Cell Reports
Article

Misregulation of an Adaptive Metabolic Response Contributes to the Age-Related Disruption of Lipid Homeostasis in *Drosophila*

Jason Karpac,[1,2,*] Benoit Biteau,[1] and Heinrich Jasper[1,2,*]
[1]Department of Biology, University of Rochester, Rochester, NY 14627, USA
[2]Buck Institute for Research on Aging, Novato, CA 94945, USA
*Correspondence: jkarpac@buckinstitute.org (J.K.), hjasper@buckinstitute.org (H.J.)
http://dx.doi.org/10.1016/j.celrep.2013.08.004

SUMMARY

Loss of metabolic homeostasis is a hallmark of aging and is commonly characterized by the deregulation of adaptive signaling interactions that coordinate energy metabolism with dietary changes. The mechanisms driving age-related changes in these adaptive responses remain unclear. Here, we characterize the deregulation of an adaptive metabolic response and the development of metabolic dysfunction in the aging intestine of *Drosophila*. We find that activation of the insulin-responsive transcription factor Foxo in intestinal enterocytes is required to inhibit the expression of evolutionarily conserved lipases as part of a metabolic response to dietary changes. This adaptive mechanism becomes chronically activated in the aging intestine, mediated by changes in Jun-N-terminal kinase (JNK) signaling. Age-related chronic JNK/Foxo activation in enterocytes is deleterious, leading to sustained repression of intestinal lipase expression and the disruption of lipid homeostasis. Changes in the regulation of Foxo-mediated adaptive responses thus contribute to the age-associated breakdown of metabolic homeostasis.

INTRODUCTION

Insulin/Insulin Growth Factor Signaling/Foxo Function and Metabolic Adaptation

In complex organisms, adaptation to changing dietary conditions is critical to maintain metabolic homeostasis (Luca et al., 2010; Roberts and Rosenberg, 2006). Adaptation has to occur at multiple levels and involves adjusting processes that control nutrient uptake, storage, and usage to dietary changes in order to properly maintain energy homeostasis (Roberts and Rosenberg, 2006). It has been proposed that modern (high-sugar/high-fat) diets can lead to misregulation of evolutionarily conserved adaptive dietary responses, resulting in metabolic dysfunction (Odegaard and Chawla, 2013). Furthermore, the age-associated decline of the ability to metabolically adapt to dietary changes is a likely cause for the increased incidence of metabolic diseases in the elderly (Roberts and Rosenberg, 2006). Our understanding of the mechanisms causing diet-induced or age-related changes in metabolic adaptive responses remains limited, and studies in model organisms are likely to provide critical insight into such mechanisms and into potential strategies for therapeutic or preventive interventions.

Insulin/insulin growth factor (IGF) signaling (IIS) is one of the best understood signaling systems involved in metabolic adaptation and the control of metabolic homeostasis. In multicellular organisms, IIS governs energy homeostasis by regulating lipid and carbohydrate metabolism (Saltiel and Kahn, 2001), promotes cell growth and proliferation (Accili and Arden, 2004; Oldham and Hafen, 2003), and influences systemic stress responses (Baumeister et al., 2006; Karpac et al., 2009, 2011; Niedernhofer et al., 2006; van der Pluijm et al., 2007). Its central role in these processes is highlighted by the fact that attenuating IIS function in invertebrates and vertebrates can extend lifespan (Barzilai et al., 2012; Karpac and Jasper, 2009; Taguchi and White, 2008; Tatar et al., 2003). The transcription factor Foxo, activated in response to decreased IIS activity, is an essential mediator of this lifespan extension (Lin et al., 1997; Ogg et al., 1997; Slack et al., 2011). Foxo regulates a battery of stress response and metabolic control genes (Calnan and Brunet, 2008; Matsumoto and Accili, 2005), and its activation has selective tissue-specific consequences. Thus, activating Foxo in the intestine of worms or in the adipose tissue and muscle of flies can extend lifespan (Demontis and Perrimon, 2010; Giannakou et al., 2004; Hwangbo et al., 2004; Libina et al., 2003; Zhang et al., 2013), whereas activation in other tissues and cell-types can reduce lifespan or promote cell death (Biteau et al., 2010; Luo et al., 2007).

In mammals, loss of the insulin receptor in adipose tissue can have positive effects on lifespan (Blüher et al., 2003), whereas IIS inhibition and Foxo activation in other tissues (such as liver, brain, and muscle) contributes to metabolic and degenerative diseases (Kido et al., 2000; Michael et al., 2000; Mihaylova et al., 2011; Suzuki et al., 2010). Importantly, many age-related metabolic pathologies are associated with chronic insulin resistance in humans (Barzilai et al., 2012). In these conditions, Foxo

contributes to the breakdown of lipid and glucose homeostasis, promoting diabetes (Eijkelenboom and Burgering, 2013; Samuel and Shulman, 2012).

Importantly, Foxo can be activated independently by stress signaling pathways, in particular Jun-N-terminal Kinase (JNK) signaling, which responds to a variety of inflammatory and stress signals and is a major contributor to insulin resistance but can also increase lifespan in worms and flies (Biteau et al., 2011b; Hirosumi et al., 2002; Karpac and Jasper, 2009; Samuel and Shulman, 2012). The integration of nutritional responses through IIS with JNK-mediated stress signals by Foxo thus likely determines the beneficial or pathological consequences of attenuated insulin signaling.

IIS/Foxo and the Regulation of Lipid Metabolism

Many of the metabolic consequences of altered IIS and Foxo signaling are caused by changes in glucose and lipid metabolism. The role of IIS in regulating systemic glucose homeostasis and the function of Foxo in promoting glycogenolysis and gluconeogenesis in various tissues are well understood (Accili and Arden, 2004; Eijkelenboom and Burgering, 2013; Saltiel and Kahn, 2001; Samuel and Shulman, 2012). At the same time, altered IIS activity is also associated with abnormal lipid metabolism. Mice and flies in which IIS has been genetically manipulated have disrupted lipid metabolism (Kitamura et al., 2003; Teleman, 2010), yet the cell and tissue-specific mechanisms by which IIS regulates lipid metabolism are only partially understood. In both *Drosophila* and mammals, Foxo regulates the transcription of lipases in adipose tissue required for the lipolysis of stored lipids (Chakrabarti and Kandror, 2009; Ihervaara and Puig, 2008; Wang et al., 2011), as well as the expression of enzymes and other transcription factors involved in lipid catabolism (Deng et al., 2012; Xu et al., 2012). Regulation of Foxo thus provides a mechanism by which insulin can regulate lipid metabolism during metabolic adaptation. Changes in IIS/Foxo-regulated lipases (such as adipose triglyceride lipase) and lipogenic transcription factors (such as SREBP-1c) have been linked to the dyslipidemia associated with type 2 diabetes and other metabolic syndromes (Badin et al., 2011; Schoenborn et al., 2006; Shimomura et al., 2000).

The *Drosophila* intestine is rapidly becoming a productive model system in which to study complex questions relating to the maintenance of tissue function, immune responses, metabolic homeostasis, and stress signaling. Importantly, the intestine is key to the control of lipid and cholesterol homeostasis in the fly (Sieber and Thummel, 2012). Disruption of gut enterocyte function (cells required for nutrient uptake and innate immune function within the intestinal epithelium) can lead to organism-wide changes in lipid metabolism (Sieber and Thummel, 2009). Furthermore, as a critical barrier epithelium, the age-related dysfunction of this tissue significantly limits health- and lifespan of flies (Biteau et al., 2010; Rera et al., 2012, 2013). The adult intestine is regenerated by intestinal stem cells (ISCs), which divide to replace enterocytes (ECs) and enteroendocrine cells when needed (Biteau et al., 2011a; Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Previous studies have shown that ISCs hyperproliferate in the aging gut, resulting in loss of tissue homeostasis and the development of a general inflammatory condition characterized by excessive oxidative stress and chronic JNK activation (Biteau et al., 2008, 2010; Buchon et al., 2009; Choi et al., 2008; Rera et al., 2012). We have recently shown that the regenerative capacity of stem cells strongly influences lifespan in *Drosophila* and that improving intestinal tissue homeostasis also rescues the age-dependent systemic breakdown of lipid homeostasis (Biteau et al., 2010).

