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Fatty Acid Desaturation and the Regulation of Adiposity in *Caenorhabditis elegans*

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ABSTRACT

Monounsaturated fatty acids are essential components of membrane and storage lipids. Their synthesis depends on the conversion of saturated fatty acids to unsaturated fatty acids by Δ9 desaturases. *Caenorhabditis elegans* has three Δ9 desaturases encoded by the genes *fat-5*, *fat-6*, and *fat-7*. We generated nematodes that display a range of altered fatty acid compositions by constructing double-mutant strains that combine mutations in *fat-5*, *fat-6*, and *fat-7*. All three double-mutant combinations have reduced survival at low temperatures. The *fat-5;fat-6* double mutants display relatively subtle fatty acid composition alterations under standard conditions, but extreme fatty acid composition changes and reduced survival in the absence of food. The strain with the most severe defect in the production of unsaturated fatty acids, *fat-6;fat-7*, exhibits slow growth and reduced fertility. Strikingly, the *fat-6;fat-7* double-mutant animals have decreased fat stores and increased expression of genes involved in fatty acid oxidation. We conclude that the Δ9 desaturases, in addition to synthesizing unsaturated fatty acids for properly functioning membranes, play key roles in lipid partitioning and in the regulation of fat storage.

Δ9 desaturases, also known as stearoyl-CoA desaturases (SCDs), are key lipogenic enzymes that catalyze the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids. These monounsaturated products are the most abundant fatty acids found in phospholipids, triglycerides, and cholesterol esters (Enoch *et al.* 1976). As components of phospholipids, MUFAs are key in maintaining optimal membrane fluidity and also serve as mediators of signal transduction (Ntambi 1999). In humans, alterations in ratios of saturated to unsaturated fatty acids are associated with various diseases including diabetes, atherosclerosis, cancer, and obesity (Wang *et al.* 2003a,b; Warensjo *et al.* 2005; Bougnoux *et al.* 2006). The mechanisms in which Δ9 desaturase activity affects these disease conditions are not well understood.

The Δ9 desaturases are essential and are ubiquitous among eukaryotes. Previous work has shown that Δ9 desaturases are regulated to respond to changing environmental conditions. In poikilotherms, organisms that are physiologically unable to regulate body temperature, expression of desaturases is induced upon exposure to low temperatures to maintain fluid and functioning cell membranes (Tiku *et al.* 1996; Gracey *et al.* 2004; Los and Murata 2004). The Δ9 desaturases are also highly regulated in response to diet. In yeast, expression of the *OLE1* desaturase is repressed by exposure to exogenous unsaturated fatty acids in the growth media (Choi *et al.* 1996). Mice display a similar reduction in stearoyl-CoA desaturase 1 (SCD1) expression when unsaturated fatty acids are provided in the diet, and this isoform is also regulated by various dietary carbohydrates and by hormones such as insulin and leptin (Ntambi and Miyazaki 2003, 2004). Mouse mutants lacking SCD1 activity are lean and resistant to diet-induced obesity and insulin resistance (Dobrzyn and Ntambi 2005a).

In *Caenorhabditis elegans*, the *fat-6* and *fat-7* genes encode SCDs and a similar gene, *fat-5*, encodes a palmitoyl-CoA desaturase (Watts and Browse 2000). The pathway for unsaturated fatty acid synthesis in *C. elegans* begins with palmitic acid (16:0), obtained from the *Escherichia coli* diet or synthesized *de novo*, which is converted to palmitoleic acid (16:1) by FAT-5 (Figure 1A). This fatty acid is then elongated to *cis*-vaccenic acid (18:1Δ11), which is the most abundant fatty acid in phospholipids and triglycerides (Tanaka *et al.* 1996). Palmitic acid (16:0) can also be elongated to stearic acid (18:0), the substrate for FAT-6 and FAT-7 desaturation to oleic acid (18:1Δ9), which is further desaturated and elongated to form all of the polyunsaturated fatty acids (PUFAs), including arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3) (Watts and Browse 2002) The desaturases involved in PUFA production downstream of the Δ9 desaturases include FAT-2 (Δ12 desaturase), FAT-3 (Δ6 desaturase), FAT-4 (Δ5 desaturase), and FAT-1 (omega-3 desaturase). Long-chain

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PUFAs are components of membrane phospholipids where they play important roles in membrane function and lipid signaling (Kahn-Kirby *et al.* 2004; Kubagawa *et al.* 2006). We recently characterized mutant strains that lack each Δ9 desaturase activity and these studies revealed only slight effects on fatty acid composition due to compensation by the remaining isoforms. Although these genes display functional overlap, the Δ9 desaturation is essential because *fat-5;fat-6;fat-7* triple mutants that lack all activity are unable to survive unless they are supplemented with dietary oleic acid (Brock *et al.* 2006).

In this study, we generated Δ9 desaturase double-mutant strains to examine intermediate, nonlethal effects that arise from reduced Δ9 desaturase activity. Our characterization of the double mutants reveals striking roles for Δ9 desaturases in maintaining energy homeostasis as well as for growth and development. All three double-mutant combinations affect growth and viability at low temperatures. The *fat-5;fat-6* double mutants display relatively subtle fatty acid composition alterations under standard conditions, but extreme fatty acid composition changes and reduced survival in the absence of food. The *fat-6;fat-7* double mutant shows the greatest fatty acid composition alterations under standard conditions and exhibits the broadest range of defects, including slow growth and reduced fertility. A key finding of these studies is that the *fat-6;fat-7* double mutants have reduced fat stores and induction of genes encoding components of peroxisomal and mitochondrial β-oxidation. Other mutant strains with reduced PUFAs (*fat-2* and *fat-3* mutants) show similar defects in growth and fertility, yet do not display fat storage defects, indicating that ability to convert stearic acid (18:0) to oleic acid (18:1) is vital for proper energy partitioning and fat synthesis.

MATERIALS AND METHODS

Culture of nematodes: Unless otherwise noted, animals were grown on nematode growth media plates (NGM) at 20° with the *E. coli* (OP50) strain as a food source (Wood 1988). The wild-type strain used was N2. The axenic culture media consisted of 3% soy peptone, 3% yeast extract, 0.5 mg/ml hemoglobin in 1 m KOH, and 20% ultra-high-temperature pasteurized skim milk (Houthoofd *et al.* 2002). The liquid axenic cultures were grown at room temperature (22°–23°) with constant shaking. Plates containing dietary fatty acid supplementation were prepared fresh for each supplementation experiment as described (Watts *et al.* 2003).

Generation of double mutants: Single-worm PCR (Wicks *et al.* 2001) was used to determine the genotype of worms during crossing to generate *fat-5(tm420);fat-6(tm331)*, *fat-5(tm420);fat-7(wa36)*, and *fat-6(tm331);fat-7(wa36)* double-mutant lines. For *fat-5(tm420)* and *fat-6(tm331)* identification, standard PCR was used (for primer sequences see Brock *et al.* 2006). For *fat-7(wa36)* identification, real-time quantitative PCR was used with two primer sets. One primer set was designed to preferentially amplify the wild-type allele and the other primer set to preferentially amplify the *fat-7(wa36)* mutation (Drenkard *et al.* 2000).

