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REVIEW

Caenorhabditis elegans as a model for obesity research

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Caenorhabditis elegans (*C. elegans*) is a small nematode that conserves 65% of the genes associated with human disease, has a 21-day lifespan, reproductive cycles of 3 days, large brood sizes, lives in an agar dish and does not require committee approvals for experimentation. Research using *C. elegans* is encouraged and a *Caenorhabditis* Genetics Center (CGC, Minnesota) is funded by the National Institutes of Health–National Center for Research Resources. Many genetically manipulated strains of *C. elegans* are available at nominal cost from the CGC. Studies using the *C. elegans* model have explored insulin signaling, response to dietary glucose, the influence of serotonin on obesity, satiety, feeding and hypoxia-associated illnesses. *C. elegans* has also been used as a model to evaluate potential obesity therapeutics, explore the mechanisms behind single gene mutations related to obesity and to define the mechanistic details of fat metabolism. Obesity now affects a third of the US population and is becoming a progressively more expensive public health problem. Faster and less expensive methods to reach more effective treatments are clearly needed. We present this review hoping to stimulate interest in using the *C. elegans* model as a vehicle to advance the understanding and future treatment of obesity.
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Introduction

Obesity now affects one-third of the US population, is rising in prevalence around the world, is associated with insulin resistance and is associated with an increased risk for developing diabetes and cardiovascular diseases.[1,2] The prevalence of diabetes follows the prevalence of obesity with a 10-year lag.[3] Obesity, diabetes and cardiovascular diseases are also expensive to the healthcare system.[4,5] Thus, finding a solution to the obesity epidemic in an accelerated and cost-efficient manner has gained increasing importance.

The traditional animal model for the study of obesity has been a rodent, primarily mice or rats. These animals have life spans measured in years. Their genetic manipulations using knockdown, knockout and knock-in strategies are labor- and time-intensive. Research using rodents and higher animals are regulated by Animal Care and Use Committees. Although the field of obesity has learned much from rodent research and will certainly learn much more in the future, a clear need for a less expensive and more time-efficient model for initial high-throughput screening and detailed mechanistic studies has emerged. Using a *Caenorhabditis elegans* (*C. elegans*) model will reduce the number of rodents or higher animals required, and serve as an intermediate step before moving to higher animal models.

C. elegans is a small nematode about 1 mm in length when it reaches adulthood. This is a eukaryotic, multi-organ animal for which research approval by Animal Care and Use Committees is not required. Wild-type *C. elegans* (N2) has a lifespan of about 21 days and reaches adulthood within 38–46 h after hatching. *C. elegans* has a generation time of 3 days and produces large broods of 300 progeny per hermaphrodite. This rapid growth and quick turnover allows rapid screening protocols looking for bioactive compounds. *C. elegans* grows on agar plates and uses non-pathogenic *Escherichia coli* (*E. coli*, OP50) as a standard food. *C. elegans* is the first animal to have its genome completely sequenced and more than 65% of the genes relating to human disease are conserved in *C. elegans*.[6,7] Genetic manipulations promote the evaluation, facilitate the acquisition of mechanistic information and define the behavioral aspects of obesity. Many lipid biosynthetic and catalytic enzymes are present in the epithelial cells of the cuticle and the intestinal cells. *C. elegans* stores fat mainly within the hypodermal and the intestinal cells, which can be stained with lipid affinity dyes such as Nile Red,[8] Sudan black and Oil Red O, and quantified through the transparent body by measuring the intensity of the accumulated dye.[9–11] Although fat is most

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frequently measured by the fluorescence intensity of lipid affinity dyes imaged through the transparent body of the worms, fat has been measured in *C. elegans* using biochemical assays, fat uptake, rate of *de novo* fat synthesis, fat oxidation and other methods.[9] Analysis of the disruption of the expression of each of the ~20 000 genes can be used to evaluate a loss of function mutant phenotype. A search has been made for the genes involved in normal fat storage in *C. elegans* using gene deletions and genome-wide RNA interference technologies.[12] Over 300 genes have been shown to cause a reduction in body fat when inactivated, and inactivation of more than 100 genes has shown an increased fat storage.[9]

Compared with a rodent model, the small size, the short lifespan, the quick turn over, the complete genetic information and their easy maintenance in the laboratory make *C. elegans* a valuable animal model while greatly reducing the time and costs required to obtain answers to research questions. The National Institutes of Health encourages the use of *C. elegans* by funding the Caenorhabditis Genetics Center (CGC) since 1978 through the University of Michigan and Columbia University, and since 1992 at the University of Minnesota. The CGC requests that all genetic mutations made using *C. elegans* obtained from the CGC be shared. This allows one to obtain specific strains quickly. Only recently has the CGC required a nominal fee for a partial cost recovery. This article will review the information that shows the insights gained into obesity physiology and behavior through research using the *C. elegans* model. We hope that this review will stimulate more interest in the use of the *C. elegans* model. We believe that the *C. elegans* model can serve as an intermediate whole-animal model between *in vitro* methods and *in vivo* approaches in a rodent model.

