Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase

Xun Shi,1*, Juan Li,1,8 Xiaoju Zou,∗∗∗ Joel Greggain, ∗ Steven V. Rødker,†† Nils J. Færgeman,†† Bin Liang,∗∗∗ and Jennifer L. Watts∗∗∗

School of Molecular Biosciences,∗ Washington State University, Pullman, WA 99164-7520; Key Laboratory of Animal Models and Human Disease Mechanisms,† Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan 650223, China; School of Life Science,‡ University of Science and Technology of China, Hefei 230036, China; Department of Life Sciences and Biotechnology,*** Kunming University, Kunming, Yunnan 650214, China; and Department of Biochemistry and Molecular Biology,†† University of Southern Denmark, 5230 Odense M, Denmark

Abstract Fatty acid desaturation regulates membrane function and fat storage in animals. To determine the contribution of stearoyl-CoA desaturase (SCD) activity on fat storage and development in the nematode Caenorhabditis elegans, we analyzed the lipid composition and lipid droplet size in the fat-6;fat-7 desaturase mutants independently and in combination with mutants disrupted in conserved lipid metabolic pathways. C. elegans with impaired SCD activity displayed both reduced fat stores and decreased lipid droplet size. Mutants in the daf-2 (insulin-like growth factor receptor), rsk-1 (homolog of p70S6kinase, an effecter of the target of rapamycin signaling pathway), and daf-7 (transforming growth factor β) displayed high fat stores, the opposite of the low fat observed in the fat-6;fat-7 desaturase mutants. The metabolic mutants in combination with fat-6;fat-7 displayed low fat stores, with the exception of the daf-2;fat-6;fat-7 triple mutants, which had increased de novo fatty acid synthesis and wild-type levels of fat stores. Notably, SCD activity is required for the formation of large-sized lipid droplets in all mutant backgrounds, as well as for normal ratios of phosphatidylcholine (PC) to phosphatidylethanolamine (PE). These studies reveal previously uncharacterized roles for SCD in the regulation of lipid droplet size and membrane phospholipid composition.— Shi, X., J. Li, J. Greggain, S. V. Rødker, N. J. Færgeman, B. Liang, and J. L. Watts. Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. J. Lipid Res. 2013. 54: 2504–2514.

Supplementary key words fatty acid synthesis • Caenorhabditis elegans • phosphatidylcholine • phosphatidylethanolamine • fatty acid oxidation • oleic acid

During the past 50 years, humans world-wide have increased their caloric intake beyond what is required for body mass maintenance. Consequently, the rates of obesity, metabolic syndrome, and type 2 diabetes have steadily increased. Stearoyl-CoA desaturase (SCD), also known as Δ9 desaturase, is a key enzyme in the de novo lipogenic pathway. SCD1 deficiency in mice leads to decreased fat stores and increased fat oxidation; consequently, deficient mice are resistant to diet-induced obesity and are protected from lipotoxicity induced by saturated fats (1, 2). Human studies have identified genetic variations in human SCD1 associated with body fat distribution, insulin sensitivity, and metabolic syndrome (3, 4).

SCD is responsible for the formation of monounsaturated fatty acids from saturated fatty acids by catalyzing the insertion of a double bond into the ninth carbon of saturated C16 and C18 substrates. Monounsaturated fatty acids are preferred substrates for the synthesis of triacylglycerol (TAG), as well as for membrane phospholipids and sphingolipids (5). The C. elegans fat-5;fat-6;fat-7 triple-mutant strain, which is completely deficient in SCD activity, is lethal, but the fat-6;fat-7 double mutants, similar to their mouse SCD1 counterparts, are viable but have decreased fat stores and increased expression of fat oxidation genes (6, 7). The fat-6;fat-7 strain also exhibits slow growth, reduced brood size, cold sensitivity, and greatly altered fatty acid composition (7). Unlike most animals, C. elegans...

Support for this work was provided by National Institutes of Health Grant R01 DK-74114 (to J.L.W.) and National Natural Science Foundation of China Grants 31171134 (to B.L.) and 31160216 (to X.Z.). Some C. elegans strains were provided by the Caenorhabditis Genetics Center, which is funded by Office of Research Infrastructure Programs, National Institutes of Health Grant P40 OD-010440.

*Author’s Choice—Final version full access.

Manuscript received 1 May 2013 and in revised form 4 June 2013. Published, JLR Papers in Press, June 20, 2013
DOI 10.1194/jlr.M039669

Abbreviations: AMPK, AMP-activated protein kinase; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; IIS, insulin/insulin growth factor signaling; NGM, nematode growth medium; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; SCD, stearoyl-CoA desaturase; TAG, triacylglycerol; TL, total lipid; TGFβ, transforming growth factor β; TOR, target of rapamycin; WT, wild-type.

1 X. Shi and J. Li contributed equally to this work.
2 To whom correspondence should be addressed.
3 e-mail: jwatts@wsu.edu (J. L. W.); liangb@mail.kiz.ac.cn (B. L.)
5 The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of two figures and three tables.

Copyright © 2013 by the American Society for Biochemistry and Molecular Biology, Inc.
contains a Δ12 fatty acid desaturase (FAT-2) that enables it to synthesize polyunsaturated fatty acids from oleic acid (8). Polyunsaturated fatty acids (PUFA) are not present in the C. elegans laboratory diet, because they are not synthesized by E. coli. Thus, in C. elegans, SCD is the first desaturase required for the biosynthesis of a wide range of PUFAs (9).

Lipid synthesis and oxidation are regulated by various nutrient and energy sensing pathways. These include the conserved insulin/insulin growth factor-1 (IIS) signaling pathway, the target of rapamycin (TOR) pathway, the transforming growth factor β (TGFβ) pathway, and the AMP-activated protein kinase pathway (AMPK). C. elegans mutants with deficient IIS or TGFβ signaling, such as daf-2 and daf-7, tend to arrest during development as dauer larvae, and under conditions where the mutants reach adulthood, they are resistant to environmental stresses, have a greatly extended lifespan, and store excess fat compared with wild-type (WT) (10–14). Similarly, the C. elegans TOR mutant let-63, along with Raptor mutant daf-15, arrest in larval stages with excess fat stores (15–17). Mutants in the gene encoding the TOR complex 2 component Rictor, rict-1, do not arrest as larvae, but they exhibit slow growth, increased fat stores, and shortened lifespan (18,19). Similarly, rsk-1 mutants in the downstream ribosomal S6 kinase also exhibit slow growth and altered lifespan (20). Finally, overexpression of the C. elegans AMPK gene aak-2 increases lifespan (21), and AAK-2 activity is necessary for proper energy expenditure during dauer larval stages (22).