Here, we identify a mechanism by which Foxo activity in the intestinal epithelium regulates metabolic adaptation to dietary changes in *Drosophila*. We show that Foxo activity in the intestine is required to inhibit the expression of evolutionarily conserved lipases as part of an adaptive metabolic response to changes in dietary protein/lipids. On a standard diet, Foxo-mediated repression of these lipases is required to prevent excessive lipid uptake, thus promoting systemic metabolic homeostasis. Strikingly, we find that this regulatory mechanism is constitutively activated in aging animals, contributing to metabolic imbalances and the age-associated breakdown of lipid homeostasis. This activation is caused by chronic activation of JNK and reflects the development of a general inflammatory condition in the aging gut. Our findings define a distinct case in which an adaptive mechanism promoting metabolic homeostasis is deregulated by an age-associated increase in stress signaling, thus contributing to the breakdown of metabolic homeostasis.

RESULTS

Foxo-Mediated Regulation of Intestinal Lipid Metabolism

To explore potential mechanisms within the gut that could mediate IIS-dependent adaptive responses to changes in nutrient availability and therefore promote metabolic homeostasis, we focused on the response of the *Drosophila* intestine to a dietary shift from a standard yeast/sugar-based (St. SY) diet to an isocaloric high-sugar/low-yeast (HSLY) diet. The HSLY diet greatly reduces the amount of dietary protein and lipids while maintaining calories through excessive carbohydrates (Skorupa et al., 2008). HSLY diet feeding leads to the transcriptional downregulation of insulin ligands *dilp2* and *dilp5* (Figure 1A). These insulin-like peptides are secreted by neurosecretory insulin-producing cells in the brain and have previously been described as responsive to nutrient imbalance (Garofalo, 2002; Géminard et al., 2009; Rulifson et al., 2002). This repression of *dilp* transcription is sufficient to systemically attenuate IIS activity in the gut, as HSLY diet feeding results in decreases in peripheral phospho-Akt (p-Akt) levels (Figure 1B). Total Akt levels are also reduced in the intestine during HSLY diet feeding (Figure 1B). This suggests that other insulin-independent feedback mechanisms might also contribute to changes in Akt activity under these dietary conditions. The decrease in Akt/p-Akt levels was accompanied by the activation of Foxo target genes (evidenced by increased expression of lacZ from a *thor* [4E-BP] promoter [thor-lacZ]; Figure 1C) in the intestine, confirming that IIS-dependent signaling is suppressed in this tissue.

To assess whether the decrease in dietary lipids and proteins in the HSLY diet influences the expression of genes involved in metabolic adaptation to dietary changes, we measured the

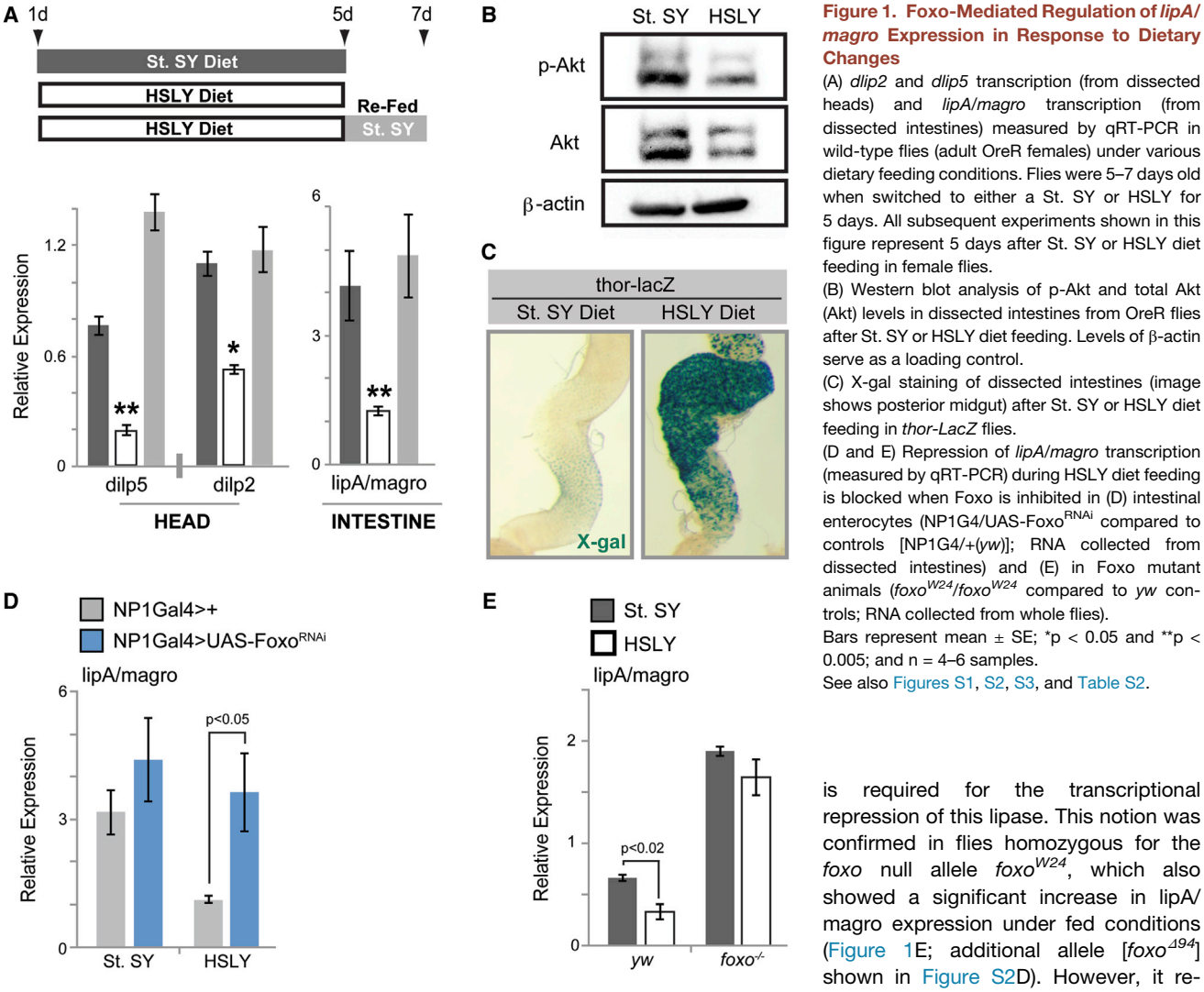

Figure 1. Foxo-Mediated Regulation of *lipA/magro* Expression in Response to Dietary Changes

(A) *dilp2* and *dilp5* transcription (from dissected heads) and *lipA/magro* transcription (from dissected intestines) measured by qRT-PCR in wild-type flies (adult OreR females) under various dietary feeding conditions. Flies were 5–7 days old when switched to either a St. SY or HSLY for 5 days. All subsequent experiments shown in this figure represent 5 days after St. SY or HSLY diet feeding in female flies.

(B) Western blot analysis of p-Akt and total Akt (Akt) levels in dissected intestines from OreR flies after St. SY or HSLY diet feeding. Levels of β-actin serve as a loading control.

(C) X-gal staining of dissected intestines (image shows posterior midgut) after St. SY or HSLY diet feeding in *thor-LacZ* flies.

(D and E) Repression of *lipA/magro* transcription (measured by qRT-PCR) during HSLY diet feeding is blocked when Foxo is inhibited in (D) intestinal enterocytes (NP1G4/UAS-Foxo^{RNAi} compared to controls [NP1G4/+(*yw*)]; RNA collected from dissected intestines) and (E) in Foxo mutant animals (*foxo*^{W24}/*foxo*^{W24} compared to *yw* controls; RNA collected from whole flies).

Bars represent mean ± SE; *p < 0.05 and **p < 0.005; and n = 4–6 samples.

See also Figures S1, S2, S3, and Table S2.

expression of *lipA/magro*, a gene that encodes an intestine-specific lipase with a described role in fat and cholesterol absorption in the gut (Sieber and Thummel, 2009, 2012). Magro (LipA in mammals) is expressed in distinct set of enterocytes in the adult fly intestine (Figure S1A) and is secreted into the intestinal lumen to digest dietary triacylglycerol (TAG) (Sieber and Thummel, 2012). Strikingly, the HSLY diet results in strong repression of *lipA/magro* in the intestine (Figure 1A), whereas refeeding flies a St. SY diet after 5 days on a HSLY diet could normalize their expression (Figure 1A), suggesting that the transcriptional regulation of this lipase is part of an adaptive response to dietary changes.