It should be pointed out that *C. elegans* has peculiarities that need to be taken into account when using it as a model for studying obesity. First, *C. elegans* lives on a diet of nontoxogenic *E coli*. The strain of *E coli* can modify the fatty acid composition and the staining characteristics of the body fat. This must be kept in mind and the same strain of *E. coli* should be used as food for *C. elegans* in a series of experiments in which body fat is an endpoint.[13] Second, during times of limited food or other situations in which conditions are unfavorable for growth such as overcrowding, the *C. elegans* produces a pheromone that induces the larvae to enter a dauer stage. Dauers contain large amounts of fat, a thin body and do not age, allowing them to endure adverse conditions for up to several months. *C. elegans* enters its life cycle again when conditions are favorable for growth.[14] Thus, studies of obesity using *C. elegans* should be conducted exclusively during the life cycle.

Initiation of obesity research with C. elegans

The genome sequence of *C. elegans* was described in 1998 followed by the discovery that gene function is preserved for important physiological variables.[6] Fatty acid transporters transport long-chain fatty acids intra-cellulary across the cell plasma membrane. Humans have six fatty acid transport proteins, five homologs of which have been identified in the mouse, whereas two fatty acid transport proteins in *C. elegans* (CeFATPa and CeFATPb) have homologs in higher animals. Overexpression of CeFATPb in human COS cells has been shown to increase fat deposition, determined by BODIPY staining, indicating a homologous function.[10] Reports of the conservation of proteins important to the physiology of obesity from nematodes to humans started to appear early after the sequencing of the *C. elegans* genome. NHR-49 in *C. elegans* has been shown to be the human homolog of peroxisome proliferator-activated receptor-α (PPARα).[15]

C. elegans *regulation of feeding*

The *C. elegans* model has been used to show the effect of calorie restriction for increasing lifespan. Pharyngeal movements enable *C. elegans* to pump food into its body and feed. The *eat-2* mutant is associated with a decreased pumping rate owing to a mutation of the pharyngeal nicotinic acetylcholine receptor sub-unit, and is associated with an increased lifespan due to reduced ingestion of nutrients.[16] The pumping rate is not always directly related to fat stores. Serotonin increases the pumping rate and decreases fat stores, whereas dauer formation-7 gene (*daf-7*) and transforming growth factor-β (TGF-β) mutants decrease the pumping rate and increase fat stores.[17] These findings show that the nervous system in *C. elegans* regulates the fate of nutrients and not feeding alone.[18]

Studying satiety in C. elegans

Quiescent behavior with cessation of food intake in *C. elegans* has been equated by You *et al*. to satiety in higher species.[13] In general, the quiescence is induced by high-quality food, requires nutritional signals from the intestine and depends on a prior feeding history, as re-feeding after fasting evokes quiescence as well. The quiescence is regulated by the dauer formation-2 gene (*daf-2*) in the insulin signaling pathway and the *daf-7* gene in the TGF-β signaling pathway. The *daf-2* mutant has a shorter quiescence than the wild-type, suggesting involvement of the insulin receptor. This quiescence is missing in *daf-7* mutants, showing that the homolog of the human TGF-β pathway is involved.[19] Expression of the wild-type *daf-7* gene in the pair of amphid sensilla chemosensory neurons (ASI) of *daf-7*-mutant *C. elegans*, however, completely rescues the quiescence defect. Thus, the quiescence after fasting and re-feeding appears to be mediated by *daf-7*. The egg laying defective (*egl-4*) gene codes for a cyclic-GMP-dependent protein kinase needed for olfaction adaptation and also controls the quiescence response to food intake. As occurs with replacement of the DAF-7 gene product in *daf-7* mutants, the EGL-4 or protein kinase-G also restores quiescence when replaced

in the pair of amphid sensilla chemosensory neurons of *elg-4* mutants. The *eat-2* mutant that pumps food at 15% of the pumping rate of wild-type animals has decreased quiescence. The *act-5* gene encodes a microvillus-specific actin required for absorbing nutrients from the intestine, and an *act-5* mutation decreases quiescence.[19] The *elg-3* or *elg-21* gene codes for proprotein convertase (PC1/3) or carboxypeptidase (CPA), respectively. Mutations of *egl-3* and *elg-21* eliminate most peptide signals and abolish the quiescence, suggesting a dependence of quiescence on the signaling of the PC1/3 and/or CPA protein(s). The *unc-31* gene codes for the calcium-dependent activator protein for secretion, which functions in synapses to secrete protein. Mutation of *unc-31* also abolishes the quiescence.