Lipid droplets are fat-storing organelles consisting of a hydrophobic core of TAG and cholesterol ester surrounded by a phospholipid monolayer containing various proteins (23). Proteomic studies of lipid droplets from various organisms, including C. elegans, reveal that lipid droplets are associated with a complex mixture of proteins that are predicted to play roles in lipid synthesis and degradation, membrane trafficking, and protein degradation (24, 25). C. elegans contains lipid droplets in intestinal, hypodermal, and gonadal tissues (9, 26).

The fat-6;fat-7 strain exhibits opposite fat storage phenotypes compared with the daf-2 (IIS), daf-7 (TGFβ), and previously characterized mutants in TOR signaling. The SCD-deficient fat-6;fat-7 double mutants have reduced fat stores, whereas the other mutants cause nematodes to store higher amounts of TAG. We constructed triple-mutant strains to determine whether SCD activity is necessary for high fat stores in these strains. We found a striking requirement for endogenous SCD activity for the regulation of lipid droplet size and discovered that SCD activity influences the relative ratios of membrane phospholipid species.

### MATERIALS AND METHODS

#### Nematode strains and growth conditions

Nematode growth media (NGM) was used to maintain C. elegans with the E. coli (OP50) at 20°C. The WT strain was N2. The strains used in this study were BX106 fat-6(tm331), BX107 fat-5(tm420), BX110 fat-5(tm420);fat-6(tm331), BX1156 fat-6(tm331);fat-7(wa36), CB1370 def-2(e1370), CB1372 def-7(e1372), RB754 aak-2(ok524), KQ6 rict-1(mg360), RB1206 rsk-1(ok1255), and HA1947 sams-1(ok3033). The RB strains were outcrossed four times to N2. Double- and triple-mutant strains constructed for this study were BX168 def-2(e1370);fat-6(tm331), BX250 def-7(e1370);fat-5(tm420), BX251 def-2(e1370);fat-5(tm420);fat-6(tm331), BX177 def-2(e1370);fat-6(tm331);fat-7(wa36), BX217 aak-2(ok524);fat-6(tm331);fat-7(wa36), and BX218 rsk-1(ok1255);fat-6(tm331);fat-7(wa36). Fatty acid supplementation was achieved by adding sodium oleate (NuChek Prep) at a final concentration range of 0.1–0.5 mM to NGM media containing 0.1% tergitol (NP40). Fatty acid stock solutions were added after autoclaved media cooled to 50°C. Feeding RNAi was performed on NGM plates supplemented with 100 μg/ml ampicillin and 2 mM isopropyl-β-D-thiogalactopyranoside (ITPG) and E. coli strain HT115 (27).

#### Fatty acid composition and lipid analysis

Fatty acid composition of young adult nematodes was determined by gas chromatography/mass spectrometry (GC/MS) as previously described (6,28). Separation of the TAG and phospholipid fractions used a two-solvent TLC protocol. Approximately 10,000 young adult stage C. elegans were washed from NGM plates several times in water. Most of the water was removed, and worm pellets were frozen in liquid nitrogen. Lipids were extracted by adding 5 ml of ice-cold chloroform:methanol (1:1) and incubating overnight at −20°C with occasional shaking. A solution of 0.2M H3PO4 and 1M KCl was added to samples, which resulted in phase separation of the organic and aqueous phases. The organic phase was removed and dried under argon, then resuspended in chloroform. Samples were loaded in triplicate, and TLC plates were developed two thirds of the way up the plate in the first solvent system: chloroform: methanol:water:acetic acid (65:43:9:2,5), dried, and then the second solvent system hexane:diethylther:acetic acid (80:20:2) was developed to the top of the plate. Lipids were visualized under UV light after spraying the plate with 0.005% primuline, and spots corresponding to TAG and the major phospholipids were scraped, spiked with a known standard (15:0), and transesterified for GC/MS analysis to determine the fatty acid composition as well as to determine the relative levels of TAG and phospholipid (PL) fractions. At least three biological replicates were used for TLC analysis. Significance was determined with one-way ANOVA analysis and Tukey’s multiple comparison posttest using GraphPad Prism 5 software.

Stable isotope labeling of fatty acids was performed essentially as described (29). Briefly, equal amounts of bacteria grown in either LB (13C media) or isogrow (98.5%, 13C-enriched, Sigma) were mixed and plated onto agarose plates. For each sample, approximately 30,000 synchronized L1 nematodes prepared from hypochlorite treatment of gravid adults were added to the plates and grown for 48 h at 20°C (worms reached L4 larval stage). Nematodes were washed off the plates, their lipids were extracted, and fatty acids were analyzed by GC/MS as described (29). Isotopomers were monitored in a scanning ion mode corresponding to the fatty acid species of interest: 16:0 was scanned from m/z 270–286, 18:0 was scanned from m/z 298–316, and 18:1(n-7) was scanned from m/z 296–314.

#### Quantification of lipid droplet size

For measurement of lipid droplet size, at least ten young adult worms of each genotype stained with postfixed Nile Red were photographed (13). For each worm photograph, a 26 × 26 μm square was placed arbitrarily over the mid-intestinal region, and within the square, each visible Nile Red-stained droplet was manually traced using the circle tool of Image Pro Plus software, which recorded the diameter of each droplet. For each worm, the average lipid droplet diameter
was calculated. Statistical comparisons (one-way ANOVA and Tukey’s multiple comparison test) were performed using GraphPad Prism 5 software.

Fatty acid oxidation assay

Fatty acid oxidation was performed on L4 nematodes essentially as described (30), except that tritiated palmitic acid (30 Ci/mmole, Perkin Elmer, Waltham, MA) was used as substrate.

Quantitative RT-PCR analysis

WT, fat-6(fat-7), and daf-2(fat-6(fat-7) nematodes were synchronized and harvested at L4 stage. RNA and cDNA was prepared as described (7). Real-time PCR assays were run and monitored with an ABI Prism 7000 Sequence Detection system (Applied Biosystems, Foster City, CA). The real-time PCR was conducted on three treatment groups, with each individual treatment group in triplicate. Threshold values (Ct) for the gene of interest and a housekeeping gene tib-2 were determined using ABI Prism SDS software version 1.1 (Applied Biosystems). The expression level of the gene of interest was evaluated using the $2^{-\Delta\Delta \text{Ct}}$ method (31).

