Reproduction, Fat Metabolism, and Life Span: What Is the Connection?

Malene Hansen,[1,5,*] Thomas Flatt,[2,3,5,6,*] and Hugo Aguilaniu[4,5,*]
[1]Sanford-Burnham Medical Research Institute, Del E. Webb Neuroscience, Aging and Stem Cell Research Center, Development and Aging Program, La Jolla, CA 92037, USA
[2]Institute of Population Genetics, Vetmeduni Vienna, 1210 Vienna, Austria
[3]Wissenschaftskolleg zu Berlin, Institute for Advanced Study, 14193 Berlin, Germany
[4]Ecole normale supérieure de Lyon; CNRS; Université de Lyon Claude Bernard, Molecular Biology of the Cell Laboratory/UMR5239, 69342 Lyon, France
[5]These authors contributed equally to this work
[6]Present address: Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland
*Correspondence: mhansen@sanfordburnham.org (M.H.), thomas.flatt@unil.ch (T.F.), hugo.aguilaniu@ens-lyon.fr (H.A.)
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Reduced reproduction is associated with increased fat storage and prolonged life span in multiple organisms, but the underlying regulatory mechanisms remain poorly understood. Recent studies in several species provide evidence that reproduction, fat metabolism, and longevity are directly coupled. For instance, germline removal in the nematode *Caenorhabditis elegans* promotes longevity in part by modulating lipid metabolism through effects on fatty acid desaturation, lipolysis, and autophagy. Here, we review these recent studies and discuss the mechanisms by which reproduction modulates fat metabolism and life span. Elucidating the relationship between these processes could contribute to our understanding of age-related diseases including metabolic disorders.

Introduction

Reproduction is an energetically costly process that has profound effects on the metabolism of fat, the major form of energy storage in animals (Bronson, 1989). During reproduction, animals mobilize their fat reserves; reduced or abolished reproduction can increase lipid storage and lead to weight gain in many species (Corona et al., 2009; Judd et al., 2011; McElroy and Wade, 1987). This inverse relationship between reproduction and fat storage seems to reflect an inevitable energetic trade-off. In this so-called "cost of reproduction" scenario, depletion of energy reserves to support reproduction is thought to compromise the organism's ability to support somatic maintenance and survival (Kirkwood, 1977; Williams, 1966). Hence, lipids apportioned to reproduction, for instance, would be unavailable for other life-sustaining processes to support somatic maintenance and survival (Shanley and Kirkwood, 2000). Indeed, in most animals, reproduction trades off with maintenance and survival so that individuals with reduced or curtailed reproduction survive better and live longer than those with higher reproductive effort and vice versa (Bell and Koufopanou, 1986; Partridge et al., 2005). Interestingly, in a variety of organisms, increased life span is associated with reduced reproduction but markedly increased lipid storage and thus improved survival under starvation conditions (Gems et al., 1998; Judd et al., 2011; Rion and Kawecki, 2007; Tatar et al., 2001). Despite these observations, the mechanisms connecting reproduction, fat metabolism, and life span remain poorly understood.

Recent evidence suggests that the three processes might be causally linked through a reproductive-endocrine signaling axis. In the nematode *C. elegans* and the fruit fly *Drosophila melanogaster*, for example, ablation of the germline increases life span (Flatt et al., 2008; Hsin and Kenyon, 1999) and signifi-

cantly alters lipid metabolism (O'Rourke et al., 2009; Parisi et al., 2010). Moreover, studies in *C. elegans* have begun to uncover the molecular mechanisms by which signals from the reproductive system regulate lipid metabolism and life span (Goudeau et al., 2011; Lapierre et al., 2011; McCormick et al., 2012; Wang et al., 2008).

Here, we review these recent findings on the complex interplay between reproduction, fat metabolism, and aging. We first summarize seminal observations and recent findings that suggest that the processes are physiologically linked in many organisms (Figure 1). We then discuss recent insights into the molecular mechanisms that may underlie these connections (Figure 2). Finally, we highlight the implications of these findings for our understanding of aging and age-related diseases, including metabolic disorders, and summarize some of the future challenges (Figure 3).

Reproduction, Fat Metabolism, and Life Span Are Interconnected

Animal physiologists have long known that reproduction affects the storage and mobilization of fat, and similarly, evolutionary biologists have made numerous observations suggesting that reproduction influences organismal life span and aging. It is thus tempting to speculate that fat might be a causal, physiological link coupling reproduction and life span. Indeed, several lines of evidence indicate that reproduction, resistance to starvation stress, and life span might be energetically linked through lipid metabolism (Figure 1).