A complex eating behavior separate from quiescence and satiety has been shown in *C. elegans*. *C. elegans* engage in sophisticated food-related behaviors such as social feeding. The decision to eat alone or with others is determined by a homolog of the neuropeptide-Y receptor in *C. elegans*.[20]

The insulin signaling pathway

The next area of research linking the study of obesity to *C. elegans* involved showing that many components of the insulin signaling pathways are conserved from nematodes to humans.[21] *C. elegans daf-2* has been determined to encode 35, 34 or 33% identical proteins to the human insulin receptor, the human insulin-like growth factor-1 receptor or the human insulin receptor-related receptor, respectively. *daf-2* mutations shift the metabolism to increased fat and glycogen production. In humans, an insulin receptor mutation in a morbidly obese 14-year-old patient has been confirmed to be identical to the *C. elegans daf-2* mutation and shows a similar phenotype. *C. elegans* AGEing-related gene-1 (*age-1*) is the homolog of human phosphatidylinositol-3-hydroxy kinase and is the signaling molecule downstream from the insulin receptor.[22] DAF-18 inhibits the *age-1* downstream protein PDK-1 which phosphorylates the SGK-1/*akt-1/-2* complex that acts as an inhibitor of *daf-16*. *C. elegans* DAF-16 has a central role in mediating the downstream insulin signaling pathways and is the major target of the *daf-2* pathway. *daf-16* codes for the Forkhead family of transcription factors (FOXO). A parallel pathway, *daf-7*, is the homolog of TGF-β and a parallel insulin signaling pathway, which is also able to increase glucose transport and fatty acid synthesis (Figure 1). Both *daf-2* and *age-1* suppress *daf-16* through the SGK-1/*akt-1/-2* complex, which is the homolog of human serine/threonine protein kinase (AKT/PKB) and regulates multiple cellular process as well as glucose metabolism. Consequently, the metabolic effects of *daf-2* and *age-1* mutations are suppressed by *daf-16* mutations. Encoding the TGF-β, the *daf-7* gene mediates a parallel pathway to the insulin-like signaling pathway in *C. elegans*. Mutations of *daf-7* or *daf-3* show that the two genes negatively regulate each other to control glucose metabolism, and that *daf-3* is suppressed by *daf-7* in a manner similar to the suppression of *daf-16* by *daf-2*.[23–25]

The insulin signaling pathway has also been shown to be involved with female fertility. In *C. elegans*, mutations in the *daf-2* and *age-1* genes reduce fertility.[26] Reduced fertility, increased food intake, obesity and leptin resistance characterize mice lacking insulin receptor substrate-2. Burks *et al.*[27] have suggested that these observations from animal models may have implications for polycystic ovary disease in humans, where obesity, insulin resistance, infertility and leptin resistance co-exist.

The *C. elegans daf-2* gene encodes the homolog of the mammalian insulin receptor, which has preserved ligand-binding and tyrosine kinase domains. Deletion of *daf-2* increases lifespan in a manner similar to caloric restriction, with a decrease in insulin signal transduction and a decrease in fertility.[28] DAF-2 is also the homolog of the insulin-like growth factor-1 receptor (IGF-1). Low levels of insulin-like growth factor-1 or congenital insulin-like growth factor-1 deficiency prolong lifespan in rodents or in humans, respectively.[29] Conversely, insulin-like growth factor-1

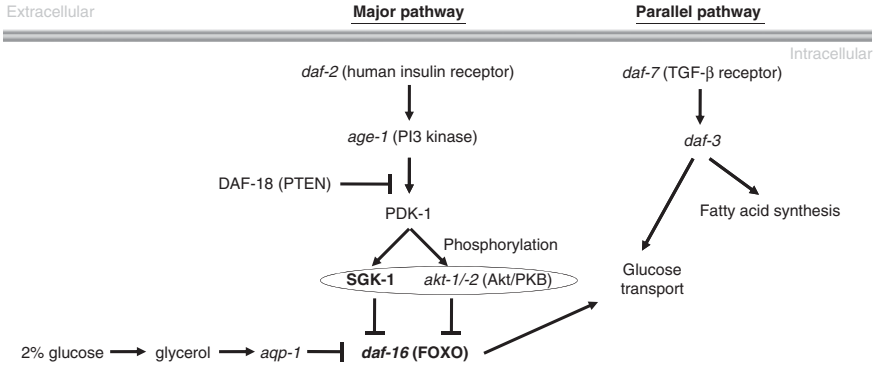

Figure 1 Summary of the insulin signaling pathway in *C. elegans*. *daf-16* is the major target of the *daf-2* pathway. DAF-18 inhibits PDK-1, which phosphorylates the SGK-1/*akt-1/-2* complex. A 2% glucose diet may activate the glycerol channel and *aqp-1*, which in turn inhibits *daf-16*. *daf-7* is the homolog of TGF-β and a parallel insulin signaling pathway, which is as well able to increase glucose transport and fatty acid synthesis. An SGK-1/*akt-1/-2*-independent pathway was omitted from this graphical representation. The human homologs are described in parentheses. Activations are indicated by '→' and inhibitions are indicated by '⊥'. *aqp*, aquaporin gene; *daf-16*, dauer formation-16 gene; TGF-β, transforming growth factor-β.