Physiological assays

Growth rate. Eggs were isolated from gravid adults using hypochlorite treatment and then plated onto NGM plates seeded with E. coli strain OP50. The number of adults and total number of nematodes were determined at various time points.

Brood size. For analysis of total progeny produced per worm, 10–20 L4s were transferred individually to fresh NGM plates seeded with E. coli strain OP50. Worms were transferred daily until they did not produce any more progeny. Two days after removal of the adult, the live progeny of each genotype were counted.

RESULTS

SCD activity is necessary for large-sized lipid droplets

In the nematode C. elegans, the lack of SCD activity in the fat-6(fat-7) double-mutant strain has profound consequences for the fatty acid composition of membrane phospholipids and TAG storage lipids (7). In our previous study, we showed that Nile Red staining in fat-6(fat-7) animals, which increased in intensity when the animals were supplemented with 0.1 mM sodium oleate (7). However, recent work in many labs has demonstrated that Nile Red fluorescence in live animals does not correlate with actual TAG stores but instead stains lysosome-related organelles (13, 32–34). In contrast, the intensity of Nile Red, Sudan black, or Oil-Red O staining in nematodes that have been fixed with isopropanol or paraformaldehyde corresponds well with biochemical measurements of TAG (13, 33) and stains lipid droplets, not lysosome-related organelles (35). We therefore analyzed the lipid-staining pattern of fixed, young-adult-stage nematodes (with 0–2 eggs), and we found that the SCD-deficient fat-6(fat-7) double mutants have fewer and smaller lipid droplets than WT controls (Fig. 1A). We used imaging software to measure lipid droplet diameter of cross-sections of the mid-intestinal region to determine the frequency of lipid droplets of various sizes. The individual lipid droplets in WT ranged in diameter from 0.5 μm to 3.7 μm, with an average size of 1.4 μm, while lipid droplets in fat-6(fat-7) were much smaller, with a range from 0.2 μm to 1.5 μm, and an average diameter of 0.5 μm (Fig. 1B). In addition, we examined lipid droplet sizes from the single SCD mutant strains fat-5, fat-6, and fat-7 single mutants, as well as the fat-5(fat-6) double mutant, and we found that the lipid droplet diameters in these strains do not appreciably differ from WT (supplementary Fig. 1-A). Thus, the C. elegans SCD strain with the most severe lipid droplet-size defect is fat-6(fat-7), which also has the most severe fatty acid composition defect among the SCD single- and double-mutant strains (6, 7).

Next, we analyzed the fatty acid composition of individual membrane phospholipids in the fat-6(fat-7) double mutants. In WT, PUFA is detected mostly in the phospholipid fraction, in contrast to TAG, which contains only small amounts of PUFA (7, 29, 36). When SCD activity is blocked by simultaneous mutations in fat-6 and fat-7, normal PUFAs are not formed, because the FAT-6 and FAT-7 desaturases are required to synthesize PUFA de novo from acetyl CoA or to synthesize them from fatty acid precursors (primarily palmitic acid) derived from the bacterial diet (7, 29). In the absence of FAT-6 and FAT-7, unusual C18 PUFAs are synthesized via desaturation and elongation of palmitoleic acid (16:1n-7), which is synthesized from palmitic acid by the $\Delta 9$ desaturase FAT-5 (7, 29). For this study, we examined the fatty acid composition of TAG fraction, as well as the membrane phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylethanolamine (PS), and phosphatidylinositol (PI) in the fat-6(fat-7) double mutants. Similar to the distribution of PUFAs in WT lipids, we detected the unusual C18 PUFA in PI and PS, as well as PC, but they are present to a lesser extent in PE and in the TAG fraction (supplementary Table 1). In agreement with our previous studies, fat-6(fat-7) had overall lower levels of TAG than WT (Fig. 1C, Table 1) and a greatly increased composition of saturated fatty acids and decreased composition of polyunsaturated fatty acids (Fig. 1D).

Dietary oleic acid promotes PUFA production but does not restore lipid droplet size to WT levels

Our previous work showed that the slow growth and reduced brood size in fat-6(fat-7) worms were partially rescued by dietary oleic acid (7). To see whether dietary oleic acid can rescue the lipid droplet and fat storage defects of fat-6(fat-7) we grew the worms on plates containing 0.1–0.5 mM sodium oleate. We examined the lipid droplet size in the oleate-supplemented worms and found they had a larger size distribution compared with unsupplemented fat-6(fat-7) worms, indicating that dietary oleate induces large-sized lipid droplets in SCD-deficient worms, although oleate levels up to 0.5 mM cannot fully rescue lipid droplet size to that of WT (Fig. 1A, B, supplementary Fig. 1-B). GC/MS analysis revealed that fatty acid uptake did not increase significantly above that seen in 0.2 mM oleate; therefore, the 0.2 mM concentration was
Interactions between SCD and conserved metabolic pathways regulating fat storage and fat oxidation

To examine the role of SCD in the formation of lipid droplets, TAG storage, and growth and development, we generated the triple-mutant strains that allowed us to examine the role of SCD activity in the background of mutations that confer high fat stores or altered fat oxidation. AMPK mutants have WT lipid composition and do not suppress low fat in SCD-deficient worms. Mutations in the *C. elegans* aak-2 gene, which encodes the α subunit of AMPK, affect the regulation of fat oxidation, especially under conditions of starvation (22). Because experiments in *C. elegans* indicate that the low fat stores in SCD mutants are associated with increased expression of fat oxidation genes (7) and because experiments in mice indicate that SCD inhibition leads to activation of AMPK (37), we tested whether the low fat stores in the *fat-6;fat-7* double mutants could be suppressed by a mutation in *aak-2*. We first examined the lipid droplet size and measured the lipid composition of unsaturated fatty acids for optimal lipid droplet size and TAG stores.