Effects of the Reproductive System on Fat Metabolism

Numerous studies in mice, rats, cats, monkeys, and other mammals have reported that gonadectomy leads to weight gain and increased fat mass (Crane, 1991; Fettman et al., 1997; McElroy and Wade, 1987; Pallier et al., 1980; Salmeri et al., 1991;
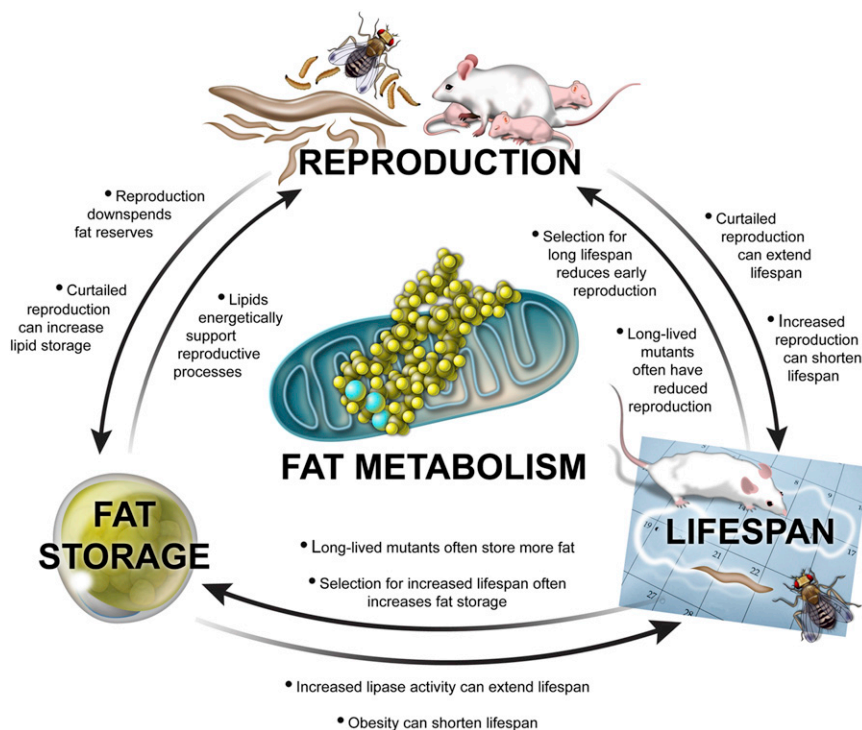
Figure 1. Reproduction, Fat Metabolism, and Life Span are Intimately Interconnected
Although the mechanistic cause-and-effect relationships are not yet clear, multiple lines of experimental evidence point to tight links between reproduction, fat metabolism, and aging (see bullet points in figure). For example, observations in worms, insects, and rodents indicate that reproduction (top center) can directly affect fat storage and life span. Moreover, increased life span (bottom right) is often negatively correlated with reproduction and positively correlated with increased fat storage, whereas fat metabolism (bottom left) influences the energetic cost of reproduction and may directly modulate life span.

Effects of the Reproductive System on Life Span

Surgical or genetic ablation of the reproductive system significantly extends life span in many animals (Drori and Folman, 1976; Flatt, 2011; Flatt et al., 2008; Hatle et al., 2008; Hsin and Kenyon, 1999; Leroi, 2001; Maynard Smith, 1958; Partridge et al., 2005). Ovariectomized grasshoppers, for example, live significantly longer than sham-operated control animals (Drewry et al., 2011; Judd et al., 2011), and gonadectomy of Pacific salmon also increases their life span (Robertson, 1961). Similarly, castration of male rats extends their life span by a short but significant period (Drori and Folman, 1976), and castrated men have been reported to live significantly longer than fertile men (Hamilton and Mestler, 1969; Min et al., 2012).

The effect of interventions that abolish reproduction is particularly dramatic in *C. elegans* and *Drosophila*, where targeted ablation of germline stem cells significantly extends life span (Arantes-Oliveira et al., 2002; Flatt et al., 2008; Hsin and Kenyon, 1999; Maynard Smith, 1958). Notably, this effect is abrogated in *C. elegans* when the somatic gonad is also removed (Hsin and Kenyon, 1999), suggesting that life span extension is not simply a result of sterility but is regulated by opposing signals produced by the germline and the somatic gonad.

Together, these findings suggest that gonadal signals not only affect fat metabolism in a number of species but also modulate their rate of aging. Although these signals have not yet been unambiguously identified, steroid hormones are promising candidates (Gáliková et al., 2011; Gerisch et al., 2007; Tatar et al., 2003). Thus, given that reproduction profoundly affects both fat metabolism and life span, it is plausible to hypothesize that the three physiological functions might be causally interconnected.

The tripartite interconnection between reduced reproduction, increased fat storage, and improved adult survival (see Figure 1) is perhaps most clearly seen in experiments with *C. elegans* and *Drosophila*, although similar observations have been made in other animals (Reznick, 1985; Bell and Koufopanou, 1986). For example, several independent studies have shown that wild-type flies artificially selected for increased life span also exhibit impaired early fecundity and enhanced starvation resistance