To determine if changes in IIS/Foxo activity are responsible for this response, we further monitored the expression of *lipA/magro* in various Foxo loss-of-function genetic backgrounds. Reducing Foxo activity in intestinal enterocytes (using the NP1Gal4 driver [Figure S1B] and upstream activating sequence [UAS]-Foxo RNAi [Figures S2A–S2C]) prevented the downregulation of *lipA/magro* expression during HSLY diet feeding (Figure 1D), suggesting that Foxo activity is required for the transcriptional repression of this lipase. This notion was confirmed in flies homozygous for the *foxo* null allele *foxo*^{W24}, which also showed a significant increase in lipA/magro expression under fed conditions (Figure 1E; additional allele [*foxo*$^{\Delta 94}$] shown in Figure S2D). However, it remains unclear how changes in Foxo activation and *lipA/magro* expression affect intestinal lipid metabolism during HSLY diet feeding.

The regulation of *lipA/magro* by Foxo suggests that Foxo-mediated responses to dietary changes are critical for metabolic adaptation and the control of lipid homeostasis. To test this hypothesis, we temporally manipulated Foxo activity in intestinal enterocytes using an adult-onset driver. To prevent developmental effects of the expression of UAS-driven transgenes, we used a heat-inducible system, in which NP1Gal4 is combined with a temperature-sensitive Gal80 (TARGET system [Osterwalder et al., 2001]). Reducing Foxo activity (using UAS-Foxo RNAi) in enterocytes for 5 days leads to upregulation of intestinal *lipA/magro* expression (Figure 2A). This correlates with a significant increase (28%) in stored TAG levels in the whole animal (Figures 2A and S3D). Elevated TAG levels are not due to changes in feeding behavior, as inhibiting Foxo function in enterocytes does not lead to significant changes in activity/rhythmicity or food intake (Figures S3A–S3C). These data suggest that Foxo is required in enterocytes to limit intestinal lipase expression and maintain lipid homeostasis in the organism. Conversely, chronic

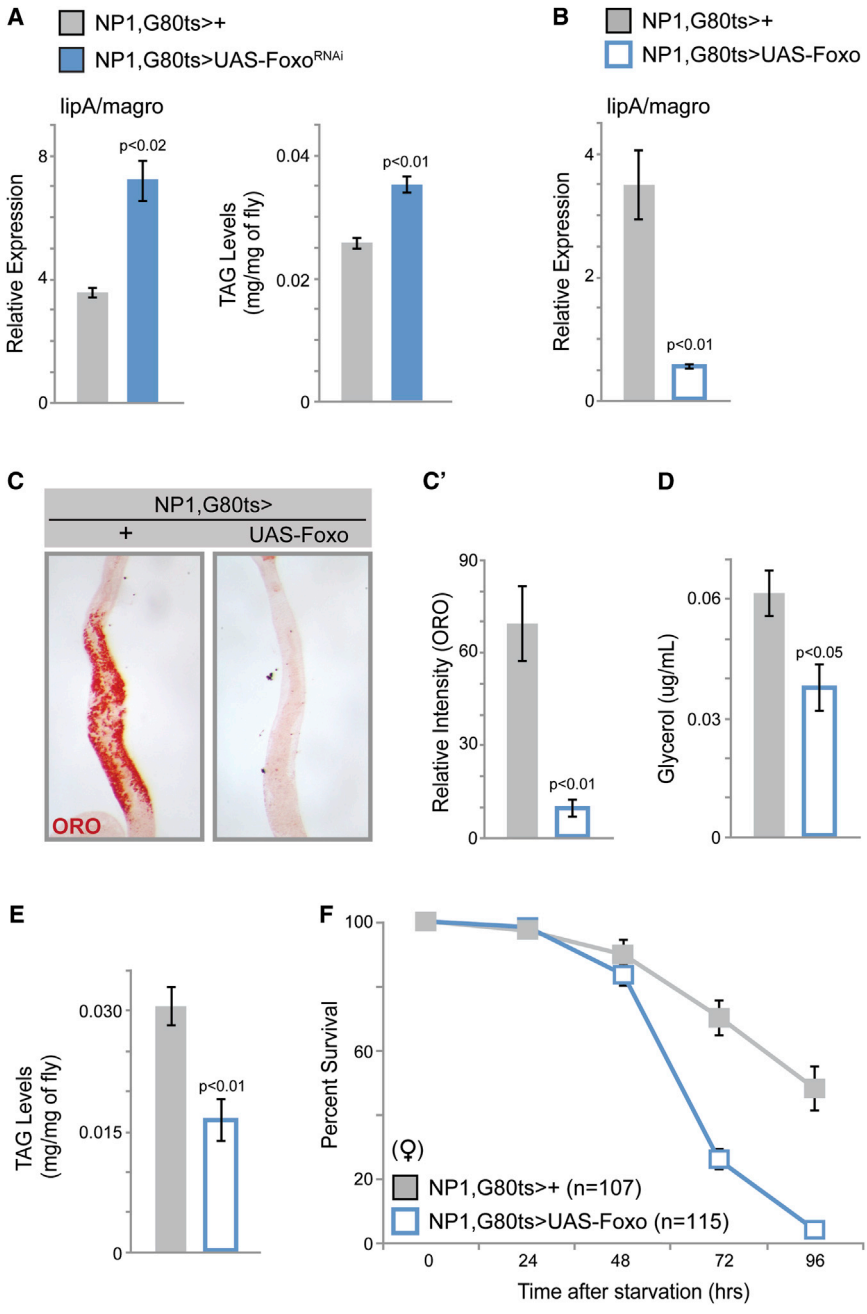

Figure 2. Foxo-Dependent Regulation of Intestinal Lipid Metabolism

(A) Elevated *lipA/magro* transcription (measured by qRT-PCR in dissected intestines) and TAG levels (from whole animals) when Foxo is conditionally knocked down in intestinal enterocytes (NP1G4,tubG80^{ts}/UAS-Foxo^{RNAi} compared to NP1G4,tubG80^{ts}/+ [*yw*] controls). Five- to seven-day-old adult females were placed at 29°C for 5 days to induce conditional expression of transgenes for all experiments represented in this figure.

(B) Repression of *lipA/magro* transcription when Foxo is overexpressed in enterocytes (NP1G4, tubG80^{ts}/UAS-Foxo compared to NP1G4, tubG80^{ts}/+ [w^{1118}] controls, 5 days at 29°C).

(C and C′) Oil red O (ORO) staining of dissected intestines shows reduced lipid accumulation (image shows anterior midgut) in enterocyte-specific Foxo gain-of-function conditions. ORO intensity measurements represent mean ± SE of ten guts.

(D and E) Lipolytic activity from intestinal lysates (measured as the release of glycerol from a lipid emulsion) (D) and whole animal TAG levels (E) are also decreased.

(F) After 5 days at 29°C to induce Foxo expression, female flies were subjected to wet starvation at 25°C. Results are represented as percent survival (mean ± SE) of six cohorts of 17–20 flies form individual populations. Similar results were obtained with male flies.

Bars represent mean ± SE and n = 4–6 samples. See also Figures S2, S3, S4, and Table S2.

overexpression of wild-type Foxo in intestinal enterocytes strongly represses *lipA/magro* transcription and disrupts intestinal lipid storage (Figures 2B–2C′). Importantly, intestinal overexpression of Foxo induces *thor* expression but does not lead to the disruption of epithelial morphology (Figures S4A and S4B), showing that these effects are truly a response to Foxo activity in enterocytes and not due to global tissue degeneration. This downregulation of intestinal lipase transcription correlates with a reduction in intestinal lipolytic activity (Figure 2D) and a strong decrease (nearly 50%) in stored lipid levels in the whole organism (Figures 2E and S3D). Furthermore, flies with chronic intestinal Foxo activation are extremely sensitive to starvation, confirming a breakdown of lipid homeostasis in these animals (Figures 2F and S3E). Overexpression of Foxo in enterocytes does lead to a small but significant decrease in food intake (Figure S3C). Further studies are needed to establish whether this change in feeding behavior is a cause or a consequence of the observed decrease in intestinal lipase expression and lipid storage. However, because loss of Foxo in this tissue did not influence feeding behavior, it is likely that the small change observed in Foxo gain-of-function conditions is a consequence rather than a cause of tissue-specific metabolic changes elicited by Foxo.

