REVIEW

The Nematode *Caenorhabditis elegans* as a Model Organism to Study Metabolic Effects of ω-3 Polyunsaturated Fatty Acids in Obesity

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ABSTRACT

Obesity is a complex disease that is influenced by several factors, such as diet, physical activity, developmental stage, age, genes, and their interactions with the environment. Obesity develops as a result of expansion of fat mass when the intake of energy, stored as triglycerides, exceeds its expenditure. Approximately 40% of the US population suffers from obesity, which represents a worldwide public health problem associated with chronic low-grade adipose tissue and systemic inflammation (sterile inflammation), in part due to adipose tissue expansion. In patients with obesity, energy homeostasis is further impaired by inflammation, oxidative stress, dyslipidemia, and metabolic syndrome. These pathologic conditions increase the risk of developing other chronic diseases including diabetes, hypertension, coronary artery disease, and certain forms of cancer. It is well documented that several bioactive compounds such as omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are able to reduce adipose and systemic inflammation and blood triglycerides and, in some cases, improve glucose intolerance and insulin resistance in vertebrate animal models of obesity. A promising model organism that is gaining tremendous interest for studies of lipid and energy metabolism is the nematode *Caenorhabditis elegans*. This roundworm stores fats as droplets within its hypodermal and intestinal cells. The nematode's transparent skin enables fat droplet visualization and quantification with the use of dyes that have affinity to lipids. This article provides a review of major research over the past several years on the use of *C. elegans* to study the effects of ω-3 PUFAs on lipid metabolism and energy homeostasis relative to metabolic diseases. *Adv Nutr* 2019;10:165–178.

Keywords: *C. elegans*, obesity, ω-3 fatty acids, metabolism, inflammation, microRNA, gene regulation

Introduction to the Limitations of Current Omega-3 Fatty Acid Research Due to Imperfect Model Organisms

During the last few decades, the prevalence of obesity has increased in many countries across the world with >600 million adults reported as obese and >1.9 billion as overweight. This complex disease has attained epidemic proportions projected to reach, by 2015, 700 million adults with obesity and 2.3 billion overweight adults (1). The United States is among the countries with the highest obesity incidence and prevalence (2). Obesity in the United States affects ~40% of the adult population and ~19% of the youth population, and close to 70.2% of US adults are categorized as affected by obesity or overweight (3, 4). The number of Americans suffering from obesity has progressively increased since 1960. In 2013, the American Medical Association recognized obesity as a disease, emphasizing its importance to public health (5). The estimated annual medical cost in the United States was $147 billion in 2008, and the annual medical costs for those suffering from obesity were $1429 higher than those of normal weight (6). Clearly, novel and creative public health population-based strategies for preventing and treating obesity are needed and represent an urgent health care challenge (7).

A hallmark of obesity is the expansion of fat mass, primarily in what is referred to as white adipose tissue (WAT). By contrast, brown adipose tissue (BAT) is primarily responsible for thermogenesis and energy expenditure. WAT is the principal adipose tissue type associated with metabolic complications of obesity. This tissue functions as an endocrine organ; it produces various bioactive metabolites and substances such as free fatty acids and adipose cytokines

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Abbreviations used: AA, arachidonic acid; AGO, argonaute; ALA, α-linolenic acid; BAT, brown adipose tissue; *daf*, dauer larva formation abnormal; DR, dietary restriction; *elo*, fatty acid elongation; *fat*, fatty acid desaturase; GPR, G protein–coupled receptor; HFD, high-fat diet; IR, insulin resistance; LA, linoleic acid; *let*, larval-lethal; *lin*, lineage abnormal; MaR, maresin; miRNA, microRNA; NF-κB, nuclear factor kappa-B; NHR, nuclear hormone receptor; OA, oleic acid; OS, oxidative stress; RV, resolvin; SA, stearic acid; WAT, white adipose tissue.

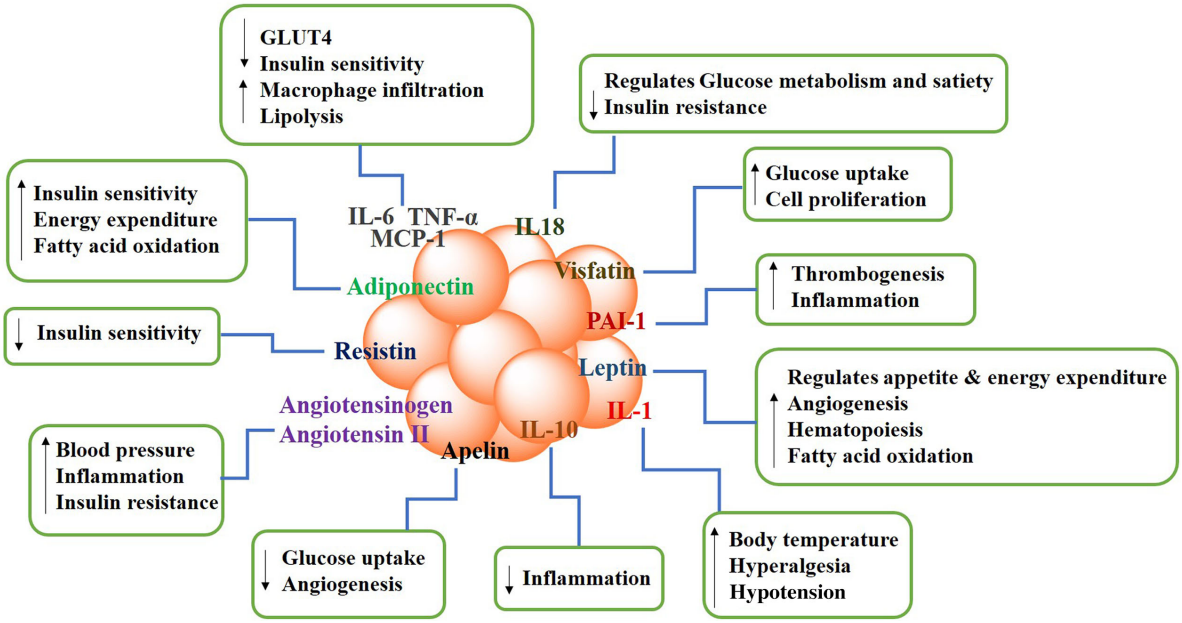

FIGURE 1 Major adipokines and adipose-derived soluble factors in regulating energy homeostasis and immune status. A wide variety of WAT-produced molecules contribute to regulation of lipid and carbohydrate metabolism in health and disease. In obese white adipose tissue (WAT), activation of energy deposition pathways is coupled with elevated proinflammatory signaling, causing obesity-associated chronic inflammation. GLUT4, glucose transporter type 4 protein; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; IL, interleukin; TNF-α, tumor necrosis factor-α.

(adipokines) (8). In particular, increased production of adipose tissue–derived proinflammatory adipokines, such as Tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein (MCP-1), plasminogen activator inhibitor-1 (PAI-1), Interleukins IL-1, IL-6, and IL-18; the hormones resistin and leptin; along with reduced secretion of the anti-inflammatory and insulin-sensitizing adipokines, such as IL-10 and adiponectin, have been reported to partially cause obesity-related insulin resistance (IR) (9–12). Increased adipokine levels in obesity stimulate the production of reactive species of oxygen and nitrogen by resident myeloid cells. Elevated reactive species of oxygen and nitrogen levels are accountable for increasing the process of oxidative stress (OS) (13). **Figure 1** summarizes the major adipocyte-derived factors that are engaged in energy homeostasis.