Stotsenburg, 1913, and references therein). In humans and other mammals, hypogonadism, a deficiency in gonadal hormone production and function (reviewed in Wilson and Roehrborn, 1999), is often associated with accumulation of excess fat (obesity), insulin resistance (inability of cells to properly respond to insulin), and metabolic syndrome (diabetes and other metabolic disorders) (Corona et al., 2009). The link between hypogonadism and excess fat seems to be bidirectional: while it is clear that visceral obesity can cause hypogonadism, gonadectomy experiments in animals and other data suggest that hypogonadism can also cause fat accumulation and insulin resistance (Corona et al., 2009).
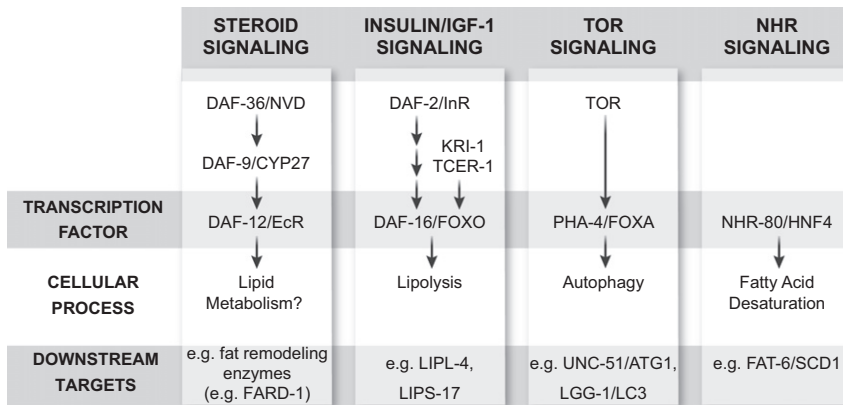
The connection between impaired or reduced reproduction and excess fat storage is not restricted to mammals. In a variety of insects, including blow flies, bugs, migratory locusts, and grasshoppers, ovariectomy causes hypertrophy of the fat body, the insect equivalent of mammalian adipose and liver tissue (Judd et al., 2011; Socha et al., 1991; Strong, 1967; Thomsen and Hamburger, 1955). Such fat-body hypertrophy is also seen in two female sterile mutants of *D. melanogaster*, called *mama*[1] (Doane, 1961) and *Rbp9* (Butterworth and Bodenstein, 1968). Remarkably, implantation of wild-type ovaries restores the fat-body tissue to its normal size in both mutants. Consistent with these findings, transgenic ablation of the germline by over-expression of *bag of marbles* (*bam*), a protein involved in germline differentiation and maintenance, increases fat storage and starvation resistance in adult female flies (M. Galikova, H.A., and T.F., unpublished data). Similarly, germline-less *C. elegans glp-1* mutants, which carry a mutation in the GLP-1/Notch receptor (Arantes-Oliveira et al., 2002), have greatly increased fat stores (McCormick et al., 2012; O'Rourke et al., 2009; M.H. and H.A., unpublished data).

Figure 2. Model of Germline Signaling and Its Impact on Fat Metabolism

Ablation of the germline increases life span in *C. elegans* and *D. melanogaster* and profoundly alters fat metabolism. To date, our understanding of the underlying molecular mechanisms is mainly based on findings in *C. elegans*; however, *Drosophila* homologs are indicated where possible. For functional parallels between steroid signaling in *C. elegans* and *D. melanogaster*, see Gáliková et al. (2011). Each of the four longevity-promoting transcription factors (DAF-12/EcR, DAF-16/FOXO, PHA-4/FOXA, and NHR-80/HNF4) either target fat-remodeling enzymes or activate cellular processes that can affect fat metabolism. Life span extension through removal of the germline therefore provides insights into the mechanisms that link reproduction, fat metabolism, and life span.

due to increased fat storage (Chippindale et al., 1993; Djawdan et al., 1996, 1998; Service et al., 1985; Zwaan et al., 1995). Similarly, many long-lived single-gene mutants of *C. elegans* and *Drosophila* (e.g., mutations in the insulin/IGF-1 and TOR [target of rapamycin] signaling pathways) exhibit reduced fecundity, increased lipid storage, and improved starvation stress resistance (Broughton et al., 2005; Gems et al., 1998; Tatar et al., 2001; Tissenbaum and Ruvkun, 1998). Conversely, artificial selection for increased starvation resistance in *Drosophila* is often correlated with increased lipid storage, reduced fecundity, and prolonged life span (reviewed in Hoffmann and Harshman, 1999; Rion and Kawecki, 2007). In mice, most but not all long-lived models exhibit increased fat levels (Wolf, 2010). While enhanced fat storage in response to life span-promoting mutations may be necessary to support somatic maintenance (Liao et al., 2011), excessive fat is also an important risk factor for metabolic syndrome, diabetes, and cardiovascular disease, which can significantly curtail life span (Das et al., 2004). Thus, the role of fat in long-lived mouse models remains unclear.

Although the association between reproduction, lipid storage, and life span is not always observed, suggesting that these processes can be dissociated (Flatt, 2011; Force et al., 1995; Gems et al., 1998; Hoffmann and Harshman, 1999; Rion and Kawecki, 2007; Vermeulen et al., 2006), it is clear that such an association—be it correlative or causal—very often exists. In the following sections, we discuss our current understanding of the molecular underpinnings of the links between the three processes.

