## Title

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## Authors

Lemieux, George A
Ashrafi, Kaveh

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# Investigating connections between metabolism, longevity, and behavior in C. elegans

**George A Lemieux, PhD** and **Kaveh Ashrafi, PhD**

UCSF, San Francisco, CA

Kaveh Ashrafi: kaveh.ashrafi@ucsf.edu

## Abstract

An overview of C. elegans as an experimental organism for studying energy balance is presented. Some of the unresolved questions that complicate the interpretation of lipid measurements from C. elegans are highlighted. We review studies that show both lipid synthesis and breakdown pathways are activated and needed for the longevity of hermaphrodites that lack their germ lines. These findings illustrate the heterogeneity of triglyceride rich lipid particles in *C. elegans* and reveal specific lipid signals that promote longevity. Finally, we provide a brief overview of feeding behavioral responses of *C. elegans* to varying nutritional conditions and highlight an unanticipated metabolic pathway that allows for the incorporation of experience in feeding behavior.

### Keywords

*C. elegans*; lipids; germ-line; aging; feeding; yolk; satiety

## Advantages of an invertebrate experimental system for understanding energy balance

Having diverged from a common ancestor with mammals approximately a billion years ago, *C. elegans* has evolved distinct behaviors and organismal survival strategies, lacks certain easily identifiable orthologous molecules (e.g. leptin, PGC-1α) and specialized tissues (e.g. white or brown adipose tissues, macrophages) that are dominant themes in the mammalian metabolic biology literature. As such, *C. elegans* might seem to be a peculiar experimental model for studying metabolism, its regulators, and its consequences on physiology and behavior. A proposal to identify genes that regulate entry of *C. elegans* into an organism-specific, alternative developmental state induced by various nutrient and environmental stresses could easily be dismissed as unlikely to yield anything of relevance to human disease. However, it was genetic studies of just such a state, that identified components of TGF-β like and insulin-like signaling pathways and revealed that antagonism of a program under control of a FOXO-family transcription factor is a major consequence of insulin

signaling [1]. At the time of this discovery, insulin was one of the most intensively studied signal transduction pathways in history, yet investigations in other systems had failed to identify aspects of its signaling related to transcriptional control, a now well established and critical aspect of insulin signaling in mammals [2].

Among the reasons fundamental discoveries related to insulin signaling pathways were possible in *C. elegans*, despite lacking an obvious equivalent of the pancreas and blood in which sugar levels need to be regulated, are the experimental advantages of this organism. These include investigation of heritable phenotypes through genetic screening, the efficiency in determining the molecular bases of such phenotypes, and the experimental ease with which multiple mutations can be investigated relative to each other. While the relative simplicity of *C. elegans* is often viewed as a weakness, it is precisely this feature that helps illuminate conserved principles of energy balance and deconvolutes connections between processes previously considered disparate. Even at a time that technological advances make comparable approaches increasing feasible in other experimental systems, these decisive experimental advantages remain for *C. elegans*.

Here, we highlight several recent advances in linking metabolism to aging and behavior in *C. elegans* but also emphasize challenges in interpreting lipid phenotypes in this organism. Additional information pertaining to *C. elegans* fat and feeding regulatory pathways have appeared in several recent reviews [3–5].

## Overview of *C. elegans* energy balance pathways

In the hermaphroditic *C. elegans*, significant amounts of neutral lipids are seen in the intestinal and skin-like epidermal cells as well as oocytes and embryos within the germ line. *C. elegans* do not have a specialized adipose tissue or even a liver, nevertheless, many functions of these organs and associated molecular mechanisms are found in *C. elegans*. Its intestine, in addition to performing enterocytic functions, expresses many genes that perform liver-like functions including lipid synthesis and lipoprotein secretion [6]. The germ line receives nutrients in the form of lipid rich lipoprotein particles, namely yolk, that are synthesized in the intestine, secreted into the body cavity, and taken up by the developing oocytes through receptor mediated endocytosis [7,8]. Although the *C. elegans* intestine has received most of the attention when visualizing lipids in intact animals, the triglyceride depots in the skin-like epidermal cells may be more akin in function to those stored in mammalian adipocytes.

In spite of significant differences, many known metabolism genes are conserved between *C. elegans* and humans including lipid, sugar and amino acid synthesis and catabolism genes, lipogenesis regulators such as SREBP, and nutrient sensing kinase complexes such as TOR and AMPK [3]. Additional similarities include neuroendocrine pathways such as insulin and steroid hormone signaling as well as neuromodulators, for example, serotonin and dopamine, and feeding regulatory neuropeptides such as oxytocin and opioid-like peptides [4,5,9]. As in mammals, the nervous system of *C. elegans* integrates both external and internal cues to modulate behavior but also peripheral physiology via hormone secretion

[5,10,11]. In turn, inputs from the periphery modulate feeding behavior in *C. elegans* [12,13].