Fatty acid and lipid analysis: Fatty acid composition of adult nematodes was determined as previously described (Watts and Browse 2002; Brock *et al.* 2006). Thin-layer chromatography and lipid analysis was performed as described in Ashrafi *et al.* (2003) and Watts and Browse (2006). Nile Red staining was performed as described in Ashrafi *et al.* (2003). Images were captured at ×40 magnification using identical settings and exposure time for each image. To identify the unusual fatty acids in the *fat-6;fat-7* double mutants, the fatty acid 4,4-dimethyloxazoline (DMOX) derivatives were prepared from fatty acid methyl esters to stabilize them for analysis by gas chromatography (GC)/mass spectroscopy (MS). For the DMOX reaction, the fatty acid methyl esters were evaporated using argon (Ar). A solution of 9:1 ethanol: benzene was added and after evaporation warmed 2-amino-2-methylpropanol was added. The reaction was capped and incubated 6 hr at 190°. After cooling, the DMOX derivatives were dissolved in hexane and washed twice with water. The hexane layer was then passed through a drying column of glass wool and Na_2SO_4. After evaporation of the solvent with Ar, 9:1 ethanol:benzene was added and then evaporated. The DMOX derivatives were then dissolved in hexane and separated on a 30 m × 0.25 mm AT-WAXms column (Alltech) with an HP6890 series GC system (Hewlett Packard) and the mass spectra were determined on the HP 5973 Mass Selective Detector (Hewlett Packard) (Watts and Browse 1999). The mass spectra of the peaks identified as DMOX-13-octadecenoate, DMOX-6,9,12-hexadecatrienoate, and DMOX-8,11,14,17-octadecatetraenoate matched the spectra presented by W. W. Christie on the lipid library website (http://www.lipidlibrary.co.uk/index.html). The mass spectrum of the peak identified as DMOX-5,8,11,14-octadecatetraenoate contained a prominent peak at *m/z* 153, which is diagnostic of a double bond at the Δ5 position, in addition to the characteristic gaps of 12 atomic mass units at *m/z* 182 and 194, *m/z* 222 and 234, and *m/z* 262 and 274, which correspond to double bonds at the 8, 11, and 14th carbon of DMOX-derived 18-carbon fatty acids.

Quantitative RT–PCR analysis: Adult nematodes were harvested and RNA was prepared using TRIzol Reagent (Invitrogen, San Diego). A DNA-FREE RNA kit (Zymo Research) was used for Dnase treatment and purification. After quantification, 1 μg of RNA was used in a reverse-transcription reaction with SuperScriptIII (Invitrogen) to generate cDNA. Primer sequences for the metabolism genes were obtained from Marc Van Gilst (Van Gilst *et al.* 2005a). The PCR mixture consisted of 0.3 μm primers, cDNA, ROX, and 1× SYBR green mix (Invitrogen Platinum SYBR green qPCR Supermix UDG). The quantitative RT–PCR (QRT–PCR) was run and monitored on a MX3000P machine (Stratagene, La Jolla, CA). Relative abundance was determined using the ΔΔCt method and the reference genes *tbb-2* and *ubc-2* to control for template levels (Wong and Medrano 2005).

Growth and development phenotype analysis: *Life-span analysis:* Aging experiments were performed on adult nematodes grown at 20°. Worms were moved to plates containing 5-fluoro-2′-deoxyuridine (Sigma, St. Louis) at the fourth larval stage of development (L4). Live animals were assayed for movement in response to touch every 1–2 days (Apfeld and Kenyon 1998). Movement assays were performed with 1-day-old adults as described in Miller *et al.* (1996) and Watts *et al.* (2003).

Growth rate analysis: Eggs were isolated from gravid adults using hypochlorite treatment and plated onto NGM plates. Twice a day the number of worms at each life stage was counted.

Fertility analysis: For analysis of total progeny produced per worm, L4 (nonreproductive) worms were isolated and moved

TABLE 1

Fatty acid composition of wild-type and Δ9 desaturase double mutants

Fatty acid	Wild type	*fat-5;fat-7*	*fat-5;fat-6*	*fat-6;fat-7*
14:0	1.6 ± 0.1	2.3 ± 0.1***	1.9 ± 0.3	1.4 ± 0.3
16:0	5.5 ± 0.5	7.1 ± 0.6***	6.4 ± 0.6*	1.6 ± 0.3***
18:0	8.5 ± 1.0	9.9 ± 1.2*	9.4 ± 0.5	22.2 ± 1.3***
Total saturated	15.6	19.3	17.7	25.2
16:1	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1**	3.4 ± 0.8***
18:1Δ9	3.2 ± 0.2	4.2 ± 0.4**	3.2 ± 0.2	—***
18:1Δ11	14.3 ± 1.3	13.5 ± 0.6	12.6 ± 1.3*	24.0 ± 1.6***
Total MUFA	18.9	18.9	16.9	27.4
18:2	3.1 ± 0.1	3.5 ± 0.3*	3.0 ± 0.3	—***
20:3	4.4 ± 0.4	4.1 ± 0.3	3.9 ± 0.5	—***
20:4n-6	2.0 ± 0.4	1.7 ± 0.1	1.5 ± 0.2	—***
20:4n-3	5.0 ± 0.6	4.5 ± 0.6	4.4 ± 0.6	—***
20:5	11.7 ± 1.7	11.0 ± 1.4	10.2 ± 1.2	—***
Total PUFA	26.2	24.8	23.0	—
18:1 Δ13	—	—	—	2.0 ± 0.3***
18:3 Δ8,11,14	—	—	—	8.0 ± 2.0***
18:4 Δ5,8,11,14	—	—	—	4.1 ± 0.3***
18:4 Δ8,11,14,17	—	—	—	3.7 ± 0.3***
Total unusual	—	—	—	17.8
C15iso	3.9 ± 0.3	3.6 ± 0.2	4.3 ± 0.4	0.7 ± 0.3***
C17iso	3.1 ± 0.3	2.4 ± 0.2*	3.5 ± 0.2*	1.5 ± 0.6***
Total branched	7.0	6.0	7.4	2.2
17Δ	19.9 ± 0.9	19.1 ± 1.0	20.9 ± 1.8	18.8 ± 2.1
19Δ	13.2 ± 0.9	11.7 ± 0.7*	13.8 ± 0.9	8.8 ± 1.5*
Total cyclopropane	33.1	30.8	34.7	27.6

Data are weight percentages (mean ± SD) of four to six independent determinations of total worm fatty acids measured by gas chromatography. 17Δ, 9,19-methylenehexadecanoic acid; 19Δ, 11,12-methyleneoctadecanoic acid. Dash indicates fatty acids <0.5%. Values determined to be significantly different from wild-type worms using an unpaired *t*-test are *$P < 0.05$, **$P < 0.001$, and ***$P < 0.0001$.

to fresh plates. After reaching reproductive viability, adults were moved to fresh plates twice daily, as needed. Two days after removal of the adult, the live progeny were counted. For analysis of biochemical complementation of fertility, worms were grown from hatching on supplemented plates. They were moved as young adults to supplemented plates of the same type and allowed to lay eggs for 2 days after which the adult was removed. The number of live progeny was counted on the following day.

Cold temperature growth: Equal numbers of synchronous L1 worms were placed on plates at 20°, 15°, and 10°. The number of live nonarrested worms was counted on each plate when the wild-type population reached adulthood. These values are expressed relative to the number of live nonarrested worms counted at 20°.

L1 starvation survival: Embryos were collected from adult worms by hypochlorite treatment and hatched on unseeded NGM plates without peptone. This produced a population of *C. elegans* arrested in the first larval stage. These larvae were washed from the plate and incubated at room temperature in M9 buffer with cholesterol (10 μg/ml). Every 48 hr, aliquots were transferred to standard NGM plates seeded with *E. coli* (OP50) bacteria. After 3 days of growth at 20°, viable adult nematodes were counted (Derry *et al.* 2001).

RESULTS

Fatty acid composition is altered in Δ9 desaturase double mutants: To examine the roles of the Δ9 desaturases and the effects of altered saturated and monounsaturated fatty acid compositions, we generated double-mutant strains for all combinations of the three *C. elegans* Δ9 desaturase genes. The *fat-5;fat-7*, *fat-5;fat-6*, and *fat-6;fat-7* double-mutant strains are all capable of reaching adulthood and reproducing under standard growth conditions even though they rely on only one of the three Δ9 desaturase isoforms. GC was used to measure the fatty acid composition of worms grown under standard conditions feeding on *E. coli* bacteria (Table 1). The *fat-5;fat-7* and *fat-5;fat-6* double mutants displayed subtle alterations compared to wild type, with increased saturated fatty acid content (19.3 and 17.7% saturated fatty acids compared to 15.6% in wild type) and slightly decreased MUFA and PUFA content.