excess, as in acromegaly, is associated with a reduced lifespan in humans. *C. elegans* with mutations of *daf-2* live three times longer than wild-type N2.[29,30] In fact, mutations in most of the insulin signaling pathway genes *daf-2* (insulin receptor), *age-1* (phosphatidylinositol-3-hydroxy kinase) or *akt-1/2* (AKT) all increase lifespan more than 50%. As *daf-2* mutations are associated with increased fat deposition, there seems to be no inverse connection of longevity and fat mass in *C. elegans* as there appears to be in humans.[31]

C. elegans responds to a 2% glucose diet with a shortened lifespan, and the authors postulate a connection with insulin resistance.[32] Mutations of *daf-2* increase lifespan by removing the inhibition of the FOXO transcription factor, DAF-16, and the heat-shock transcription factor (HSF-1). Thus, it would be expected that inhibitors of DAF-16 and HSF-1 would decrease lifespan. A 2% glucose diet reduces lifespan in *C. elegans* by inhibiting DAF-16 and HSF-1.[32] A downstream aquaporin gene (*aqp-1*) encodes for a glycerol channel, which is downregulated by glucose feeding. Consequently, *aqp-1* downregulation increases the glycerol and eventually shortens lifespan (Figure 1). Aquaporin genes 7 (*AQP7*) and 9 (*AQP9*) in mammals have features common to *C. elegans aqp-1*, and have been suggested to be its homolog.[32] The mRNA of AQP7 and AQP9 in mammals decreases with insulin secretion, and AQP7-knockout mice show insulin resistance and obesity.[33] Expression of the *AQP7* gene is reduced in obese women, and polymorphisms of this gene are associated with obesity and diabetes.[34,35] Not only may a decrease in sugar intake and a reduction in glycemic index improve insulin resistance in humans, but feeding *C. elegans* with glucose may also be able to function as an animal model of insulin resistance based upon the cited studies with aquaporin. For example, an elevated glycemic index with a 2% glucose diet may offer a possibility to observe an increase in lifespan of *C. elegans* using drugs that effectively reduce insulin resistance.

Serotonin

Tryptophan hydroxylase (TPH-1) is the enzyme responsible for serotonin synthesis. In humans, serotonin receptor agonists like fenfluramine and serotonin reuptake inhibitors such as fluoxetine reduce appetite and promote body fat reduction.[17,36] Mice lacking serotonin eat more, gain weight and develop diabetes. In *C. elegans*, serotonin feeding causes a reduction in fat and an increased feeding rate. *C. elegans* with deletion of *tph-1* lacks serotonin, accumulates fat, has reduced egg laying, decreased feeding and a diminished metabolic rate, but lives longer. This metabolic dysregulation is due, in part, to the downregulation of TGF-β and insulin-like neuroendocrine signals. The DAF-7 (TGF-β) pathway has a serotonin input in pair of amphid sensilla chemosensory neurons.[17] Conversely, antidepressants that are serotonin antagonists such as mianserin and methiothepin reduce lifespan and increase body fat in *C. elegans* and humans.[37] *daf-16(mgDf47)* mutation, however, did not affect 5-HT-induced fat reduction, indicating that fat loss in *C. elegans* is independent of insulin signaling, but is related to an increase in the β-oxidation of fat and an increase in energy expenditure.[38]

Evaluating single gene mutations associated with obesity

Obesity can be related to a disorder of ciliated cells, as a defect at the basal body that impairs twitching and mobility of the ciliary pilus is thought to be responsible for Bardet–Biedl syndrome (BBS). The first description of BBS was in 1865 in a 7-year-old short and obese girl who had a phenotype that included a visual defect owing to retinal degeneration associated with polydactyly, renal malformations and learning disabilities.[39] Several genes have been associated with BBS, and the *BBS-8* gene has been shown to encode a protein with a prokaryotic domain (pilF), which is involved in pilus formation. *C. elegans* has human BBS homologs that are expressed exclusively in ciliated neurons containing regulatory factor-X (RFX). RFX is a transcription factor that modulates the expression of genes associated with ciliogenesis and intra-flagellar transport.[40] Each *C. elegans* BBS ortholog is regulated by the same X box located about 100 bp pairs upstream from the start codon. The transcription factor regulating the X boxes in *C. elegans* is DAF-19, a member of the RFX protein family required for cilia formation. Moreover, a human GTP-binding protein of the Ras super-family, ADP-ribosylation factors-like proteins (ARL6), has also been associated with the BBS. The *C. elegans osm-5* gene is an ortholog of the mouse *Tg737* gene that causes polycystic kidney disease and is restricted to ciliated cells.[40] Cilia are also needed to sense motion. Associated with situs inversus, sinusitis and bronchiectasis, Kartagener's syndrome is because of a ciliary defect that interferes with sensing fluid flow in the embryo needed to produce left–right asymmetry. Cilia are needed for reproduction as well. *C. elegans lov-1* and *pkd-2* are homologs for the human genes *PKD1* and *PKD2*, respectively. These two genes are essential for male mating behavior and are restricted to the cilia.[41] Likewise, the *C. elegans* ARL6 homolog *arl-6* is restricted to ciliated cells and is involved in ciliary transport.[42]