Investigation of *C. elegans* lipid metabolic pathways has identified molecular mechanisms that have proved to be conserved in mammals, for example, the relationship between the SREBP transcription factor and the mediator complex [14] as well as the role of SREBP in controlling aspects of 1-carbon metabolism [15]. Investigation of other conserved regulators of organismal energy balance, for instance that of serotonin signaling and the *C. elegans* counterparts of the genes that underlie the obesity associated Bardet-Biedl syndrome, have led to different conclusions that those coming from mammalian studies (TEXT BOX 1). Whether these discrepancies highlight species differences or suggest a need for re-evaluating the mammalian paradigms remains to be determined. Finally, some of the pharmacological tools that affect fat in mammals are similarly effective in changing lipid content of *C. elegans* and this organism has been used to identify novel small molecules that alter fat through mechanism that are conserved in mammals [16].

## Methods and considerations when interpreting lipid phenotypes in *C. elegans*

The transparency and small size of *C. elegans* allows for visualization of lipid depots in all tissues of intact animals. Vital dyes, fixed staining methods, and label free Raman microscopy techniques have been employed to examine lipid depots of *C. elegans* [17–24]. Biochemical techniques have also been applied to profile individual fatty acid species, total lipid content, and investigate rates of lipid uptake, *de novo* fatty acid synthesis, and fat oxidation from *C. elegans* [25,26]. Regardless of the specific methods used, several basic considerations that have long been resolved in mammalian systems remain mostly unacknowledged in *C. elegans* (TEXT BOX 2). In our opinion, this has been the basis of a great deal of confusion in interpretation of lipid related measurements from *C. elegans.* Some or maybe even most triglycerides in *C. elegans* are unlikely to be in the service of somatic fat storage. This issue is illustrated below by the studies linking lipids to longevity in germ line deficient animals.

## Lipid metabolism as a link between germ line stem cells and longevity

In organisms ranging from fruit flies [27] to the women of the British aristocracy [28] longevity correlates with either reduced or delayed fertility. It is thought that the metabolic cost of reproductive maintenance comes at the expense of diminished somatic longevity. Understanding the mechanisms that link longevity and reproduction in *C. elegans* has implicated lipid metabolism as a key element.

The *C. elegans* reproductive system consists of cells of the somatic gonad as well as germ line stem cells and their descendants. In 1999 Hsin and Kenyon reported that ablating the precursor cells that specifically give rise to the *C. elegans* germ line results in a significant increase in longevity [29]. Similarly, animals with loss-of-function mutations in *glp-1*, which lack a functional germ line due to loss-of-function mutations in a LIN-12/Notch family receptor required for cell fate specification were found to be long-lived [30]. These mutant

animals are referred to as GSC(−) due to their germ line stem cell deficiency. The long lifespans of GSC(−) animals could not simply be attributed to the loss of fecundity since animals deficient in both the somatic gonad and the germ line, while sterile, were not long lived [29]. These observations lead to the notion that the noted changes in longevity are not simply metabolic trade-offs between reproduction and longevity, but instead are consequences of distinct, pro- and anti- longevity signals originate in various regions of the gonad. Ever since, the hunt has been on to identify these signals. While no specific signals from the gonad have yet been identified, a number of genes and transcriptional programs that function in somatic tissues have been reported to be required for this longevity program [31–37].

In 2008, Wang and colleagues noted a link between the longevity of GSC(−) animals and lipid metabolism [31], a finding that has since been bolstered by numerous additional molecular connections [32,34,35,37,38]. However, this relationship is far from simple: relative to wild type animals, the total fat content of GSC(−) animals has been reported to be increased anywhere from 20 to 400 percent as adults [18,34,37–40]. Consistent with this extensive triglyceride content, GSC(−), animals exhibit a DAF-16/FOXO transcription factor dependent increase in expression of fatty acid synthesis genes including increased expression of Δ9 fatty acyl CoA desaturases [35,38], conserved lipogenesis genes associated with the production of unsaturated fatty acids such as oleic acid [8]. Indeed, oleic acid supplementation is sufficient to bypass the requirement of desaturase genes in the longevity of GSC(−) animals [35]. It is worth considering that while the activities of a variety of lipogenesis genes are required for the enhanced longevity of GSC(−) animals [34,35,37,38], the genetic studies cannot easily ascertain whether the requirement for lipogenic programs in GSC(−) longevity is confined to the rise seen in these programs. In addition to the lipogenic programs, lipid catabolic pathways are also upregulated and required for longevity of GSC(−) animals [31,32,34,36,37]. For example, longevity of GSC(−) animals depends on fatty acid catabolic programs that are under the control NHR-49, a nuclear hormone receptor [34], or that of SKN-1, a Nrf family transcription factor [37]. The fat catabolism program of GSC(−) animals includes autophagy in epidermal and intestinal cells [32,36]. Accordingly, the longevity of GSC(−) animals depends on PHA-4, a FOXA family transcription factor, and HLH-30, the *C. elegans* ortholog of mammalian TFEB, transcription factors that broadly regulate autophagy programs [36]. Finally, a key effector that links fat catabolism and autophagy in GSC(−) animals is the lysosomal acid lipase encoded by *lipl-4*. Overexpression of *lipl-4* promotes longevity and induces autophagy in animals with intact germ lines [31,32]. Several acid lipases distinct from *lipl-4* are also regulated by HLH-30 and overexpression of a subset of these promotes longevity [74]. Together, analyses of the pathways required for longevity of GSC(−) animals suggests a number of feed-forward and feedback regulatory loops among the different lipolytic and lipogenic arms, although many of the precise connections remain to be elucidated (FIGURE 1). It is also currently unclear whether the lipogenic and lipolytic programs act on the very same or distinct pools of lipids.