In contrast, the fatty acid composition of *fat-6;fat-7* double mutants was dramatically altered from wild type. The *fat-6;fat-7* double mutants accumulated very high levels of 18:0 (22.2% of total fatty acids compared to 8.5% in wild type) and completely lack oleic acid (18:1Δ9) and PUFAs derived from this fatty acid, such as linolenic acid (18:2) and eicosapentaenoic acid (20:5n-3). In addition, the mono-methyl branched-chain fatty acids were reduced approximately threefold below wild-type levels. GC traces for *fat-6;fat-7* contained

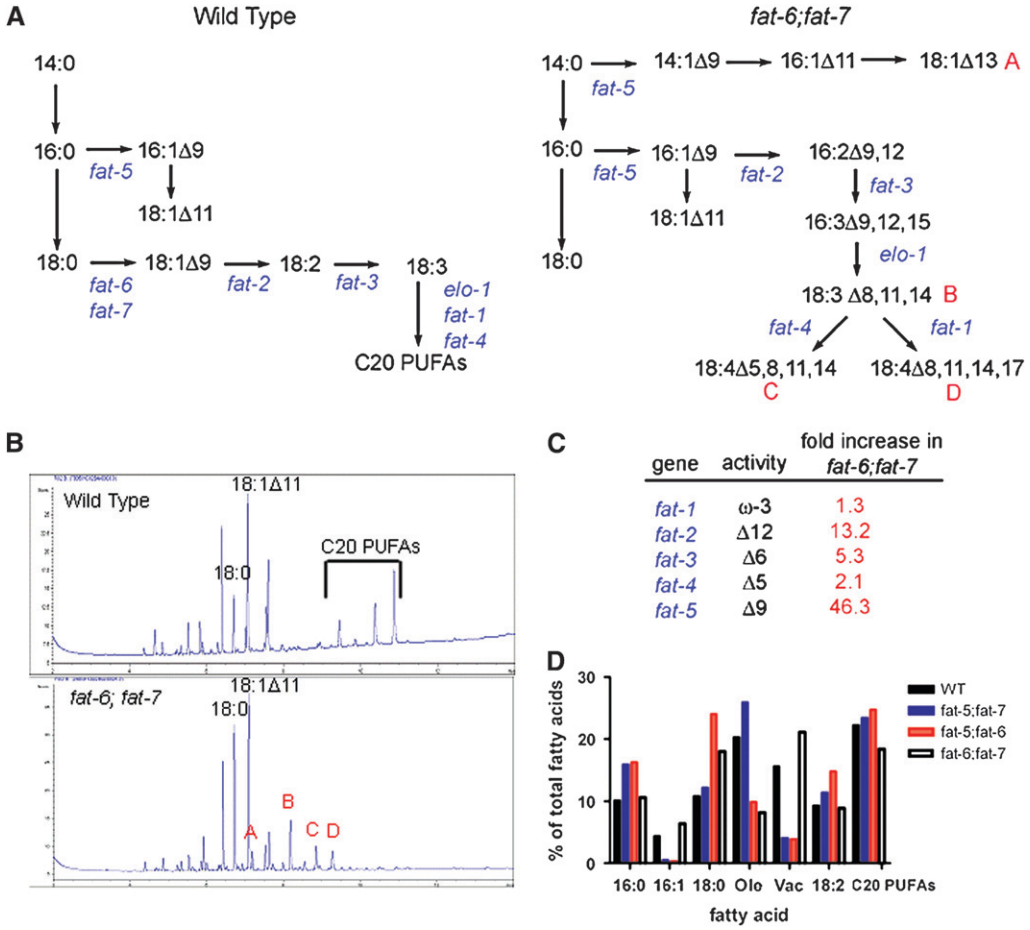

FIGURE 1.—Fatty acid composition is altered in Δ9 desaturase double mutants. (A) Simplified scheme of fatty acid desaturation in wild-type *C. elegans* highlighting the roles of the fatty acid desaturases *fat-1*–*fat-7* (left). (Right) The proposed pathway for generation of the unusual fatty acids produced by the *fat-6; fat-7* double mutant. Fatty acid nomenclature: X:YΔZ, as in 18:1Δ11, fatty acid chain of X carbon atoms and Y methylene-interrupted *cis* double bonds; Z indicates the position of a double bond relative to the carboxyl end of the molecule. (B) Gas chromatography traces showing the fatty acid profiles of wild-type and *fat-6; fat-7* double mutants. The *fat-6;fat-7* double mutants lack 18:1Δ9 and the 20-carbon PUFAs. In addition, they accumulate higher levels of 18:0 as well as unusual fatty acids, labeled A–D in red. The identities of these fatty acids are A-18:1Δ13, B-18:3(Δ8,11,14), C-18:4 (Δ5,8,11,14), and D-18:4 (Δ8,11,14,17). (C) Changes in desaturase gene expression in *fat-6;fat-7* double mutants compared to wild type. Gene expression was measured by QRT–PCR. (D) Simplified fatty acid composition of Δ9 desaturase double mutants grown in axenic culture. Ole, oleic acid (18:1Δ9); Vac, vaccenic acid (18:1Δ11).

novel peaks that were not present in wild type. To identify the novel peaks, fatty acid methyl esters were converted to DMOX derivatives to stabilize them for analysis of double-bond position by GC/mass spectroscopy (see MATERIALS AND METHODS). We found that these peaks correspond to four unusual isomers of C18 unsaturated fatty acids that make up nearly 18% of total fatty acids (Figure 1B, Table 1).

Quantitative real time PCR (QRT–PCR) was used to monitor the expression of fatty acid desaturase genes in the *fat-6;fat-7* double mutants (Figure 1C). We found that, compared to wild-type nematodes grown under the same conditions, adult *fat-6;fat-7* double mutants showed induction of four of five fatty acid desaturases. The biggest change in expression was seen for the *fat-5* gene, which encodes the only remaining Δ9 desaturase. This gene showed 46-fold greater expression in the *fat-6;fat-7* double mutants than in wild type. The increased *fat-5* expression likely leads to the increased vaccenic acid (18:1Δ11) content of the *fat-6;fat-7* double mutants, since this fatty acid is elongated from 16:1 and cannot be further modified into PUFAs. In addition, the *fat-2* Δ12 desaturase showed 13-fold induction and the *fat-3* Δ6 desaturase showed 5-fold induction. Increased expression of desaturases, together with the absence of their normal substrates, likely leads to the formation of unsaturated fatty acids that are not normally produced. Presumably these unusual unsaturated fatty acids compensate for some of the functions that are ordinarily provided by C20 PUFAs. A proposed pathway for the formation of the unusual C18 unsaturated fatty acids in *fat-6;fat-7* double mutants is shown in Figure 1A.

To determine if altered growth conditions affected fatty acid composition of the double mutants, we grew the worms in liquid axenic media. While the *E. coli* diet contains saturated fatty acids [myristic and palmitic acids (14:0 and 16:0)] as well as the MUFAs palmitoleic and *cis*-vaccenic acids (16:1 and 18:1Δ11) (TANAKA *et al.* 1996), the axenic media consists of yeast extract, peptone, and skim milk, which provides small amounts of palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1Δ9), and linoleic acid (18:2) (BROCK *et al.* 2006). We found that both the *fat-5;fat-7* and the *fat-5; fat-6* double mutant showed amplified fatty acid composition changes when grown in axenic culture, with palmitoleic acid (16:1) and vaccenic acid (18:1Δ11)