The reason for the association of obesity with BBS has been elusive. However, it has been discovered that *tub-1* is a single gene mutation, which induces obesity that crosses species and results in changes consistent with Tubby mice, human BBS and *C. elegans* through increases in fat storage and decreases in fat oxidation. *C. elegans* with *tub-1* mutation has defects in ciliated neuronal function and obesity.[43] Mutation of the 3-ketoacyl-coA thiolase (*kat-1*) gene is associated with increased fat storage in tissue owing to impairment of fat oxidation, and decreased *kat-1* activity appears to be responsible for the increased fat accumulation in *tub-1*-mutant *C. elegans*.[44] Specifically, *tub-1* interacts with a Rab-GTPase-activating protein (RBG-3) to regulate fat storage by controlling receptor or sensory molecule degradation in neurons, and by upregulating the activity of a small

Rab-GTPase (RAB-7) to mediate an endocytic pathway that increases fat storage.[45] Taken together, mutations of *tub-1* result in increased fat storage in *C. elegans* through a decrease in KAT-1 activity, which impairs fat oxidation and through an increase in fat storage by enhanced endocytosis.[44]

The human adipose gene (Adp) dose dependently suppresses obesity. The othologs of the Adp gene are conserved in other species as well as in *C. elegans* (Y73E7A.9), in flies (*adpr-1*) and in mice (Wdtc1). *C. elegans* treated by RNA interference to the Y73E7A.9 showed an increase in fat deposition, which is correlated with the effect of human Adp, which has an anti-obesity function.[46] Studies of yeast and mice suggest that an *adpr-1* mutation appears to exert its effect by binding to histones and histone deacetylase-3, which inhibits PPARγ in fat tissue and regulates fat accumulation.[46]

Studying adipogenesis and lipogenesis in C. elegans

C. elegans obtains most of its fat directly from its bacterial diet, but is also capable of fat synthesis. *C. elegans* synthesizes palmitic acid (16:0) through acetyl Co-A carboxylase (ACC) and fatty acid synthetase (FAS). Palmitic acid can then be integrated into triglycerides or be modified by fatty acid elongases and desaturases into long-chain polyunsaturated fatty acids (Figure 2). Branched-chain fatty acids are synthesized through bacteria degradation from valine to isobutyryl-CoA[25,47] (Figure 2). Wild-type *C. elegans* synthesize 7% of their palmitic acid, but the rest is absorbed from the bacterial diet.[48] Twenty percent of C18 monounsaturated fatty acids and polyunsaturated fatty acids are derived from *de novo* synthesis. Almost all of the branched-chain fatty acids are derived from *de novo* synthesis, as *E. coli* do not contain any of them. FAT-5, FAT-6 and FAT-7 are three Δ9 desaturases important in producing monounsaturated fatty acids (Figure 2).[18]

Adipogenesis and lipogenesis in *C. elegans* involve PPARs, sterol-binding element proteins (SREBPs) and Kruppel-like factors. The Kruppel-like factors are an important family of zinc-finger DNA-binding proteins that are involved in gene activation, repression or both. All of the Kruppel-like factors bind to similar DNA sequences. Eight members of the family are key components of the transcription network responsible for adipocyte differentiation. They bind to the DNA CCAAT gene sequence (C/EBPs) areas where the binding of PPARs and proteins is located.[49]

Located in the endoplasmic reticulum and regulated by insulin and glucagon, SREBPs govern lipid synthesis in humans. Insulin causes increased transcription of SREBP-1, which binds to its own promoter and initiates a feed-forward amplification of the transcriptional response. This consequence of insulin signaling stimulates lipogenesis and glycolysis through the expression of FAS, ACC, steroyl-CoA desaturase and glucokinase. At the same time, it represses genes encoding phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and insulin receptor substrate-2. Through its counter-regulatory effect on insulin's action, glucagon represses the SREBP-1 in the course of an accumulation of cAMP.[50] These processes are conducted in *C. elegans* by *sbp-1*, which is the homolog of human SREBP-1. *sbp-1* is expressed in the intestine. When *C. elegans* is exposed to glucose, *sbp-1* causes increased fat accumulation. With RNA interference downregulation, the animals show reductions in body size, fat storage and egg-laying.