These results raise several conundrums: *first*, extended lifespan has most often been associated with caloric restriction and conditions of leanness but GSC(−) are laden with lipids, and *second*, why components of both lipid synthesis and lipid breakdown pathways are simultaneously required for the longevity of GSC(−) mutants is not well understood.

One intriguing explanation offered has been that all fats are not equal and that the balance between beneficial and harmful lipids in GSC(−) animals is in favor of the beneficial variety [35]. Supporting this view, administration of oleic acid is sufficient to bypass the requirement for some of the lipid desaturation enzymes in the longevity of GSC(−) animals [35]. Another pro-longevity lipid identified in GSC(−) animals is oleoyl ethanolamide (OEA) [41]. The initial link between the longevity of GSC(−) animals and lipid metabolism emerged from identification of a lysosomal acid lipase, LIPL-4 [31]. OEA was identified in a metabolomic analysis of long-lived animals overexpressing *lipl-4* [41]. LBP-8, a lipid binding protein capable of binding OEA, was reported to function as a relay mechanism between OEA that is generated by lysosome localized LIPL-4 and NHR-80 [41], a nuclear localized transcription factor required for the longevity of GSC(−) animals [35]. Application of a hydrolytically stable analog of it to wild type animals enhances their mean lifespans by 14% [41].

A recent study pertaining to the origins of lipid accumulation in GSC(−) animals further illuminates the relationship of lipids in GSC(−) animals and their longevity. It is important to consider that the *C. elegans* intestine is the major site of yolk production (TEXT BOX 2). Germ line loss does not silence the program of yolk formation. Rather, it results in aberrant accumulation of yolk within the intestine as well as the body cavity of hermaphrodites [37]. In their study, Steinbaugh and colleagues provided compelling evidence that accumulation of yolk in GSC(−) animals provokes a compensatory fat catabolic response controlled by the transcription factor SKN-1 [37]. They found this compensatory response is orchestrated by SKN-1 resulting in upregulation of programs of lipid catabolism, proteostasis, stress response, and detoxification, which collectively promote longevity [37].

The relationship of yolk accumulation and longevity, however, remains complicated. For example, animals in which the receptor involved in yolk uptake by the oocytes, RME-2, is downregulated, have a normal lifespan [42], despite elevated yolk levels and activated SKN-1 [37]. One possibility is that in *rme-2* mutants yolk levels do not rise to that of GSC(−) animals and that this difference determines the extent of compensatory, pro-longevity responses. Over-expression of individual vitellogenins, protein components of yolk, does not compromise the longevity of otherwise wild-type animals, but interferes with the longevity of several mutants including GSC(−) animals [42]. Yet, inactivation of vitellogenins elevates intestinal fat content and extends the lifespan of animals with intact germ lines [42]. Finally, SKN-1 has recently been proposed to promote the partition of intestinal fat into yolk, which would place it both upstream and downstream of yolk production [43].

Despite many unanswered questions regarding the precise relationship of yolk and longevity, identification of yolk as a source of aberrant intestinal lipid accumulation highlights several issues. *First*, it is unclear whether the original hypothesis that various gonadal regions emanate specific longevity signals is still tenable. While the existing data certainly do not rule the existence of gonad-derived longevity signals, they offer a simpler explanation: longevity of GSC(−) arises from protective programs that are activated in the face of aberrant accumulation of yolk due to anatomical perturbations. *Second*, identification of OEA and dependency of SKN-1 activation on desaturation enzymes suggest that elevated

lipid content in the absence of specific lipid signals is insufficient for pro-longevity responses [42]. However, it remains to be seen whether activation of various transcriptional programs that collectively promote longevity reflect the presence of beneficial lipids or that of lipid-based stress signals specifically when lipids cannot be adequately packaged into storage droplets. *Third*, while simultaneous upregulation of fat synthesis and breakdown pathways may appear paradoxical, such a scenario is exactly what would be expected if conditions warrant conversion of lipids from one neutral lipid rich entity into another. Together, these issues highlight the need for better understanding the distinct functions and relationship between various neutral lipid-rich entities in *C. elegans*. GSC(−) animals also illustrate the utility of methods that selectively probe of minor lipid compartments since such a strategy initially led the link between LIPL-4 and GSC(−) longevity [31].