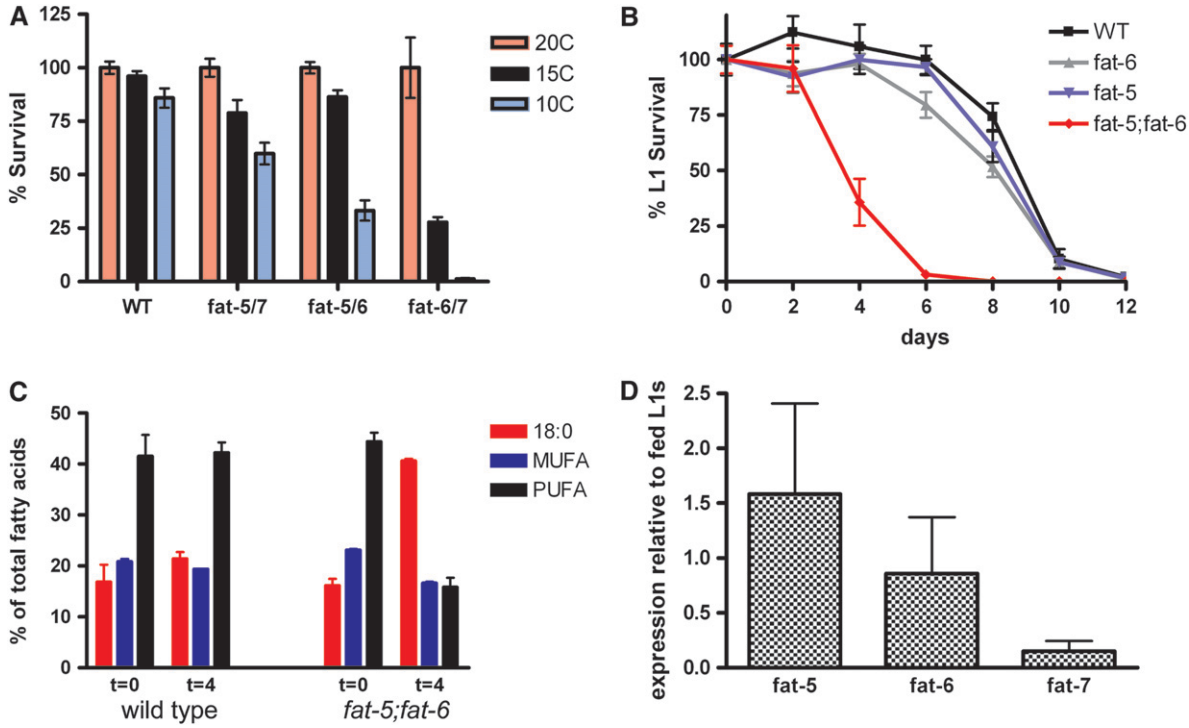

FIGURE 2.—Δ9 desaturase double mutants have reduced survival at low temperature and during L1 starvation. (A) Survival at 15° and 10° relative to that at 20° for *fat-5;fat-6*, *fat-5;fat-7* and *fat-6;fat-7* double mutants and wild type. Synchronized L1 larvae of each genotype were plated at various temperatures and live animals were counted several days later when wild-type nematodes reached adulthood. (B) Survival as L1's for *fat-5*, *fat-6*, *fat-5;fat-6*, and wild type in the absence of food. Synchronized L1 larvae were incubated in M9 buffer + 0.01% cholesterol. Larvae were plated every 48 hr and allowed to develop on plates for 3 days, after which viable animals were counted. Error bars represent the standard error. (C) Simplified fatty acid composition of newly hatched L1 larvae ($t = 0$) and starved L1 larvae that have been incubated in M9 buffer for 4 days ($t = 4$). (D) Change of expression of the *fat-5*, *fat-6*, and *fat-7* genes in 4-day-starved wild-type L1 larvae relative to expression in newly hatched L1 larvae. Data shown are the average of three determinations, each from four biological replicates; error bars represent the standard deviation.

decreasing more than threefold compared to wild type in both double mutants and with 18:0 increasing twofold in the *fat-5;fat-6* double (Figure 1D). This indicates that both *fat-5* and *fat-6* are particularly important for maintaining fatty acid composition during axenic growth. In contrast, the *fat-6;fat-7* double mutant grown in axenic media had a less severe fatty acid composition change than when grown on *E. coli* plates. In axenic media, the *fat-6;fat-7* double mutant accumulated 18:2 and C20 PUFAs, due to the presence of oleic and linoleic acids in the media. This, together with the 18:1 and 20:5 supplementation studies (see below), illustrates the importance of dietary contribution to fatty acid composition.

Δ9 desaturase activity is necessary for survival at low temperatures: As a poikilothermic animal, *C. elegans* is sensitive to environmental temperatures. To determine if the Δ9 desaturases and the MUFAs that they produce play a role in survival at low temperatures, we plated *fat-5;fat-6*, *fat-5;fat-7*, and *fat-6;fat-7* double mutants and wild-type L1 larvae at 20°, 15°, and 10°. When the wild-type larvae had reached adulthood, the nonarrested living worms on the plates were counted. With survival at 20° set at 100%, wild-type animals had a 96 and 85% survival rate at 15° and 10°, respectively. All three of the Δ9 desaturase double mutants exhibited reduced survival at 10° (Figure 2A). The *fat-5;fat-7* double mutant displayed only 60% survival at 10° while the *fat-5;fat-6* double mutant was more strongly affected with only 33% of the population surviving. The *fat-6;fat-7* double mutant had the most extreme cold-sensitive phenotype. Only 1% of the worms survived at 10° and only 28% survived when grown at 15°. Thus, higher saturated fatty acid and lower unsaturated fatty acid composition is detrimental to nematodes at low temperatures.

***fat-5;fat-6* double mutants reveal a role for Δ9 desaturation in survival of L1 arrest:** In the course of maintaining our stocks, we noted that the *fat-5;fat-6* nematodes were difficult to revive from old plates containing starved worms. Several days after *C. elegans* worms had depleted their *E. coli* food, adult animals died and the surviving nematode population typically consisted of numerous L1 larvae that arrested development until food is reintroduced (JOHNSEN *et al.* 1984). We examined the ability of wild-type and mutant L1 larvae to survive in the absence of food by counting viable animals that had been incubated as L1 larvae for increasing periods of time in the absence of food. We

found a shorter survival time for the *fat-5;fat-6* L1 larvae compared to wild type. The time for half of the L1 larva population to die in the absence of food for wild type was ~9 days, while the time for half of the *fat-5;fat-6* L1 larva population to die in the absence of food was only 3.5 days (Figure 2B). The single *fat-5* and *fat-6* mutants, as well as other Δ9 desaturases double mutants, *fat-5; fat-7* and *fat-6;fat-7*, displayed a similar ability to survive L1 arrest as wild type (Figure 2B and data not shown). Thus, the *fat-5;fat-6* double mutant displays a specific defect in surviving starvation.

In an attempt to explain the reduced survival time of starved *fat-5;fat-6* larvae, we characterized the fatty acid composition of fed and starved L1 animals. We found that starved wild-type L1 larvae showed very small changes in fatty acid composition compared to fed L1 larvae, with only a 4% increase in 18:0 and little change in MUFAs and PUFAs (Figure 2C). In contrast, the *fat-5; fat-6* larvae showed greatly increased 18:0 accumulation (41% in starved larvae compared to 16% in fed L1 larvae) and decreased MUFAs and PUFAs (16% each of MUFAs and PUFAs in starved animals compared to 23% MUFAs and 44% PUFAs in fed L1 larvae). A recent study showed that the *fat-7* gene is repressed 10-fold in wild-type adults after removal from food for 12 hr (Van Gilst *et al.* 2005b). We performed QRT–PCR on the three Δ9 desaturase genes using wild-type RNA isolated from fed and 4-day-old starved L1 larvae and found that the *fat-7* gene was repressed >6-fold in the starved larvae while the *fat-5* and *fat-6* genes were unchanged or slightly induced (Figure 2D). While FAT-7 compensates for the lack of FAT-5 and FAT-6 under standard conditions, it appears that FAT-7 alone cannot provide the level of desaturation needed for survival in the absence of food.

MUFAs or PUFAs are required for proper movement, growth, and fertility: Compared to the *fat-5;fat-7* and *fat-5;fat-6* strains, both phenotypes of the *fat-6;fat-7* double mutants are more varied and more severe, which correlates with the more severe alterations in fatty acid composition. Relative to wild type, the *fat-6;fat-7* worms grow slowly and produce fewer progeny, a phenotype similar to that of the other PUFA-deficient *C. elegans* strains *fat-2* and *fat-3* (Watts and Browse 2002; Watts *et al.* 2003). While the *fat-6;fat-7* double mutants do not display the dumpy body shape observed in *fat-2* and *fat-3* mutants, the *fat-6;fat-7* double mutants appear to move more sluggishly than wild type, a phenotype shared with *fat-3* and *fat-2* mutants. Indeed, when we quantified movement by assaying thrashing in liquid, we found that the *fat-6;fat-7* double mutants generated less than half the number of thrashes per minute observed for wild type (Figure 3A). This rate is comparable to that of *fat-3* mutants, but substantially higher than that of *fat-2*.