TABLE 1. Relative percentage of phospholipid classes and TAG in WT (N2) and *fat-6;fat-7* double mutants, grown without fatty acids and supplemented with 0.2 mM sodium oleate (18:1n-9)

| Relative % of Phospholipids | PC     | PE     | PI     | PS     | PC:PE   | % TAG/TL |
|-----------------------------|--------|--------|--------|--------|---------|----------|
| WT                          | 54.1% (1.2)* | 35.7% (0.9)* | 5.1% (0.6)* | 5.1% (0.1)* | 1.51   | 52.0% (0.6)* |
| WT 0.2 mM oleate            | 53.5% (2.3)* | 34.4% (2.0)* | 6.4% (0.2)* | 5.8% (0.2)* | 1.55   | 53.2% (2.7)* |
| *fat-6;fat-7*               | 45.5% (0.8)* | 40.1% (0.1)* | 5.6% (0.2)* | 7.8% (0.6)* | 1.13   | 42.2% (0.7)* |
| *fat-6;fat-7* oleate        | 44.1% (0.2)* | 42.4% (0.9)* | 5.7% (0.3)* | 7.1% (1.6)* | 1.04   | 48.8% (1.2)* |

Values are the average (SEM) from three independent lipid extractions of young adult stage *C. elegans*. Those not sharing a common letter within the same column differ (*P* < 0.05)
is influenced by the TOR pathway, and mutants in conserved members of TOR complexes 1 and 2 show alterations in fat storage (15, 16, 18, 19). In mammals, a downstream target of TOR signaling is the p70 ribosomal S6 kinase, which in C. elegans promotes germline proliferation and is required for the proper regulation of lifespan (20, 38). We examined lipid droplet size and fatty acid composition in the TOR complex 2 component rict-1 mutants as well as in rsks-1 mutants. We found that, in both strains, the formation of large lipid droplets depends on FAT-6 and FAT-7 (Fig. 2A, B, supplementary Fig. II-B). In contrast, the requirements for RICT-1 and RSKS-1 for producing optimal growth rate and brood size act in parallel to SCD, because the triple mutants have a more severe growth and brood size reduction than the fat-6;fat-7 strain (Fig. 2E, F, supplementary Fig. II-C).

Because fat composition had not been previously studied in the rsks-1 mutant, we examined the lipid composition in this mutant strain, as well as in the rsks-1;fat-6;fat-7 triple-mutant strain. We discovered that rsks-1 mutants, similar to other mutants in the TOR pathway, have increased TAG content compared with WT (Fig. 2C, supplementary...
Table II). However, in combination with SCD deficiency, TAG accumulation was similar to the fat-6;fat-7 double mutant, indicating that SCD activity is required for the accumulation of high fat stores and large lipid droplets in the rsks-1 mutants (Fig. 2A–D, Table 2, supplementary Table II). Interestingly, while rict-1 mutants have a nearly WT fatty acid composition (supplementary Fig II-D), all of the lipids in the rsks-1 strain showed an increase in C20 omega-6 PUFAs (20:3n-6 and 20:4n-6) and a decrease in the C20 omega-3 fatty acids (20:4n-3 and 20:5n-3) (supplementary Fig. II-E, supplementary Table II). We found a fatty acid composition defect similar to rsks-1 in RNAi treatment of more than 30 ribosomal proteins that we screened [C20 fatty acid composition of rps-9(RNAi) is shown in supplementary Fig. II-F]. This indicates that for fatty acid composition, rsks-1 mutants, not rict-1, phenocopy the altered omega-6/omega-3 fatty acid composition changes that result from the disruption of protein translation.

Overlapping developmental requirements of DAF-7 and SCD. The C. elegans TGFβ mutant daf-7, similar to daf-2 mutants, tends to arrest as dauer larvae (39). However, when grown under conditions that allow the worms to develop to adulthood, the daf-7 mutants store excess fat (13, 40). Our attempts to construct the daf-7;fat-6;fat-7 triple-mutant strain were unsuccessful. We observed that the daf-7;fat-6 double mutants showed increased incidence of dauer formation at 20°C compared with the daf-7 strain (data not shown), and after numerous attempts to obtain the triple mutant, only one worm was confirmed by PCR to possess the daf-7;fat-6;fat-7 genotype, and this nematode did not produce live progeny. Therefore, we conclude that the additive developmental defects of TGFβ and SCD deficiency led to lethality of the daf-7;fat-6;fat-7 triple mutants.

Mutation in daf-2 (IIS) partially suppresses the low TAG content in SCD-deficient worms. Like the daf-7 strain, the daf-2(1370) mutants, carrying a hypomorphic allele of the IGF receptor, store high amounts of fat (41). We visualized fat stores in WT, daf-2, and daf-2;fat-6;fat-7 triple-mutant strains using Nile Red staining of fixed nematodes, and we found that lipid droplets were significantly larger in the daf-2 strain than in WT (Fig. 2A). We found that lipid droplets in daf-2 mutants ranged 0.5–7.5 μm, with an average size of 2.0 μm (Fig. 2A, B), and, as previously reported (13, 42), the daf-2 mutant strain contained high levels of TAG and slightly reduced levels of PUFAs (Fig. 2C, D, Table 2, supplementary Table II). The daf-2;fat-6;fat-7 strain had lipid droplets that ranged 0.3–3.0 μm, with an average lipid droplet size of 1.1 μm, which was closer to the size of the fat-6;fat-7 strain than was daf-2 and which was smaller than WT (Fig. 2A, B). This indicates that SCD activity is necessary to produce the large-sized lipid droplets (>3 μm) that are found in WT and daf-2 mutants. However, qualitative staining with Nile Red indicated that the lipid droplets in daf-2, fat-6;fat-7 worms were more abundant than those in the fat-6;fat-7 strain, and lipid analysis revealed that the percentage of fatty acids found in the TAG fraction was significantly higher in young adult daf-2;fat-6;fat-7 worms than in fat-6;fat-7, similar to the levels measured in similar-aged WT nematodes (Fig. 2C, Table 2). This indicates that, whereas FAT-6 and FAT-7 SCD activity is required to produce large-sized lipid droplets, it is only partially required for increased fat stores in the daf-2 mutant background. While the daf-2;fat-6;fat-7 strain contained increased PUFA in all of the lipid classes compared with the fat-6;fat-7 strain (Fig. 2D, supplementary Table II), the increased fat stores and increased PUFAs did not confer any suppression of the slow growth or reduced brood size of the fat-6;fat-7 double mutants; in fact, the daf-2;fat-6;fat-7 triple mutants grew at a slower rate than the fat-6;fat-7 double mutants (Fig. 2E, F). Taken together, these findings reveal an interaction between IGF signaling and SCD activity in the regulation of fat stores.