## Neural mechanisms of fat regulation

As in mammals, the *C. elegans* nervous system regulates energy balance. It does so through regulation of food seeking behaviors and feeding. An emerging theme from the *C. elegans* studies is that the nervous system can also regulate fat metabolism through a series of neuroendocrine signaling pathways [12,44–50]. Importantly, while these neuroendocrine mechanisms are coordinated with the feeding regulatory processes, the fat regulatory effects of the *C. elegans* nervous system are not simply secondary consequences of its neural regulation of feeding (FIGURE 2) [12,44,45,51]. For instance, both serotonin and a TGF-β like signaling mechanisms operate in *C. elegans* to coordinate a vast array of behavioral, physiological, and metabolic responses that animals exhibit in the context of various external and internal cues of food availability and energy demand. In both cases, these signaling cascades initiate in specific regions of the *C. elegans* nervous system, but the fat and feeding effects of changes in these signaling pathways ultimately map to distinct molecular relays in distinct neurons [12,44,45,47,48,51]. This organization allows for coordinating numerous organism-wide responses to changes in nutritional status but preserves the capability for modulating each response differently, if warranted. This flexibility gives an animal the capability to tune its responses to a variety of conditions. The same organization of fat and feeding regulatory pathways also exists in mammals. For example, engagements of either leptin or MC4R receptors have profound effects on both fat and feeding, but for each receptor, these effects involve relatively distinct brain regions [52].

## Serotonin regulates fat and feeding through distinct neural circuits

In *C. elegans* as in mammals elevation of serotonin signaling promotes fat loss [75]. In mammals serotonin-induced fat reduction has been largely attributed to the effects of this neuromodulator on feeding reduction. In *C. elegans*, however, increasing serotonin signaling causes fat reduction despite feeding increase [12]. In *C. elegans*, elevation of peripheral mechanisms of fat oxidation in response to enhanced neural serotonin signaling accounts for the fat reducing effects of serotonin [12]. At least three states of serotonin signaling exist in *C. elegans*: reduced levels seen when animals are off of food, intermediate levels when animals are well fed, and transiently elevated levels seen when fasted animals are returned to food [16]. Fat reducing effects of serotonin are noted when serotonin levels are experimentally increased beyond those seen in well-fed animals. The fat reducing effects of

serotonin are dependent on MOD-1, a chloride channel on neurons that sense both internal fat status and oxygen [12,47,48]. Octopamine, the invertebrate functional analog of norepinephrine, is also required in serotonin promoted fat loss, signaling through the SER-6 G-protein coupled receptor expressed on a pair of sensory neurons which induces the expression of the serotonin biosynthesis gene *tph-1* [47,50]. These components are proposed to function in a neural regulatory loop whose unknown endocrine output signals through NHR-76, a peripherally expressed nuclear receptor, to promote breakdown of peripheral fat stores [47].

The feeding promoting effects of serotonin depend on a molecular and neural circuit that is distinct from the one through which serotonin promotes fat loss [12,51,76]. For example, serotonergic feeding increase but not fat reduction depends on *hlh-34*, an ortholog of *SIM1*, a critical hypothalamic regulator of energy balance in mammals [51]. A molecular component common to both serotonergic fat reduction and feeding increase is AAK-2, a catalytic subunit of the AMPK. Serotonergic inactivation of AAK-2 in distinct neurons underlies the fat and feeding effects of serotonin [51,44]. There is cross talk between serotonin-induced mechanisms of fat reduction and feeding increase in that peripheral metabolic signals generated upon elevation of serotonin signaling can abrogate serotonin induced feeding increase [12]. Such mechanisms may be operable in mammals as well, and may underlie the perceived anorectic effects of serotonin in these systems.

## Intersection of metabolic and food sensory pathways with feeding regulation

The intersection of sensory cues derived from food availability with the internal metabolic state of the organism is a task mediated by the nervous system that tunes the organism's behaviors in response to physiological needs and environmental conditions. Feeding behavior in *C. elegans* is modulated by food availability, composition and past experience [5]. *C. elegans* ingests its microbial diet by action of a muscular pharynx [53]. In the presence of its typical laboratory food source *E. coli* OP50 the pharynx is highly active, pumping at a regular frequency. Upon immediate withdrawal of food, pumping is initially strongly suppressed but within minutes begins to increase although to a level far below that seen when animals have *ad libitum* access to food. Upon returning to food, pharyngeal pumping rapidly elevates. If *C. elegans* have been cultivated on one species of bacteria, then transferred to another their pharyngeal pumping rate is depressed which appears to involve a type of conditioning of sensory neurons to the odors and taste of different foods [54]. A large array of neuropeptide and biogenic amine neuromodulators regulate pharyngeal pumping in the presence and absence of food [5,9,53].

As in mammals, nutrient status of *C. elegans* has a strong influence on feeding behavior. Animals fasted for a few hours hyper-activate their pumping immediately after returning to food [13]. After two hours of feeding post-fast the feeding rate subsides to a stable rate characteristic of *ad libitum* fed animals. The mechanism underlying this hyper-activated state involves depletion of kynurenic acid, a neuro-inhibitory metabolite of tryptophan [55], during fasting, which unleashes a peptidergic signaling axis in the extra-pharyngeal nervous

system that enhances serotonin secretion in response to food [13]. As animals continue to eat, kynurenic acid accumulates, reducing the activity of this peptidergic axis and attenuating the hyper-activated state. Most aspects of the kynurenine pathway are conserved between *C. elegans* and mammalian systems. This behavior appears to maximize the organism's ability to recover from a brief period of nutrient deprivation when it is reproductively committed. The kynurenine pathway is particularly notable in that it is perturbed in multiple neurodegenerative and psychiatric diseases in humans [55]. Peripheral metabolic perturbations influence the function of this pathway in the brain in multiple mammalian disease models [55,56].