We determined the growth rate of the *fat-6;fat-7* double mutants by measuring the time that it takes isolated early stage embryos to develop and reach the fourth larval stage of development (L4). The *fat-6;fat-7* double mutants reached the L4 stage well after wild-type animals (Figure 3B). The time for half of the population to reach L4 ($L4^{50}$) was 57 hr for wild type and 77 hr for *fat-6;fat-7* double mutants. In comparison, *fat-2* mutants display an even greater growth retardation than *fat-6; fat-7*, not reaching the $L4^{50}$ until 110 hr at 20°. The *fat-3* worms grew considerably faster than the *fat-6;fat-7* animals, reaching $L4^{50}$ at 64 hr.

We observed reduced brood size in the *fat-6;fat-7* double mutants. By counting the progeny of single animals, we found that the *fat-6;fat-7* double mutants produced an average of only 37 ± 9 live progeny/adult worm compared with 260 ± 10 live progeny for wild type (Figure 3C). The number of eggs laid by *fat-6;fat-7* double mutants was only 36% of the number of eggs laid by wild type and only 34% of the eggs laid by *fat-6;fat-7* double mutants that hatched into viable larvae. Microscopic examination revealed abnormalities in eggshells or absent eggshells for 58% of the unhatched eggs. Similarly, *fat-2 (wa17)* mutants layed an average of 53 eggs, and only 29% of these hatched. An abnormal eggshell was observed in ~10% of the unhatched eggs produced by *fat-2* mutants. In comparison, *fat-3* mutants have greater reproductive success, as they lay an average of 160 eggs and 94% of them hatched (Watts *et al.* 2003). Interestingly, in spite of the movement, growth, and fertility defects observed in the *fat-6;fat-7* strain, we found that the double mutants have a life span only slightly shorter than that of wild type (Figure 3D).

Fatty acid supplementation with 18:1Δ9 or 20:5 resulted in the amelioration of some, but not all, of the defects observed in *fat-6;fat-7* animals. Supplementation with 0.1 mm 18:1 or 0.1 mm 20:5 fully restored movement (Figure 3A). The slow growth rate of *fat-6; fat-7* could be partially complemented by dietary supplementation of 18:1 or 20:5 (Figure 3B). Fertility requirements were somewhat more specific, as the number of progeny produced by the *fat-6;fat-7* double mutants was partially rescued by dietary oleic acid (18:1Δ9) but eicosapentaenoic acid (20:5n-3) did not improve either the number of eggs laid or their hatch rate (Figure 3E).

***fat-6;fat-7* double mutants display reduced fat stores and induced expression of mitochondrial and peroxisomal β-oxidation genes:** In addition to the decreased growth rate, reduced fertility, and altered fatty acid profile, we noted that the *fat-6;fat-7* double mutants grown on *E. coli* have a clear intestine, a phenotype often associated with reduced fat stores (McKay *et al.* 2003). We used the lipophilic dye Nile Red to stain the lipid droplets in the intestine of adult worms. The staining in *fat-6;fat-7* mutants was qualitatively different from wild type: it was fainter and more diffuse, and the Nile Red-staining granules common in wild type were not distinct (Figure 4). To quantify this decrease in fat storage, we used thin-layer chromatography and gas chromatography to characterize the lipid profile of the *fat-6;fat-7*

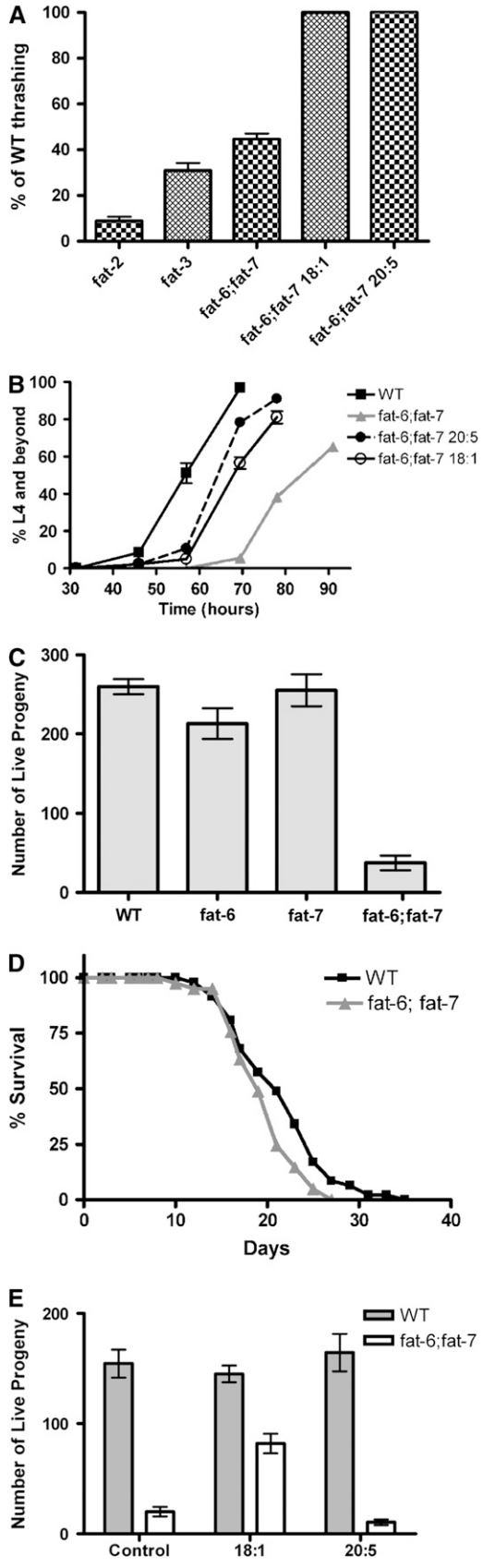

FIGURE 3.—Movement, growth, and fertility defects in the *fat-6;fat-7* double mutants. (A) Movement assayed by thrashing in liquid. (B) Time in hours for animals to reach the L4 stage. Nonsupplemented wild type are compared to *fat-6;fat-7* double mutants grown without supplementation or supplementation with 18:1Δ9 or 20:5. (C) Average number of progeny produced per adult for wild type, *fat-6*, *fat-7*, and *fat-6;fat-7* individuals. (D) Life span of wild type and *fat-6;fat-7* at 20°. (E) Average progeny produced by individual adults over 2 days for wild-type and *fat-6;fat-7* double mutants grown with control and 18:1Δ9 or 20:5 supplementation. All error bars represent the standard error.

double mutants and found a decrease in triglyceride stores. The average percentage of lipids as triglycerides in three independently grown samples was 51 ± 1% in wild-type animals and 41 ± 2% in *fat-6;fat-7* double mutants. Analysis of the fatty acid composition of the various lipid classes revealed that the unusual 18-carbon PUFAs found in the *fat-6;fat-7* double mutants were mainly partitioned to phospholipids and may replace the C20 PUFAs that are a major component of phospholipids (Table 2). The triglyceride fraction showed a dramatic increase in saturated fatty acids, containing 24.8% saturated fatty acids compared to only 9.9% in wild type.

The *fat-2* and *fat-3* mutants, which have normal Δ9 desaturase activity and are capable of synthesizing MUFAs, but are deficient in the synthesis of downstream PUFAs, do not show reduced Nile Red staining (ASHRAFI *et al.* 2003). Therefore, it appears that the reduced synthesis of monounsaturated fatty acids or the reduction of Δ9 desaturase activity specifically, rather than the reduction in PUFAs, leads to lower adiposity in nematodes. Dietary supplementation with oleic acid (18:1) restored normal Nile Red staining to the *fat-6;fat-7* double mutants (Figure 4). Thus, dietary fats are able to compensate for the fatty acid desaturation defect and restore the normal fat accumulation.