Molecular Connections between Reproduction, Fat Metabolism, and Life Span

The most direct molecular evidence for a causal connection between reproduction, fat metabolism, and life span comes from recent studies in *C. elegans* showing that germline removal not only extends life span but also profoundly affects fat metabolism (Goudeau et al., 2011; Lapierre et al., 2011; Wang et al., 2008). Moreover, several fat-remodeling enzymes were found to be necessary, and sometimes sufficient, to promote longevity through removal of the germline (Goudeau et al., 2011; Lapierre et al., 2011; O'Rourke et al., 2009; Wang et al., 2008; Xie, 2008). Much progress has been made in identifying genes involved in both fat metabolism and life span determination that are acti-

vated by germline ablation in *C. elegans* (Berman and Kenyon, 2006; Gerisch et al., 2007; Ghazi et al., 2009; Goudeau et al., 2011; Hsin and Kenyon, 1999; Lapierre et al., 2011; McCormick et al., 2012; Yamawaki et al., 2008, 2010). Together with work in other systems, including *Drosophila*, these studies provide a first glimpse of the molecular mechanisms (Figure 2) that might form the regulatory framework linking reproduction, fat metabolism, and life span.

Insulin/IGF-1 and Steroid Signaling Pathways: Common Regulators of Reproduction, Fat Metabolism, and Life Span

The first genes found to affect life span and metabolism in germline-less animals were identified in *C. elegans* and are examples of genes that function through a process we refer to as germline signaling. The impact of germline ablation on life span was first shown to be mediated by two *dauer formation* (*daf*) genes: *daf-16*, a FOXO transcription factor in the insulin/IGF-1 signaling pathway, and *daf-12*, a nuclear hormone receptor that functions in steroid hormone signaling (Hsin and Kenyon, 1999). These pathways are described in detail below.

Insulin/IGF-1 Signaling. Insulin/IGF-1 signaling is well known to affect both life span and fat metabolism in fertile animals (Kenyon et al., 1993; Perez and Van Gilst, 2008; Wolf, 2010). The positive impact of reduced insulin/IGF-1 signaling on life span and fat storage has been observed in worms, flies, and mice, suggesting an evolutionarily conserved association (Clancy et al., 2001; Holzenberger et al., 2003; Kenyon et al., 1993; Wolf, 2010; Wang et al., 2008); however, the role of insulin/IGF-1 signaling in germline-ablated animals has only recently been uncovered. In *C. elegans*, several components of the insulin/IGF-1 signaling pathway, such as *daf-16*/FOXO and *daf-18*/PTEN, are required for life span extension upon germline loss (Hsin and Kenyon, 1999; Larsen et al., 1995). Despite this connection, it should be noted that germline-mediated life span extension is influenced by many factors that are not components of the insulin/IGF-1 signaling pathway. For instance, genes such as *kri-1*, an ankyrin-repeat protein orthologous to the human gene KRIT1, and *tcer-1*, a transcription elongation factor related to TCERG1, promote life span extension in germline-less animals by interacting with *daf-16*/FOXO but are not involved in canonical insulin signaling (Ghazi et al., 2009). Other observations support the existence of a noncanonical insulin/IGF-1 signaling pathway in

Conceptual Questions

- Are the effects of the germline and somatic gonad on lifespan and fat metabolism, respectively, evolutionarily conserved?

- How are reproduction, lifespan, and fat metabolism causally connected? For example, do steroid hormones play a critical role in this interconnection, and if so, in which tissues?

- What are the relative roles of fat content, composition, and localization in modulating lifespan and reproduction?

- Can autophagy contribute to lifespan extension by modulating fat metabolism in flies and mammals?

- Do parallels exist between NHR-80/DAF-12 in worms and PPARα/LXR in flies and mammals?

Technical Questions

- Produce mammalian models of germline ablation.

- Determine whether a combination of gonadectomy/ovariectomy and reduced insulin/IGF-1 signaling extends lifespan in mammals and flies, as it does in worms.

- Combine mutations affecting lifespan and fat metabolism to determine which aspects of fat metabolism are required for lifespan extension.

- Similarly, combine mutations affecting reproduction and fat metabolism to determine which aspects of fat metabolism are required for reproduction.

- Compare which fat-modifying enzymes/processes affect lifespan in fertile and in reproductively impaired animals.

- Create mouse models with tissue-specific deletion of fat-modifying enzymes.

- Measure specific lipids and lipid metabolites in long-lived models using metabolomics and/or biochemical assays.

Figure 3. Outstanding Questions and Future Perspectives
The links between fat metabolism, reproduction, and life span are only starting to be uncovered. The figure summarizes some of the key questions that are likely to significantly improve our understanding of these links at the molecular level and highlights current technical challenges such as engineering germline-less mice.

germline-less *C. elegans*. For example, many *daf-16*/FOXO target genes are nonoverlapping in insulin/IGF-1 signaling-deficient versus germline-less mutants (McCormick et al., 2012; Murphy et al., 2003). Furthermore, *kri-1* mediates DAF-16/FOXO translocation into intestinal nuclei upon germline ablation, but not under conditions of reduced insulin signaling in *daf-2* (insulin/IGF-1-like receptor, InR) mutants (Berman and Kenyon, 2006). Similarly, intestinal DAF-16/FOXO is activated (Lin et al., 2001; Libina et al., 2003) cell nonautonomously by microRNAs such as *mir-71* that are produced in the neurons of germline-less animals, but not of *daf-2*/InR mutants (Boulias and Horvitz, 2012). Some of these regulatory connections may be evolutionarily conserved because long-lived germline-ablated *Drosophila* also exhibit altered insulin/IGF-1 signaling (Flatt et al., 2008).