Additional satiety-like and sleep-like states in *C. elegans* have also been reported. After returning to food following a fast of 12 hours *C. elegans* actively feeds for approximately an hour, but then is increasingly susceptible to falling into a quiescent state peaking 6 hr after re-feeding [57]. This quiescent state is characterized by a complete cessation of body movements, including pharyngeal contractions and is likely a sleep state [58]. Sleep has been described as an ultimate satiety state, perhaps allowing the organism to cope with nutrient stress incurred in moving from a nutrient poor environment to one of excess nutrients. No individual metabolic pathway has been characterized that provides this stress signal, but the neural mechanism requires a TGF-β signaling axis that emanates from a pair of sensory neurons and targets a pair of motor neurons [57,59]. The same motor neurons also regulate steady-state fat and feeding pathways in the animal [45].

## Concluding Remarks and Future Perspectives

*C. elegans* is a versatile experimental organism that allows for investigation of metabolic, physiological, and behavioral responses that together determine energy balance in an intact organism. There are, of course, many fat and feeding regulatory pathways that are of great importance to mammalian biology that simply do not exist in *C. elegans*. Moreover, some *C. elegans* pathways may be organism-specific. Nonetheless, *C. elegans* studies have already led to identification of new molecular regulators of energy balance, some of which similarly function in mammals. In other cases, the *C. elegans* findings present challenges to existing mammalian paradigms. While such challenges can easily be dismissed as peculiarities of the *C. elegans* system, the existing data in mammals is insufficient to rule out the *C. elegans* findings as organism-specific.

*C. elegans* studies have revealed unanticipated connections between elevated lipid levels and activation of pro-longevity mechanisms. *C. elegans* also display a surprisingly complex array of feeding behavioral responses to various cues of food availability, quality, and past experience. In one case, these studies have identified a specific, neuromodulatory metabolite that allows for the incorporation of experience in behavioral responses.

Despite its many experimental advantages, the small size and difficulty in easily dissecting out some of its tissues has presented vexing challenges when it comes to interpretation of lipid results. Certain basic questions that were settled long ago in mammalian systems remain poorly addressed in *C. elegans* (**OUTSTANDING QUESTIONS BOX**). Two basic issues that are critical for a correct interpretation of lipid measurements from *C. elegans*-

recognizing that not all triglycerides of *C. elegans* have the same physiological functions and that no single methodological approach provides an accurate distribution of triglycerides between vastly differently moieties- remain frequently overlooked. Resolving these basic, technical issues is arduous and unlikely to be motivated by significant funding or publication potential. Yet, an accurate and clear resolution to these issues is fundamental to the promise of *C. elegans* in helping decipher complex relationships between lipid metabolism and processes such as longevity or behavior, where *C. elegans* findings have often led the way.

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**TEXT BOX 1**

## Does hyperactive secretion underscore clinical manifestations of Bardet Biedl syndrome?

Bardet-Biedl syndrome (BBS) is a human genetic syndrome that results in obesity, renal dysfunction and blindness as well as incidences of hypertension and diabetes [60]. The diverse multi-organ alterations of BBS are thought to be due to a mis-localization of different signaling molecules that normally reside in the primary cilium, specialized organelles that contain a variety of signaling receptors [61]. Disruption of cilia formation due to mutations affecting proteins involved in intraflagellar transport causes phenotypes similar to BBS mutations, a finding that has been taken to mean that manifestations of BBS disruptions are due to ciliary defects [62]. In *C. elegans* only sensory neurons are ciliated. These cells selectively express all of the homologs of the proteins that form a complex known as the BBSome [63,64]. *C. elegans* bearing mutations in BBSome components exhibit defective cilia [65,66], have reduced body size, a feeding mechanism desensitized to food withdrawal, and an elevated accumulation of vital dyes that stain lipids in select intestinal depots [46,67]. However, the ciliary defects were found to be separable from the body size, feeding and lipid phenotypes of these mutants [46]. Mutant *bbs C. elegans* exhibit excessive secretion of hormones via enhanced dense core vesicle release [46]. Similarly, inactivations of BBSome components in a human pancreatic β-cell line enhance insulin secretion. Interventions that abrogate this excessive secretion in *C. elegans* are sufficient to revert the body size, feeding and lipid phenotypes to that of wild type animals, without correcting the underlying cilia defects of these mutants [46]. Finally, while in *C. elegans* as in mammals disruption of intraflagellar transport machinery results in phenotypes that mimic those of *bbs* mutants, distinct molecular and cellular mechanisms underlie these superficially similar outcomes [46]. Together these results suggest that hyperactive hormone secretion whether due to direct actions of the BBSome on dense core vesicle secretion or indirect effects of cilia defects may underlie many of the clinical manifestations of this syndrome.