**Increased fat stores in daf-2;fat-6;fat-7 mutants are not a consequence of reduced fat oxidation but are associated with increased de novo fatty acid synthesis and increased FAT-5 activity**

In mice and nematodes, SCD deficiency leads to increased expression of β-oxidation genes, which may lead to increased fat oxidation and reduced fat stores (2, 7). Therefore, we hypothesized that the increased fat stores in the daf-2;fat-6;fat-7 mutants may be a result of decreased expression of β-oxidation genes in the daf-2 mutant background. We used real-time quantitative RT-PCR to examine expression of mitochondrial and peroxisomal

### Table 2. Relative percentage of lipid classes in WT (N2), aak-2, rsks-1, daf-2, fat-6;fat-7, aak-2;fat-6;fat-7, rsks-1;fat-6;fat-7, and daf-2;fat-6;fat-7

| Lipid Class | Relative % of Phospholipids |
|-------------|----------------------------|
|             | PC/PE | PE/PL | PL/PE | PS/PL | PC:PE | % TAG/TL |
| N2          |       |       |       |       |       |         |
| aak-2       |       |       |       |       |       |         |
| rsks-1      |       |       |       |       |       |         |
| daf-2       |       |       |       |       |       |         |
| fat-6;fat-7 |       |       |       |       |       |         |
| aak-2;fat-6;fat-7 |       |       |       |       |       |         |
| rsks-1;fat-6;fat-7 |       |       |       |       |       |         |
| daf-2;fat-6;fat-7 |       |       |       |       |       |         |

Values are the average (SEM) of three independent lipid extractions of young adult stage C. elegans. 

*C* Values not sharing a common letter within the same column differ (P < 0.05).
β-oxidation genes as well as fatty acid-binding protein genes in WT, fat-6;fat-7, and daf-2;fat-6;fat-7 worms. We found that, compared with WT, 12 of 29 genes tested showed increased expression in fat-6;fat-7 worms. However, the expression remained high in the daf-2;fat-6;fat-7 worms, indicating that reduced expression of genes encoding β-oxidation machinery is unlikely to be the mechanism for high fat content in daf-2;fat-6;fat-7 worms (supplementary Table III). Furthermore, a direct assay of fatty acid oxidation activity (30) demonstrated that both the fat-6;fat-7 and daf-2;fat-6;fat-7 strains showed higher rates of oxidation of palmitic acid (16:0) and does not desaturate stearic acid (18:0; reference (6, 43)). The fat-6;fat-7 strain contained a palmitoyl-CoA desaturase that only is only active on saturated fatty acids or the low content of polyunsaturated fatty acids influencing the relative amounts of PC and PE in membranes.

Reduced amounts of PC are associated with large lipid droplets in Drosophila S2 cells (45) and fatty livers in mice (46). Therefore, we were surprised that the small lipid droplets in fat-6;fat-7 mutants were associated with reduced amounts of PC relative to PE. In C. elegans, depletion of sams-1 leads to diminished PC (47). The sams-1 gene encodes S-adenosylmethionine synthase, which is required to transfer methyl groups onto PE to form PC, one pathway of PC synthesis. Because the sams-1 mutants have large lipid droplets (47), consistent with reduced PC content, we used RNAi to deplete sams-1 in the fat-6;fat-7 double mutant (Fig. 4B). The large lipid droplets induced by depletion of sams-1 did not form in the SCD-deficient fat-6;fat-7 worms, indicating that SCD is essential for lipid droplet expansion, even when PC levels are reduced. Taken together, these studies reveal an important role for SCD activity in the regulation of lipid droplet size, independent of fat accumulation and membrane phospholipid ratios.

SCD deficiency leads to low PC:PE ratio

Our extensive lipid analysis allowed us to compare the ratios of the two major membrane phospholipids, PC and PE, in all of the strains. The ratio of PC to PE is important for proper membrane function. We found that the ratio of PC:PE among independently grown batches of young adult WT nematodes ranged 1.41–1.65, with an average ratio of 1.51 (Fig. 4A, Table 1). The ratio of PC:PE was somewhat higher than WT in the aak-1, rsks-1, and daf-2 mutant strains, ranging 1.57–2.26, with an average of 1.78 for aak-2, 1.96 for rsks-1, and 1.69 for daf-2 mutants. In contrast, the ratio of PC:PE was less variable and significantly lower in the fat-6;fat-7 mutant strain, ranging 1.11–1.17, with an average of 1.13. Strikingly, in combination with aak-2, rsks-1, and daf-2, the ratio remained low, ranging 0.99–1.25 in the triple-mutant strains, with an average of 1.08 for aak-2;fat-6;fat-7, 1.04 for rsks-1;fat-6;fat-7, and 1.13 for daf-2;fat-6;fat-7 (Fig. 4A, Table 2). This finding indicates that the low PC:PE ratio correlates with the fatty acid composition of the animals, with either the high content of saturated fatty acids or the low content of polyunsaturated fatty acids influencing the relative amounts of PC and PE in membranes.
SCD regulates lipid droplet size 2511

polyunsaturated fatty acids in the membrane lipid components (supplementary Fig. I, supplementary Table II). Although upstream components of the TOR signaling pathway, such as daf-15/RAPTOR, rict-1/RICTOR, and let-363/TOR, also have increased fat stores (15, 19), we did not identify the alteration in the membrane omega-3:omega-6 ratios in rict-1 mutants, although we detected similar fatty acid composition changes in ribosomal protein RNAi knockdown worms (supplementary Fig. II). Therefore, the rsks-1 mutants and knockdowns in proteins required for translation have an altered fatty acid composition that is not observed in rict-1 mutants.

In spite of the decreased lipid droplet size, the abundance of lipid droplets, as well as the total TAG levels in the worms, was increased in the daf-2 mutant background. This was not due to a decrease of β-oxidation conferred by the daf-2 mutation, because we found that fatty acid oxidation rates were increased in both the fat-6;fat-7

Fig. 3. Increased de novo synthesis and FAT-5 activity, not decreased β-oxidation, mediates fat storage in the daf-2 background. (A) Fatty acid oxidation rates are increased in fat-6;fat-7 and daf-2;fat-6;fat-7 compared with WT (P < 0.05). Nematodes were incubated in 20 μM 3H-palmitate (16:0) complexed to BSA, and the amount of tritiated water generated was determined. Values shown are the mean and SEM of 4–6 biological replicates. (B) Isotopomers associated with de novo synthesis (MW302-312) are increased in stearate (18:0) isolated from the total lipids of daf-2;fat-6;fat-7 (pink) compared with fat-6;fat-7. Data are the mean of three experiments (± SD). (C) The percentage of de novo synthesized fatty acids in total lipids is shown for WT, fat-6;fat-7, and daf-2;fat-6;fat-7. Data are the mean of three experiments (± SEM). (D and E) FAT-5 activity in TAG (D) and PC (E) lipid fractions. Shown is the ratio of the FAT-5 product (16:1) to the FAT-5 substrate (16:0). Error bars are SEM, **P < 0.01, *P < 0.05.