Several approaches are used to alleviate inflammation and OS in obesity and metabolic disorders. These include dietary and pharmacologic interventions, such as caloric restriction and antiobesity drugs, which ameliorate some of the metabolic dysfunctions in obesity (14, 15). In addition, several bioactive compounds found in foods and botanicals possess anti-inflammatory properties and are attractive means to treat and/or prevent obesity-related inflammation. Other diet-based treatment strategies use bioactive food components, such as polyphenols and long-chain ω-3 PUFAs, namely EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). These fatty acids possess well-documented anti-inflammatory properties, but although a body of data exists about their potential anti-inflammatory benefits in metabolic disorders (16), limited information exists about specific mechanisms mediating the effects of (ω-3) PUFAs on metabolic inflammation, energy balance, and cell signaling pathways.

Long-chain ω-3 PUFAs are potent dietary anti-inflammatory compounds. Importantly, they represent an essential fatty acid type. Mammals and, in particular, human organisms are not capable of synthesizing them de novo due to the lack of Δ-12 desaturase and Δ-15 desaturase endogenous enzymes, required for ω-3 desaturation (17, 18). For this reason, supplementing these PUFAs with the diet is necessary. The short-chain fatty acid α-linolenic acid (ALA; 18:3ω-3) serves as a metabolic precursor for longer-chain ω-3 fatty acids. The most critically important for human diet and health of all the long-chain ω-3 PUFAs are DHA (22:6ω-3) and EPA (20:5ω-3) due to their ability to modify the cellular membrane composition and to modulate gene transcription and cellular signaling, thus exerting numerous and versatile biological effects (19).

Certain amounts of DHA and EPA can be synthesized by mammals via elongation of ALA supplemented with diet, but the capacity of this function is limited. These long-chain ω-3 fatty acids have important therapeutic and nutritional benefits in humans.

Linoleic acid (LA; 18:2ω-6) is the most common ω-6 fatty acid in the human diet (20). LA is an essential dietary component. All long-chain ω-3 fatty acids in human cells are synthesized from ALA and all long-chain ω-6 fatty acids are synthesized from LA. As summarized in **Figure 2**, the dietary essential PUFAs ALA (ω-3) and LA (ω-6) are metabolized

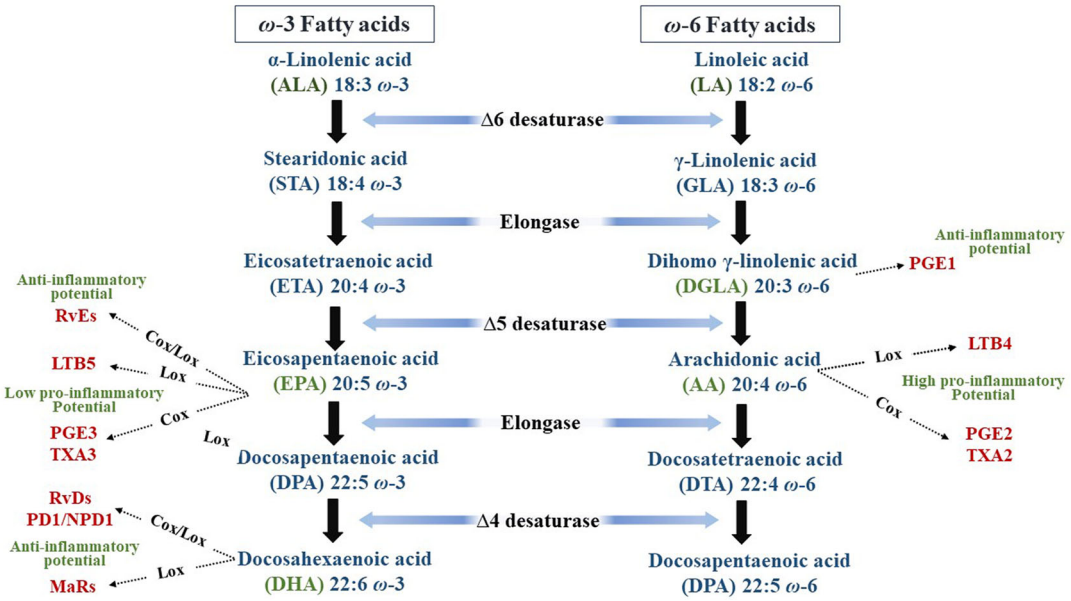

FIGURE 2 Metabolism of ω-3 and ω-6 PUFAs in humans. ω-3 Fatty acids are synthesized from the ALA precursor and ω-6 fatty acids are synthesized from the LA precursor. LA is converted to AA. Eicosanoids derived from AA have proinflammatory properties. ALA is subsequently converted to EPA and DHA. The metabolites of EPA and DHA have anti-inflammatory properties. AA, arachidonic acid; ALA, α-linolenic acid; Cox, cyclooxygenase; LA, linoleic acid; Lox, lipoxygenase; LTB4, leukotriene B4; LTB5, leukotriene B5; MaR, maresin; NPD1, neuroprotectin D1; PD1, protectin D1; PGE2, prostaglandin E2; PGE3, prostaglandin E3; RvD, resolvin D; RvE, resolvin E; TXA2, thromboxane A2; TXA3, thromboxane A3.

to become longer carbon chains (a range of 20- and 22-carbon ω-6 and ω-3 fatty acids) with higher double-bond numbers by successive reactions catalyzed by the same set of desaturase and elongase enzymes. Many of these are further metabolized into other lipid mediators such as prostanoids for ω-6 PUFA metabolites and resolvins (RVs), protectins, or maresins (MaRs) for ω-3 PUFA metabolites (21).

The biosynthesis of lipid mediators occurs at sites of inflammation and tissue injury. MaRs' biosynthesis is initiated in macrophages by lipoxygenation of DHA, whereas protectins and RVs are formed by a wide range of cells and tissues (22, 23). Overall, PUFAs generate proresolving lipid mediators, including arachidonic acid (AA)–derived lipoxins, n–3 EPA–derived RVs of the E-series, DHA-derived RVs of the D-series, protectins, and MaRs, during the resolution phase of acute inflammation. These endogenous hormone-like bioactive compounds are contributory factors of the regulation of pathologic inflammation and various physiologic activities in humans and animals (17, 24, 25). These effects have been extensively studied and reviewed in mammals (17, 26–31). We will only provide a summary of the major mechanisms in mammals. The rest of this review will focus on applications of the nematode model *Caenorhabditis elegans* to ω-3 fatty acid studies.

Numerous hypotheses have been proposed regarding antiobesity and anti-inflammatory protective effects of ω-3 PUFAs (**Figure 3**) (32, 33). These fatty acids modulate the expression of genes associated with lipid oxidation in adipose, cardiac, and liver tissues (34). This is consistent with reduced adipogenesis by DHA and EPA and increased mitochondrial carnitine palmitoyl transferase 1 enzyme (CPT1), which controls fat oxidation in adipocytes, skeletal muscle, and cardiac cells. These catabolic effects are mediated in part by activation of PPAR-γ and AMP-activated protein kinase, the energy-sensing enzyme. This activation results in the inhibition of malonyl-CoA decarboxylase, a key lipid metabolism enzyme implicated in fatty acid biosynthesis (35). On the other hand, as ligands for PPAR-α and PPAR-γ, DHA and EPA stimulate the expression of lipoprotein lipase and adipose triacylglycerol lipase, the lipolysis-mediating enzymes, essential for lipid utilization, further enhancing their antiadipogenesis effects (36).