Taken together, these data show that Foxo can inhibit the expression of intestinal lipases and therefore control organismal lipid homeostasis through the regulation of intestinal lipid metabolism.

Age-Related Disruption of Intestinal Lipid Metabolism

In a previous study, we found that an age-related decline in gut function is associated with changes in lipid metabolism in

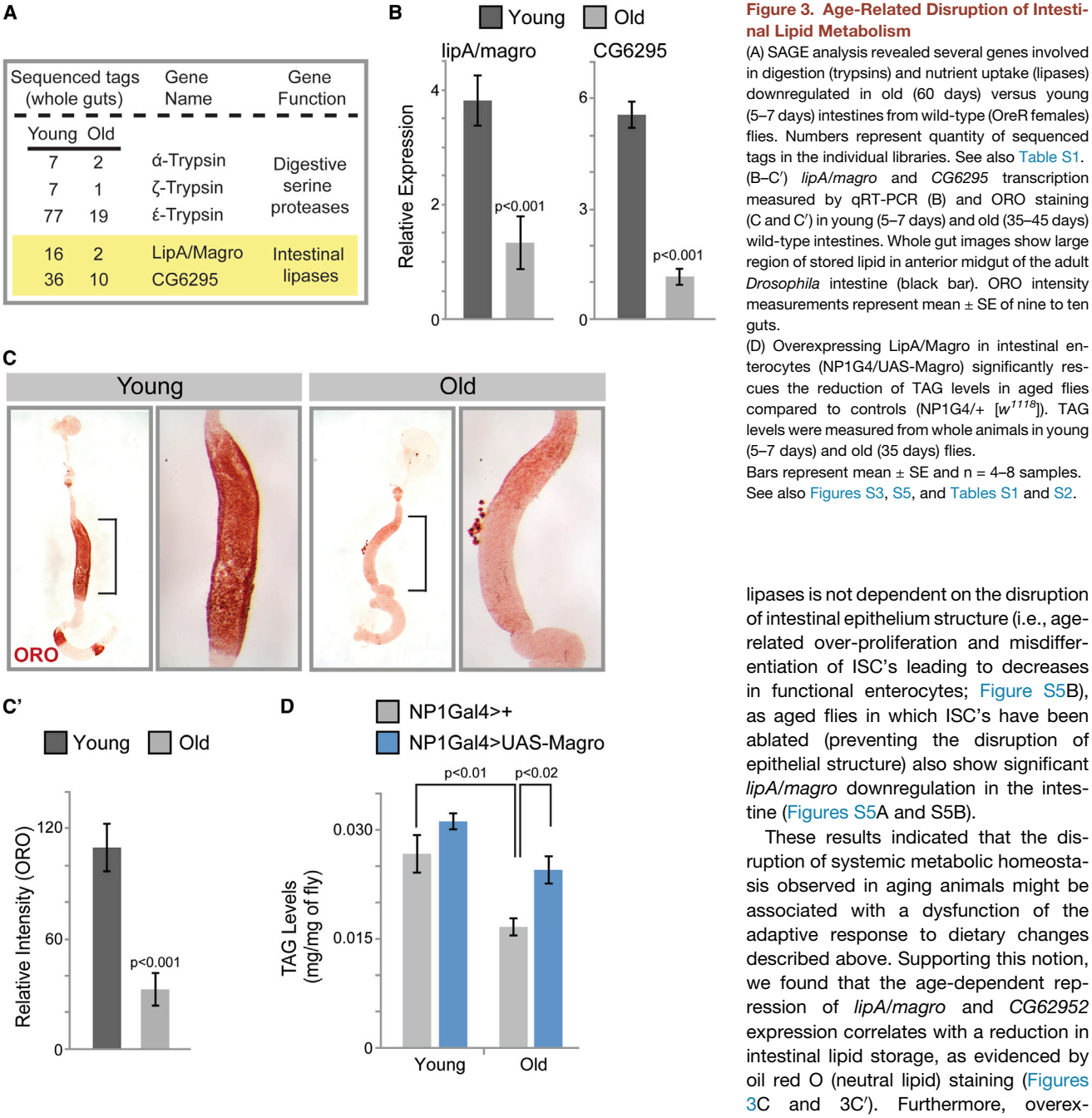

Figure 3. Age-Related Disruption of Intestinal Lipid Metabolism

(A) SAGE analysis revealed several genes involved in digestion (trypsins) and nutrient uptake (lipases) downregulated in old (60 days) versus young (5–7 days) intestines from wild-type (OreR females) flies. Numbers represent quantity of sequenced tags in the individual libraries. See also Table S1.

(B–C′) *lipA/magro* and *CG6295* transcription measured by qRT-PCR (B) and ORO staining (C and C′) in young (5–7 days) and old (35–45 days) wild-type intestines. Whole gut images show large region of stored lipid in anterior midgut of the adult *Drosophila* intestine (black bar). ORO intensity measurements represent mean ± SE of nine to ten guts.

(D) Overexpressing LipA/Magro in intestinal enterocytes (NP1G4/UAS-Magro) significantly rescues the reduction of TAG levels in aged flies compared to controls (NP1G4/+ [w^{1118}]). TAG levels were measured from whole animals in young (5–7 days) and old (35 days) flies.

Bars represent mean ± SE and n = 4–8 samples. See also Figures S3, S5, and Tables S1 and S2.

Drosophila (Biteau et al., 2010). In genome-wide expression profiles generated from young and old guts of wild-type flies (using serial analysis of gene expression [SAGE] in combination with next-gen sequencing), we further found significant transcriptional downregulation of trypsins (digestive serine proteases, see also Biteau et al. [2008]) and of *lipA/magro* and *CG6295* (confirmed by qRT-PCR; Figures 3A and 3B; also see Table S1). CG6295 is another putative lipase that has over 30% amino acid homology to mammalian pancreatic TAG lipase-related proteins, which are gastric lipases important for fat absorption in the intestine (Lowe, 2000). Importantly, the repression of these lipases is not dependent on the disruption of intestinal epithelium structure (i.e., age-related over-proliferation and misdifferentiation of ISC's leading to decreases in functional enterocytes; Figure S5B), as aged flies in which ISC's have been ablated (preventing the disruption of epithelial structure) also show significant *lipA/magro* downregulation in the intestine (Figures S5A and S5B).

These results indicated that the disruption of systemic metabolic homeostasis observed in aging animals might be associated with a dysfunction of the adaptive response to dietary changes described above. Supporting this notion, we found that the age-dependent repression of *lipA/magro* and *CG62952* expression correlates with a reduction in intestinal lipid storage, as evidenced by oil red O (neutral lipid) staining (Figures 3C and 3C′). Furthermore, overexpression of LipA/Magro, specifically in intestinal enterocytes, can significantly rescue the age-related reduction of TAG levels in whole animals (Figures 3D and S3C). These data support a role for the deregulation of intestinal lipase expression in the age-related disruption of lipid homeostasis.

Increased Foxo Activation in the Aging Intestine

Because we found that LipA/Magro can be regulated by Foxo activity in the intestine, we tested whether aging modulates Foxo activity in the gut and found that Foxo activity indeed significantly increases in the intestine as the animal ages. Foxo activation was measured using both the Foxo reporter thor-lacZ, as

well as by measuring the expression of the Foxo target genes *thor*, *InR* (insulin receptor), and *dlip4* (lipase 4) (Figures 4A and 4B). Intestinal activation of Foxo is first observed in a significant number of flies at around 20 days of age and progressively spreads through the population to reach around 85% at age 40 (Figure 4C). These data do not rule out that thor-lacZ induction could be invariant with age, but individuals without induction die at younger ages and are eliminated from the population. However, we did not observe significant changes in mortality at 20 days of age (compared to 5-day-old flies; data not shown), suggesting that the incidence of intestinal thor-lacZ induction within a population of flies does increase with age. Immunohistochemistry for β-Gal revealed that this age-related increase in Foxo activity occurs specifically in intestinal enterocytes and not in intestinal stem cells (marked by esgGal4 > UAS-GFP; Figure 4A). Furthermore, reducing Foxo activity in enterocytes was sufficient to prevent the age-associated repression of *lipA/magro* and *CG6295* (Figures 5D and 5E). Elevated Foxo activity in the aging gut thus contributes to the inhibition of intestinal lipase expression.

