long-chain fatty acid phospholipid 20:3n3, with no significant increase in EPA concentrations. There was an increase in the concentration of both EPA and total n3 fatty acid in triglycerides and their precursors of EPA. There were no other significant changes in fatty acid concentrations.

Table 3. Phospholipid Fatty Acid Concentrations of WT *C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.005 ± 0.003	0.006 ± 0.003	0.011 ± 0.006
16:0	0.030 ± 0.010	0.044 ± 0.009	0.052 ± 0.024
16:1n7	0.006 ± 0.004	0.003 ± 0.003	0.008 ± 0.004
18:0	0.132 ± 0.052	0.181 ± 0.016	0.162 ± 0.040
18:1n9	0.046 ± 0.019	0.044 ± 0.001	0.049 ± 0.016
18:1n7	0.260 ± 0.107	0.243 ± 0.022	0.276 ± 0.084
18:2n6	0.162 ± 0.081	0.179 ± 0.018	0.166 ± 0.045
18:3n6	0.009 ± 0.005	0.005 ± 0.003	0.011 ± 0.006
18:3n3	0.009 ± 0.009	0.020 ± 0.004	0.023 ± 0.008
20:0	0.017 ± 0.007	0.019 ± 0.001	0.018 ± 0.005
20:1n9	0.008 ± 0.005	0.005 ± 0.002	0.004 ± 0.002
20:2n6	0.028 ± 0.012	0.033 ± 0.004	0.020 ± 0.002
20:3n6	0.069 ± 0.027	0.047 ± 0.004	0.042 ± 0.010
20:4n6	0.025 ± 0.011	0.012 ± 0.003	0.010 ± 0.004
20:3n3	nd	0.010 ± 0.001 a	0.004 ± 0.002
20:5n3	0.320 ± 0.153	0.414 ± 0.046	0.280 ± 0.075
22:0	0.022 ± 0.010	0.022 ± 0.001	0.019 ± 0.004
22:6n3	nd	nd	0.141 ± 0.057 b
SFA	0.206 ± 0.081	0.273 ± 0.028	0.261 ± 0.079
MUFA	0.320 ± 0.133	0.294 ± 0.023	0.336 ± 0.103
PUFA	0.622 ± 0.297	0.720 ± 0.071	0.697 ± 0.202
n3	0.328 ± 0.161	0.443 ± 0.048	0.307 ± 0.081
n6	0.293 ± 0.137	0.276 ± 0.023	0.249 ± 0.065
n7	0.266 ± 0.110	0.246 ± 0.024	0.284 ± 0.089
n9	0.054 ± 0.024	0.049 ± 0.001	0.052 ± 0.015

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

Table 4. Triglyceride Fatty Acid Concentrations in WT *C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.013 ± 0.007	0.022 ± 0.002	0.036 ± 0.018
16:0	0.036 ± 0.004	0.054 ± 0.010	0.072 ± 0.038
16:1n7	0.011 ± 0.005	0.008 ± 0.005	0.019 ± 0.008
18:0	0.067 ± 0.009	0.088 ± 0.010	0.096 ± 0.027
18:1n9	0.026 ± 0.013	0.039 ± 0.008	0.051 ± 0.016
18:1n7	0.117 ± 0.040	0.136 ± 0.029	0.187 ± 0.049
18:2n6	0.062 ± 0.022	0.079 ± 0.013	0.071 ± 0.010
18:3n6	0.002 ± 0.002	nd	0.001 ± 0.001
18:3n3	nd	nd	nd
20:0	0.008 ± 0.004	0.013 ± 0.001	0.015 ± 0.004
20:1n9	0.002 ± 0.002	nd	0.004 ± 0.002
20:2n6	0.009 ± 0.005	0.013 ± 0.002	0.007 ± 0.004
20:3n6	0.002 ± 0.002	nd	0.003 ± 0.002
20:4n6	nd	nd	nd
20:3n3	nd	nd	nd
20:5n3	0.013 ± 0.007	0.032 ± 0.002 b	0.016 ± 0.002
22:0	0.003 ± 0.003	0.007 ± 0.004	0.006 ± 0.003
22:6n3	nd	nd	0.025 ± 0.008 a
SFA	0.127 ± 0.025	0.186 ± 0.027	0.225 ± 0.085
MUFA	0.155 ± 0.058	0.183 ± 0.042	0.261 ± 0.070
PUFA	0.088 ± 0.034	0.124 ± 0.015	0.124 ± 0.011
n3	0.013 ± 0.007	0.032 ± 0.002 b	0.016 ± 0.002
n6	0.075 ± 0.027	0.092 ± 0.014	0.083 ± 0.004
n7	0.128 ± 0.045	0.144 ± 0.033	0.207 ± 0.056
n9	0.028 ± 0.015	0.039 ± 0.008	0.055 ± 0.014

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

Lipid analysis of *fat-3* mutants grown at 20 °C revealed lower concentrations of 20:3n6, 20:4n6, and 20:5n3, which is in agreement with the results of Watts et al. (*10*) (**Tables 5−7**). There was also a decrease in 20:3n6 and 20:4n6 in total phospholipids along with increases in 16:0, 18:1n9, 18:2n6, 18:

Table 5. Total Lipid Fatty Acid Concentrations in *fat-3 C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.055 ± 0.002	0.025 ± 0.004	0.037 ± 0.008
16:0	0.087 ± 0.005	0.059 ± 0.007	0.073 ± 0.007
16:1n7	0.119 ± 0.008 a	0.026 ± 0.006	0.038 ± 0.009
18:0	0.147 ± 0.011	0.215 ± 0.009	0.174 ± 0.019
18:1n9	0.215 ± 0.021 b	0.148 ± 0.014	0.154 ± 0.032
18:1n7	0.846 ± 0.055	0.482 ± 0.074	0.550 ± 0.076
18:2n6	0.571 ± 0.022	0.502 ± 0.066	0.399 ± 0.047
18:3n6	0.019 ± 0.001	0.004 ± 0.004 a	0.006 ± 0.003 a
18:3n3	0.464 ± 0.023 a	0.195 ± 0.032 a	0.311 ± 0.050 a
20:0	0.024 ± 0.002	0.025 ± 0.001	0.023 ± 0.003
20:1n9	0.025 ± 0.004	0.020 ± 0.004	0.022 ± 0.004
20:2n6	0.044 ± 0.007	0.103 ± 0.010 b	0.052 ± 0.007
20:3n6	nd b	nd a	nd a
20:4n6	0.011 ± 0.007	nd a	nd a
20:3n3	0.038 ± 0.013	0.066 ± 0.008 a	0.058 ± 0.009 a
20:5n3	0.008 ± 0.006 a	0.476 ± 0.062	0.148 ± 0.016
22:0	0.009 ± 0.007	0.028 ± 0.001	0.024 ± 0.004
22:6n3	nd	nd	0.353 ± 0.042 a
SFA	0.322 ± 0.014	0.352 ± 0.021	0.331 ± 0.040
MUFA	1.205 ± 0.088	0.675 ± 0.097	0.763 ± 0.121
PUFA	1.155 ± 0.043	1.347 ± 0.162	1.327 ± 0.057
n3	0.509 ± 0.004	0.737 ± 0.084	0.518 ± 0.044
n6	0.645 ± 0.040	0.610 ± 0.078	0.457 ± 0.057
n7	0.966 ± 0.063	0.507 ± 0.080	0.587 ± 0.085
n9	0.240 ± 0.025	0.168 ± 0.018	0.176 ± 0.036

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

Table 6. Phospholipid Fatty Acid Concentrations in *fat-3 C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.009 ± 0.002	0.006 ± 0.001	0.003 ± 0.002
16:0	0.036 ± 0.003	0.028 ± 0.001	0.028 ± 0.003
16:1n7	0.029 ± 0.003 a	0.004 ± 0.002	0.004 ± 0.003
18:0	0.105 ± 0.001	0.139 ± 0.007	0.114 ± 0.002
18:1n9	0.110 ± 0.005 a	0.063 ± 0.007	0.070 ± 0.001
18:1n7	0.474 ± 0.014	0.235 ± 0.022	0.287 ± 0.003
18:2n6	0.370 ± 0.012 b	0.234 ± 0.016	0.220 ± 0.002
18:3n6	0.010 ± 0.001	nd	nd
18:3n3	0.301 ± 0.006 a	0.096 ± 0.012 a	0.177 ± 0.006 a
20:0	0.018 ± 0.000	0.016 ± 0.000	0.016 ± 0.001
20:1n9	0.013 ± 0.002	0.007 ± 0.001	0.007 ± 0.000
20:2n6	0.033 ± 0.008	0.053 ± 0.006	0.027 ± 0.004
20:3n6	nd b	nd b	nd b
20:4n6	nd b	nd b	nd b
20:3n3	0.046 ± 0.007 a	0.033 ± 0.002 a	0.035 ± 0.003 a
20:5n3	0.013 ± 0.000	0.291 ± 0.047	0.074 ± 0.002
22:0	0.017 ± 0.001	0.018 ± 0.000	0.017 ± 0.001
22:6n3	nd	nd	0.180 ± 0.013 a
SFA	0.184 ± 0.004	0.207 ± 0.010	0.178 ± 0.007
MUFA	0.626 ± 0.010 b	0.310 ± 0.031	0.369 ± 0.008
PUFA	0.772 ± 0.024	0.708 ± 0.067	0.713 ± 0.010
n3	0.359 ± 0.005	0.421 ± 0.047	0.286 ± 0.005
n6	0.413 ± 0.020	0.287 ± 0.020	0.247 ± 0.002
n7	0.503 ± 0.012 b	0.240 ± 0.024	0.291 ± 0.007
n9	0.123 ± 0.005 a	0.070 ± 0.008	0.078 ± 0.001

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

3n3, monounsaturated fatty acids (MUFAs), n7s, and n9s (**Table 6**). In the triglyceride fraction, there was an increase in 14:0, 16:0, 18:1n9, 18:1n7, 18:3n6, 18:3n3, 20:1n9, 20:3n3, MUFAs, PUFAs, n3s, n7s, and n9s compared with WT control concentrations (*6*).