Long-chain ω-3 PUFAs exert a wide spectrum of antiobesity effects involving numerous molecular pathways. These include stimulating a unique fat oxidation pathway that results in generating heat instead of ATP biosynthesis. This heat-generating pathway is specifically associated with BAT, which has physiologic functions different from those of WAT, which serves the energy storage of the organism. ω-3 PUFAs modulate the expression of uncoupling protein-1 (UCP-1), which mediates the thermogenesis function. Activating this pathway is associated with adipose tissue mass reduction (37, 38). Moreover, DHA and EPA suppress fat synthesis and increase metabolism in adipose tissue via suppression of sterol regulatory element-binding protein-1 (SREBP-1) (39). Long-chain ω-3 PUFAs ameliorate obesity-induced IR and

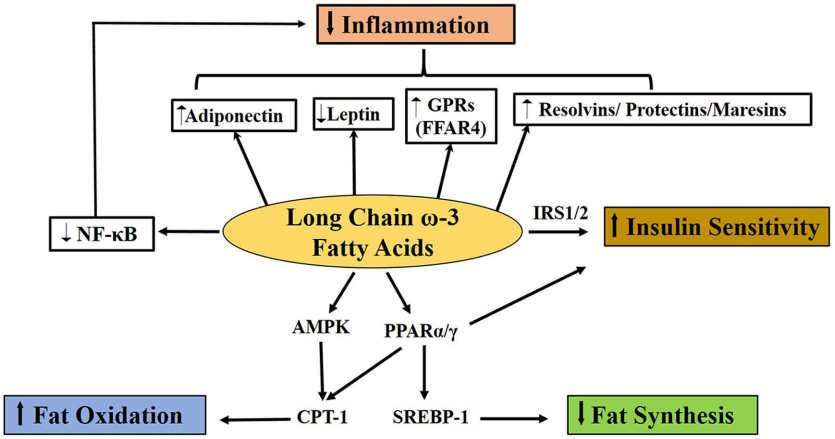

FIGURE 3 Molecular mediators of the effects of long-chain ω-3 PUFAs. In adipocytes, ω-3 PUFAs modulate gene expression and promote biosynthesis of regulatory proteins, which enhance the utilization of carbohydrates and fats, reduce adipogenesis, increase insulin sensitivity, and ameliorate inflammation. AMPK, 5′ AMP-activated protein kinase; CPT-1, carnitine palmitoyl transferase-1; FFAR4, free fatty acid receptor 4; GPR, G-protein–coupled receptor; IRS, insulin substrate receptor; NF-κB, nuclear factor kappa-B; SREBP-1, sterol regulatory element-binding protein-1.

metabolic syndrome by activating the AMP-activated protein kinase pathway and enhancing the expression of adiponectin, an insulin-sensitizing adipokine (40). Both IR and obesity-associated inflammation are further improved by DHA and EPA via generation of the protective lipid mediators. Their mechanistic roles appear interestingly more potent than their ω-3 PUFA precursors (40).

Some of the anti-inflammatory effects of ω-3 fatty acids are receptor mediated. Polymorphonuclear leukocytes, monocytes and macrophages, and blood vessel endothelium all have been implicated in the systemic anti-inflammatory effects triggered by ω-3 PUFAs (16). Given the potent and stereo-selective actions of the specific lipid mediators generated from ω-3 fatty acids, they act via specific high-affinity receptors, G protein–coupled receptors (GPRs), present in the membranes of the relevant cell types, including GPR32, lipoxin A4 receptor/formyl peptide receptor 2, chemokine-like receptor 1, leukotriene B4 receptor type 1, and cannabinoid receptor 2 (22, 41). Activation of these receptors directly affects different anti-inflammatory pathways that can further mediate the timely resolution of inflammation in mammals (27, 42). The receptors GPR40 and GPR120 (43) also mediate some of the effects of ω-3 fatty acids. Oh and Walenta (30) found that anti-inflammatory effects exerted by DHA and EPA are mediated by intracellular signaling transmitted through the GPR120 receptor [also named free fatty acid receptor 4 (FFAR4)], highly expressed in mature adipocytes and proinflammatory macrophages. Upon binding its ligand, GPR120 couples to β-arrestin 2, thus providing the inhibition of both the Toll-like receptor-4 (TLR4) and TNF-α proinflammatory signaling pathways associated with NF-κB (43). The NF-κB pathway is also inhibited via activation of PPARs (26). The activation of GPR120 by supplementing the diet with ω-3 PUFAs increases cell sensitivity to insulin and reduces the incidence of diabetes in vivo by repressing macrophage-induced tissue inflammation (43).

Clinical data regarding the beneficial effects ω-3 PUFAs are consistent with numerous animal studies demonstrating that enriching a mouse high-fat diet (HFD) with ω-3 PUFAs, particularly EPA, prevents and even reverses the development of fatty liver, glucose intolerance, and IR; reduces adiposity and OS; lowers serum and tissue lipids; and reduces the blood levels of such systemic inflammation markers as TNF-α, IL-6, and C-reactive protein (CRP) (40, 44). The anti-OS protective effects of ω-3 fatty acids were previously reviewed in an article by Puglisi et al. (44). These studies reported that consumption of ω-3–rich fish oil results in a significant reduction in F_2-isoprostanes, a gold-standard marker of systemic OS levels in adipose tissue.

Overall, dietary intake of DHA and EPA has been proven to be directly associated with human health and development.

All of this suggests that science-based dietary interventions, using ω-3 PUFAs or their combinations, represent a promising soft therapeutic approach to prevention and treatment of diet-induced obesity and its associated comorbidities and provide insight into the mechanisms of ω-3 fatty acids and their actions (27). Dissecting molecular and physiologic mechanisms mediating the biological effects of ω-3 PUFAs as well as the mechanisms regulating their metabolism has become a hot topic in biomedical research. The next section will focus on the *C. elegans* nematode as a novel and exciting model organism for ω-3 PUFA–related studies. Unlike most other animals, *C. elegans* is capable of de novo synthesis of long-chain PUFAs. This model may yield new insights for the nutritional scientist interested in evaluating the therapeutic potential of ω-3 PUFAs and using them for developing science-based diets for prevention and/or treatment of obesity and its associated metabolic disorders.

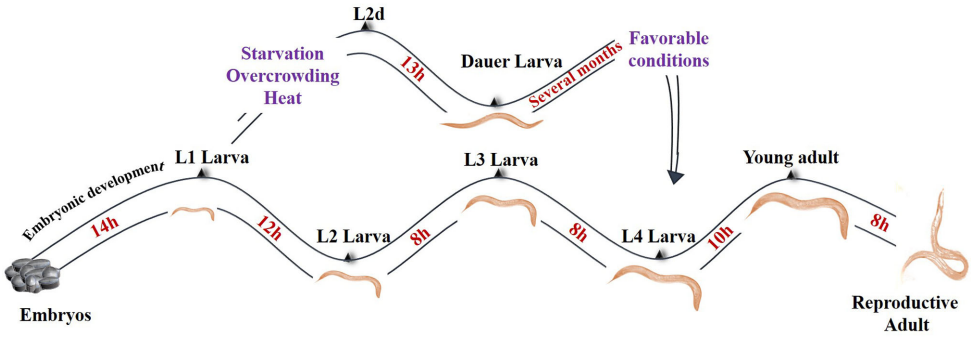

FIGURE 4 *Caenorhabditis elegans* life cycle at 20°C. The life cycle of this nematode is ∼3.5 d. Under standard laboratory conditions, reproductive adult worms survive for ∼3 wk. The regular ontogenesis includes embryonic stage, 4 larval stages (L1–L4; separated by molts), and adulthood. Under stress conditions (starvation, crowding, high temperature), the roundworm can enter an alternative L3 stage called the Dauer state, which can last for several months. The Dauer larva develops from a pre-Dauer L2 (L2d). Numbers in red underneath the arrows show the time span that the worm stays at the indicated stage.