Intriguingly, three independent studies have found that DAF-16/FOXO activates fat-processing enzymes in response to germline ablation in worms (Goudeau et al., 2011; McCormick et al., 2012; Wang et al., 2008). Indeed, genes involved in fat metabolism are overrepresented among *daf-16*/FOXO targets in germline-less animals (McCormick et al., 2012). In this study, *daf-16*/FOXO was found to regulate the transcription of genes involved in fat catabolism (e.g., lipases) and anabolism (e.g., diacylglyceride transferases, desaturases). As discussed below, at least two of these *daf-16*/FOXO targets, the lipases *lipl-4* and

lips-17, have been directly linked to the long life span of germline-less animals (Wang et al., 2008; McCormick et al., 2012). Thus, alterations in fat mobilization may represent one mechanism by which longevity and fat content are linked, perhaps as a means to optimize sustained energy consumption. Finally, *daf-16*/FOXO also regulates many genes involved in steroid hormone metabolism (e.g., steroid hormone dehydrogenases, cytochrome P450s), suggesting the possibility of crosstalk between the insulin and steroid hormone signaling pathways. The production of hydrophobic signaling molecules such as hormones would be an efficient way to trigger systemic responses. Thus, it is possible that changes in fat metabolism occurring in response to altered reproductive status may induce hormonal signaling that in turn elicits life span extension.

Steroid Signaling. The second pathway known to be relevant for life span extension in response to germline loss, at least in *C. elegans*, is a steroid hormone pathway that includes *daf-12*, a nuclear hormone receptor (NHR) related to the vitamin D, pregnane X, and liver X receptors. This pathway also includes *daf-36*, a Rieske-like oxygenase; *daf-9*, a cytochrome P450 similar to CYP27A1; and *dhs-16*, a 3-hydroxysteroid dehydrogenase, all of which are involved in the production of dafachronic acids, the bile acid-like steroid hormone ligands of DAF-12/NHR (Hsin and Kenyon, 1999; Jia et al., 2002; Rottiers et al., 2006; Motola et al., 2006; Wollam et al., 2012). Of note, other genes

involved in steroid signaling have not been reported to be required for germline-mediated life span extension in *C. elegans*. The longevity-promoting effects of *daf-9*/CYP27 and *daf-12*/NHR are specific to germline signaling because loss-of-function mutations in *daf-9*/CYP27 extend the life span of fertile animals (Jia et al., 2002), and some *daf-12*/NHR alleles increase the life span of males and mutants that exhibit reduced insulin/IGF-1 signaling (Gems et al., 1998; Hochbaum et al., 2011). The detailed mechanisms by which these genes govern germline-mediated longevity remain to be clarified. Interestingly, a recent study has suggested that dafachronic acid might be produced in the somatic gonad (Yamawaki et al., 2010), which could explain the requirement for these tissues in life span extension upon germline ablation (Hsin and Kenyon, 1999). The somatic gonad requirement is alleviated in animals carrying reduction-of-function alleles of *daf-2*/InR that result in markedly reduced insulin/IGF-1 signaling (Hsin and Kenyon, 1999; Yamawaki et al., 2008). One potential explanation for this observation is that low insulin/IGF-1 signaling might induce ectopic production of longevity-promoting steroid hormones in tissues other than the somatic gonad.

The impact of steroid signaling on fat metabolism has been demonstrated most clearly in mammals. Gonadectomy in mammals (mice, rats, and humans) causes a significant redistribution of fat to the periabdominal area and a gross increase in fat mass (Colombel and Charbonnel, 1997; Hausberger and Hausberger, 1966; McElroy and Wade, 1987; O'Rourke et al., 2009). Conversely, steroid hormone treatment restores normal fat metabolism in mice with reproductive deficits (Björntorp, 1997; Wohlers and Spangenburg, 2010). Worms also exhibit increased fat storage upon germline ablation (O'Rourke et al., 2009), although the impact of steroid hormones on fat storage has not yet been directly assessed. In fact, microarray studies show that fat-remodeling enzymes are not overrepresented among DAF-12/NHR targets (McCormick et al., 2012). However, at least one DAF-12/NHR target, predicted to be the fatty acyl reductase *fard-1*, is essential for life span extension in germline-depleted animals (McCormick et al., 2012).

Although our limited understanding of the role of steroid signaling in fat metabolism makes it difficult to identify parallels among the molecularly tractable models, some noteworthy similarities do exist. For example, DAF-12/NHR interacts with another nuclear hormone receptor, NHR-80, to promote fatty acid desaturation in response to germline ablation (Goudeau et al., 2011). Fatty acid desaturation is also upregulated in ovariectomized mice and requires the presence of the nuclear receptors liver X receptor (LXR) and peroxisome proliferator-activated receptor (PPAR)α (Chu et al., 2006; Paquette et al., 2008). Thus, it will be interesting to explore the functional parallels between PPARα/LXR and NHR-80/DAF-12.