Table 7. Triglyceride Fatty Acid Concentrations of *fat-3 C. elegans* Grown at 20 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.044 ± 0.004 a	0.016 ± 0.002	0.035 ± 0.007 b
16:0	0.061 ± 0.003 a	0.038 ± 0.002	0.059 ± 0.002 a
16:1n7	0.063 ± 0.003 a	0.004 ± 0.004	0.021 ± 0.005
18:0	0.053 ± 0.002	0.067 ± 0.005	0.072 ± 0.004
18:1n9	0.082 ± 0.006 a	0.029 ± 0.010	0.061 ± 0.013
18:1n7	0.312 ± 0.013 a	0.090 ± 0.032	0.180 ± 0.022
18:2n6	0.103 ± 0.003	0.060 ± 0.016	0.095 ± 0.015
18:3n6	0.008 ± 0.001 b	nd	0.002 ± 0.002
18:3n3	0.037 ± 0.005 a	0.007 ± 0.007	0.054 ± 0.016 a
20:0	0.010 ± 0.001	0.011 ± 0.001	0.012 ± 0.000
20:1n9	0.015 ± 0.003 b	0.002 ± 0.002	0.006 ± 0.003
20:2n6	0.014 ± 0.003	0.013 ± 0.004	0.014 ± 0.003
20:3n6	nd	nd	nd
20:4n6	nd	nd	nd
20:3n3	0.011 ± 0.001 a	0.002 ± 0.002	0.008 ± 0.005
20:5n3	nd	0.006 ± 0.003	0.008 ± 0.004
22:0	0.008 ± 0.004	0.009 ± 0.002	0.011 ± 0.001 b
22:6n3	nd	nd	0.037 ± 0.010 a
SFA	0.176 ± 0.001	0.141 ± 0.012	0.189 ± 0.015
MUFA	0.473 ± 0.008 a	0.125 ± 0.048	0.268 ± 0.043
PUFA	0.174 ± 0.007 b	0.088 ± 0.030	0.218 ± 0.045 b
n3	0.048 ± 0.004 a	0.015 ± 0.011	0.070 ± 0.023 b
n6	0.126 ± 0.006	0.073 ± 0.020	0.111 ± 0.019
n7	0.375 ± 0.011 a	0.094 ± 0.036	0.201 ± 0.027
n9	0.098 ± 0.003 a	0.031 ± 0.012	0.067 ± 0.016

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 20 °C ($P < 0.05$); b, significantly different from WT control at 20 °C ($P < 0.1$). nd, none detected.

Supplementation of *fat*-3 mutants with EPA at 20 °C resulted in a lipid profile similar to that of *fat-3* controls for fatty acids upstream of EPA in total lipids and phospholipids; however, downstream of 18:3n3 fatty acid profiles more closely resembled those of WT controls (**Tables 5** and **6**). Triglyceride fatty acid concentrations were similar to those of WT controls (**Table 7**). Addition of DHA to *fat-3* mutant cultures increased DHA concentrations in all three lipid classes. Total lipid and phospholipid fatty acid profiles were nearly identical to those of the *fat-3* mutant samples grown in the presence of EPA, with the notable exception of the presence of DHA (**Tables 5** and **6**). Triglycerides were increased in 14:0, 16:0, 18:3n3, 22:0, DHA, PUFAs, and n3 fatty acids in DHA-supplemented worms as compared with WT controls (**Table 7**). Although EPA restored triglyceride concentrations to those of WT worms, it failed to restore concentrations of 18:3n6, 20:3n6, and 20:4n6 (**Table 7**).

WT cultures supplemented with EPA and grown at 16 °C showed no significant changes in the total lipid fatty acid profile compared with that of controls from the same temperature (**Table 8**). Fatty acids in phospholipids from the same culture had decreased concentrations of 14:0, 16:1n7, 18:0, 18:1n9, 20:2n6, 20:3n6, 22:0, 20:4n6 SFA, MUFA, n6, and n9 compared with the control (**Table 9**). In these cultures, the concentrations of 14:0, 16:0, 16:1n7, 18:0, 18:1n9, 18:1n7, 18:2n6, 20:0, 20:2n6, SFAs, MUFAs, PUFAs, n6, n7, and n9 in triglycerides were all lower than in the control (**Table 10**). DHA supplementation of WT worms at 16 °C increased DHA in all three classes (**Tables 8**−**10**). Total lipids were lower in 14:0, 16:1n7, 18:1n9, and 20:3n6, as well as n9 fatty acids, than the control (**Table 8**). Additionally, phospholipids were lower in 20:3n6 compared with the control (**Table 9**). The concentrations of 14:0 and 16:0 in triglycerides were decreased when worms were supplemented with DHA at 16 °C (**Table 10**).