Furthermore, a decade ago, an emerging hot topic of interest for research that had been found to be involved in a wide range of biological processes was the microRNA (miRNA) field. Originally, these molecules were discovered in *C. elegans* and the first 2 known miRNAs are lineage abnormal (*lin*-4) and larval-lethal (*let*-7) (45). They are found in most eukaryotes including humans. *C. elegans* has proven to be the ideal organism for miRNA research. Currently, there is evidence that miRNAs play a potent role in the regulation of gene expression, controlling diverse cellular and metabolic pathways including lipid metabolism and adipocyte differentiation processes, which are both related to obesity (46). Thus, these fascinating molecules can be used as promising biomarkers via the dysfunction and dysregulation of several proteins (47).

As discussed previously, given the prevalence of obesity, understanding its pathogenesis is becoming critical in order to develop better dietary and pharmacologic therapies. Hence, ω-3 fatty acids, especially EPA, may reduce or prevent complications linked to obesity by regulating specific miRNAs (48, 49).

Review of *C. elegans* as a Model Organism and Its Advantages

In 1963, the South African biologist Sydney Brenner introduced *Caenorhabditis elegans* (*Caeno*, recent; *rhabditis*, rod; *elegans*, nice), commonly named *C. elegans*, as a model organism to pursue research in developmental biology and neurology (50, 51). *C. elegans* is a free-living, nonparasitic, nonhazardous, noninfectious, and nonpathogenic soil nematode that has been widely used in laboratories worldwide. This roundworm is transparent throughout its life cycle and is ∼1 mm in length at adulthood. In different regions of the world, *C. elegans* lives in the soil, mainly in rotting vegetation, where it can feed on bacteria. Under laboratory conditions, it is routinely cultured in an agar petri dish seeded with *Escherichia coli* as a food source providing carbohydrates, proteins, SFAs, and MUFAs derived from digestion of bacterial membranes (52). Advantages of using these bacteria include their ability to form a thin layer after multiplying that allows for optimal visualization of the worms' development.

Another important advantage of *C. elegans* as a model organism is its short life span. As illustrated in **Figure 4**, an egg develops to an adult within ∼3.5 d at 20°C. As with other nematodes, *C. elegans* develops through 4 larval stages (L1–L4), separated by molts (53, 54) (Figure 4). The whole life span is ∼3 wk. The period required for generating adult nematodes capable of producing progeny is as short as 3.5 d, which is ∼15-fold shorter than in mice and 3-fold shorter than in *Drosophila melanogaster* fruit flies or *Danio rerio* (zebrafish). *C. elegans* can be easily and cheaply cultivated in large numbers, i.e., ∼10,000 worms/plate in the laboratory (300–350 progenies/nematode). There are 2 sexual forms of the worm: a self-fertilizing hermaphrodite and a male that is smaller in size and rare. The male can be recognized by its fan-shaped tail. The adult hermaphrodite, which is the first higher organism that had its genome completely sequenced, harbors ∼17,800 distinct genes, 65% of which are associated with human diseases (55). Despite the genetic homology between humans and *C. elegans* being lower than that between humans and the mouse, *D. melanogaster*, or zebrafish, *C. elegans* still represents a physiologically relevant model for studying lipid metabolism due to its completely sequenced genome and ease of genetic manipulation and screening for mutants having necessary metabolic deviations. For instance, genetic defects in fatty acid desaturation and elongation provide a set of mutant worm strains (fatty acid desaturase (*fat*)*-1*, *fat-2, fat-3, fat-4, fatty acid elongation (elo)-1*) incapable of PUFA synthesis, which makes the lipid metabolism in these strains similar to that of humans (56). Interestingly, *C. elegans* has only 959 somatic cells, of which 302 are neurons and 95 are muscle cells (57). Last but not least, no ethics constraints are associated with culturing *C. elegans*. It reduces significantly the "indirect workload" related to getting quick answers for emerging research questions related to human or animal health. The same is true for *D. melanogaster* and zebrafish, but these

otherwise simple model species are not as easily manageable and convenient for housing and reproduction purposes. Tables 1 and 2 provide comparisons between *C. elegans* and other model organisms as well as differences and similarities in lipid metabolism between *C. elegans* and mammals.

Taken together, these unique characteristics make *C. elegans* an effective model and an attractive pool of resources for scientists to use in a variety of biomedical research areas. Key *C. elegans* advantages as a model organism include the following (50, 58):

- its small size and transparent body that enable noninvasive imaging and scaled screening of the effects of treatments through the use of microscopy techniques;
- constant cell numbers and position between individual worms;
- rapid growth, quick turnover, and large brood sizes, which reduce the experimental timeline compared with rodent and other animal studies;
- simple nervous system, which dissects neural circuits that govern metabolism, nutrient perception, and food-related behaviors;
- *C. elegans* culture can be placed in long-term storage as frozen stock and avoid the expenses associated with long-term colony maintenance;
- because *C. elegans* is a nematode, its use does not require research ethics approval unlike with rodents and higher animals, further facilitating research on this organism;
- the small size of its well-annotated genome, which facilitates genetic analyses as well as producing genetically modified roundworm strains. The mutations can be easily introduced into the *C. elegans* genome by a variety of mutagens. Thus, many highly affordable genetically modified strains, such as dumpy, small, and long mutated worms are available for biomedical research from the Caenorhabditis Genetics Center (Minnesota). This center is funded by the NIH—National Center for Research Resources.

Current studies report the use of *C. elegans* as a model organism for exploring a variety of biological processes including apoptosis, insulin signaling, gene regulation, metabolism, aging, and satiety. Importantly, in obesity, *C. elegans* has been used for unraveling the mechanisms of dyslipidemia, for ascertaining endocrine regulation deviations, as well as for evaluating the effects of dietary interventions and other obesity therapies (55). Table 3, based on the National Center for Biotechnology Information (59), summarizes some key *C. elegans* metabolic genes.

Related Research on ω-3 Fatty Acid Metabolism That Has Been Conducted in *C. elegans* and Knowledge Gained

Wild-type *C. elegans* nematode stores fat mainly in droplets within its hypodermal and intestinal cells (Figure 5). The quantification of fats accumulated in the *C. elegans* body may be conducted via biochemical assays, measuring fat uptake and fat oxidation, and via other methods appropriate for most model organisms (55). But, in addition, due to the transparency of the *C. elegans* body, its fat depositions can be easily visualized in the intact nematode with the use of lipid-specific dyes, such as Nile Red, Oil Red O, or Sudan Black, which do not affect the *C. elegans* brood size, growth rate, or life span (60). The quantification of visualized fats can be performed simply by measuring the intensity of the accumulated dye (61, 62).

Like in mammals, and as discussed already, a wide range of SFAs, MUFAs, and PUFAs such as ω-6 AA (20:4ω-6) and ω-3 EPA (20:5ω-3), as well as monomethyl branched-chain fatty acids, are present in *C. elegans* (60, 63).

Triglycerides in *C. elegans* represent ~40–55% of total body lipids depending on diet and growth stage (60, 64). The principal phospholipids are ethanolamine (~55%), choline (32%), and sphingomyelin (8%). Cardiolipin, inositol, and lyso-choline account for the remaining 5% of phospholipids (65, 66).