We used QRT–PCR to characterize the expression of a panel of genes involved in lipid and glucose metabolism in the *fat-6;fat-7* double mutant compared to wild type (VAN GILST *et al.* 2005a). In addition to the increased expression of desaturase genes mentioned earlier, this analysis revealed a dramatic increase in expression of genes predicted to be involved in the mitochondrial and peroxisomal β-oxidation pathways (Figure 5). Notably, genes predicted to encode an acyl-CoA synthetase (*acs-2*) and a trifunctional β-oxidation enzyme (*ech-1*) showed increased expression (7.7-fold and 18.4-fold, respectively) in the *fat-6;fat-7* double mutants. Previous studies have shown that the expression of these two genes is downregulated in high-fat *nhr-49* mutants and that reducing their activity by RNA interference leads to increased fat stores (VAN GILST *et al.* 2005a). Another group of genes that are induced in the *fat-6;fat-7* double mutants are the fatty-acid-binding proteins *lpd-1–lpd-7*. These proteins are predicted to encode cytosolic fatty-acid-binding proteins but their precise function is unknown. Genes involved in glucose metabolism were largely unaffected, with the exception of one isoform of phosphofructo kinase that was repressed 13-fold in the

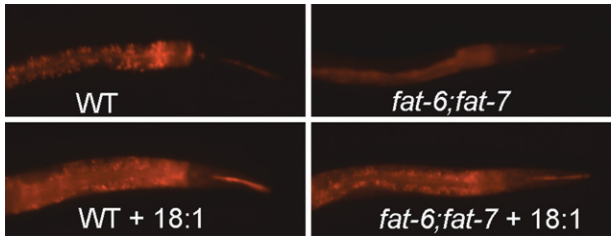

FIGURE 4.—Reduced adiposity in *fat-6;fat-7* double mutants. Wild-type and *fat-6;fat-7* 1-day-old adults stained with Nile Red. Animals were grown on unsupplemented plates (top) or on plates containing 0.1 mM 18:1 (bottom).

fat-6;fat-7 double mutants. The induction of enzymes involved in fatty acid oxidation indicates that, in addition to maintaining optimal unsaturation in membranes, Δ9 desaturase activity plays an important role in fat storage and energy homeostasis.

DISCUSSION

The generation of three Δ9 desaturase double-mutant strains enabled us to examine the consequences of altering the content of saturated and monounsaturated fatty acids in an animal. Our previous studies determined that the deletion of each of the single Δ9 desaturase genes had only subtle effects on fatty acid composition and no physiological consequences for the mutant animals, while the deletion of all three Δ9 desaturases led to lethality (BROCK *et al.* 2006). The double-mutant strains created for this study showed a range of fatty acid compositions as well as a range of physical defects that provide clues to the biological processes that depend most on the synthesis of MUFAs.

To maintain membrane fluidity at low temperatures, plants and poikilothermic animals alter their fatty acid composition by increasing the level of unsaturation of their membrane phospholipids (LOS and MURATA 2004). We found that the *fat-6;fat-7* double mutant, which lacks all 20-carbon PUFAs, is not able to survive at 10° and shows reduced survival at 15°. Other PUFA-deficient mutants, *fat-2* and *fat-3*, also show decreased survival at low temperatures (WATTS *et al.* 2003 and our unpublished observations). Thus, growth at low temperature is very sensitive to changes in fatty acid composition. The only apparent defect in the *fat-5;fat-7* double mutants is a small reduction in survival at 10°. The lack of phenotypic consequences in the *fat-5;fat-7*

TABLE 2

Fatty acid composition of lipid fractions from wild-type and *fat-6;fat-7* double mutants

Fatty acid	Wild-type TAG	*fat-6;fat-7* TAG	Wild type PL	*fat-6;fat-7* PL
14:0	2.3 ± 0.3	2.4 ± 0.6	0.7 ± 0.1	0.5 ± 0.1
16:0	4.0 ± 2.5	2.2 ± 1.9	4.1 ± 0.5	1.8 ± 1.3*
18:0	3.6 ± 0.1	20.2 ± 3.1**	9.3 ± 1.8	23.5 ± 4.0*
Total saturated	9.9	24.8	14.1	25.8
16:1	2.3 ± 0.2	3.2	0.9 ± 0.1	3.0 ± 0.1***
18:1Δ9	6.0 ± 0.1	—***	2.2 ± 0.2	—***
18:1Δ11	14.6 ± 2.6	16.8 ± 0.9	15.0 ± 1.7	26.0 ± 3.9*
Total MUFA	21.8	20.0	16.8	29.0
18:2	3.0 ± 0.6	—*	4.6 ± 0.5	—***
20:3	2.5 ± 0.3	—**	6.8 ± 0.5	—***
20:4n-6	0.8 ± 0.4	—	2.5 ± 0.4	—*
20:4n-3	1.5 ± 0.3	—*	9.4 ± 0.1	—***
20:5	3.1 ± 0.5	—**	22.0 ± 1.1	—***
Total PUFA	11.5	—	46.1	—
18:1 Δ13	—	2.2 ± 0.2**	—	1.7 ± 0.1***
18:3 Δ8,11,14	—	0.8 ± 0.7	—	8.3 ± 0.9***
18:4 Δ5,8,11,14	—	0.5 ± 0.5	—	5.6 ± 0.8**
18:4 Δ8,11,14,17	—	0.5 ± 0.5	—	6.3 ± 1.3***
Total unusual	—	4.0	—	21.9
C15iso	5.7 ± 0.2	2.0 ± 0.5**	1.8 ± 0.1	—***
C17iso	3.2 ± 0.2	2.1 ± 0.4*	2.6 ± 0.1	0.9 ± 0.3**
Total branched	8.9	4.1	4.5	0.9
17Δ	28.8 ± 0.6	31.1 ± 2.4	6.7 ± 0.7	10.6 ± 1.7*
19Δ	16.8 ± 1.2	12.2 ± 1.5*	6.8 ± 0.5	7.7 ± 2.9
Total cyclopropane	45.6	43.3	13.5	18.3

Data are weight percentages of total fatty acids (mean ± SD) of lipid extractions from three independently grown nematode populations measured by gas chromatography. 17Δ, 9,19-methylenehexadecanoic acid; 19Δ, 11,12-methyleneoctadecanoic acid; TAG, triacylglyceride fraction; PL, phospholipid fraction. Dashes indicate fatty acids <0.5%. Values determined to be significantly different from wild-type lipid fractions using an unpaired *t*-test are $*P < 0.05$, $**P < 0.001$, and $***P < 0.0001$.