Table 8. Total Lipid Fatty Acid Concentrations of WT *C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.050 ± 0.015	0.098 ± 0.076	0.036 ± 0.006 b
16:0	0.100 ± 0.023	0.248 ± 0.190	0.100 ± 0.025
16:1n7	0.058 ± 0.029	0.078 ± 0.071	0.043 ± 0.007 a
18:0	0.340 ± 0.064	0.653 ± 0.408	0.347 ± 0.070
18:1n9	0.204 ± 0.049	0.364 ± 0.262	0.155 ± 0.033 a
18:1n7	0.743 ± 0.291	1.372 ± 1.033	0.793 ± 0.182
18:2n6	0.452 ± 0.184	0.971 ± 0.696	0.576 ± 0.178
18:3n6	0.025 ± 0.014	0.030 ± 0.030	0.015 ± 0.009
18:3n3	0.050 ± 0.017	0.129 ± 0.096	0.068 ± 0.020
20:0	0.038 ± 0.009	0.077 ± 0.052	0.038 ± 0.007
20:1n9	0.029 ± 0.016	0.035 ± 0.028	0.035 ± 0.005
20:2n6	0.084 ± 0.017	0.129 ± 0.068	0.085 ± 0.018
20:3n6	0.118 ± 0.045	0.144 ± 0.098	0.071 ± 0.023 b
20:4n6	0.047 ± 0.027	0.029 ± 0.029	0.013 ± 0.013
20:3n3	0.011 ± 0.011	0.038 ± 0.030	0.021 ± 0.012
20:5n3	0.866 ± 0.323	1.864 ± 1.228	0.804 ± 0.242
22:0	0.038 ± 0.022	0.058 ± 0.046	0.041 ± 0.011
22:6n3	nd	nd	0.401 ± 0.043 a
SFA	0.566 ± 0.123	1.133 ± 0.771	0.562 ± 0.118
MUFA	1.033 ± 0.369	1.850 ± 1.394	1.026 ± 0.225
PUFA	1.654 ± 0.626	3.333 ± 2.274	2.053 ± 0.553
n3	0.927 ± 0.351	2.030 ± 1.354	1.293 ± 0.314
n6	0.727 ± 0.284	1.303 ± 0.920	0.760 ± 0.239
n7	0.801 ± 0.313	1.450 ± 1.105	0.836 ± 0.189
n9	0.232 ± 0.064	0.400 ± 0.290	0.190 ± 0.037 a

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

Table 9. Phospholipid Fatty Acid Concentrations of WT *C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.013 ± 0.003	nd a	0.006 ± 0.006
16:0	0.052 ± 0.016	0.093 ± 0.061	0.053 ± 0.017
16:1n7	0.016 ± 0.004	nd a	0.005 ± 0.005
18:0	0.216 ± 0.035	0.417 ± 0.283 b	0.228 ± 0.064
18:1n9	0.104 ± 0.014	0.092 ± 0.058 a	0.057 ± 0.020
18:1n7	0.367 ± 0.123	0.526 ± 0.401	0.354 ± 0.132
18:2n6	0.265 ± 0.095	0.369 ± 0.273	0.235 ± 0.108
18:3n6	0.015 ± 0.008	nd	nd
18:3n3	0.019 ± 0.006	0.040 ± 0.033	0.021 ± 0.013
20:0	0.024 ± 0.005	0.053 ± 0.039	0.018 ± 0.011
20:1n9	0.009 ± 0.005	nd	0.010 ± 0.005
20:2n6	0.049 ± 0.009	0.056 ± 0.032 a	0.041 ± 0.013
20:3n6	0.099 ± 0.017	0.080 ± 0.054 a	0.046 ± 0.018 b
20:4n6	0.042 ± 0.009	nd	0.007 ± 0.007
20:3n3	nd	nd	0.007 ± 0.007
20:5n3	0.483 ± 0.210	0.800 ± 0.559	0.407 ± 0.177
22:0	0.030 ± 0.009	0.038 ± 0.031 b	0.022 ± 0.014
22:6n3	nd	nd	0.152 ± 0.051 a
SFA	0.335 ± 0.067	0.601 ± 0.414 b	0.327 ± 0.109
MUFA	0.496 ± 0.127	0.618 ± 0.459 b	0.426 ± 0.161
PUFA	0.972 ± 0.344	1.346 ± 0.951 b	0.917 ± 0.394
n3	0.502 ± 0.215	0.840 ± 0.592	0.588 ± 0.248
n6	0.470 ± 0.132	0.505 ± 0.359 b	0.328 ± 0.146
n7	0.383 ± 0.126	0.526 ± 0.401	0.359 ± 0.138
n9	0.113 ± 0.015	0.092 ± 0.058 a	0.066 ± 0.024

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

All three lipid classes measured were decreased in 18:0 and EPA and increased in 18:3n3 and 20:3n3 in *fat-3* mutants cultured at 16 °C with no supplementation as compared with WT worms grown at the same temperature (**Tables 11**−**13**). Additionally, total lipids were increased in 16:1n7 and decreased in 20:3n6 (**Table 11**). Phospholipid 20:3n6 and 20:4n6 concentrations were lower in *fat-3* mutant worms than in WT worms (**Table 12**). The triglycerides in these same cultures also had decreased SFAs and increased 16:1n7, 18:1n7, 20:1n9, MUFAs, PUFAs, n3s, and n7s (**Table 13**). The *fat-3* mutants supple-

Table 10. Triglyceride Fatty Acid Concentrations in WT *C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.049 ± 0.011	0.017 ± 0.003 a	0.024 ± 0.002 b
16:0	0.070 ± 0.006	0.043 ± 0.002 a	0.055 ± 0.001 b
16:1n7	0.035 ± 0.003	0.005 ± 0.005 a	0.020 ± 0.014
18:0	0.116 ± 0.007	0.072 ± 0.005 a	0.110 ± 0.004
18:1n9	0.076 ± 0.014	0.024 ± 0.012 b	0.039 ± 0.012
18:1n7	0.227 ± 0.028	0.078 ± 0.039 a	0.175 ± 0.051
18:2n6	0.096 ± 0.009	0.036 ± 0.014 a	0.069 ± 0.012
18:3n6	0.003 ± 0.003	nd	nd
18:3n3	nd	nd	0.005 ± 0.005
20:0	0.018 ± 0.003	0.010 ± 0.001 a	0.016 ± 0.001
20:1n9	0.007 ± 0.003	nd	0.007 ± 0.004
20:2n6	0.021 ± 0.002	0.003 ± 0.003 a	0.012 ± 0.006
20:3n6	0.003 ± 0.003	0.002 ± 0.002	nd
20:4n6	nd	nd	nd
20:3n3	nd	nd	nd
20:5n3	0.022 ± 0.002	0.017 ± 0.011	0.026 ± 0.016
22:0	0.009 ± 0.004	0.003 ± 0.003	0.004 ± 0.004
22:6n3	nd	nd	0.031 ± 0.019 a
SFA	0.261 ± 0.024	0.144 ± 0.008 a	0.208 ± 0.008
MUFA	0.344 ± 0.009	0.107 ± 0.056 a	0.242 ± 0.080
PUFA	0.146 ± 0.014	0.058 ± 0.030 b	0.143 ± 0.056
n3	0.022 ± 0.002	0.017 ± 0.011	0.062 ± 0.039
n6	0.124 ± 0.012	0.041 ± 0.019 a	0.081 ± 0.017
n7	0.262 ± 0.025	0.083 ± 0.044 a	0.196 ± 0.064
n9	0.082 ± 0.017	0.024 ± 0.012 b	0.046 ± 0.016