In addition to mammals, Δ-12 and Δ-15 desaturases have been identified in some plants (*Arabidopsis thaliana*), lower eukaryotes, and animals such as nematodes. These enzymes introduce a double bond at the 12th and 15th carbon-carbon positions in fatty acid molecules (17). Figure 6 indicates the synthetic pathways of ω-3 and ω-6 PUFAs from their common precursors stearic and oleic acids (stearic acid (SA) and oleic acid (OA)) to 20-carbon fatty acids in *C. elegans*. It is noteworthy that, unlike other animals including humans, the *C. elegans* organism contains both Δ-12 and Δ-15 enzymes (57, 67). For this reason, *C. elegans* does not require essential fatty acids supplemented with the diet.

Excluding the nematodes, all other mammals including humans lack ω-3 desaturase genes that convert ω-6 fatty acids to ω-3 fatty acids. Among nematodes, the roundworm *C. elegans*, unlike most other animals, possesses the genes for the protein products mediating the endogenous biosynthesis of long-chain ω-3 PUFAs including the *fat-1* gene, coding the ω-3 desaturase (Δ-15 desaturase) required for converting the ω-6 fatty acids 18-carbon LA and γ-linolenic acid into ω-3 ALA and stearidonic acid, respectively (68). Another unique fatty acid metabolism gene carried by *C. elegans* is *fat-2*, coding the Δ-12 enzyme, which mediates the synthesis of 18-carbon ω-6 LA from its precursor, 18-carbon ω-9, the MUFA OA (69, 70). The other 2 enzymes, Δ-6 desaturase encoded by the *fat-3* gene and Δ-5 desaturase encoded by the *fat-4* gene, are involved in the biosynthesis of 20-carbon PUFAs (Figure 6). However, as in mammals, *C. elegans*' Δ-9 fatty acid desaturases, encoded by the *fat-5* and *fat-6*/*fat-7* genes, are regulated by the transcriptional regulator sterol response element binding protein (SREBP) and nuclear hormone receptors (NHRs). Δ-9 Fatty acid desaturases convert SFAs into MUFAs. However, *C. elegans* lacks the specific enzyme required for fatty acid elongation to 22-carbon PUFAs (60). Similar to mammals, *C. elegans* possesses key enzymes (e.g., acetyl CoA carboxylase and fatty acid synthase) for fatty acid biosynthesis (60, 67).

TABLE 1 Comparisons between *Caenorhabditis elegans* and other model organisms

Organism	Advantages	Drawbacks
Nematode (*Caenorhabditis elegans*)	- Small size/959 somatic cells - Simple anatomy - Transparent body - Short life span: 2–3 wk - Short generation time: 2–3 d - Inexpensive and easy to grow - Cultures can be frozen - Genome is sequenced and annotated	- Only 65% of worm genes homologous to human genes - Lacking some organs and tissues: blood, brain, and internal organs - Cultures may be subject to contamination
Fruit fly (*Drosophila melanogaster*)	- Small size - Short generation time: 10 d - Inexpensive and easy to grow - Genome is sequenced and annotated	- Only 50–80% of fly genes homologous to human genes - Lack of physiologic similarity with humans - Cultures cannot be frozen
Zebrafish (*Danio rerio*)	- Small size - Transparent embryos - Draft genome is available	- Long generation time: 2–4 mo - Isogenic strains are not available
Pufferfish (*Fugu rubripes*)	- Genome is sequenced and annotated - Very small genome for a vertebrate	- Produces lethal toxin - No transgenic technology exists
Mouse (*Mus musculus*)	- Strong genetic, physiologic overlap with humans - Genome is sequenced and annotated	- Comparatively expensive - Comparatively long life span - Comparatively long generation time: 2–3 mo - Ethical concerns
Chimpanzee (*Pan troglodytes*)	- Genome is sequenced and annotated - Most closely related to humans	- Long life span - Long generation time - Very expensive and labor-consuming housing - Ethical concerns

The presence of a complete set of fatty acid metabolism genes that code all enzymes, found in plants and animals, required for desaturating and elongating fatty acid molecules, makes *C. elegans* a unique model organism for lipid metabolism studies. In addition, core fat and sugar metabolic pathways in *C. elegans* are similar to their mammalian analogs.

Genetic alterations of metabolic enzymes affect fat deposition in *C. elegans*. Inactivation of specific proteins belonging to the desaturase and elongase families (encoded by *fat* and *elo* genes, respectively) results in lipid metabolism deviations (60), which may mimic different aspects of dyslipidemia in humans. In worms, inactivation of these genes is associated with metabolic, physiologic, and behavioral nematode phenotypes, such as reduced body size, deviated body adiposity, growth retardation, reproductive defects, changes in physiologic rhythms, slowed movement, reduced adult life span, as well as defects in sensory signaling and neurotransmission (71–77).

Mutant *C. elegans* strains, lacking the activity of certain desaturases, were used as a model organism in research devoted to the anti-inflammatory effects of PUFAs in reproduction, neurobiological studies, as well as in experiments on the nematode life span and ontogenesis (67). Importantly, *C. elegans* does not express such mediators of inflammation as TNF-α and NF-κB, extensively used for evaluating the severity of inflammation in other vertebrate animal models and humans. Because *C. elegans*' body does not have blood vessels, the roles of PUFAs can be studied independently of their inflammatory functions (67). Furthermore, the impacts

TABLE 2 Differences and similarities in lipid homeostasis between *Caenorhabditis elegans* and mammals[1]

Lipid metabolism regulators	*C. elegans*	Mammals
Δ-9 Desaturase	Fatty acid synthesis	Fatty acid synthesis
Δ-12 Desaturase	Fatty acid synthesis	Not available
ω-3 Fatty acyl desaturase	Fatty acid synthesis	Not available
Insulin-like pathway	Lipid metabolism	Lipid metabolism
AMP-activated protein kinase	Fat storage and use	Fat storage and use
Serotonin signaling	Fat metabolism and feeding behavior	Fat metabolism and feeding behavior
SREBP	Fat storage	Fat storage
TUB-1 protein	Peripheral lipid storage	Peripheral lipid storage
Leptin	Not available	Food intake and energy balance
LDL family receptors	Fatty acid transport	Fatty acid transport

[1] SREBP, sterol response element binding protein; TUB-1, Tubby protein homolog 1.

TABLE 3 Genes related to nutrition and obesity research in *Caenorhabditis elegans* mutants, strain: Bristol N2