**TEXT BOX 2**

## What do the standard strategies of *C. elegans* fat measurement capture and miss?

Despite potentially divergent physiological roles, the triglyceride pools of *C. elegans* have usually been treated as a single entity. A major but often overlooked source of triglycerides in *C. elegans* is yolk, lipoprotein particles through which nutrients are transferred from the hermaphrodite to its oocytes. Given that a *C. elegans* hermaphrodite can produce its body weight in progeny in a day [68], a substantial fraction, if not the majority of the somatic lipid content of a gravid animal is in the service of yolk production and transport [7,8]. Mutations that disrupt yolk formation and/or deposition result in severely elevated lipid phenotypes. For example, inactivation of Krüppel-like factor 3, which promotes the transcription of protein components of yolk known as vitellogenins, causes highly elevated fat levels [69].

Importantly, none of the existing methods of visualizing neutral lipids can conclusively distinguish the triglycerides that are part of yolk from those that are in storage droplets. Since *C. elegans* tissues are refractory to large-scale dissection, biochemical analyses of lipid content is conducted on extracts derived from whole organism populations, which makes it impossible to know whether the measured triglycerides are derived from yolk or storage depots. As one remedy, certain protein markers are being used as indicators of yolk or lipid droplets [8,70–73]. However, the specificities of these markers or the fraction of yolk or lipid droplets that are captured by each are unknown. Similarly, whether there is a continuum between lipid storage depots and yolk is also poorly understood.

Too often, changes in total fat content rather than changes in minor depots or fat regulatory molecular mechanisms are held as the criteria for connecting gene function to lipid metabolism in *C. elegans*. As noted in this review, the links between lipids and longevity of germ line deficient animals initially emerged from studying lipases that act in acidic compartments [31,32,36,37,41,74]. Reliance on standard methods would have made identification of such lipases difficult since their presence or absence results in very little change in total fat mass of animals [39]. There are likely many other processes where minor pools of *C. elegans* fat have profound physiological and behavioral effects but are unlikely to be found by the standard fixed staining and biochemical strategies prevalent in the field.

## OUTSTANDING QUESTIONS BOX

- **Which tissue serves as the main site of fat storage?** Traditionally, the focus has been on the intestine. However, this tissue may be more liver-like while the *C. elegans* epidermis may have roles that more closely mimic those of adipocytes.
- **What are the molecular and cell biological mechanisms that underlie production of lipid-rich yolk particles?** This investigation has the potential of revealing mechanisms that similarly underlie mammalian lipid-rich lipoprotein particles, which are tied to cardiovascular disease.
- **What fraction of yolk can be captured by protein markers such as VIT-2::GFP and can the protein markers used to label lipid droplets unambiguously distinguish them from yolk?** None of the existing methods can definitively distinguish between various triglyceride rich moieties of *C. elegans*, even though these distinct moieties may have dramatically different functions.
- **What mechanisms distribute lipids from the intestine to tissues other than the germ line?** While the existence of yolk has been recognized, albeit mostly overlooked, it is likely that lipoprotein-like processes are also involved in transport of lipids to a variety of tissues beyond the germ line.
- **Why are elevated fat levels in certain circumstances detrimental while in other circumstances beneficial?** One possibility is the ratio between beneficial and harmful lipids. Another possibility is that when lipids that cannot be put into storage pathways accumulate, specific lipid stress signals are generated that then activate compensatory stress-resistance, pro-longevity pathways.
- **What are the functions of internal acid compartments, lysosomes and lysosome related organelles, in lipid homeostasis?** Feedback and feed-forward loops between lipolysis, autophagy, and other pro-longevity mechanisms are being identified and linked to lipases that function in these acidic compartments but the functions of these compartments in lipid homeostasis remain poorly understood.
- How do different durations of food deprivation affect subsequent responses of animals to food availability and food choices?
- What are the molecular circuits and mechanism by which a multitude of and perhaps conflicting food related cues are integrated to elicit a given behavioral response?

**TRENDS BOX**

- *C. elegans* allows for investigation of numerous evolutionarily conserved fat and feeding regulatory mechanisms.
- *C. elegans* studies have led to deciphering of regulatory connections that were subsequently shown to similarly function in mammals, but also to findings that challenge certain paradigms of mammalian fat biology.
- One difficulty in studying lipids in *C. elegans* is that there are currently no easy methods to unambiguously distinguish lipids in storage depots from those that are in lipoprotein-like yolk particles.
- Both lipid synthesis and breakdown pathways are activated in long-lived animals that lack their germ lines and specific, signaling lipids that promote activity of pro-longevity pathways have been identified.
- Levels of a specific tryptophan-derived metabolite underlie the incorporation of experience in feeding behavior.