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

Table 11. Total Lipid Fatty Acid Concentrations in *fat-3 C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.052 ± 0.008	0.024 ± 0.018	0.035 ± 0.019
16:0	0.102 ± 0.013	0.099 ± 0.053	0.126 ± 0.048
16:1n7	0.137 ± 0.019 a	0.019 ± 0.019 a	0.055 ± 0.015
18:0	0.183 ± 0.023 a	0.341 ± 0.153	0.270 ± 0.085
18:1n9	0.256 ± 0.041	0.158 ± 0.098	0.195 ± 0.067
18:1n7	1.189 ± 0.164	0.695 ± 0.352	0.857 ± 0.234
18:2n6	0.718 ± 0.151	0.684 ± 0.384	0.580 ± 0.255
18:3n6	0.022 ± 0.006	nd a	0.002 ± 0.002 a
18:3n3	0.611 ± 0.124 a	0.235 ± 0.131	0.408 ± 0.135 b
20:0	0.033 ± 0.005	0.039 ± 0.018	0.027 ± 0.015
20:1n9	0.042 ± 0.003	0.021 ± 0.014	0.019 ± 0.010
20:2n6	0.059 ± 0.010 a	0.139 ± 0.067	0.070 ± 0.029
20:3n6	nd a	nd a	nd a
20:4n6	nd a	nd a	nd a
20:3n3	0.115 ± 0.009 a	0.087 ± 0.043	0.090 ± 0.022 a
20:5n3	0.022 ± 0.006 b	1.086 ± 0.530	0.356 ± 0.193
22:0	0.013 ± 0.007 b	0.023 ± 0.023	0.022 ± 0.013
22:6n3	nd	nd	0.832 ± 0.419
SFA	0.466 ± 0.042 a	1.054 ± 0.264	0.814 ± 0.178
MUFA	2.074 ± 0.226	1.854 ± 0.482	1.712 ± 0.325
PUFA	2.154 ± 0.305	4.524 ± 1.152	4.418 ± 1.044
n3	1.022 ± 0.138	2.806 ± 0.702	1.541 ± 0.344
n6	1.132 ± 0.167	1.718 ± 0.450	1.208 ± 0.282
n7	1.688 ± 0.182	1.453 ± 0.371	1.371 ± 0.249
n9	0.385 ± 0.044	0.401 ± 0.111	0.342 ± 0.077

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

Table 12. Phospholipid Fatty Acid Concentrations in *fat-3 C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.008 ± 0.002	0.006 ± 0.006	0.008 ± 0.005
16:0	0.039 ± 0.005	0.047 ± 0.022	0.065 ± 0.031
16:1n7	0.025 ± 0.008	nd a	0.009 ± 0.005
18:0	0.123 ± 0.023 b	0.229 ± 0.119	0.199 ± 0.091
18:1n9	0.109 ± 0.036	0.068 ± 0.046	0.083 ± 0.039
18:1n7	0.526 ± 0.143	0.337 ± 0.196	0.415 ± 0.169
18:2n6	0.403 ± 0.129	0.305 ± 0.197	0.302 ± 0.147
18:3n6	0.006 ± 0.006	nd	nd
18:3n3	0.363 ± 0.106 a	0.110 ± 0.068	0.229 ± 0.092 b
20:0	0.024 ± 0.005	0.027 ± 0.014	0.025 ± 0.010
20:1n9	0.013 ± 0.003	0.007 ± 0.007	0.008 ± 0.005
20:2n6	0.030 ± 0.010	0.071 ± 0.041	0.037 ± 0.020
20:3n6	nd a	nd a	nd a
20:4n6	nd a	nd a	nd a
20:3n3	0.050 ± 0.015 a	0.055 ± 0.021 b	0.058 ± 0.036
20:5n3	0.018 ± 0.006 b	0.536 ± 0.320	0.215 ± 0.127
22:0	0.016 ± 0.004	0.025 ± 0.014	0.023 ± 0.012
22:6n3	nd	nd	0.427 ± 0.242
SFA	0.288 ± 0.039	0.682 ± 0.174	0.615 ± 0.149
MUFA	1.052 ± 0.191	0.908 ± 0.248	0.939 ± 0.219
PUFA	1.413 ± 0.271	2.369 ± 0.646	2.585 ± 0.662
n3	0.686 ± 0.127	1.518 ± 0.409	1.008 ± 0.254
n6	0.727 ± 0.144	0.851 ± 0.237	0.667 ± 0.166
n7	0.852 ± 0.151	0.728 ± 0.196	0.764 ± 0.175
n9	0.199 ± 0.039	0.180 ± 0.053	0.175 ± 0.044