C. elegans gene name	Description	Location
daf-2 (Dauer formation-2)	Insulin-like growth factor receptor subunit β	Chromosome III, NC_003281.10 (2995919..3028702, complement)
daf-7 (Dauer formation-7)	Dauer larva development regulatory growth factor daf-7	Chromosome III, NC_003281.10 (811938..813275)
daf-16	Forkhead box protein O	Chromosome I, NC_003279.8 (10750498..10775050)
daf-18	Hypothetical protein	Chromosome IV, NC_003282.8 (420426..425148, complement)
tub-1	Tubby protein homolog 1	Chromosome II, NC_003280.10 (8154771..8156937, complement)
tph-1	Tryptophan hydroxylase	Chromosome II, NC_003280.10 (7549358..7551777)
egl-30	Hypothetical protein	Chromosome I, NC_003279.8 (1835936..1840888)
fat-1	ω-3 Fatty acid desaturase fat-1	Chromosome IV, NC_003282.8 (13315646..13318606)
fat-2	Δ(12)-Fatty-acid desaturase fat-2	Chromosome IV, NC_003282.8 (13323880..13326278)
fat-3	Δ(6)-Fatty-acid desaturase fat-3	Chromosome IV, NC_003282.8 (9803063..9805999)
fat-5	Δ(9)-Fatty-acid desaturase fat-5	Chromosome V, NC_003283.11 (17725005..17726717)
fat-6	Δ(9)-Fatty-acid desaturase fat-6	Chromosome IV, NC_003282.8 (11913819..11915668, complement)
fat-7	Δ(9)-Fatty-acid desaturase fat-7	Chromosome V, NC_003283.11 (7151447..7153131, complement)
acdh-11	Acyl-CoA dehydrogenase family member 11	Chromosome III, NC_003281.10 (10566286..10571549)
eat-2	Neuronal acetylcholine receptor subunit eat-2	Chromosome II, NC_003280.10 (14166888..14171484, complement)
act-5	Actin	Chromosome III, NC_003281.10 (13604554..13606066, complement)
nhr-49	Nuclear hormone receptor family	Chromosome I, NC_003279.8 (9869718..9874124)
acs-2	Fatty acid CoA synthetase family	Chromosome V, NC_003283.11 (15567408..15569685)
mai-2	ATPase inhibitor mai-2, mitochondrial	Chromosome IV, NC_003282.8 (3385911..3386765)
mdt-15	Mediator of RNA polymerase II transcription subunit 15	Chromosome III, NC_003281.10 (5830222..5833337, complement)
SCD-1 (stearoyl-CoA desaturase)	Suppressor of constitutive Dauer formation	Chromosome X, NC_003284.9 (12504620..12509217, complement)
SCD-2 (stearoyl-CoA desaturase)	ALK tyrosine kinase receptor homolog scd-2	Chromosome V, NC_003283.11 (6633269..6639420)
kat-1	Acetyl-CoA acetyltransferase homolog, mitochondrial	Chromosome II, NC_003280.10 (7073815..7075409)
SBP-1	Sterol regulatory element binding protein	Chromosome III, NC_003281.10 (11428418..11442565, complement)
let-767	Very-long-chain 3-oxooacyl-coA reductase let-767	Chromosome III, NC_003281.10 (6338598..6339955, complement)

of specific fatty acids on life span and aging processes in *C. elegans* were recently evaluated (78, 79). Previous studies have demonstrated that synthesis of PUFA-derived mediators (F-series prostaglandins) in this worm is mediated by insulin signaling in the intestine and TGF-β signaling in sensory neurons to ensure the process of reproduction (67, 80). Furthermore, dietary supplementation of dihomo-γ-linolenic acid (DGLA; 20:3ω-6) resulted in sterility associated with germ cell death due to apoptosis, caused by production of specific epoxy- and hydroxyl-toxic metabolites through the activity of CYP-33E2, one of the major isoforms of the cytochrome-P450 protein family, involved in the production of long-chain fatty acid metabolites (81). On the contrary, high amounts of EPA-rich fish oil intake did not affect *C. elegans*' fertility (71). However, it led to a shorter life span due to OS caused by accumulation of reactive lipid peroxidation products, damaging cell proteins and nucleic acids. A recent study reported that OA derivatives, ethanolamide and oleoylethanolamide, accumulate in *C. elegans*, over expressing lysosomal acid lipase 4 (LIPL-4) and lacking the germline. LIPL-4 triggered nuclear translocation of LPB-8 (lysosomal lipid chaperone), thereby increasing longevity by activating NHR-49 and NHR-80 and regulating Δ-9 desaturase expression (82).

Moreover, adding low amounts of glucose to *C. elegans*' diet shortens the life span of the worm by inhibiting the DAF-16 and HSF-1 transcription factors (83). In humans, high sugar content leads to excessive lipid accumulation and eventually causes obesity, diabetes, and heart disease (84, 85). Supplementing the *C. elegans* diet with OA (ω-9, monounsaturated), AA (ω-6, polyunsaturated), and EPA (ω-3, polyunsaturated) influences both the reproduction and longevity of these animals (86). Regarding the neural function of *C. elegans*, Watts (67) reported that 20-carbon PUFAs are required for synaptic vesicle formation and accumulation, and both ω-6 and ω-3 PUFAs perform the required cellular functions but they have different roles in neurologic processes; ω-3 fatty acids are specifically required for maintaining neuroplasticity. In particular, they are capable of compensating the effects of alcohol intoxication in this model (87).

Clinical and animal model studies are very valuable in understanding the mechanisms mediating the effects of bioactive food compounds such as ω-3 fatty acids. However, these are both expensive and time consuming, and are often limited for research studies, due to ethical concerns. Therefore, there is a critical need for affordable and efficient animal models for scaled screening of various bioactive compounds or pharmacologic agents in various diseases including obesity. In this review, we focused on the *C. elegans* nematode as an affordable, convenient, and metabolically relevant model organism to understand the mechanisms mediating the effects of ω-3 PUFAs in obesity and inflammation. *C. elegans* can be further expanded and applied

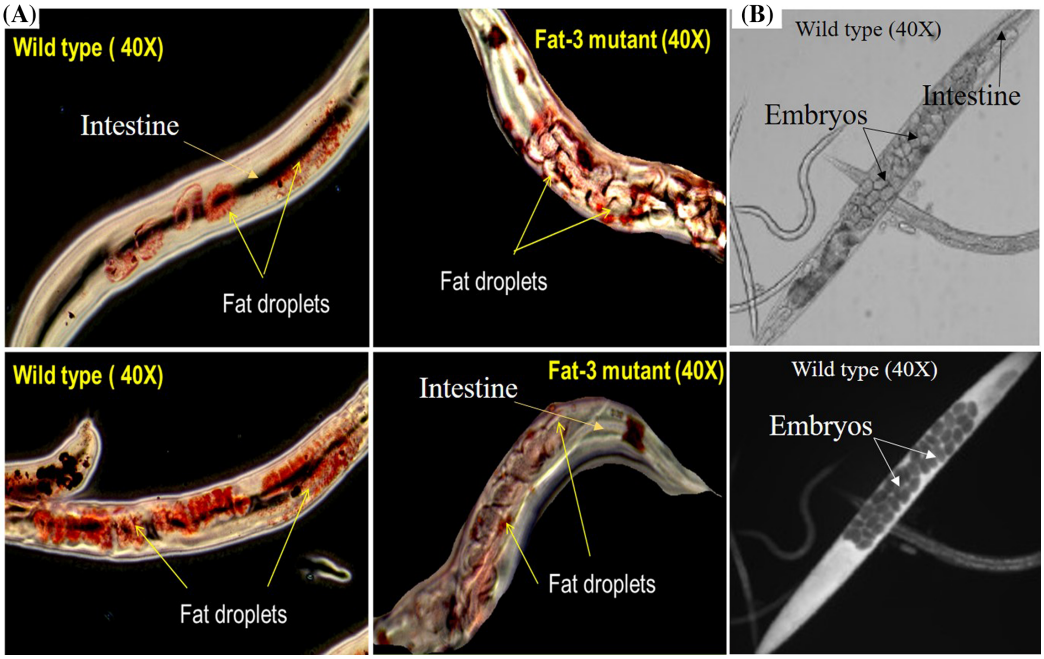

FIGURE 5 Visualization of intestinal fat droplets in the *Caenorhabditis elegans* body. (A) Oil Red O staining in N2 wild-type and *fat-3* mutant worms. (B) Nile Red staining in N2 wild-type nematode. Because of the transparency of *C. elegans'* body, lipid droplets, intestine, and embryos are easily visualized through the use of conventional staining protocols. *fat-3* mutants lack delta 6 desaturase.

to other metabolic studies that use dietary compounds and botanicals. However, despite all the excellent features discussed so far, there are also numerous drawbacks for this nematode as a model organism. The long evolutionary distance between *C. elegans* and humans, including the fact that the simple body of *C. elegans* lacks such essential tissues and organs for human physiology as blood, brain, and defined fat cells, and is subjected to significantly different mechanisms of central regulation, poses a question: whether the data generated through the use of this worm can be directly extrapolated to humans. Most likely, the lipid metabolism data generated through the use of *C. elegans* will require further validation via a mouse or another model organism more closely related to humans. Also, the small size of *C. elegans* can be an issue when comparatively high amounts of tissues or cells are required for biochemical and/or molecular analyses.