Our extensive lipid analysis revealed previously unknown lipid composition defects in the S6kinase-deficient rsks-1 strain. We found that the overall TAG levels were high in this strain, consistent with larger lipid droplets observed by Nile Red staining of fixed worms. We also identified increased omega-6 and decreased omega-3

fat storage as well as in ensuring proper growth and development. Notably, the FAT-6 and FAT-7 SCDs regulate the size of lipid droplets and ratios of cellular phospholipids in every strain examined. Fig. 5 depicts a model summarizing the pathways examined in these studies. A link between SCD activity and lipid droplet size was previously observed in cell lines cultured from patients with Berardinelli-Seip congenital lipodystrophy (48). This study showed that patients carrying mutations in the Seipin gene had increased proportions of saturated fatty acid in their lipids, indicating decreased SCD activity and decreased size and abundance of lipid droplets.

Our extensive lipid analysis revealed previously unknown lipid composition defects in the S6kinase-deficient rsks-1 strain. We found that the overall TAG levels were high in this strain, consistent with larger lipid droplets observed by Nile Red staining of fixed worms. We also identified increased omega-6 and decreased omega-3 polyunsaturated fatty acids in the membrane lipid components (supplementary Fig. I, supplementary Table II). Although upstream components of the TOR signaling pathway, such as daf-15/RAPTOR, rict-1/RICTOR, and let-363/TOR, also have increased fat stores (15, 19), we did not identify the alteration in the membrane omega-3:omega-6 ratios in rict-1 mutants, although we detected similar fatty acid composition changes in ribosomal protein RNAi knockdown worms (supplementary Fig. II). Therefore, the rsks-1 mutants and knockdowns in proteins required for translation have an altered fatty acid composition that is not observed in rict-1 mutants.

In spite of the decreased lipid droplet size, the abundance of lipid droplets, as well as the total TAG levels in the worms, was increased in the daf-2 mutant background. This was not due to a decrease of β-oxidation conferred by the daf-2 mutation, because we found that fatty acid oxidation rates were increased in both the fat-6;fat-7
and daf-2;fat-6;fat-7 strains (Fig. 3). Instead, increased de novo synthesis correlates with the increased fat stores in strains containing the daf-2 mutation (29). Our findings indicate that the induction of the FAT-5 palmitoyl-CoA desaturase facilitates the increase in de novo fat synthesis.

Mice lacking SCD1 are lean and resistant to diet-induced obesity (2, 49). They have reduced fatty acid and TAG synthesis in response to high dietary carbohydrates (50), and they also have increased fat oxidation rates in various tissues (49). Cell culture studies of lipid droplet formation rely on oleic acid in the culture media to induce the expansion of lipid droplets (45). However, our studies in an intact organism reveal that dietary oleic acid does not fully compensate for the endogenous synthesis of monounsaturated fatty acids. This is consistent with mouse studies that revealed that diets containing up to 5% olate did not rescue the low fat stores in SCD1 mutants (50) and with human studies that showed that diets high in oleic acid, such as a Mediterranean diet, provide a protective role against obesity, whereas diets high in saturated fats and simple carbohydrates, which induce endogenous SCD activity, lead to excess fat stores (51).

Interestingly, the SCD1 was shown to colocalize with diacylglycerol acyltransferase (DGAT)2 in the mitochondrial-associated membrane subcompartment of the endoplasmic reticulum (ER) (52). DGAT enzymes are required for the formation of lipid droplets (53). The colocalization of SCD1 and DGAT2 suggests that metabolic channeling of endogenously synthesized monounsaturated fatty acids and DGAT2 optimizes TAG synthesis. Work in C. elegans demonstrated that DGAT2 and ACS-22 form a complex at the ER-lipid droplet interface during lipid droplet expansion (54). Although we neither observed FAT-6::GFP or FAT-7::GFP on the lipid droplet surface nor were these gene products identified in a proteomic analysis of C. elegans lipid droplets (25), we found that endogenous SCD activity is necessary for efficient lipid storage in C. elegans, and we found that dietary oleic acid did not restore large-sized lipid droplets, consistent with the metabolic channeling hypothesis. A recent study provides evidence that lipogenic enzymes, specifically GPAT4, relocalize from the ER to a subset of expanding lipid droplets (55). It is possible that proper membrane composition may be required for efficient relocalization of TAG synthesis enzymes to expanding lipid droplets.

In mice, Drosophila, and C. elegans, disruption of PC synthesis leads to increased TAG and large-sized lipid droplets (45–47, 56). Upon lipid loading of cells, PC is synthesized and its presence prevents the coalescence of lipid droplets (45). Our studies show that all of the SCD-deficient strains have a decreased amount of PC relative to PE. This data is in agreement with a recent metabolic study of C. elegans SCD mutants which revealed a reduction in some phosphocholine derivatives in SCD mutants compared with WT (57). The small-sized lipid droplets in the fat-6;fat-7 double mutants seem contradictory to the reduced PC, since reducing PC synthesis leads to large lipid droplets. Even so, we show that SCD activity is required for the large-sized
droplets in the PC-deficient sams-1 mutants. Therefore, even though PC levels are reduced in fat-6;fat-7 mutants, the levels are apparently adequate to prevent the coalescence of lipid droplets. Instead, the lipid droplet size appears to be driven by the ability of SCD to synthesize unsaturated fatty acids. It is tempting to speculate that the alteration in the PC:PE ratio compensates for the increased presence of saturated fatty acids and decreased abundance of PUFAs in the SCD-deficient strains. For example, PC molecules containing unsaturated fatty acid are typically cylinder shaped, whereas PE molecules, with a smaller headgroup, are typically cone shaped (58). Because the chain length and the relative degree of saturation influences the shape of the fatty acids, which in turn influences the phospholipid shape, we predict that the shape of the PC molecules may be altered in fat-6;fat-7 double mutants. The decrease in PC together with the increase in PE may provide a phospholipid composition that improves membrane function in the context of increased saturated fatty acids in the fat-6;fat-7 strains.

The authors thank Kylene Brooks, Veronica Windell, and Olga Shiva for technical assistance; Jason Watts and Carissa Perez Olsen for advice on stable isotope labeling of C. elegans fatty acids; and Tracy Vrablik for helpful comments on the manuscript.