To determine if this increase in Foxo activity and repression of lipase transcription correlates with attenuation of IIS, similar to the response to HSLY feeding, we assayed p-Akt levels in young and old guts. Surprisingly, both p-Akt and total Akt levels are slightly increased in aging intestines (Figure 4D). The age-related increase in total Akt protein is accompanied by increases in *akt* expression (Figure 4E), an activation that is likely driven by Foxo, as *akt* has recently been described as a Foxo target gene (Alic et al., 2011). These data suggest that the IIS pathway is activated rather than repressed in older intestines and that Foxo is activated by IIS-independent mechanisms.

Because high steady-state p-Akt levels may not reflect changes in the sensitivity of the IIS pathway to extracellular ligands, we also confirmed that the IIS pathway remains sensitive to systemic insulin ligands in aging intestines. We performed ex vivo insulin stimulation tests on young and old guts by exposing dissected guts in culture media to mammalian insulin and then measuring p-Akt levels. Insulin stimulation of both young and aged intestines revealed a strong upregulation of phosho-Akt, and older intestines appear to be even more sensitive to this stimulation than young controls (Figure 4D). This increased sensitivity is likely caused by the increased Akt and InR expression levels.

Taken together, our data reveal that Foxo becomes chronically activated in intestinal enterocytes with age, whereas the IIS pathway appears to be active and hypersensitive. We propose that this deregulation of IIS pathway control is at the center of the metabolic dysfunction observed in aging flies.

Age-Related Changes in Intestinal JNK/Foxo Activity Contribute to the Breakdown of Lipid Homeostasis

To identify alternative, IIS-independent, mechanisms responsible for the age-related increase in Foxo activity, we turned to JNK (Jun-N-terminal kinase), a stress-activated protein kinase that can activate Foxo in both vertebrates and invertebrates. JNK activity increases in older guts (Biteau et al., 2008), and we thus hypothesized that this elevated JNK activity may be responsible for the observed age-related increase in Foxo activity and the subsequent breakdown of intestinal lipid metabolism. Importantly, we confirmed that increased JNK activity in the aging intestine, like Foxo, occurs primarily in enterocytes (as determined using the JNK reporter puc-lacZ; Figure 5A). Inhibiting JNK (using a dominant-negative form of the *Drosophila* JNK basket [Bsk]) in intestinal enterocytes significantly blocked the induction of thor-lacZ in aging guts (Figures 5B and 5B′), suggesting that elevated JNK activity drives Foxo activation in the aging intestine. We further found that acute JNK activation in young guts is sufficient to repress intestinal lipase expression in a Foxo-dependent manner: overexpression of a constitutively active JNKK (hemipterous [Hep] in *Drosophila*) in intestinal enterocytes induces *thor* expression (Figure S6) and also strongly represses *lipA/magro* and *CG6295* expression (Figure 5C). This lipase repression is reduced in a Foxo mutant background (Figure 5D). Importantly, the transcriptional downregulation of intestinal lipases is not caused by JNK-mediated apoptosis of enterocytes (Figure S4) as the same suppression was observed in animals expressing the antiapoptotic molecule p35.

Supporting a role for chronic intestinal JNK/Foxo activation in the age-related disruption of lipid homeostasis, we found that reducing JNK activity (using UAS-BskDN) in enterocytes was sufficient to prevent the age-associated repression of *lipA/magro* and *CG6295* (Figure 6A). Importantly, inhibiting intestinal JNK activity also rescued the age-related reduction of lipid levels in the whole organism (Figure 6B). These data support the notion that JNK activation in enterocytes is a major contributor to the overall breakdown of lipid homeostasis that occurs in the aging fly. To further test this concept, we assayed starvation sensitivity in the young and old flies described above. Aging flies are more sensitive to starvation, which correlates with age-related changes in glucose and lipid metabolism. Inhibiting JNK activation in enterocytes had no effect on starvation resistance in young animals but led to significantly improved starvation resistance in older flies (as compared to wild-type controls; Figures 6C and 6C′).

Together, these results suggest that JNK-mediated Foxo activation (independent of IIS) in the aging intestine promotes the deregulation of intestinal lipase expression and subsequent disruption of intestinal and organismal lipid homeostasis in *Drosophila*.

DISCUSSION

Our work identifies Foxo-mediated repression of intestinal lipases as a critical component of an adaptive response to dietary changes in *Drosophila*. Interestingly, misregulation of this metabolic response also contributes to the age-associated breakdown of lipid homeostasis, as elevated JNK signaling leads to chronic Foxo activation and subsequent disruption of lipid metabolism due to chronic repression of lipases. This age-related deregulation of an adaptive metabolic response is reminiscent of insulin resistance-like phenotypes in vertebrates, which can also be triggered by chronic activation of JNK (Hirosumi et al., 2002; Samuel and Shulman, 2012), and thus highlights the antagonistic pleiotropy inherent in metabolic regulation. The adaptive nature of signaling interactions that drive pathology (such as JNK-mediated insulin resistance) has

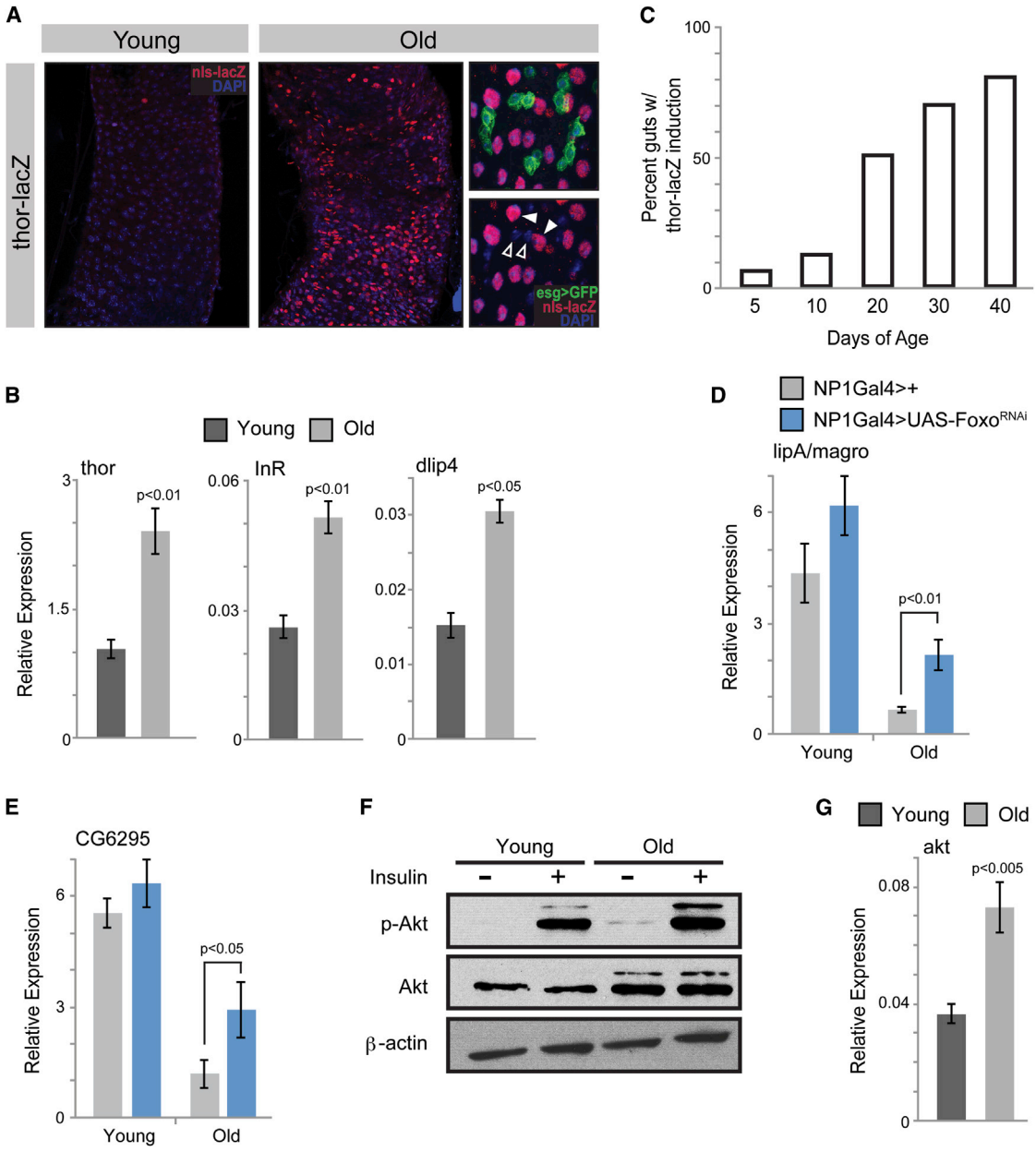

Figure 4. Increased Foxo Activation in the Aging Intestine

(A) Nuclear β-galactosidase (nls-lacZ) accumulates in old *thor-lacZ* intestines (35 days). Small panels show (at higher magnification) nls-lacZ is detected in large enterocytes (white arrowheads) but not in intestinal stem cells (open arrowheads, cells marked by esg > GFP [green]). Genotype of fly: esgG4, UAS-GFP/*thor-lacZ*; β-galactosidase (nls-lacZ; red); and DAPI (blue) detected by immunostaining. Images taken from posterior midgut (similar to the location of *lipA/magro* expression; Figure S1A).