Role of miRNAs in Mediating Nutritional and Obesity-Related Effects in *C. elegans* and in Other Animal Models

miRNAs are a group of small noncoding RNAs that function as specific post-transcriptional inhibitors of target gene expression (88). miRNAs are ubiquitous in both animal and plant genomes and are highly conserved between related species. By their ability to quench gene expression, miRNAs are similar to small interfering RNAs, another class of regulatory noncoding RNA molecules, but miRNA biogenesis from maternal DNA sequences is quite different from small interfering RNA synthesis (89). While still in the nucleus, the primary long transcript of the miRNA gene, a pri-miRNA, is cleaved by the protein complex, which, in *C. elegans*, includes DRSH-1 RNase (Drosha in mammals) and PASH-1 (Partner of Drosha; Pasha in mammals) (90), to produce the 60- to 70-nucleotides–long intermediate called pre-miRNA (91). After its transport to the cytoplasm, the pre-miRNA is converted to functional miRNA by additional cleavage performed by Dicer endoribonuclease as well as ALG-1 and ALG-2 proteins (92, 93), which belong to the argonaute (AGO) RNase family and are the only 2 AGOs in *C. elegans*, reported to take part in miRNA biosynthesis. Other 24 AGO proteins, described for the worm, participate in gene regulation processes, mediated by other small RNAs (94). In the cytoplasm, mature miRNA is complexed with ALG-1/ALG-2, AIN-1/AIN-2 (GW-182 in mammals), and poly(A)-binding protein (PABP) molecules to create an RNA-induced silencing complex (RISC), which binds the 3′-untranslated region of target mRNA and prevents protein biosynthesis on this molecule by interfering with the process of translation on its initiation and/or elongation phases or by destabilizing the target mRNA itself (95). Importantly, miRNAs are multipurpose molecules: a typical miRNA is capable of regulating the translation of ∼200 target mRNAs (96).

C. elegans was the first model where miRNAs were discovered. Several *C. elegans* strains as well as a few other *Caenorhabditis* species were used by Lee et al. (97) to report the first miRNA molecule in their pioneering study published in 1993. They demonstrated that a small RNA product, produced from the nonprotein encoding *lin-4* genetic locus, directly inhibits the translation of the *lin-14 C. elegans* gene, coding for the transcription factor

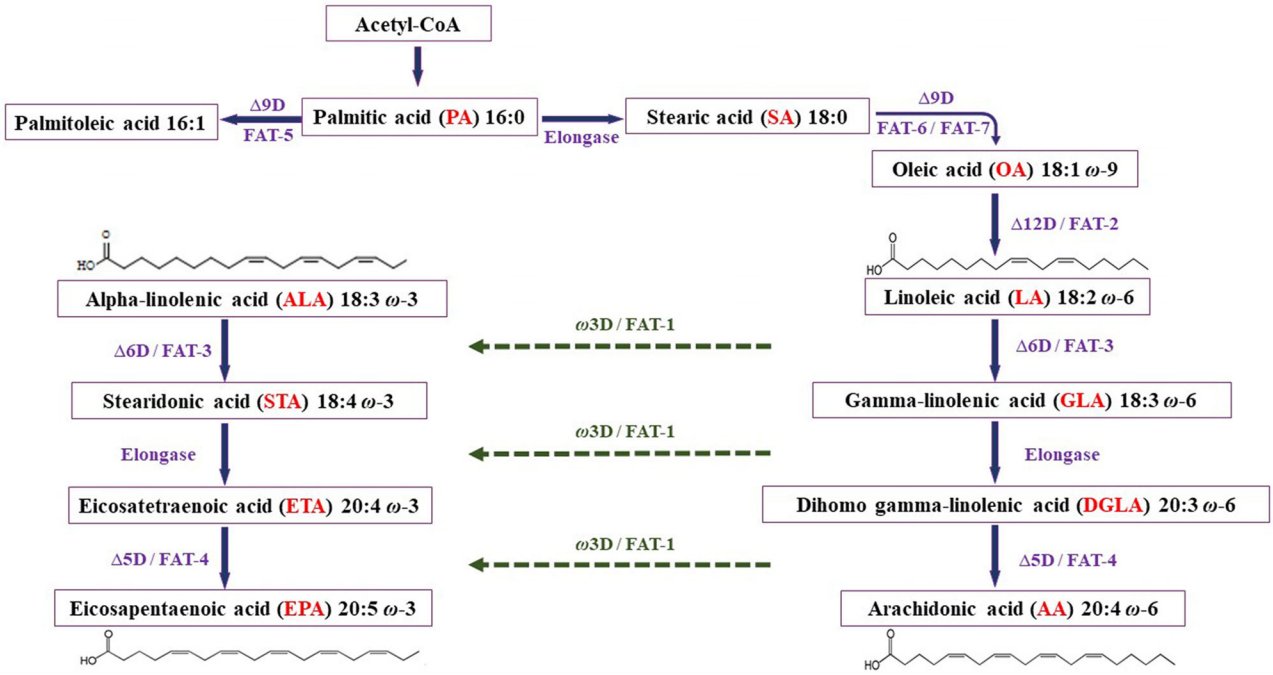

FIGURE 6 Metabolism of ω-3 and ω-6 PUFAs in *Caenorhabditis elegans*. Unlike humans, *C. elegans* is capable of producing ω-3 PUFAs via the enzymatic conversion of various ω-6 PUFAs, biosynthesized from the short-chain stearic and oleic acid precursors. Δ5D, Δ-5 desaturase (encoded by the *fat-4* gene); Δ6D, Δ-6 desaturase (encoded by the *fat-3* gene); Δ9D, Δ-9 desaturase; Δ12D, Δ-12 desaturase (encoded by the *fat-2* gene); ω3D, ω-3 desaturase (encoded by the *fat-1* gene). *fat* encodes fatty acid desaturase genes.

involved in larva development regulation. Numerous other miRNAs regulating a wide variety of genes and biological processes were discovered for *C. elegans* and other species (98–100). For humans, >2600 miRNAs have been described to date (101), and the predicted number of miRNAs encoded by the human genome is >6000 (102). The fact that, among miRNA genes harbored by the human genome, about one-half have analogs in *C. elegans* (103) makes this roundworm a fit model for studies of the role of miRNAs in regulating gene activity and physiologic functions in health and disease. Moreover, unlike when studying a specific gene, which may only relate to one or very few pathways, studying miRNAs provides a more comprehensive understanding of biological systems and functions, because they can target numerous and distinct metabolic genes across diverse biological processes. Therefore, identifying miRNAs that mediate responses to PUFAs or other nutrients can provide a better understanding of whole-tissue and possibly whole-body responses to dietary interventions.