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

Table 13. Triglyceride Fatty Acid Concentrations in *fat-3 C. elegans* Grown at 16 °C[a]

	treatment		
fatty acid	control	EPA	DHA
14:0	0.041 ± 0.009	0.016 ± 0.010 b	0.033 ± 0.008
16:0	0.069 ± 0.006	0.060 ± 0.022	0.079 ± 0.017
16:1n7	0.075 ± 0.016 b	nd a	0.013 ± 0.013
18:0	0.063 ± 0.007 a	0.098 ± 0.034	0.086 ± 0.019
18:1n9	0.124 ± 0.023	0.030 ± 0.025	0.046 ± 0.030
18:1n7	0.478 ± 0.075 a	0.106 ± 0.080	0.162 ± 0.077
18:2n6	0.136 ± 0.025	0.065 ± 0.046	0.062 ± 0.018
18:3n6	0.005 ± 0.002	nd	nd
18:3n3	0.072 ± 0.007 a	nd	0.029 ± 0.021
20:0	0.008 ± 0.004	0.016 ± 0.005	0.007 ± 0.004 b
20:1n9	0.023 ± 0.004 a	nd	0.003 ± 0.003
20:2n6	0.017 ± 0.002	0.011 ± 0.011	0.000 ± 0.000 a
20:3n6	nd	nd	nd
20:4n6	nd	nd	nd
20:3n3	0.020 ± 0.003 a	nd	0.003 ± 0.003
20:5n3	0.000 ± 0.000 a	0.011 ± 0.006	0.003 ± 0.003 a
22:0	0.008 ± 0.004	0.003 ± 0.003	nd
22:6n3	nd	nd	0.023 ± 0.023
SFA	0.212 ± 0.011 b	0.326 ± 0.069	0.276 ± 0.040
MUFA	0.919 ± 0.115 a	0.345 ± 0.105	0.131 ± 0.123
PUFA	0.317 ± 0.035 b	0.210 ± 0.061	0.061 ± 0.065
n3	0.110 ± 0.009 a	0.019 ± 0.006	0.023 ± 0.027
n6	0.207 ± 0.025	0.191 ± 0.057	0.061 ± 0.018 a
n7	0.722 ± 0.088 a	0.264 ± 0.080	0.097 ± 0.090
n9	0.196 ± 0.027	0.080 ± 0.025	0.034 ± 0.034

[a] Concentrations are in mg of fatty acids/g of worm wet mass ± SEM. a, significantly different from WT control at 16 °C ($P < 0.05$); b, significantly different from WT control at 16 °C ($P < 0.1$). nd, none detected.

mented with EPA exhibited a decrease in 20:3n6 and 20:4n6 in both total lipids and phospholipids (**Tables 11** and **12**). In all three lipid classes, 16:1n7 was decreased (**Tables 11−13**). Also, phospholipids were increased in 20:3n3 (**Table 12**). Triglyceride showed a decrease in 14:0 compared to WT control levels (**Table 13**). Supplementing the *fat-3* mutants grown at 16 °C with DHA increased the concentrations of 18:3n3 and 20:3n3 and decreased those of 20:3n6 and 20:4n6 in total lipids compared with WT controls (**Table 11**). There was also an increase in 18:3n3 in phospholipids and a decrease in 20:3n6 and 20:4n6 in the same lipid class (**Table 12**). Triglycerides were decreased in 20:0, 20:2n6, EPA, and n6s under these conditions (**Table 13**).

In all cultures supplemented with DHA, DHA concentrations increased relative to control; however, only those specifically mentioned above were significant at $P < 0.1$. Interestingly, *fat-3* mutant triglyceride concentrations from cultures supplemented with DHA at 16 °C showed the least significant increase in DHA. Additionally, all *fat-3* mutant cultures except those grown in the presence of EPA showed some decrease in EPA, but not all changes were significant at $P < 0.1$.

An interesting finding in this study was that supplementation of the *fat-3* mutant worms grown with DHA or EPA did not significantly affect the life span of this mutant strain, as there was a difference between the average life spans of *fat-3* mutants and WT, regardless of treatment. There are two possible explanations for the lack of effect of DHA or EPA supplementation in *fat-3* mutant worms. One explanation may be that because the fatty acid supplementation that failed to rescue the mutant worms was initiated after the nematodes reached adulthood, there may be a critical function of EPA that is necessary during development that cannot be corrected after adulthood is reached. Watts and Browse (*10*) showed that supplementation with EPA after *fat-3* reached adulthood rescued all of the pleiotropic phenotypes of *fat-3* mutants except the increased susceptibility of their cuticle to disintegration by alkaline hypochlorite solution (sodium hydroxide and bleach). However, they also discovered that supplementation of the worms at an earlier developmental stage did rescue this phenotype. It is possible that a weakened cuticle could contribute to a shortened life span under the conditions of the longevity assay. Individual worms were transferred at least every other day by physically lifting them off the plates with a platinum wire pick and placing them onto fresh plates. This "man handling" of the worms may cause physical trauma to the worms that is amplified by a compromised cuticle that would otherwise serve as a protective measure against such harm. Another possibility is that fatty acid intermediates between 18:2n6 or 18:3n3 [both fatty acids are substrates for the δ-6 desaturase (*7*)] and EPA may be required by *C. elegans*. Because the insertion of a double bond into a fatty acid is not a reversible biochemical reaction, feeding exogenous EPA would not satisfy a requirement for an EPA precursor molecule that contains the δ-6 double bond (*11*). Arachidonic acid (20:4n6) is one fatty acid that is not produced by the *fat-3* mutant, but is found in WT worms and is necessary for proper human neuron function; it may be necessary for the worms as well. There may be other, unknown roles for EPA during development in early larval stages, but it seems likely that a lack of long-chain n-6 fatty acids such as arachidonic acid is the most plausible reason that supplementation of EPA and DHA failed to restore *fat-3* mutant life span to WT levels. However, this hypothesis has not been tested.