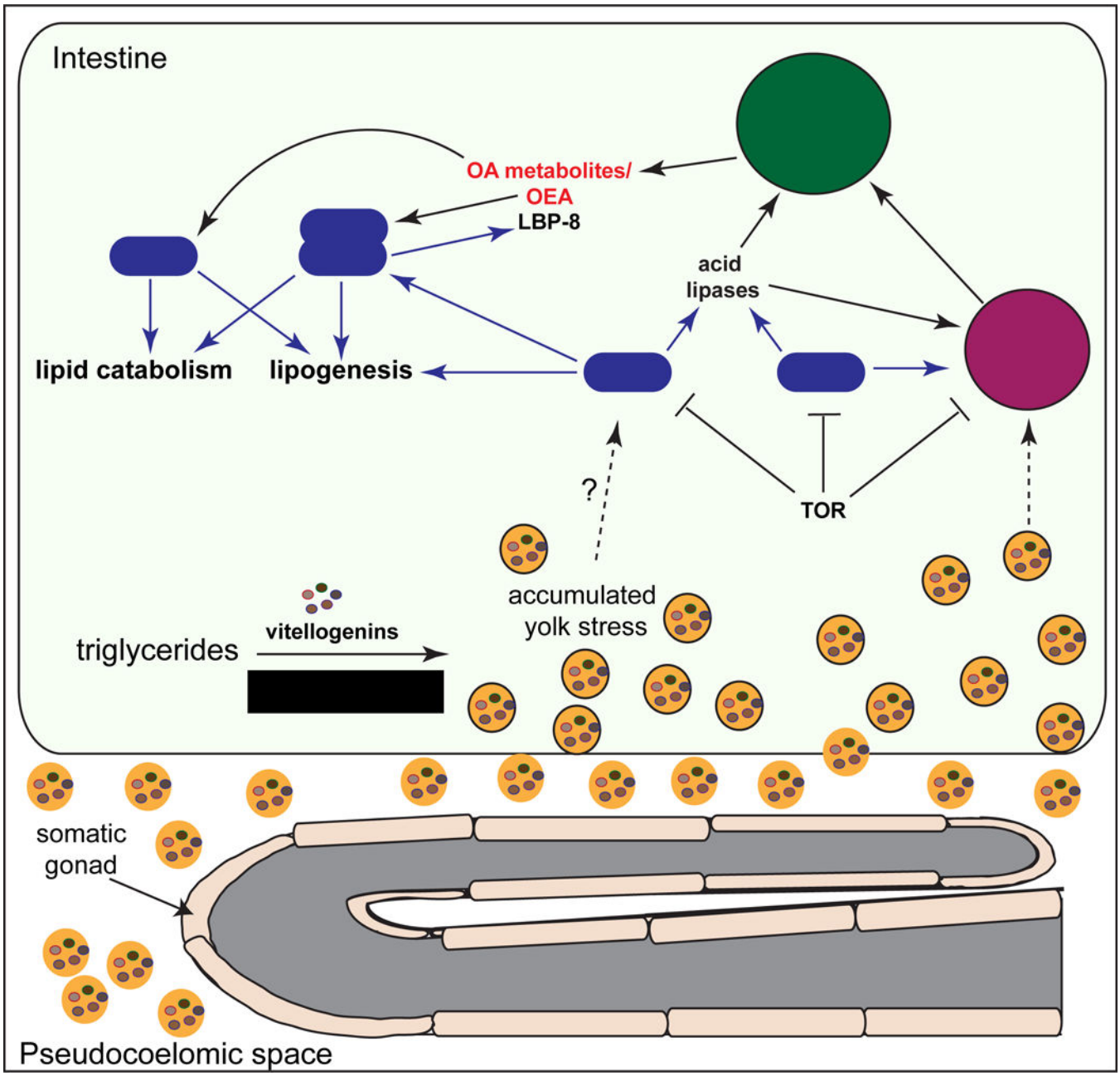


**Figure 1. Transcriptional programs linking the gonad, fat metabolism and somatic longevity**

In wild type animals, yolk, composed of triglycerides and vitellogenin proteins, is made in the intestinal cells and deposited in the oocytes through receptor mediate endocytosis. The source of lipids for yolk is unknown. In animals that maintain their somatic gonad but lack their germ line, GSC(−), yolk continues to be made but accumulates in excess in the pseudocoelomic space and the intestinal cells. Despite highly elevated triglyceride levels of GSC(−) animals, their TOR activity is depressed leading to activation of DAF-16/FOXO and HLH-30/TFEB transcription factors. In turn, DAF-16 promotes the expression of lysosomal acid lipases, NHR-49/PPARα and numerous other lipogenesis but also fat catabolism genes. HLH-30 and PHA-4/FOXA (not shown) regulate the expression of autophagy genes and

lysosomal acid lipases required for the longevity associated with germ line loss. The lysosomal lipases stimulate the production of oleic acid (OA) derived metabolites including oleoyl ethanolamide (OEA). OEA stimulates transcriptional programs driven by a nuclear hormone transcriptional complex comprised of NHR-49 and NHR-80/HNF-4α while OA drives activation of the SKN-1/NRF transcription factor, which in turn drives lipid catabolism programs. The lipid binding protein LBP-8 shuttles OEA between the lysosome (LYS) and the nuclear localized NHR-80. The accumulated yolk lipids in GSC(−) animals might provide a stress signal that activates DAF-16 or provide a source of OA metabolites that are liberated by autophagy (AΦ)/lysosomal digestion. Please note that the indicated genes are only a subset of those identified to be required for longevity of GSC(−) animals. For example, LXR-like steroid signaling components, microRNAs, heat shock factor, a Kringle domain containing protein, and an ortholog of mammalian transcriptional elongation and splicing factor have also been found as required for the extended longevity of GSC(−) but are not shown.