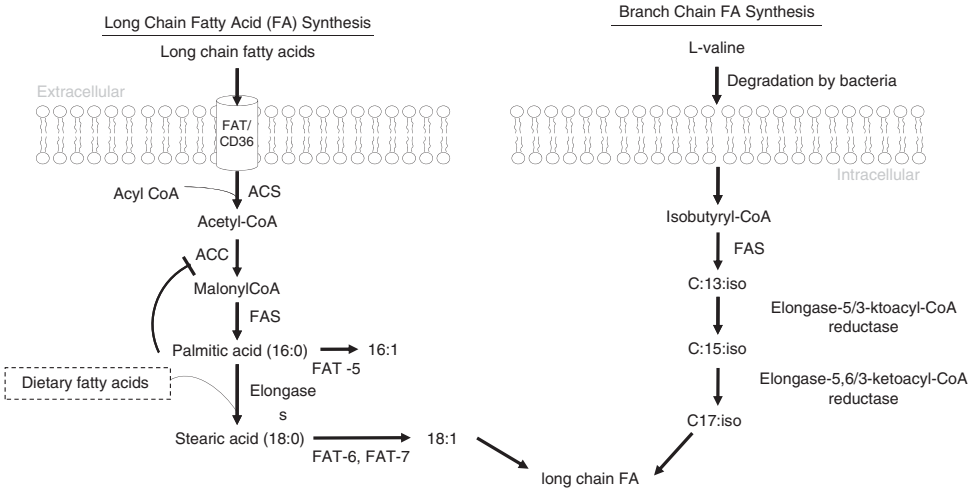

Figure 2 Summary of fatty acid synthesis. Long-chain fatty acid synthesis requires the membrane fatty acid transporter cluster differentiation transport protein (CD36) to be transported intra-cellularly, binding to acyl-CoA to be converted to acetyl-CoA by ACS. Through ACC, which is negatively regulated by palmitic acid, acetyl-CoA is converted to malonyl-CoA. The palmitic acid will then be converted to stearic acid with elongases or 16:1 with a desaturase. The stearic acid will be converted to 18:1 by desaturases. The branched-chain FAs are synthesized from isobutyryl-CoA that is depredated from valine by bacteria. It further requires FAS and elongases. Eventually, both 18:1 from the long-chain FA pathway or C17:iso from the branched-chain FA pathway will be converted into long-chain FAs. Activations are indicated by '→' and inhibitions are indicated by '⊥'. ACC, acetyl-CoA carboxylase; ACS, acetyl CoA synthetase; CD36, cluster differentiation protein; FA, fatty acid; FAS, fatty acid synthetase; FAT-5, Δ9 desaturase; FAT-6, Δ9 desaturase; FAT-7, Δ9 desaturase.

Normal egg laying and expression of the starvation-inducible gene *asc-2* can be restored on exposure to polyunsaturated fatty acids. This suggests that both humans and *C. elegans* regulate the amount and composition of body fat, and the response to starvation in a similar manner.[51]

C. elegans *and fat oxidation*

Adiposity depends on fat oxidation as much as it does on fat synthesis. The Δ9 desaturase double mutants of *fat-6* and *fat-7* decrease fat synthesis and activate genes associated with mitochondrial β-oxidation. Mammalian Δ9 desaturase-1 (SCD-1) is a potential therapeutic target for the treatment of obesity on both a wild-type and a leptin-deficient background.[52] SCD-1-knockout rodents show dramatic reductions in body fat. SCD-1 deficiency increases β-oxidation and decreases lipogenesis in liver and muscles. The mechanism is thought to be that SCD-1 inhibition results in an accumulation of acetyl-CoA, causing inhibition of the rate-limiting enzyme of fatty acid synthesis, ACC. The consequence of ACC inhibition is a reduction in malonyl-CoA, which releases carnitine palmitoyltransferase (CPT) from inhibition. This causes the transport of fatty acids into the mitochondria for β-oxidation (Figure 3). Both Δ9 desaturases and the SCD-1 are transcriptionally regulated by SEBPs in *C. elegans*.[25,53]

Conversely, the high-fat stores in nuclear hormone receptor (*nhr*) mutants correlate with a decreased expression of the mitochondrial β-oxidation genes. Mammalian nuclear hormone receptors (NHRs) such as the liver X receptor, the farnesoid X receptor and PPARs control energy metabolism. Deletion of the *C. elegans* NHR gene *nhr-49* results in an elevated fat content and a shortened lifespan. The *nhr-49* influences the expression of at least 13 genes involved in energy metabolism. The high-fat phenotype is because of a reduction in the expression of genes involved in fatty acid oxidation, and the shortened lifespan is due to impaired expression of steroyl-CoA desaturase. The activity of *nhr-49* is most similar to mammalian PPARs and can provide insights into how the NHR governs fat metabolism.[54] Serotonin is also associated with increased fat oxidation in *C. elegans*. A neural serotonin channel (MOD-1) and a G-protein-coupled receptor (SER-6) ultimately increase fat oxidation in peripheral tissues.[18]