The finding that DHA had no negative effect on average life span of the *fat-3* mutant, and may have slightly increased life span, argues against a role for DHA in premature aging. This result is not entirely conclusive, however, due to the apparent retroconversion of DHA by *C. elegans* to form EPA (*11*). It would be useful to construct a double mutant in which a knockout of the peroxisomal β-oxidation system, as opposed to the mitochondrial β-oxidation pathway, is combined with the *fat-3* gene knockout. Using such a mutant strain, DHA

supplementation could be investigated further without the complication of retroconversion to EPA. With DHA as the only source of LC PUFA in the organism, it would be possible to more directly ascertain if DHA causes any significant differences, either positive or negative, compared with the effects of EPA in *C. elegans*.

The large proportion of EPA and other LC PUFAs (20:3n6, 20:4n6, and 20:3n3) in the phospholipid pool suggests that these highly unsaturated molecules are necessary for proper membrane function under certain conditions. Because EPA is found at a concentration 10-fold higher in phospholipid relative to other LC PUFAs, it is tempting to conclude that it is the most important fatty acid in the membrane of *C. elegans* for maintaining proper membrane function. On the other hand, mutants lacking a functional n3 desaturase do not exhibit any significant defect despite a complete lack of LC n3s (*7*). It is possible that other molecules, especially arachidonic acid (20:4n6), are vital for proper function of the membrane or, perhaps, a yet undiscovered signaling pathway. The increase in *cis*-11,14,17-eicosatrienoic acid (20:3n3) in phospholipids in WT cultures supplemented with EPA is consistent with the idea that EPA inhibits the production of EPA, resulting in a buildup of α-linolenic acid (18:3n3) and subsequent elongation rather than desaturation to produce 20:3n3. The increase in EPA in triglycerides in these cultures indicates that EPA was taken up by the worms in excess of immediate needs and was being stored as triglyceride for later use. This is further supported by the finding that DHA was taken up by WT worms, but did not affect the concentrations of other fatty acids in any of the lipid classes to a significant degree.

The lipid analysis results for the *fat-3* mutant control cultures grown at 20 °C are in agreement with the results of Watts et al. (*7*). The fatty acids in *fat-3* mutants found in concentrations below those in WT controls all contained the n12 unsaturation, indicating a decrease in activity of the δ-6 desaturase, but not a complete loss of function. The increased concentrations observed were in fatty acids that do not contain the n12 unsaturation, and therefore were enriched due to the decreased activity of the δ-6 desaturase. Interestingly, an increase in total PUFAs was observed in triglycerides, but not in any of the other lipid classes. It is possible that the acyl transferases responsible for the production of phospholipids have a lower specificity for PUFAs that do not contain the n12 double bond. Alternatively, the phospho headgroup transferase may have low specificity for diglyceride molecules with the enriched fatty acids. Also of interest is the lack of incorporation of 14:0 and 16:0 into phospholipids even though they were increased in triglyceride. This was likely due to a lack of LC PUFAs available for incorporation into the phospholipid pool. It appears that n7 and n9 MUFAs, specifically 16:1n7 and 18:1n9, replaced the SFAs in this lipid pool. A lack of LC PUFAs would in essence have a similar affect on the membrane as lowering the growth temperature. At lower temperatures, *C. elegans* has a mechanism for creating phospholipids with two unsaturated fatty acids, even going so far as to produce di-EPA phospholipids (*17*). The accumulation of the 14:0 and 16:0 in triglyceride may be an indication that the membrane was lacking unsaturation and, therefore, incorporation of UFAs into phospholipids was greater than that of SFAs.

Supplementation of *fat-3* mutants grown at 20 °C with EPA appears to only partially rescue the fatty acid profile of these worms as compared with WT control worms. A persistent lack of 18:3n6, 20:3n6, and 20:4n6 in these cultures is expected because EPA cannot be retrograded to form these molecules. The presence of EPA in the culture media inhibited the enrichment of precursor fatty acids seen in *fat-3* mutant worms without supplementation. This indicates that a feedback mechanism for LC PUFAs to inhibit fatty acid synthesis is in operation. Similarities between EPA- and DHA-supplemented *fat-3* mutant cultures indicate that DHA functions in a similar manner to EPA in *C. elegans*, especially in phospholipids where lipid profiles between the two treatments are nearly identical, with the obvious exception of DHA being present in one but not the other culture. For this to occur, one of two possibilities must be true. It is possible that DHA functions through a feedback inhibition pathway by directly inhibiting enzymatic function, essentially shutting down fatty acid synthesis. A second, more intriguing, possibility is that the inhibition of fatty acids occurs through a membrane viscosity-sensitive enzyme or set of enzymes. Eukaryotic fatty acid desaturation is carried out by membrane-bound enzymes (*18*) that may be sensitive to the viscosity of the membrane. If such an enzyme were activated by decreasing viscosity (which could be achieved by decreasing PUFA concentration in the membrane or decreasing the temperature), then the addition of DHA or EPA would deactivate the enzyme. With a desaturase, deactivation would result in an increase in saturated fatty acids (SFAs), which are produced via a cytosolic fatty acid synthetase (*18*), and a decrease in UFAs. The increase in 14:0 and 16:0 and near WT concentrations of UFAs as compared with mutant controls could be explained by this mechanism; however, it could also be explained by an enzymatic inhibition by DHA or EPA of fatty acid desaturases.