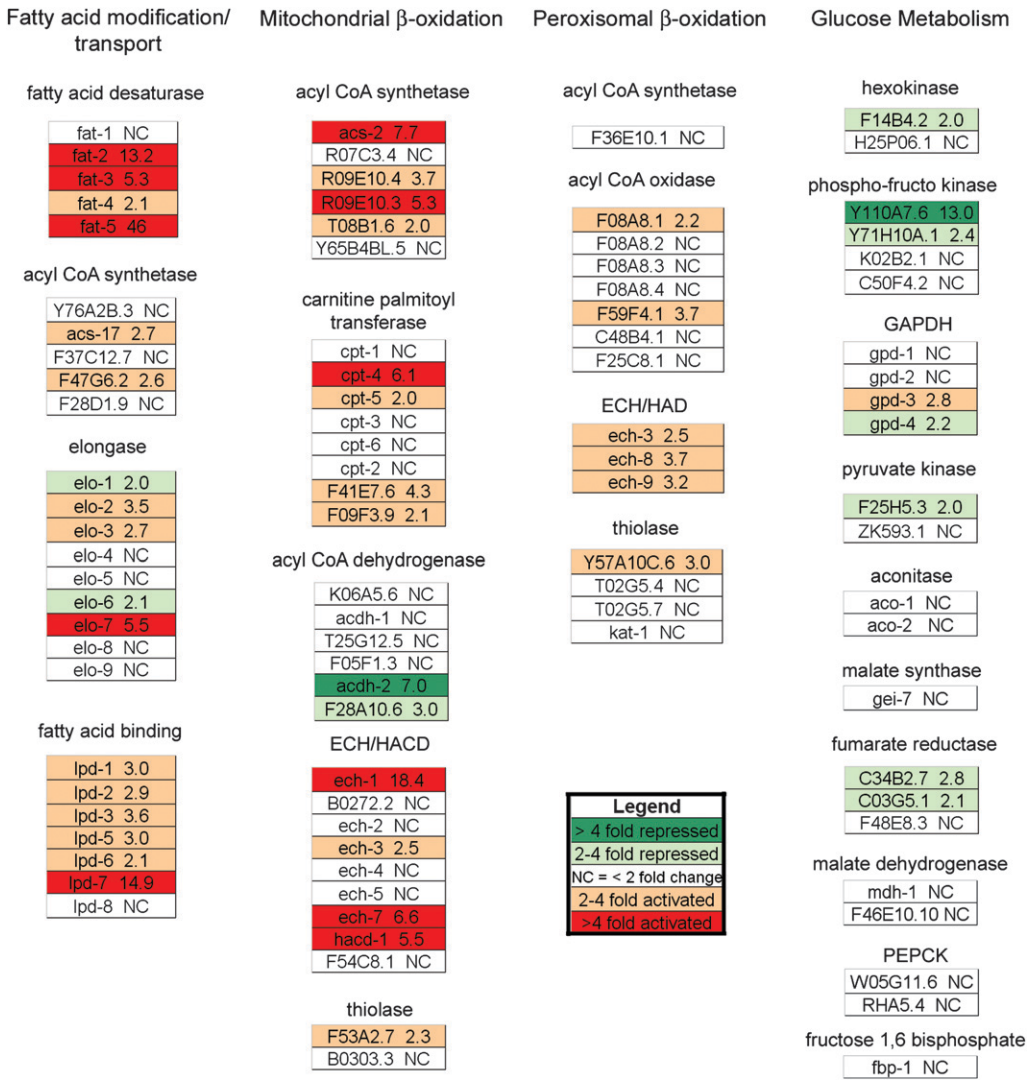

FIGURE 5.—Changes in energy metabolism gene expression in *fat-6;fat-7* double mutants compared to wild type. Gene expression was measured by QRT–PCR. The genes shown are predicted by homology to participate in *C. elegans* fatty acid and glucose metabolism pathways (VAN GILST *et al.* 2005a). Genes expressed at lower levels in *fat-6; fat-7* are shown in green and genes with higher expression in *fat-6;fat-7* are shown in orange (two to fourfold higher) and red (more than fourfold higher).

double mutants indicates that the FAT-6 Δ9 desaturase isoform, the most highly expressed of the three *C. elegans* Δ9 desaturases (BROCK *et al.* 2006), provides sufficient production of MUFAs and PUFAs under standard growth conditions.

Characterization of the *fat-5;fat-6* double mutants revealed roles for FAT-5 and FAT-6 in maintaining fatty acid composition during axenic growth conditions and in surviving L1 starvation arrest. While the FAT-7 Δ9 desaturase compensates for the loss of the other Δ9 desaturases when grown on *E. coli*-seeded plates, more extreme fatty acid composition changes occur in *fat-5; fat-6* mutants when the bacterial food source is removed or when worms are grown in axenic media. In addition, the *fat-5;fat-6* L1 larvae are severely compromised in their ability to survive periods of starvation. Under starvation conditions, the expression of the *fat-7* gene in wild-type adults and L1-arrested animals falls to very low levels (Figure 2D and VAN GILST *et al.* 2005b). Thus, when food is absent, the *fat-5;fat-6* double mutants experience a lethal deficiency in Δ9 desaturation activity and a rapid accumulation of stearic acid. This lethality is consistent with the inability of the *fat-5;fat-6;fat-7* triple mutants to survive unless they are supplemented with dietary unsaturated fatty acids . Presumably, signals from food sources positively regulate *fat-7* expression. This regulation is apparently complex, as previous studies have shown that FAT-7 expression depends on at least three transcription factors—NHR-49, NHR-80, and SREBP—as well as the transcriptional mediator MDT-15 (VAN GILST *et al.* 2005a; BROCK *et al.* 2006; TAUBERT *et al.* 2006; YANG *et al.* 2006).

The *fat-6;fat-7* double mutant displays the most extensive physiological defects of the Δ9 desaturase double mutants. The fatty acid composition of the *fat-6;fat-7* double mutant confirms that FAT-5 is a true palmitoyl-CoA desaturase and cannot use stearic acid (18:0) as a substrate for desaturation. Like *fat-2* and *fat-3*, *C. elegans* mutants with severely reduced PUFA production, the *fat-6;fat-7* double mutants grow slowly and show reduced movement and low fertility (Figure 3). Slow growth is a symptom of essential fatty

acid deficiency in humans (Heird 2005; Heird and Lapillonne 2005). Growth defects in humans with essential fatty acid deficiency as well as the *C. elegans* mutants can be rescued by dietary supplementation with PUFAs. The mechanisms linking PUFAs and growth in humans are not yet known (Smit *et al.* 2004). The reduced movement in all three strains is likely due to lack of C20 PUFAs that are required for efficient neurotransmission (Lesa *et al.* 2003). A reduction in fertility in the *fat-6; fat-7* double mutants also occurs in *fat-3* mutants but the *fat-6;fat-7* double-mutant phenotype is more severe. The *fat-3* mutants produce oleic (18:1Δ9) and linoleic acids (18:2) that the *fat-6;fat-7* mutants cannot produce (Figure 1A), indicating that these may be crucial fatty acids for the maintenance of optimal fertility. In spite of the greatly reduced growth rate and fertility of the *fat-6; fat-7* double mutants, their life span is nearly as long as that of wild type, indicating that a high proportion of saturated fatty acids (>23% compared to 8% in wild type) does not significantly reduce life span as has been suggested previously (Van Gilst *et al.* 2005a).

A key finding of these studies is the low-fat phenotype of the *fat-6;fat-7* double mutant (Figure 4). The *fat-2* and *fat-3* mutants generate normal fat stores, indicating that the low adiposity is caused by the inability to synthesize oleic acid (18:1Δ9), rather than by a lack of PUFAs (see Figure 1A). Consistent with this, wild-type triacylglyceride stores contain <12% PUFAs, with the remaining fatty acids consisting of saturated, monounsaturated, branched, and cyclopropane fatty acids (Table 2). While *fat-6;fat-7* triacylglyceride stores contain a similar proportion of monounsaturated fatty acids as wild type, they contain a more than fourfold greater proportion of stearic acid (18:0). Previous studies show that stearic acid is a poor substrate for triglyceride synthesis in cultured hepatocytes (Pai and Yeh 1996); thus it is possible that the kinetics of triglyceride synthesis are affected by the altered substrate availability. If the rate of incorporation of stearic acid is reduced, this may trigger increased catabolism of the accumulating substrates. Indeed, we find an increase in the expression of genes that encode the mitochondrial β-oxidation machinery, leaving open the possibility that the low-adiposity phenotype of the *fat-6;fat-7* double mutant may be a consequence of increased energy expenditure as well as a consequence of reduced triglyceride synthesis.

The reduced-adiposity phenotype is similar to the mouse SCD1 targeted knockout, in which the SCD1-deficient mice have low fat stores, increased insulin sensitivity, and resistance to diet-induced obesity (Dobrzyn and Ntambi 2005a). Mice lacking SCD1 consume 25% more food than wild-type mice even though they accumulated less fat than their wild-type counterparts (Cohen *et al.* 2002). Indirect calorimetry studies indicate that the SCD1-deficient mice consume higher rates of oxygen and have increased rates of β-oxidation in the liver and in brown adipose tissue (Ntambi *et al.* 2002; Lee *et al.* 2004). Like the *fat-6;fat-7* double mutants, SCD1-deficient mice display increased expression of mitochondrial β-oxidation genes (Ntambi *et al.* 2002).

Thus, in both vertebrates and invertebrates, Δ9 desaturases play a key role in cellular lipid partitioning and homeostasis. It has been suggested that Δ9 desaturases may be a potential therapeutic target in the treatment of obesity and metabolic syndrome (Dobrzyn and Ntambi 2005b). In support of this suggestion, studies in humans demonstrate that elevated Δ9 desaturase activity in skeletal muscle contributes to excess lipid accumulation and is associated with obesity, insulin resistance, and diabetes (Hulver *et al.* 2005). Targeting Δ9 desaturases directly may lead to unwanted side effects due to their essential function in maintaining proper membrane fluidity; however, the *C. elegans* model should allow rapid identification of factors that modify Δ9 desaturase gene expression and enzyme activity. Lowering the Δ9 desaturase activity in *C. elegans* mutants with increased fat stores in combination with genome-wide reverse genetics screens promises to clarify the contributions of metabolic and neuroendocrine signaling pathways in the regulation of fat storage.