(B) Transcription of Foxo target genes *thor*, *InR*, and *dlip4* in young (5–7 days) and old (35–45 days) wild-type (OreR female) intestines.

(C) Quantification of lacZ induction (X-gal staining of dissected intestines) in *thor-lacZ*/+ flies at various time points during aging. Bars represent percent of intestines displaying X-gal staining in the posterior midgut, n = 15. Intestinal LacZ induction was scored independent of intensity.

(D and E) *lipA/magro* (D) and *CG6295* (E) transcription (from dissected intestines) measured by qRT-PCR in young (5–7 days) and old (35 days) female flies in which Foxo has been inhibited in enterocytes (NP1G4/UAS-Foxo^{RNAi} compared to controls, NP1G4/+ [w^{1118}]).

(F) Western blot analysis of p-Akt and total Akt (Akt) levels in dissected young (5–7 days) and old (35 days) wild-type intestines after ex vivo insulin stimulation. Levels of β-actin serve as a loading control.

(G) Transcription of *akt* was measured by qRT-PCR from young and old wild-type intestines.

Bars represent mean ± SE and n = 4–8 samples.

See also Table S2.

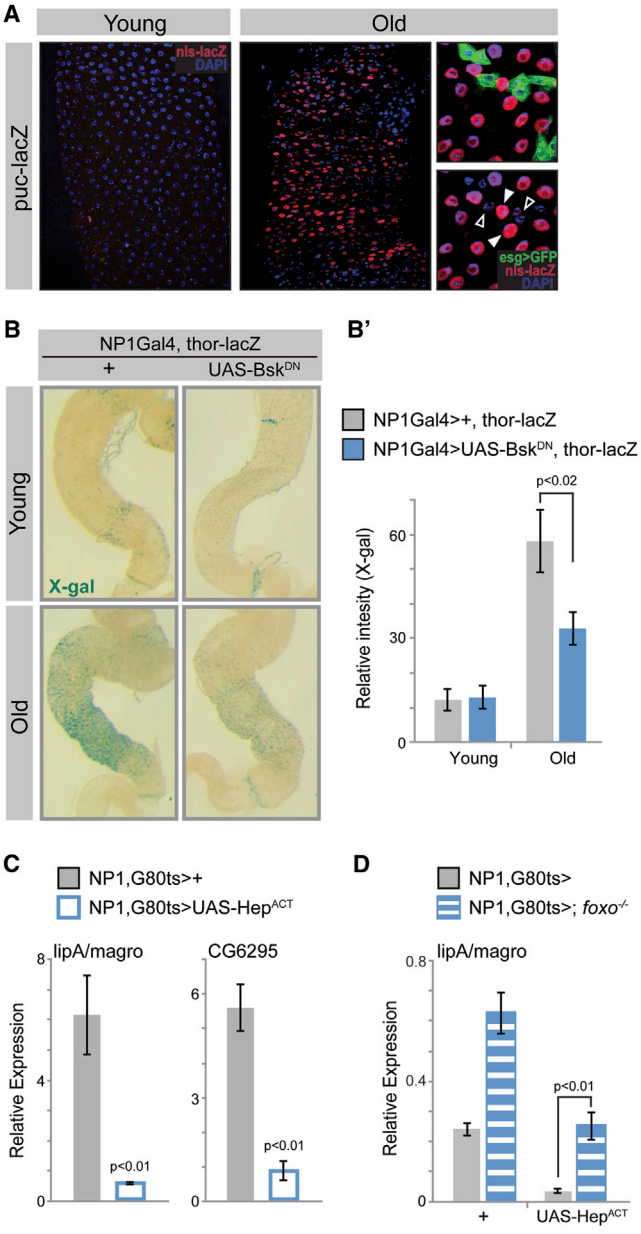

Figure 5. JNK-Dependent Foxo Activation in the Aging Intestine

(A) Nuclear β-galactosidase (nls-lacZ) accumulates in old *puc-lacZ* intestines (35 days). Small panels show (at higher magnification) nls-lacZ is detected in large enterocytes (white arrowheads) but not in intestinal stem cells (open arrowheads, cells marked by esg > GFP [green]). Genotype of fly: esgG4, UAS-GFP/+; *puc-lacZ*/+; β-galactosidase (nls-lacZ; red); and DAPI (blue) detected by immunostaining. Images taken from posterior midgut (similar to the location of *lipA/magro* expression; Figure S1A).

(B and B′) Expression of lacZ in the *thor-lacZ* line was monitored to detect Foxo activation (X-gal staining of dissected intestines; image shows posterior midgut) in young and old female flies (35 days) when JNK is inhibited in enterocytes (NP1G4, *thor-lacZ*; UAS-Bsk^{DN}, compared to controls NP1G4, *thor-lacZ*; +/+ [w^{1118}]). X-gal intensity measurements represent mean ± SE of ten (young) or 21 to 22 (old) guts.

(C) Attenuated *lipA/magro* and *CG6295* transcription (measured by qRT-PCR in dissected intestines) when JNK is conditionally overexpressed in intestinal enterocytes (NP1G4,tubG80^{ts}/UAS-Hep^{ACT} compared to NP1G4,tubG80^{ts}/+ [w^{1118}] controls). Five- to seven-day-old adult females were placed at 29°C for 16 hr to induce conditional expression of UAS-Hep^{ACT}.

(D) Inhibition of *lipA/magro* expression by enterocyte JNK activation is significantly rescued in a *foxo* mutant background. Genotypes: (1) NP1G4, tubG80^{ts} (NP1, G80)/+ (w^{1118}); (2) NP1, G80/+; $foxo^{W24}/foxo^{W24}$; (3) NP1, G80/UAS-Hep^{ACT}; and (4) NP1, G80/UAS-Hep^{ACT}; $foxo^{W24}/foxo^{W24}$; samples collected from whole flies.

Bars represent mean ± SE and n = 5 samples.

See also Figure S6 and Table S2.

remained elusive in many instances, and our work provides a model for age-related changes in an adaptive regulatory process that ultimately lead to a pathological outcome. We believe that this system can serve as a productive model to address a number of interesting questions with relevance to the loss of metabolic homeostasis in aging organisms.

Systemic Control of Lipid Metabolism by JNK/Foxo Interactions

In mammals, JNK has been shown to promote insulin resistance both cell-autonomously and systemically (through inflammation), subsequently affecting lipid homeostasis in various tissues (Samuel and Shulman, 2012). Our results further introduce a mechanism by which JNK can alter cellular and systemic lipid metabolism through the regulation of lipases, independent of changes in IIS. Thus, JNK-mediated Foxo activation in select tissues may be able to alter intracellular lipid metabolism, changing metabolic fuel substrates and disrupting metabolic homeostasis in other tissues without altering insulin responsiveness.