Steroid hormones also affect fat metabolism in mammals through the estrogen receptor (ERα). Indeed, the importance of this receptor in regulating fat metabolism is clearly demonstrated by ERα-deficient mice, which are prone to obesity (Heine et al., 2000). Moreover, it was recently shown that hypothalamic neurons are important sites of action for the regulation of fat metabolism by the ERα (Xu et al., 2011). The *Drosophila* ER homolog, dERR, has been found to regulate energy and carbohydrate metabolism (Tennessen et al., 2011), whereas the role

of *nhr-14*—the closest worm ER homolog—remains unknown (Mimoto et al., 2007). Hence, it is not yet clear whether the relationship between estrogen signaling, fat metabolism, and reproduction can be easily modeled in simpler genetic systems.

Several reports suggest that insulin/IGF-1 and steroid hormone/NHR signaling interact to promote life span extension of germline-ablated animals. For instance, DAF-16/FOXO translocation into intestinal nuclei following germline removal is impaired in the absence of *daf-12*/NHR (Berman and Kenyon, 2006), suggesting that DAF-12/NHR signaling promotes longevity, at least in part, by promoting insulin/IGF-1 signaling. Collectively, the available evidence suggests that the insulin/IGF and steroid hormone signaling pathways play pivotal roles in modulating the effects of germline ablation on life span extension and lipid metabolism in *C. elegans*. The precise role played by each pathway in regulating fat metabolism in nematodes clearly requires further investigation, as does their contribution to life span and fat metabolism in other model systems. Preliminary work in *Drosophila* suggests that the effect of germline ablation on life span and fat metabolism might be conserved between worms and flies (Flatt et al., 2008; Gáliková et al., 2011; M. Galikova, H.A., and T.F., unpublished data). Moreover, insulin/IGF-1 and steroid hormone (ecdysone) signaling also appear to regulate fat metabolism in *Drosophila* and other insects (DiAngelo and Birnbaum, 2009), and it is noteworthy that these hormonal signaling pathways also control germline stem cell proliferation and maintenance (Ables and Drummond-Barbosa, 2010; LaFever and Drummond-Barbosa, 2005). The greatest challenge, however, will be to determine if a mammalian model with curtailed reproduction has increased life span (see Figure 3 for outstanding questions and future perspectives; also see, for example, Drori and Folman, 1976).

Role of Lipolysis in Germline-Mediated Longevity

The first identified target gene of the DAF-16/FOXO transcription factor in germline-ablated animals was the predicted triacylglycerol lipase and cholesterol esterase LIPL-4 (Wang et al., 2008). *lipl-4* is induced in germline-less *glp-1* mutants in a *daf-16*/FOXO-dependent fashion. Interestingly, the induction of *lipl-4* requires *kri-1*/KRIT but is independent of *daf-12*/NHR, suggesting the existence of a steroid hormone signaling-independent pathway for *lipl-4* expression induced by germline ablation. In turn, inactivation of *lipl-4* shortens the long life span of germline-less *glp-1*/Notch animals (Wang et al., 2008), supporting a crucial role for LIPL-4 activity in life span extension in these animals. Consistent with this notion, overexpression of LIPL-4 is sufficient to extend life span when expressed in wild-type animals from a ubiquitous promoter (Lapierre et al., 2011) or an intestinal promoter (Wang et al., 2008); the latter finding provided one of the first direct links between lipid metabolism and longevity.

LIPL-4 has lipase activity in vitro (Lapierre et al., 2011) and is most prominently expressed in the gut and hypodermal seam cells (Wang et al., 2008; Lapierre et al., 2011); notably, these tissues are fat storage sites in *C. elegans* (Mak, 2012). The cellular substrate(s) and the subcellular localization of LIPL-4 are not yet known, but it is interesting to note that LIPL-4 has the highest homology to human lysosomal acid lipase (LAL), an enzyme involved in cholesterol ester processing and trafficking from autophagic and endocytic vesicles (Ouimet et al., 2011).

Other lipases are also upregulated in germline-less animals, including *lips-17*, which is required for germline-less *glp-1*/Notch mutants to live long (McCormick et al., 2012). Similarly, germline-less *Drosophila tudor* mutants exhibit increased expression of four triacylglycerol lipases (CG8093, CG6277, CG6271, CG2772) and one lipid storage gene (*lipid storage droplet protein 1*), suggesting that lipid metabolism might be enhanced in germline-less flies (Parisi et al., 2010). Despite these effects, little difference was observed between the lipid profiles of germline-less and fertile control flies in this study (Parisi et al., 2010). It will be interesting to determine whether lipolysis also plays a role in germline-mediated longevity and reproduction in other organisms and whether changes in lipolysis and expression of longevity-relevant lipases are observed during normal aging.