Lipolysis and other attributes of fat metabolism in C. elegans

Lipin-1 (LPIN-1) expression is associated with insulin sensitivity and oxygen consumption in humans. These changes correlate with lipid oxidation, lipid uptake and lipolysis. In *C. elegans* the LPIN-1 mammalian homolog *lpin-1* is involved in the maintenance of the nuclear envelope. Downregulation

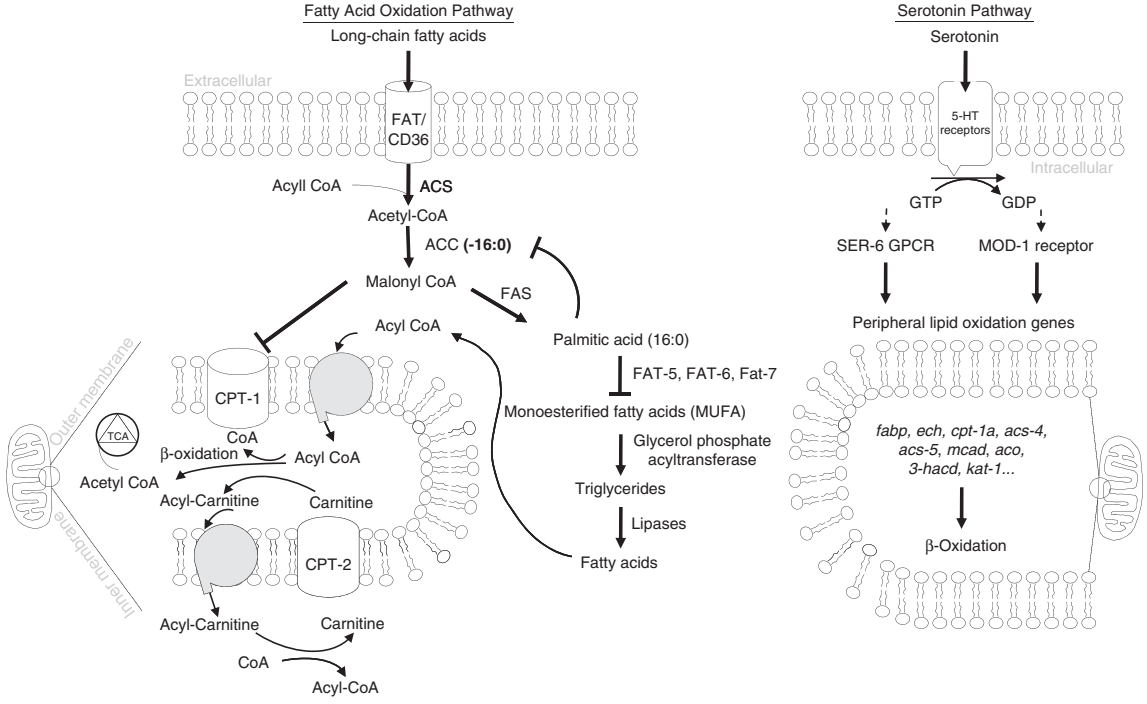

Figure 3 Summary of fatty acid oxidation. Metabolism of long-chain fatty acids first requires the membrane fatty acid transporter cluster differentiation transport protein (CD36) to be transported intra-cellularly. The fatty acids need to bind to acyl-CoA forming acetyl-CoA through the action of the enzyme ACS. The fatty acid must be bound to carnitine through CPs (CPT-1 and CPT-2) to move across the two mitochondrial membranes and enter the tricarboxylic acid cycle (TCA cycle) as acetyl-CoA or the electron transport chain for energy production. Synthesized by ACC, malonyl-CoA downregulates CPT-1 based on the need for lipids synthesis. The key enzyme CPT-1 neither stimulates fat oxidation in the absence of ACS or ACC, nor functions without CD36. The serotonin-mediated fatty acid oxidation is through peripheral and mitochondrial lipid oxidation genes. Activations are indicated by '→' and inhibitions are indicated by '⊥'. ACC, acetyl CoA carboxylase; ACS, acetyl CoA synthetase; CP, carnitine palmitoyltranferases (CPT-1, CPT-2); CD36, cluster differentiation protein; FAS, fatty acid synthetase; FAT-5, Δ9 desaturase; FAT-6, Δ9 desaturase; FAT-7, Δ9 desaturase; GPCR, G-protein-coupled receptor; MOD-1, modulation of locomotion-defective: a 5-HT-gated chloride channel.

of *lpin-1* causes a breakdown of the nuclear envelope, abnormal chromosomal segregation and an irregular nuclear morphology. This suggests that *lipin-1* has a role in the lipid synthesis of the nuclear envelope in *C. elegans*.[55]