REFERENCES

1. Sampath, H., and J. M. Ntambi. 2011. The role of stearoyl-CoA desaturase in obesity, insulin resistance, and inflammation. *Ann. N. Y. Acad. Sci.* 1243:47–53.
2. Ntambi, J. M., M. Miyazaki, J. P. Stoehr, H. Lan, C. M. Kendzierski, B. S. Yandell, Y. Song, P. Cohen, J. M. Friedman, and A. D. Attie. 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc. Natl. Acad. Sci. USA.* 99:11482–11486.
3. Warenjo, E., E. Ingelsson, P. Lundmark, L. Lammfelt, A. C. Syvanen, B. Vessby, and U. Riserus. 2007. Polymorphisms in the SCD1 gene: associations with body fat distribution and insulin sensitivity. *Obesity (Silver Spring).* 15:1732–1740.
4. Gong, J., H. Campos, S. McGarvey, Z. Wu, R. Goldberg, and A. Baylin. 2011. Genetic variation in stearoyl-CoA desaturase 1 is associated with metabolic syndrome prevalence in Costa Rican adults. *J. Nutr.* 141:2211–2218.
5. Patton, C. M., and J. M. Ntambi. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. *Am. J. Physiol. Endocrinol. Metab.* 297:E28–E37.
6. Brock, T. J., J. Browse, and J. L. Watts. 2006. Genetic regulation of unsaturated fatty acid composition in C. elegans. *PLoS Genet.* 2:e108.
7. Brock, T. J., J. Browse, and J. L. Watts. 2007. Fatty acid desaturation and the regulation of adiposity in Caenorhabditis elegans. *Genetics.* 176:865–875.
8. Peyou-Ndi, M. M., J. L. Watts, and J. Browse. 2000. Identification and characterization of an animal delta(12) fatty acid desaturase gene by heterologous expression in Saccharomyces cerevisiae. *Arch. Biochem. Biophys.* 376:399–408.
9. Watts, J. L. 2009. Fat synthesis and adiposity regulation in Caenorhabditis elegans. *Trends Endocrinol. Metab.* 20:58–65.
10. Kenyon, C., J. Chang, E. Gensch, A. Rudner, and R. Tabin. 1993. A C. elegans mutant that lives twice as long as wild type. *Nature.* 366:461–464.
11. Baumeister, R., E. Schaffitzel, and M. Hertweck. 2006. Endocrine signaling in Caenorhabditis elegans controls stress response and longevity. *J. Endocrinol.* 196:191–202.
12. Panowski, S. H., and A. Dillen. 2009. Signals of youth: endocrine regulation of aging in Caenorhabditis elegans. *Trends Endocrinol. Metab.* 20:259–264.
13. Brooks, K. K., B. Liang, and J. L. Watts. 2009. The influence of bacterial diet on fat storage in C. elegans. *PLoS ONE.* 4:e7545.
14. Mair, W., I. Morante, A. P. Rodrigues, G. Manning, M. Montminy, R. J. Shaw, and A. Dillen. 2011. Lifespan extension induced by AMPK and calcineurin is mediated by CRTG-1 and CRED. *Nature.* 470:404–408.
15. Jia, K., D. Chen, and D. L. Riddle. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. *Development.* 131:3987–3996.
16. Long, X., C. Spycher, Z. S. Han, A. M. Rose, F. Muller, and J. Arrvuch. 2002. TOR deficiency in C. elegans causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. *Curr. Biol.* 12:1448–1461.
17. Haras, K., T. Maruki, X. Long, K. Yoshino, N. Oshiro, S. Hidayat, C. Tokumaga, J. Arrvuch, and K. Yonezawa. 2002. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell.* 110:177–189.
18. Jones, K. T., E. R. Greer, D.Pearce, and K. Ashrafi. 2009. Rictor/TORC2 regulates Caenorhabditis elegans fat storage, body size, and development through sgk-1. *PLoS Biol.* 7:e60.
19. Soukas, A. A., E. A. Kane, C. E. Cauf, J. A. Melo, and G. Ruvkun. 2009. Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. *Genes Dev.* 23:496–511.
20. Pan, K. Z., J. E. Palter, A. N. Rogers, A. Olsen, D. Chen, G. J. Lithgow, and P. Kapahi. 2007. Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. *Aging Cell.* 6:111–119.
21. Apfeld, J. G., O’Connor, T. McDonagh, P. S. DiStefano, and R. Curtis. 2004. The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in C. elegans. *Genes Dev.* 18:3004–3009.
22. Narbonne, P., and R. Roy. 2009. Caenorhabditis elegans dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature* 457:210–214.
23. Walther, T. G., and R. V. Farese, Jr. 2012. Lipid droplets and cellular lipid metabolism. *Annu. Rev. Biochem.* 81:687–714.
24. Yang, L., Y. Ding, Y. Chen, S. Zhang, C. Hua, Y. Wang, J. Yu, P. Zhang, H. Na, H. Zhang et al. 2012. The proteomics of lipid droplets: structure, dynamics, and functions of the organelle conserved from bacteria to humans. *J. Lipid Res.* 53:1245–1255.
25. Zhang, P., H. Na, Z. Liu, S. Zhang, P. Xue, Y. Chen, J. Pu, G. Peng, X. Huang, F. Yang, et al. 2012. Proteomic study and marker protein identification of Caenorhabditis elegans lipid droplets. *Mol. Cell. Proteomics.* 11:317–328.
26. Mak, H. Y. 2012. Lipid droplets as fat storage organelles in Caenorhabditis elegans: Thematic review series: Lipid droplet synthesis and metabolism: from yeast to man. *J. Lipid Res.* 53:28–33.
27. Fraser, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann, and J. Ahringer. 2000. Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. *Nature.* 408:325–330.
28. 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.
29. Perez, C. L., and M. R. Van Gilst. 2008. A 13C isotope labeling strategy reveals the influence of insulin signaling on lipogenesis in C. elegans. *Cell Metab.* 8:266–274.
30. Elle, I. C., K. T. Simonsen, L. C. Olsen, P. K. Birck, S. Ehmsen, S. Tuck, T. T. Le, and N. J. Faergeman. 2011. Tissue- and paralogue-specific functions of acyl-CoA-binding proteins in lipid metabolism in C. elegans. *Biochem. J.* 437:231–241.
31. Wong, M. L., and J. F. Medrano. 2005. Real-time PCR for mRNA quantitation. *Biotechniques.* 39:75–85.
32. Schroeder, L. K., S. Kremer, M. J. Kramer, E. Currie, E. Kwan, J. L. Watts, A. L. Lawson, and G. J. Herrmann. 2007. Function of the Caenorhabditis elegans ABC transporter PGP-2 in the biogenesis of a lysosome-related fat storage organelle. *Mol. Biol. Cell.* 18:995–1008.
33. O’Rourke, E. J., A. A. Soukas, C. E. Carr, and G. Ruvkun. 2009. C. elegans major fats are stored in vesicles distinct from lysosome-related organelles. *Cell Metab.* 10:430–435.
34. Yang, K. T. T., C. Yuan, A. Bolog, S. D. Narasimhan, J. X. Cheng, and H. A. Tissenbaum. 2010. A comparative study of fat storage quantitation.
in nematode Caenorhabditis elegans using label and label-free methods. *PLoS ONE* **5**: pii: e12810.