Role of Autophagy in Germline-Mediated Longevity

The cellular recycling process of autophagy has recently been linked to the longevity of germline-less animals. Autophagy sequesters and degrades cytosolic components and is typically induced under stress conditions such as nutrient deprivation. One type of autophagy called macroautophagy (referred to here as autophagy) has been directly linked to aging (Rubinsztein et al., 2011). Specifically, pharmacological and genetic manipulations that increase life span in model organisms, including *C. elegans* and *Drosophila*, often stimulate autophagy. Conversely, inhibition of autophagy compromises the longevity-promoting effects of multiple paradigms, suggesting that increased autophagy might function as a potent antiaging mechanism. Consistent with this notion, germline-less *C. elegans* show increased rates of autophagy (Lapierre et al., 2011), and the expression of multiple autophagy genes is induced, at least in part, by the FOXA transcription factor PHA-4, another forkhead transcription factor. This suggests that autophagy is likely to be transcriptionally regulated in germline-less animals. Finally, *pha-4*, as well as multiple genes with autophagy-related functions, is required for germline-less animals to live long (Lapierre et al., 2011). These findings imply that longevity of germline-less animals is regulated by at least two transcription factors, PHA-4/FOXA and DAF-16/FOXO, through induction of autophagy and metabolic genes, respectively.

The nutrient sensor TOR, a major conserved regulator of autophagy (Kapahi et al., 2010), may serve as a common upstream regulator of lipid metabolism and autophagy in germline-less nematodes. Lapierre et al. (2011) found that germline-less animals have lower intrinsic TOR levels (Lapierre et al., 2011), which is consistent with the possibility that reduced TOR levels constitutively induce autophagy in these animals. Moreover, TOR inactivation upregulates *lipl-4* expression in a *daf-16*/FOXO-dependent fashion (Lapierre et al., 2011). Future work examining TOR pathway components will shed light on the dual regulatory role of TOR signaling in germline-less animals.

Multiple types of autophagy have been described that are cargo specific, including one that mediates lipid breakdown and has been termed "lipophagy." Evidence for this pathway comes from observations with cultured hepatocytes and mouse liver in which inhibition of autophagy increases triglyceride storage in lipid droplets (Singh et al., 2009). Interestingly, overexpression of LIPL-4 alone is sufficient to induce autophagy in *C. elegans*, probably via downregulation of TOR (Lapierre et al., 2011), and reciprocally, autophagy activity is required for the lipase activity of LIPL-4. Likewise, autophagy genes are required for LIPL-4-overexpressing animals to live long (Lapierre et al., 2011). Taken together, these data suggest that autophagy is intimately linked to lipid hydrolysis in the adult animal, possibly through a mechanism similar to lipophagy (Singh et al., 2009). Indeed, autophagosomes can be observed close to lipid droplets in germline-less animals (Lapierre et al., 2011). Alternatively, autophagy could be triggered by a substrate produced by LIPL-4. Regardless of the exact mechanism, these findings suggest for the first time that autophagy engages lipid metabolism as a mechanism to extend life span in response to germline removal. Future experiments are needed to clarify the nature of this link and to determine whether it is conserved in higher organisms.

Autophagy is not always associated with increased lipid hydrolysis because inhibition of autophagy in adipocytes results in decreased lipid accumulation as a result of impaired differentiation of white adipocytes (Singh et al., 2009). Taken together, these observations suggest that autophagy not only mediates breakdown of triacylglycerol droplets (i.e., through the catabolic process of lipophagy) but may also facilitate fat storage. The relationship between autophagy and fat storage needs more thorough investigation, especially in germline-less *C. elegans*.

NHR Signaling and Fatty Acid Desaturation in Germline-Mediated Longevity

Germline ablation in *C. elegans* was recently shown to induce transcription of the nuclear hormone receptor NHR-80. In turn, NHR-80 triggers the induction of *fat-6*/*scd1*, a gene encoding a stearoyl coenzyme A desaturase (Goudeau et al., 2011). FAT-6/SCD1 converts saturated C16 or C18 fatty acids into their corresponding monodesaturated forms. This induction is physiologically relevant because it results in increased levels of desaturated fatty acids. Remarkably, obstructing the induction of *nhr-80* or *fat-6* abolishes life span extension through germline ablation (Goudeau et al., 2011). Although the effect of *fat-6*/*scd1* upregulation on fat content has not yet been determined in the worm, SCD1 deficiency in mice protects against lipid accumulation (Kim et al., 2011). These findings suggest that signals from the germline impinge on longevity in *C. elegans* and fat content in mice by inhibiting fatty acid desaturation through SCD1.

In mice and rats, ovariectomy causes a strong induction of SCD1 levels and an associated increase in fatty acid desaturation (Jackson et al., 2011; Paquette et al., 2008). Although the mechanism by which this occurs is not yet well understood, the finding indicates that increased fatty acid monodesaturation might be a conserved response to impaired reproduction. The observation that germline ablation in *C. elegans* and ovariectomy in mammals cause increased fat content (McElroy and Wade, 1987; O'Rourke et al., 2009) is consistent with the observations that the desaturation of stearic acid leads to the production of oleic acid, a prime substrate for triacylglycerol synthesis and that SCD1 activity correlates with overall adiposity (Chu et al., 2006; Mauvoisin et al., 2007). However, whether *scd1*(−) ovariectomized mice or *fat-6*(−)/*fat-7*(−) germline-less worms are resistant to weight gain has not yet been tested.