When *C. elegans* larvae enter the dauer stage of hypometabolism, they accumulate fat. Expression of the catalytic subunit of AMP-activated kinase-α-2 (AAK-2) is required for life-extension and fat accumulation in *daf-2*-mutant animals. Impaired expression of the homologs of human adipose triglyceride lipase (AGTL), but not hormone-sensitive lipase (HSL), suppressed the effect of *aak-2* on longevity and fat storage in *daf-2*-mutant *C. elegans*. This suggests that down-regulation of lipolysis is required during the dauer phase to sustain life and slow down energy expenditure. 5′-adenosine monophosphate-activated protein kinase phosphorylates and thus inhibits ATGL-1, slowing the mobilization of fat to insure just an adequate supply of nutrients and a controlled release of energy needed for an increased lifespan.[56]

4-Hydroxynonenal is the product of peroxidation of *n*-6 polyunsaturated fatty acids, which has been implicated in lipofuscin formation. Disruption of 4-hydroxynonenal conjugation or oxidation leads to the accumulation of fat in *C. elegans*. The mechanism of fat accumulation is an elevation of malonyl-CoA, which increases fatty acid synthesis and inhibits β-oxidation. The same effect of 4-hydroxynonenal to increase body fat has been shown in mice.[57]

Effect of intestinal peptide transport on fat accumulation in C. elegans

The proton-coupled transport of di- and tripeptides from the gut lumen into intestinal epithelial cells is mediated by the intestinal peptide transporter PEPT-1, which is interdependent with the Na^+/H^+ exchanger NHX-2.[58] Loss of PEPT-1 reduces the H^+ influx and therefore is suggested to increase the intracellular pH. This alkaline intracellular environment results in a decrease in fat synthesis and an increase in fatty acid absorption, resulting in body fat accumulation. Loss of NHX-2 results in a loss of ability to reduce the intracellular pH. This acid environment reduces fatty acid uptake and produces a lean phenotype.[59]

C. elegans *as a model for studying obesity in hypoxic illnesses*

People suffering from illnesses such as chronic obstructive pulmonary disease, sleep apnea and asthma are known to be at risk for hyper-lipidemia and fat accumulation.[60] Populations living at high altitude with reduced oxygen concentrations are shorter, broader in stature and more likely to suffer from obesity.[61] *C. elegans* adapts to hypoxia through activation of the hypoxia-inducible factor, which leads to the transcription of a number of genes, including *sbp-1*, which cause accumulation of body fat.[62]

Obesity therapeutics

C. elegans exposed to compounds that can potentially increase or decrease body weight can identify active obesity interventions.[63] Atypical antipsychotic medications are well known to result in weight gain when used clinically in humans.[64] Exposure of *C. elegans* to either clozapine or olanzipine results in fat accumulation that is apparent after only 48 h.[63] Olanzapine produces a dose-dependent increase in Nile Red accumulation in fat tissue, which reaches statistical significance at dosage of 100 μM and above.[63] Quetiapine induces fat accumulation also, but less than that with olanzapine or clozapine. Fluphenazine was not associated with fat accumulations in *C. elegans*. These findings are consistent with what is seen when patients are treated with these same drugs.[63]

C. elegans can also be used for screening compounds that cause weight loss in addition to those that cause weight gain.[65] Dietary fiber with a high content of resistant starch is known to produce weight loss in humans. We have shown a similar effect in *C. elegans*. *C. elegans* fed endogenous compounds from the filtered cecal contents of amylose starch (fermentable)-fed mice, but not from amylopectin starch (digestible)-fed mice, and the short-chain fatty acids (fermentation products of resistant starch) butyrate, propionate and acetate show reduced Nile Red staining consistent with a reduction in intestinal fat deposition. This effect is most robust in the *C. elegans* fed butyrate (Figure 4). These studies are consistent with resistant starch being fermented in the gut to butyrate, which is known to stimulate the L-cells to release glucagon-like peptide-1 and peptide-YY, which are known to reduce food intake and body fat.[65]

Figure 4 Butyrate treatment reduced the Nile Red staining of the intestinal fat deposition in *C. elegans*. (**a**) The arrows indicate intestinal fat deposition in the control animals that received only *E. coli*. (**b** and **c**) The fluorescence intensity of Nile Red-positive intestinal fat deposition in butyrate (300 μM)-treated animals was significantly reduced to 63.6% compared with that in the control group (black bar, $n=3$, 5 days, $P<0.001$) (This figure was published in *J Agric Food Chem. 2010, **58**, 4744–4748*. A copyright permission was obtained from the American Chemical Society, Washington DC.).

Conclusions

It is anticipated that much more about obesity will be learned from *C. elegans* in the future just as much has been learned from rodents in the past. *C. elegans* is a tiny animal that is not controlled by Institutional Animal Care and Use Committees as are rodents and higher animal models. *C. elegans* conserves 65% of the human disease-related genes, has a short lifespan and reproduces quickly. Despite the continued need of rodent experiments, these attributes make studies of *C. elegans* an excellent transition from *in vitro* methods into higher animal models, then into humans in a more efficient and much less expensive manner. It is hoped that this review will stimulate the application of the *C. elegans* experimental model to obesity research to a greater degree, and that doing so will advance the field more rapidly.

Conflict of interest

The authors declare no conflict of interest.

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