35. Zhang, S. O., R. Trimble, F. Guo, and H. Y. Mak. 2010. Lipid droplets as ubiquitous fat storage organelles in C. elegans. *BMC Cell Biol.* **11**: 96.

36. 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.

37. Dobrzyń, P., A. Dobrzyń, M. Miyazaki, P. Cohen, E. Asilmaz, D. G. Hardie, J. M. Friedman, and J. M. Ntambi. 2004. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *Proc. Natl. Acad. Sci. USA* **101**: 6409–6414.

38. Korta, D. Z., S. Tuck, and E. J. Hubbard. 2012. S6K links cell fate, cell cycle and nutrient response in C. elegans germline stem/progenitor cells. *Development*. **139**: 859–870.

39. Hu, P. J., Dauer (August 08, 2007), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.144.1, http://www.wormbook.org.

40. Greer, E. R., C. L. Perez, M. R. Van Gilst, B. H. Lee, and K. Ashrafi. 2008. Neural and molecular dissection of a *C. elegans* sensory circuit that regulates fat and feeding. *Cell Metab.* **8**: 118–131.

41. Kimura, K. D., H. A. Tissenbaum, Y. Liu, and G. Ruvkun. 1997. dal-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* **277**: 942–946.

42. Shmoooker Reis, R. J., L. Xu, H. Lee, M. Chae, J. J. Thaden, P. Bharill, C. Tazearlan, E. Siegel, R. Alla, P. Zinnmack, et al. 2011. Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging (Albany, NY)* **3**: 125–147.

43. 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.

44. Murphy, C. T., S. A. McCarroll, C. I. Bargmann, A. Fraser, R. S. Kamath, J. Ahringer, H. Li, and C. Kenyon. 2003. Genes that act downsteam of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* **424**: 277–283.

45. Krahmer, N., Y. Guo, F. Willfling, M. Hilger, S. Lingrell, K. Heger, H. W. Newman, M. Schmidt-Supprian, D. E. Vance, M. Mann, et al. 2011. Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP:phosphocholine cytidylyltransferase. *Cell Metab.* **14**: 504–515.

46. Walkey, C. J., L. Yu, L. B. Agellon, and D. E. Vance. 1998. Biochemical and evolutionary significance of phospholipid methylation. *J. Biol. Chem.* **273**: 27043–27046.

47. Walker, A. K., R. L. Jacobs, J. L. Watts, V. Rottiers, K. Jiang, D. M. Finnegar, T. Shiota, M. Hansen, F. Yang, L. J. Niebergall, et al. 2011. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* **147**: 840–852.

48. Routet, E., H. El Mourabit, M. Prot, M. Nemani, E. Khallouf, O. Colard, M. Maurice, A. M. Durand-Schneider, Y. Chretien, S. Gres, et al. 2009. Seipin deficiency alters fatty acid Delta9 desaturation and lipid droplet formation in Berardinelli-Seip congenital lipodystrophy. *Biochemica* **91**: 796–803.

49. Flowers, M. T., and J. M. Ntambi. 2008. Role of stearoyl-Coenzyme A desaturase in regulating lipid metabolism. *Curr. Opin. Lipidol.* **19**: 248–256.

50. Miyazaki, M., A. Dobrzyń, W. C. Man, K. Chu, H. Sampath, H. J. Kim, and J. M. Ntambi. 2004. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein 1c-dependent and -independent mechanisms. *J. Biol. Chem.* **279**: 25164–25171.

51. Attie, A. D., R. M. Krauss, M. P. Gray-Keller, A. Brownie, M. Miyazaki, J. J. Kastelein, A. J. Lusis, A. F. Stalenhoef, J. P. Stoehr, M. R. Hayden, et al. 2002. Relationship between stearoyl-CoA desaturation activity and plasma triglycerides in human and mouse hypertriglyceridaemia. *J. Lipid Res.* **43**: 1895–1907.

52. Man, W. C., M. Miyazaki, K. Chu, and J. Ntambi. 2006. Colocalization of SCAD and DGAT2: implying preference for endogenous mono-unsaturated fatty acids in triglyceride synthesis. *J. Lipid Res.* **47**: 1928–1939.

53. Harris, C. A., J. T. Haas, R. S. Streeper, S. J. Stone, M. Kumari, K. Yang, X. Han, N. Brownell, R. W. Gross, R. Zechnier, et al. 2011. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *J. Lipid Res.* **52**: 657–667.

54. Xu, N., S. O. Zhang, R. A. Cole, S. A. McKinney, F. Guo, J. T. Haas, S. Bobha, R. V. Farese, Jr., and H. Y. Mak. 2012. The FATP1-DGAT2 complex facilitates lipid droplet expansion at the ER-lipid droplet interface. *J. Cell Biol.* **198**: 895–911.

55. Willfling, F., H. Wang, J. T. Haas, N. Krahmer, T. J. Gould, A. Uchida, J. X. Cheng, M. Graham, R. Christiano, F. Frohlich, et al. 2013. Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocating from the ER to lipid droplets. *Dev. Cell* **24**: 384–399.

56. Guo, Y., T. C. Walther, M. Rao, N. Sturman, G. Goshima, K. Terayama, J. S. Wong, R. D. Vale, P. Walter, and R. V. Farese. 2008. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* **453**: 657–661.

57. Castro, C., F. Sar, W. R. Shaw, M. Mishima, E. A. Miska, and J. L. Griffin. 2012. A metabolic strategy defines the regulation of lipid content and global metabolism by Delta9 desaturases in Caenorhabditis elegans. *BMC Genomics* **13**: 36.

58. van Meer, G., D. R. Voelker, and G. W. Feigenson. 2008. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**: 112–124.