Whereas our data show that Foxo activation leads to the transcriptional repression of intestinal lipases, especially LipA/Magro, it remains unclear if this control is direct or indirect. Foxo is classically described as an activator of transcription, but recent reports have shown that Foxo can transcriptionally repress genes through direct association with promoters (Alic et al., 2011; Deng et al., 2012). The promoter regions of LipA/Magro and CG6295 do not contain conserved Foxo transcription factor binding sites, suggesting that the regulation of these genes may be indirect, potentially through Foxo-regulated expression of secondary effectors. Thus, tissue-specific control of lipid homeostasis by IIS/Foxo might be achieved through the regulation of lipogenic or lipolytic transcription factors that can elicit global and direct changes in cellular lipid metabolism. Previous reports have shown that the nuclear receptor dHR96, a critical regulator of lipid and cholesterol homeostasis, promotes *lipA/magro* expression (Sieber and Thummel, 2009). However, *dhr96* expression is upregulated in aging intestines (data not shown), suggesting that the age-related repression of intestinal lipases is not merely due to decreases in dHR96 levels. *dhr96* transcript levels are strongly induced in genetic conditions where Foxo is activated and intestinal lipases are repressed (data not shown), again suggesting that Foxo does not mediate its effects on lipase transcription by antagonizing *dhr96* expression. Furthermore, age-related changes that are independent of JNK/Foxo activation may also contribute to the repression of intestinal lipase expression and disruption of lipid metabolism, such as an age-associated decline in feeding/food intake (Wong et al., 2009).

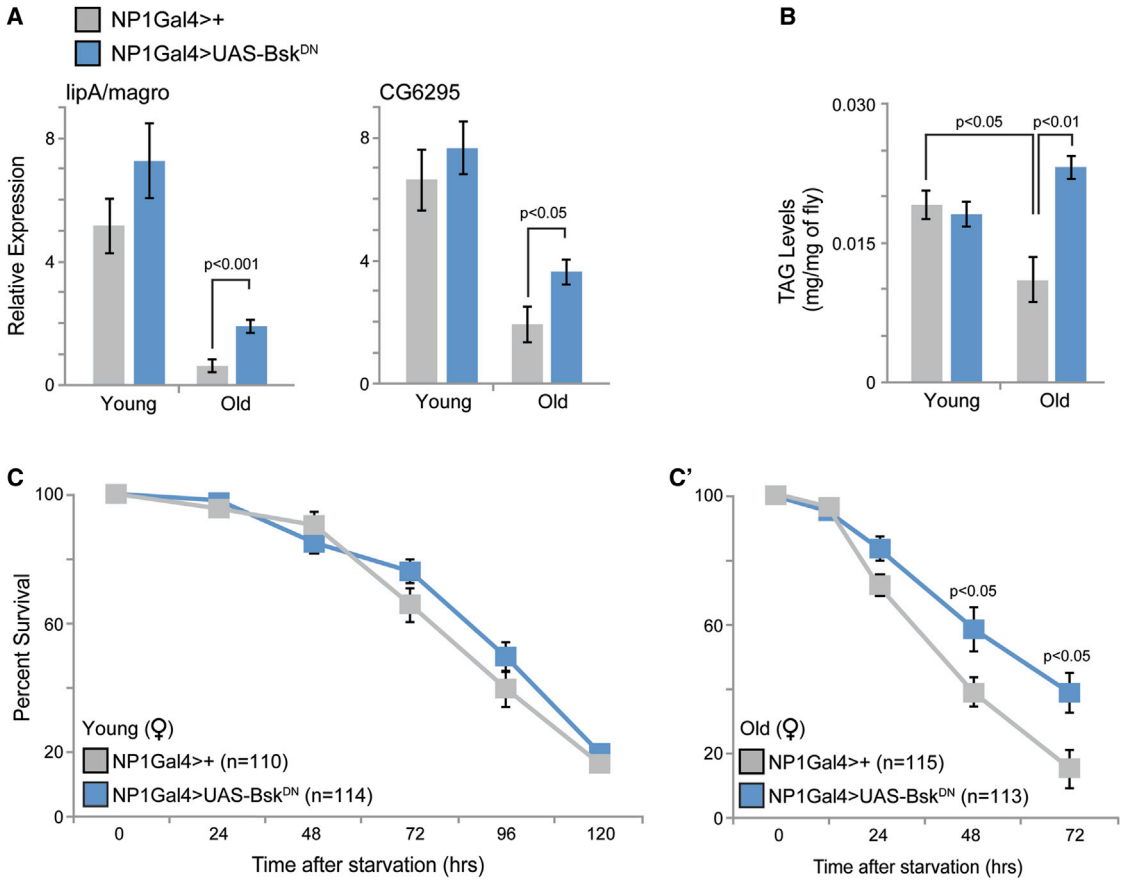

Figure 6. Age-Related Changes in Intestinal JNK Activity Contribute to the Disruption of Lipid Metabolism

(A) *lipA/magro* and *CG6295* transcription (from dissected intestines) measured by qRT-PCR in young (5–7 days) and old (35 days) female flies in which JNK has been inhibited in enterocytes (NP1G4/UAS-Bsk^{DN} compared to controls, NP1G4/+ [w^{1118}]).

(B) Whole animal TAG level measurements in young and old NP1G4/UAS-Bsk^{DN} and NP1G4/+ (w^{1118}) control female flies.

(C and C′) Increased starvation resistance in old flies (35 days, females) with JNK inhibition in intestinal enterocytes. Young (5–7 days) flies from independent populations show no change in survival after starvation. Results are represented as percent survival (mean ± SE) after wet starvation of six cohorts of 18–20 flies from individual populations.

Bars represent mean ± SE and n = 5–7 samples.

See also Table S2.

Foxo-Mediated Disruption of Metabolic Homeostasis in *Drosophila*

The reasons for the increase in JNK and Foxo activity in aging ECs remain to be explored. Buchon et al. (2009) have also shown that age-related activation of JNK in the intestinal epithelium is dependent on the presence of commensal bacteria, as maintaining animals axenically reduces activation of JNK in the first 30 days of life. Thus, bacteria-induced inflammation and subsequent JNK activation appears to be a likely cause, in part, for age-related increases in Foxo activity. In a separate study, however, we find that Foxo activation still occurs in intestines of old (40-day-old), axenically reared flies, suggesting that age-related activation of Foxo may also occur through JNK-independent processes (L. Guo, J.K., S.Tran, and H.J., unpublished data). Supporting this idea, our results show that inhibiting JNK function in enterocytes can attenuate, although not completely inhibit, this Foxo activation. Additional factors, such as sirtuins or histone deacetylases, recently shown to deacetylate and activate Foxo in response to endocrine signals (Mihaylova et al., 2011; Wang et al., 2011), may also lead to age-related increases in intestinal Foxo activity.

Interactions between JNK and IIS/Foxo are mediated by various mechanisms. In mammals, JNK phosphorylates the insulin receptor substrate (IRS), inhibiting insulin signaling transduction (Aguirre et al., 2000; Mamay et al., 2003). JNK has also been shown to directly phosphorylate and activate Foxo in mammalian cell culture (Essers et al., 2004). Whereas JNK has clearly been shown to antagonize IIS (activate Foxo) in *C. elegans* and *Drosophila* (Oh et al., 2005; Wang et al., 2005), that exact mechanism by which Foxo activation is achieved may be divergent in mammals. For example, no IRS homolog has been identified in worms, and the JNK phosphorylation site in mammalian IRS is not conserved in flies. Our data show that JNK-mediated Foxo activation in the aging fly intestine is not achieved through IIS antagonism upstream of Akt, suggesting either a direct interaction between Foxo and JNK or

changes in other regulators of Foxo. Recent studies have shown that JNK-mediated phosphorylation of 14-3-3 proteins results in the release of their binding partners, including Foxo (Sunayama et al., 2005; Yoshida et al., 2005). The conservation of 14-3-3 proteins between vertebrates and invertebrates makes 14-3-3 an interesting candidate in promoting Foxo function via JNK in the aging fly intestine (Nielsen et al., 2008). This chronic intestinal Foxo activation and subsequent metabolic changes, provide a physiological system in *Drosophila* to genetically dissect the crosstalk between IIS/Foxo and JNK signaling. Detailed analysis of these signaling interactions promises to provide important insight into the pleiotropic effects of IIS/Foxo function and the pathogenesis of age-related metabolic diseases.