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LITERATURE CITED

Apfeld, J., and C. Kenyon, 1998 Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. Cell **95:** 199–210.

Ashrafi, K., F. Y. Chang, J. L. Watts, A. G. Fraser, R. S. Kamath *et al.*, 2003 Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. Nature **421:** 268–272.

Bougnoux, P., B. Giraudeau and C. Couet, 2006 Diet, cancer, and the lipidome. Cancer Epidemiol. Biomarkers Prev. **15:** 416–421.

Brock, T. J., J. Browse and J. L. Watts, 2006 Genetic regulation of unsaturated fatty acid composition in *C. elegans*. PloS Genet. **2:** e108.

Choi, J., J. Stukey, S. Hwang and C. Martin, 1996 Regulatory elements that control transcription activation and unsaturated fatty acid-mediated repression of the *Saccharomyces cerevisiae OLE1* gene. J. Biol. Chem. **271:** 3581–3589.

Cohen, P., M. Miyazaki, N. D. Socci, A. Hagge-Greenberg, W. Liedtke *et al.*, 2002 Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science **297:** 240–243.

Derry, W. B., A. P. Putzke and J. H. Rothman, 2001 *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. Science **294:** 591–595.

Dobrzyn, A., and J. M. Ntambi, 2005a The role of stearoyl-CoA desaturase in the control of metabolism. Prostaglandins Leukot. Essent. Fatty Acids **73:** 35–41.

Dobrzyn, A., and J. M. Ntambi, 2005b Stearoyl-CoA desaturase as a new drug target for obesity treatment. Obes. Rev. **6:** 169–174.

Drenkard, E., B. G. Richter, S. Rozen, L. M. Stutius, N. A. Angell *et al.*, 2000 A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. Plant Physiol. **124:** 1483–1492.

Enoch, H. G., A. Catala and P. Strittmatter, 1976 Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. J. Biol. Chem. **251:** 5095–5103.

Gracey, A. Y., E. J. Fraser, W. Li, Y. Fang, R. R. Taylor *et al.*, 2004 Coping with cold: an integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. Proc. Natl. Acad. Sci. USA **101:** 16970–16975.

Heird, W. C., 2005 Biochemical homeostasis and body growth are reliable end points in clinical nutrition trials. Proc. Nutr. Soc. **64:** 297–303.

Heird, W. C., and A. Lapillonne, 2005 The role of essential fatty acids in development. Annu. Rev. Nutr. **25:** 549–571.

Houthoofd, K., B. P. Braeckman, I. Lenaerts, K. Brys, A. De Vreese *et al.*, 2002 Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *Caenorhabditis elegans*. Exp. Gerontol. **37:** 1371–1378.

Hulver, M. W., J. R. Berggren, M. J. Carper, M. Miyazaki, J. M. Ntambi *et al.*, 2005 Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. Cell Metab. **2:** 251–261.

Johnsen, T. E., D. H. Mitchell, S. Kline, R. Kemal and J. Foy, 1984 Arresting development arrests aging in the nematode *Caenorhabditis elegans*. Mech. Ageing Dev. **28:** 23–40.

Kahn-Kirby, A. H., J. L. Dantzker, A. J. Apicella, W. R. Schafer, J. Browse *et al.*, 2004 Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. Cell **119:** 889–900.

Kubagawa, H. M., J. L. Watts, C. Corrigan, J. W. Edmonds, E. Sztul *et al.*, 2006 Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment in vivo. Nat. Cell Biol. **8:** 1143–1148.

Lee, S. H., A. Dobrzyn, P. Dobrzyn, S. M. Rahman, M. Miyazaki *et al.*, 2004 Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis, but causes hypothermia in cold environment. J. Lipid Res. **45:** 1674–1682.

Lesa, G. M., M. Palfreyman, D. H. Hall, M. T. Clandinin, C. Rudolph *et al.*, 2003 Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans*. J. Cell Sci. **116:** 4965–4975.

Los, D. A., and N. Murata, 2004 Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta **1666:** 142–157.

McKay, R. M., J. P. McKay, L. Avery and J. M. Graff, 2003 *C. elegans*: a model for exploring the genetics of fat storage. Dev. Cell **4:** 131–142.

Miller, K., A. Alfonso, M. Nguyen, J. Crowell, C. Johnson *et al.*, 1996 A genetic selection for *Caenorhabditis elegans* synaptic transmission mutants. Proc. Natl. Acad. Sci. USA **93:** 12593–12598.

Ntambi, J., 1999 Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res. **40:** 1549–1558.

Ntambi, J. M., and M. Miyazaki, 2003 Recent insights into stearoyl-CoA desaturase-1. Curr. Opin. Lipidol. **14:** 255–261.

Ntambi, J. M., and M. Miyazaki, 2004 Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. **43:** 91–104.

Ntambi, J. M., M. Miyazaki, J. P. Stoehr, H. Lan, C. M. Kendziorski *et al.*, 2002 Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc. Natl. Acad. Sci. USA **99:** 11482–11486.

Pai, T., and Y. Y. Yeh, 1996 Stearic acid unlike shorter-chain saturated fatty acids is poorly utilized for triacylglycerol synthesis and beta-oxidation in cultured rat hepatocytes. Lipids **31:** 159–164.

Smit, E. N., F. A. Muskiet and E. R. Boersma, 2004 The possible role of essential fatty acids in the pathophysiology of malnutrition: a review. Prostaglandins Leukot. Essent. Fatty Acids **71:** 241–250.

Tanaka, T., K. Ikita, T. Ashida, Y. Motoyama, Y. Yamaguchi *et al.*, 1996 Effects of growth temperature on the fatty acid composition of the free-living nematode Caenorhabditis elegans. Lipids **31:** 1173–1178.

Taubert, S., M. R. Van Gilst, M. Hansen and K. R. Yamamoto, 2006 A Mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in *C. elegans*. Genes Dev. **20:** 1137–1149.

Tiku, P. E., A. Y. Gracey, A. I. Macartney, R. J. Beynon and A. R. Cossins, 1996 Cold-induced expression of delta 9-desaturase in carp by transcriptional and posttranslational mechanisms. Science **271:** 815–818.

Van Gilst, M. R., H. Hadjivassiliou, A. Jolly and K. R. Yamamoto, 2005a Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in C. elegans. PloS Biol. **3:** e53.

Van Gilst, M. R., H. Hadjivassiliou and K. R. Yamamoto, 2005b A Caenorhabditis elegans nutrient response system partially dependent on nuclear receptor NHR-49. Proc. Natl. Acad. Sci. USA **102:** 13496–13501.

Wang, L., A. R. Folsom and J. H. Eckfeldt, 2003a Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Nutr. Metab. Cardiovasc. Dis. **13:** 256–266.

Wang, L., A. R. Folsom, Z. J. Zheng, J. S. Pankow and J. H. Eckfeldt, 2003b Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am. J. Clin. Nutr. **78:** 91–98.

Warensjo, E., U. Riserus and B. Vessby, 2005 Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. Diabetologia **48:** 1999–2005.

Watts, J. L., and J. Browse, 1999 Isolation and characterization of a delta 5-fatty acid desaturase from *Caenorhabditis elegans*. Arch. Biochem. Biophys. **362:** 175–182.

Watts, J. L., and J. Browse, 2000 A palmitoyl-CoA-specific delta9 fatty acid desaturase from *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. **272:** 263–269.

Watts, J. L., and J. Browse, 2002 Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **99:** 5854–5859.

Watts, J. L., and J. Browse, 2006 Dietary manipulation implicates lipid signaling in the regulation of germ cell maintenance in *C. elegans*. Dev. Biol. **292:** 381–392.

Watts, J. L., E. Phillips, K. R. Griffing and J. Browse, 2003 Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *Caenorhabditis elegans fat-3* mutants. Genetics **163:** 581–589.

Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston and R. H. Plasterk, 2001 Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. Nat. Genet. **28:** 160–164.

Wong, M. L., and J. F. Medrano, 2005 Real-time PCR for mRNA quantitation. BioTechniques **39:** 75–85.

Wood, W. B. (Editor), 1988 *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Yang, F., B. W. Vought, J. S. Satterlee, A. K. Walker, Z. Y. Jim Sun *et al.*, 2006 An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature **442:** 700–704.

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