Studies of the regulation of SCD1 in mammals provide additional interesting insights. SCD1 responds to many stimuli, including hormones and nutrients, and it is striking that many important SCD regulators are known to affect longevity and/or

fat content (Mauvoisin et al., 2007). For example, SCD1 activity is strongly affected by the insulin/IGF-1 pathway and by numerous NHRs (Lundholm et al., 2004; Mauvoisin et al., 2007; Paquette et al., 2008; Waters and Ntambi, 1994). Interestingly, the increased life span observed in *C. elegans daf-2*/InR mutants has been partly attributed to the upregulation of *fat-7/SCD1* (Murphy et al., 2003), suggesting that fatty acid desaturation may also promote the life span of fertile animals. It will be interesting to discover why fatty acid desaturation is required to support extended longevity when the germline is ablated and when insulin signaling is low in other systems such as *Drosophila*.

SCD1 also appears to be regulated by NHRs in mice and worms. In mice, PPARα and LXR regulate SCD1 (Mauvoisin et al., 2007), and genes encoding for SCD1 homologs in worms are under the control of NHR-49 and NHR-80 (Goudeau et al., 2011). Moreover, although the sequences of NHR-49 and PPARα are clearly divergent, they are both involved in the regulation of fat metabolism and have been proposed to be functional analogs (Van Gilst et al., 2005). Interestingly, *nhr-80* and PPARα are upregulated in response to germline ablation in worms and ovariectomy in mice, respectively (Goudeau et al., 2011; Paquette et al., 2008).

Thus, both SCD1 and PPARα are upregulated upon ovariectomy and SCD1 and LXR are downregulated upon treatment with sex steroid hormones in mammals (Lundholm et al., 2004; Paquette et al., 2008). Together, these data suggest that SCD1 activity is regulated by reproduction through nuclear hormone receptors. Notably, fertile *nhr-80* mutants and SCD-deficient worms exhibit altered fatty acid desaturation (Brock et al., 2007) but have normal life spans (Goudeau et al., 2011).

In summary, the induction of SCD1 seems to be an evolutionarily conserved response to reduced or curtailed reproduction. Although SCD1 has been shown in the nematode system to be required for life span extension upon germline ablation, its effect on fat content in germline-less animals remains unknown. Ovariectomy in mice also induces SCD1 and increases fat content but fails to extend life span. While it is not yet clear whether SCD1 directly affects fat content in ovariectomized mice, one might expect that loss of function of SCD1 would prevent the associated weight gain. Therefore, the data gathered thus far suggest that the role of SCD1 may be conserved across species, but its effects on fat metabolism and life span require further study. One key point will be to determine whether SCD1 activity regulates fat metabolism and longevity separately or if one process is required for the induction of the other.

Summary and Future Perspectives

The work discussed here clearly supports the existence of multiple links between reproduction, lipid metabolism, and aging (Figures 1 and 2) but also raises many intriguing questions to be addressed in future work (see Figure 3 for outstanding questions and future perspectives).

Aging is often accompanied by metabolic disorders that predispose one to diabetes, cardiovascular disease, and numerous complications. Such disorders affect lipid turnover and are accompanied by an accumulation of visceral fat as well as decreased insulin sensitivity (Arner et al., 2011). Interestingly, while removal of visceral fat improves insulin sensitivity and increases longevity in rats (Barzilai et al., 1998; Gabriely et al.,

2002), wild-type mice, who mobilize fat less efficiently, live longer in response to dietary restriction (Liao et al., 2011). However, long-lived genetic mouse models often have increased fat stores, suggesting that increased fat levels might also have a positive impact on longevity (Wolf, 2010). It seems likely that the absolute quantity of stored fat could influence whether it has a beneficial or detrimental effect on the life span of these models. It will therefore be of great interest to investigate whether the fat levels of such animals are correlated with their reproductive profiles.

Because data from genetic models show that the vast majority of longevity-promoting interventions alter fat metabolism, it is legitimate to ask whether specifically targeting fat-processing enzymes will increase longevity. The clearest evidence that this might be possible was reported by Wang et al. (2008), who found that overexpression of the lipase LIPL-4 is sufficient to extend the life span of wild-type worms. Similarly, the desaturases FAT-5 and FAT-6 are required for both insulin- and germline-mediated life span extension, suggesting that fatty acid desaturation might play an important role in regulating longevity (Goudeau et al., 2011; Murphy et al., 2003). Autophagy is another effector of fat metabolism that might be critical for longevity (Lapierre et al., 2011). Although the mechanism through which autophagy modulates life span is not yet clear, the notion that it may do so in part by affecting fat metabolism is appealing (Lapierre et al., 2011; McCormick et al., 2012).

Other aspects of fat metabolism and their roles in aging remain underexplored. For example, understanding whether and how fat composition and localization (Ackerman and Gems, 2012) can modulate life span is an exciting challenge for future studies. Likewise, it will be interesting to determine whether modification of fat metabolism could be a strategy for more efficient energy consumption under stressful conditions. It may also contribute to the production of lipids that act as signaling molecules (such as hormones). Finally, it is possible that alteration of fat content and/or composition contributes to life span extension by affecting the overall metabolism of animals with impaired reproduction. To address these fascinating but largely unresolved questions, the use of powerful genetic models such as *C. elegans* and *Drosophila* will be essential.

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