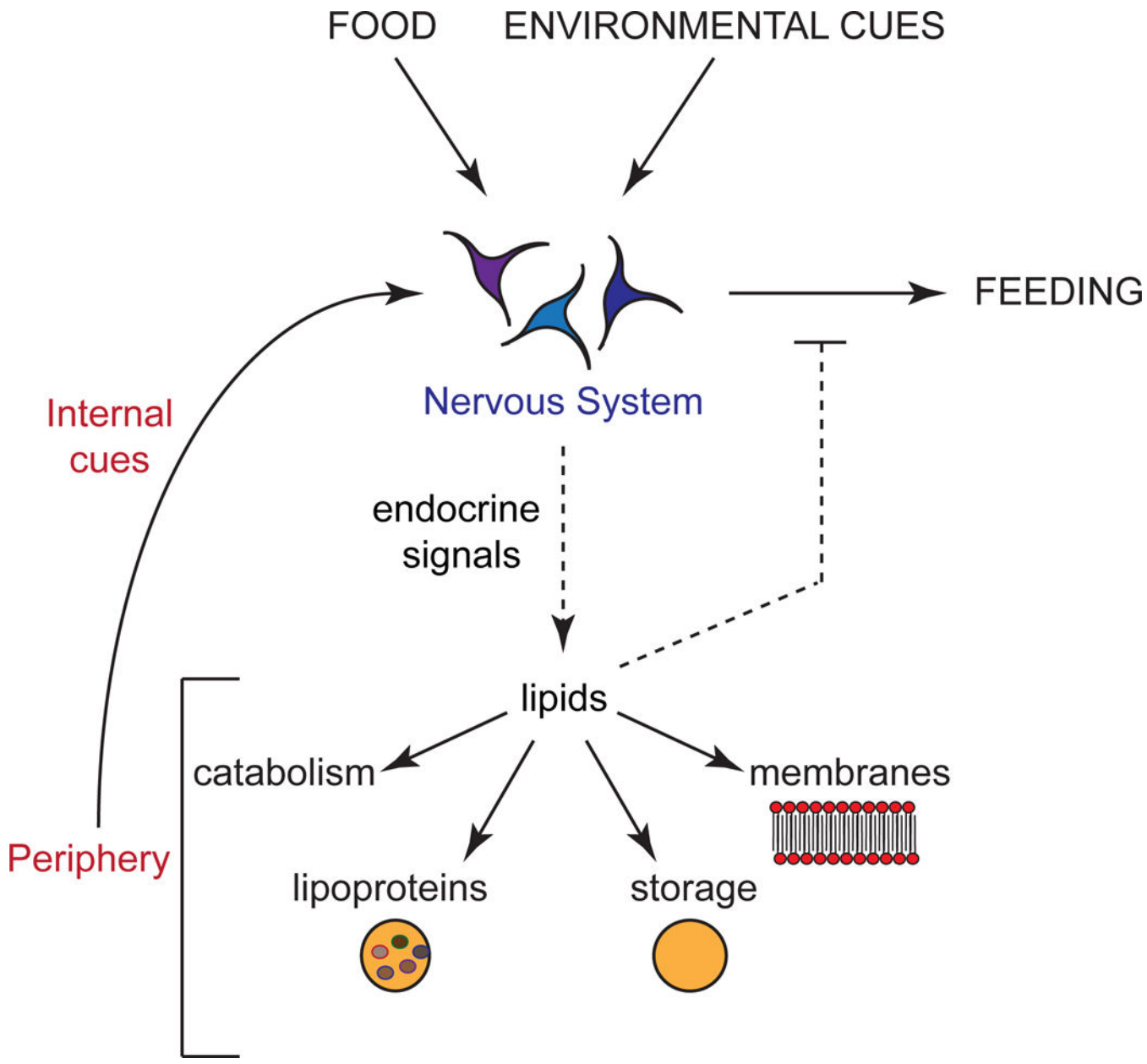


**Figure 2. Feeding and fat metabolism are independently regulated by the nervous system**

Food signals, environmental cues and internal peripheral nutrient status signals are integrated by the nervous system in *C. elegans.* While common mechanisms such as serotonin signaling regulate a range of responses to changes in nutrient status, for example feeding behavior and fat metabolism, regulation of these responses are mediated by distinct molecular mechanisms and neural circuits. Putative neuro-endocrine signals that regulate fat metabolism, and fat derived signals from the periphery that regulate feeding remain to be identified.