The results obtained from incubation of worm cultures at 16 °C are quite different from those from incubation at 20 °C. This is not unexpected, however, as it has been shown that *C. elegans* changes its fatty acid profile with different culture temperatures (*17*, *19*). The most obvious difference between *fat-3* mutants grown at 16 and 20 °C is that, compared with controls grown at the respective temperatures, the fatty acid concentrations of the 16 °C cultures were decreased, whereas those of the 20 °C cultures were increased. This was due to the fact that the WT controls grown at 16 °C had all fatty acids in all lipid classes increased relative to controls at 20 °C. An increase in total fatty acid concentrations would indicate that fatty acid synthesis is ramped up to make up for the additional EPA that is required by *C. elegans* at the lower temperature.

The lack of accumulation of PUFAs in triglyceride in worms grown at 16 °C indicates that PUFA synthesis and partitioning are tightly regulated and triglyceride is reserved primarily for excess energy storage. However, at 20 °C WT mutants supplemented with either EPA or DHA and *fat-3* mutants supplemented with DHA had an increase in the total concentrations of n3 fatty acids. This could only occur if the phospholipid pools were saturated with n3s, but the worms were still taking in either EPA or DHA from the media. The accumulated PUFAs in triglyceride could be used for rapidly and efficiently changing membrane composition in response to temperature changes or other environmental factors. As *C. elegans* was isolated as a free living nematode, it would stand to reason that it may have encountered rapid temperature fluctuations that it would have had to respond to by altering its membrane composition. Synthesizing PUFAs from precursor molecules would likely be more time-consuming and would require more energy than simply mobilizing triglyceride stores.

Even though *C. elegans* does not produce DHA, it is able to utilize it, as first reported by Lesa et al. (*8*), who demonstrated that DHA added to a *fat-3* mutant culture can restore locomotion in mutants to WT concentrations. However, due to the retroconversion of DHA into EPA in vivo, it is difficult to say whether it is DHA or EPA that causes the rescue of phenotype. It appears that a combination of DHA and EPA is responsible for the rescue

of *fat-3* mutants fed DHA. Neither DHA nor EPA concentrations alone in these cultures were at WT EPA concentrations, but, taken together, they make up for the lack of LC PUFAs in this mutant. Although it would be expected that DHA would function in the membrane in a similar manner to EPA, it would be interesting to knockout the enzyme in the worm that converts DHA into EPA and to construct a double mutant with deficiencies in the retroconversion of DHA to EPA and δ-6 desaturase activity, as stated above. In this way, it would be possible to determine if DHA itself, in the absence of EPA, is able to restore proper membrane function and rescue *fat-3* mutants.

To further research the function of LC PUFAs in the membrane and extend the principles to animals and eventually humans, it is necessary to start with the most basic of animal models. Through genetic manipulation, it is possible to manipulate the fatty acid content of *C. elegans* to meet the needs of the research. Double and possibly even triple knockouts in fatty acid metabolism will allow further manipulation of the fatty acid profile of the worm in order to investigate the function of specific LC PUFAs without complications of further anabolism or retroconversion. For instance, a double knockout of *fat-1* and *fat-3* mutant would allow a researcher to supplement growth with 20:4n6 and observe the effects of this fatty acid in the absence of other LC PUFAs. The lipid analysis presented herein should aid future research focused on the function of the LC PUFAs by providing quantitative reference fatty acid profiles for total lipids, phospholipids, and triglycerides. It should also serve as a model for further lipid analyses done in *C. elegans*. Semiquantitative, or relative, fatty acid data may be sufficient for an organism such as *E. coli*, which produces only phospholipids, but is inadequate for an organism such as *C. elegans*, with more complex lipid metabolism. This is especially true in the study of membrane-associated processes in an organism that produces non-membrane-associated lipids such as triglyceride.

Although LC PUFAs are highly oxidizable and can accumulate in mitochondria under certain conditions (*5*), it seems that any possible negative effects on an organism in terms of speeding the aging process are outweighed by the beneficial effects the organism gains from having these molecules in its membranes. These fatty acids are important in energy production and proper nerve function, as well as in generally maintaining cellular membranes in a functional state, and these processes are likely so important to an organism that it has developed tolerance to combat the increased oxidative stress in order to incorporate EPA and DHA into various membranes.

ABBREVIATIONS USED

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LC PUFAs, long-chain polyunsaturated fatty acids; UFAs, unsaturated fatty acids; MUFAs, monounsaturated fatty acids; SFAs, saturated fatty acid; WT, wild-type.

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