Despite the wealth of resources related to *C. elegans* and miRNA research in this organism, very few studies have used *C. elegans* as a model organism for dissecting the role of miRNAs in the regulation of diet-induced metabolic genes and pathways related to obesity or metabolic diseases. To date, rodents and cell systems remain a principal animal model for such studies. Numerous published studies have reported mechanisms of miRNA-mediated regulation of metabolism and other vital functions in *C. elegans* (reviewed in references 104, 105). Further, a number of studies were published regarding the role of miRNAs in obesity and inflammation in rodent models. To our knowledge, studies of protective antiobesity or anti-inflammatory miRNA-mediated effects of ω-3 PUFAs or other bioactive food components that use *C. elegans* are absent from the literature.

We now briefly review the published animal studies in this area. Zheng et al. (106) were first to use the in vivo rat model of diet-induced chronic inflammation to investigate the alterations in the miRNA transcriptome caused by supplementing the rat diet with ω-3 EPA and DHA PUFAs compared with ω-6 LA. The authors found that after 16 wk of the respective diets, the expression of 54 miRNA genes was different in animals fed the EPA and DHA (1.5:1) mix than those whose diet was enriched with ω-6 PUFAs. Feeding rats with EPA and DHA was associated with increased numbers of blood regulatory T cells as well as levels of IL-6 and TNF-α cytokine markers of inflammation. The list of pro- and anti-inflammatory pathways, presumably affected by these miRNAs, included, in particular, pattern recognition receptors, the nucleotide-binding oligomerization domain (NOD)-like and Toll-like receptors, and TGF-β. In the study, which used a mouse model of HFD-induced obesity to investigate the effects of ω-3 compared with ω-6 PUFAs on brown adipogenesis (107), upregulation of miR-30b and miR-378 miRNAs in BAT after 12 wk of the diet was reported for the HFD-fed animals whose diet was enriched with EPA but not ω-6 OA or LA. The observed increase was mediated by stimulation of FFAR4/GRP120, the ω-3 PUFA receptor expressed by adipocytes, and associated

TABLE 4 miRNAs in *Caenorhabditis elegans* related to longevity and immunity[1]

miRNA	Factor, modulating expression	Molecular targets	Biological function regulated	References
miR-48	Bacterial infection	SKN-1	Resistance to infection	(110)
miR-71	Diet restriction	PHA-4, SKN-1	Longevity	(108)
miR-80	Ad libitum feeding	CBP-1	Longevity	(106)
miR-84	Bacterial infection	SKN-1	Longevity, resistance to infection	(110)
miR-228	Diet restriction	PHA-4	Longevity	(108)
miR-241	Bacterial infection	SKN-1	Longevity, resistance to infection	(110)

[1] CBP-1, cytochrome B mRNA processing; miRNA, microRNA; PHA-4, defective PHArynx development 4; SKN-1, skinhead-1.

with elevation of cellular cAMP levels. Moreover, silencing the activity of the 2 aforementioned miRNAs in vitro by respective antisense inhibitors resulted in compromising the regular BAT gene expression pattern. The expression of UCP-1 (thermogenin), a key mitochondrial transmembrane protein mediating heat generation in brown, but not white, adipocytes was significantly reduced when these miRNAs were inhibited.

Whereas limited studies have addressed effects of dietary components in *C. elegans*, this model was used to unravel the role of miRNAs in mediating the protective effects of dietary restriction (DR). Expression of miR-80 is upregulated in well-fed worms and is reduced in conditions of food deprivation. Genetic deficiency of this miRNA mimics constitutively the conditions of DR and is associated with a life span extension in *C. elegans* (108). Consistently, when miR-80 is absent, *C. elegans* becomes hypersensitive to metformin, the antidiabetic drug that also induces a DR-like state in these nematodes (108, 109). The study authors hypothesized that miR-80 switches *C. elegans*' metabolism to DR mode by inactivating the mRNA of CBP-1 transcription factor, the *C. elegans* homolog of the mammalian cAMP response element binding protein (CREBP)/p300 family (**Table 4**). More transcription factors, involved in mediating the DR-induced longevity by miRNAs in *C. elegans*, were identified by network analysis of aging-associated miRNAs conducted by Smith-Vikos et al. (111). Defective PHArynx development 4 (PHA-4), previously described as *C. elegans*' embryonic development regulator and a homolog of mammalian hepatocyte nuclear factor 3 (HNF3) (110), is downregulated by miR-71 and miR-228. In addition, miR-71 inhibits SKN-1 (skinhead-1), the *C. elegans* homolog of Nuclear factor-erythroid–related factor (Nrf), the family of transcription factors regulating the metabolism and stress response in mammals (112). Expression of both these miRNAs is enhanced in DR. Interestingly, 2 targeted transcription factors exert different feedback regulation effects on inhibiting miRNAs. PHA-4 stimulates miR-228 expression. SKN-1 downregulates miR-228 and promotes miR-71. These findings suggest that a set of miRNAs, induced by DR, acts as a coordinated network with a complicated system of feedback loops.

A study by Liu et al. (113) illustrated that regulation of innate immunity in *C. elegans* is also mediated by miRNAs. The resistance of infected worms against *Pseudomonas aeruginosa*, an opportunistic human pathogen provided as a food, is inhibited by the group of miRNAs that belong to the *let-7-Fam* family. In particular, in wild-type nematodes exposed to *P. aeruginosa* at early ontogenesis stages, miR-241 levels are decreased ∼2-fold, indicating its negative association with the mechanisms of antibacterial defense. Moreover, 2 mutant *C. elegans* strains devoid of miR-84 or miR-241 demonstrated improved survival levels in response to challenge by *P. aeruginosa* or, to a lesser extent, by other microbial pathogens. These data are corroborated by reports that the *let-7* family of miRNAs are downregulated in mammals in response to bacterial and protozoan infections (114–116). The expression of these miRNAs in *C. elegans* is modulated via the p38 MAPK pathway, which also regulates the roundworm's developmental timing (113). Consistent with these effects of miRNAs on the worm's immunity, deficiency in the aforementioned Dicer protein, a crucial regulator of miRNA biosynthesis, is associated with significant alterations in the expression of genes involved in *C. elegans*' immune responses (117).

Taken together, the aforementioned experiments suggest that *C. elegans* has emerged as a convenient and highly relevant research tool for elucidating the cellular and molecular mechanisms of the effects of ω-3 fatty acids on animal and human health. However, further research is needed to understand the role of miRNAs in metabolic responses to diet and diseases such as obesity and for potential translation of findings from *C. elegans* to humans.

Conclusions and Future Directions of the Field

Scientific evidence is accumulating that bioactive food components, including ω-3 PUFAs discussed here, modulate the expression of genes involved in lipid metabolism, energy balance, and homeostasis. Including these fatty acids into diets may provide both antiobesity and anti-inflammatory benefits in metabolic diseases. *C. elegans* has emerged as a valuable model organism and tool for obesity and nutrition studies. Due to its short life cycle, unique fatty acid metabolism, availability and ease of generation of mutants with targeted deletions of long-chain fatty acid metabolizing genes, and simple genetics relevant to humans, *C. elegans* represents a highly relevant time-sparing model organism for biomedical and nutritional investigations. It can be conveniently used to further dissect mechanisms mediating metabolic effects of ω-3 PUFAs and other bioactive compounds, as well as in future mechanistic studies of the role of miRNAs in mediating dietary regulations and in health and disease.

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All authors contributed to this manuscript and read and approved the final manuscript.

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