Our data further reveal the pleiotropic consequences of Foxo activation in regard to healthspan and longevity in *Drosophila*. Overexpressing Foxo in the fat body or muscle of flies leads to lifespan extension (Demontis and Perrimon, 2010; Giannakou et al., 2004; Hwangbo et al., 2004). Conversely, we have recently described that overexpression of Foxo in the intestinal stem cell lineage shortens lifespan by inhibiting stem cell proliferation and thus blocking proper tissue regeneration (Biteau et al., 2010). Overexpression of selected cytoprotective Foxo target genes in stem cells, on the other hand, is sufficient to prevent age-associated dysplasia and extend lifespan (Biteau et al., 2010). The data presented here show that chronic Foxo activation in intestinal enterocytes disrupts lipid metabolism by deregulating intestinal lipases and thus highlight how cell- and tissue-specific consequences of Foxo function play an important role in determining either the beneficial (i.e., lifespan extension) or pathological (i.e., disruption of lipid metabolism) outcome of Foxo activation.

Recent work in *C. elegans* has begun to explore the relationship between lipid metabolism and longevity, revealing that increases in intestinal lipase expression can extend lifespan (O'Rourke et al., 2013; Wang et al., 2008). The beneficial effects of elevated lipase expression appear to be mediated by increases in specific types of fatty acids, which can activate autophagy and lead to lifespan extension (O'Rourke et al., 2013). Interventions that promote lipid homeostasis with age, such as JNK/Foxo inhibition in intestinal enterocytes, might thus affect healthspan and/or longevity through means other than primarily maintaining energy homeostasis.

EXPERIMENTAL PROCEDURES

Drosophila Stocks and Culture

The following strains were obtained from the Bloomington *Drosophila* Stock Center: OreR; w^{1118}; y^1w^1; *thor-lacZ*; UAS-p35; and tub-Gal80^{ts}. UAS-Foxo^{RNAi} was obtained from the Vienna *Drosophila* RNAi Center (transformant ID 106097). NP1Gal4 was kindly provided by D. Ferrandon; UAS-Magro by C.Thummel; the $foxo^{W24}$ mutant allele by M.Tatar; the $foxo^{\Delta 94}$ allele by L. Partridge; GMRGal4, UAS-Hep^{ACT}, and UAS-Bsk^{DN} by M. Mlodzik; esg-Gal4 and esg-LacZ^{C4-1} by S. Hayashi; Su(H)-GBE-lacZ by S. Bray; and *puc-lacZ* (puc^{E69}) by E. Martín-Blanco. UAS-Foxo was previously described in Puig et al. (2003). For aging and starvation sensitivity experiments, UAS-Foxo^{RNAi}, UAS-Bsk^{DN}, UAS-Magro, and UAS-Foxo transgenic lines were backcrossed 8–10 generations into the w^{1118} background. Backcrossed UAS flies and w^{1118} male siblings were used to set up the crosses in order to reduce genetic background effects. The $foxo^{W24}$ mutant was backcrossed into the *yw* background, and *yw* siblings were used as controls (Figure 1E).

All flies were raised on standard yeast- and molasses-based food at 25°C and 65% humidity on a 12 hr light/dark cycle, unless otherwise indicated. Food for HSLY diet feeding (and St. SY control food) was made with the following protocol (as described in Skorupa et al. [2008]): HSLY, 1.5 g agar/40 g sucrose/2.5 g yeast (yeast flake)/0.3 ml propionic acid/100 ml water and St. SY, 1.5 g agar/10 g sucrose/10 g yeast/0.3 ml propionic acid/100 ml water. Ingredients were combined, heated to at least 120°C, and cooled before pouring.

Analysis of Gene Expression

Total RNA from eight to ten guts or from five whole flies (without heads) was extracted using Trizol and complementary DNA synthesized using Superscript II (Invitrogen). Real-time PCR was performed using SyberGreen, a Biorad IQ5 apparatus, and the primers pairs described in the Extended Experimental Procedures (Table S2). Results are average ± standard error of at least three independent samples, and quantification of gene expression levels were measured relative to the expression of Actin5c.

Western Blot Analysis

Guts (10–20) were homogenized in protein sample buffer; proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane using standard procedures. The following antibodies (all from Cell Signaling) were used: phospho-Akt (anti-p-*Drosophila* Akt [S505]; rabbit, 1:1,000), total Akt (anti-pan Akt; rabbit, 1:1,000), and β-actin (anti-beta-actin; rabbit, 1:5,000). Signal was detected using horseradish peroxidase-conjugated anti-rabbit and chemiluminescence (Pierce) according to manufacturer instructions.

Detection of β-Galactosidase Activity

Intact guts were dissected in PBS + 2 mM $MgCl_2$ and fixed for 10 min in 0.5% glutaraldehyde. Detection of β-galactosidase activity was carried out at room temperature in staining buffer (PBS, 2 mM $MgCl_2$, 5 mM K4(Fe[CN]6), 5 mM K3(Fe[CN]6), 0.1% X-gal).

Metabolite Measurements

For TAG assays, four to five females (without the head) were homogenized in 150 μl of PBST (PBS, 0.1% Tween 20) heated at 70°C to inactivate endogenous enzymes (homogenate cleared by centrifugation). Thirty microliters of cleared extract was used to measure triglycerides concentrations according to the manufacturer instructions (StanBio Liquicolor Triglycerides). TAG levels were normalized to weight (measured using MT XS64 scale).

Oil Red O Staining

Guts were dissected in PBS and fixed in 4% formaldehyde/PBS for 20 min. Guts were then washed twice with PBS, incubated for 30 min in fresh Oil Red O solution (6 ml of 0.1% Oil Red O in isopropanol and 4 ml diethylpyrocarbonate water, and passed through a 0.45 μm syringe), followed by rinsing with distilled water.

Immunostaining and Microscopy

Intact guts were fixed at room temperature for 45 min in 100 mM glutamic acid, 25 mM KCl, 20 mM $MgSO_4$, 4 mM sodium phosphate, 1 mM $MgCl_2$, and 4% formaldehyde. All subsequent incubations were done in PBS, 0.5% BSA, and 0.1% Triton X-100 at 4°C.

The following primary antibodies were used: mouse anti-β-Gal (1:200), mouse anti-Prospero (1:250), and mouse anti-armadillo (1:100) from Developmental Studies Hybridoma Bank. Fluorescent secondary antibodies were obtained from Jackson Immunoresearch. Hoechst was used to stain DNA.

Confocal images were collected using a Leica SP5 confocal system and processed using the Leica software and Adobe Photoshop.

Ex Vivo Insulin Stimulation

Guts (8–10) were dissected in PBS and then transferred to M3+BPYE (without serum) cell culture media with or without bovine insulin (0.1 units per 40 μl media from Sigma) for 20 min. Samples were then prepared for protein extraction/western blot analysis, as previously described.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, six figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.08.004.

ACKNOWLEDGMENTS

This work was supported by the National Institute on Aging (NIH RO1 AG028127 to H.J.) and an AFAR/Ellison postdoctoral fellowship (to J.K.).

Received: February 28, 2013
Revised: July 16, 2013
Accepted: August 2, 2013
Published: September 12, 2013

REFERENCES

Accili, D., and Arden, K.C. (2004). FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell *117*, 421–426.

Aguirre, V., Uchida, T., Yenush, L., Davis, R., and White, M.F. (2000). The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J. Biol. Chem. *275*, 9047–9054.

Alic, N., Andrews, T.D., Giannakou, M.E., Papatheodorou, I., Slack, C., Hoddinott, M.P., Cochemé, H.M., Schuster, E.F., Thornton, J.M., and Partridge, L. (2011). Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. Mol. Syst. Biol. *7*, 502.

Badin, P.M., Louche, K., Mairal, A., Liebisch, G., Schmitz, G., Rustan, A.C., Smith, S.R., Langin, D., and Moro, C. (2011). Altered skeletal muscle lipase expression and activity contribute to insulin resistance in humans. Diabetes *60*, 1734–1742.

Barzilai, N., Huffman, D.M., Muzumdar, R.H., and Bartke, A. (2012). The critical role of metabolic pathways in aging. Diabetes *61*, 1315–1322.

Baumeister, R., Schaffitzel, E., and Hertweck, M. (2006). Endocrine signaling in Caenorhabditis elegans controls stress response and longevity. J. Endocrinol. *190*, 191–202.

Biteau, B., Hochmuth, C.E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell Stem Cell *3*, 442–455.

Biteau, B., Karpac, J., Supoyo, S., Degennaro, M., Lehmann, R., and Jasper, H. (2010). Lifespan extension by preserving proliferative homeostasis in Drosophila. PLoS Genet. *6*, e1001159.

Biteau, B., Hochmuth, C.E., and Jasper, H. (2011a). Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. Cell Stem Cell *9*, 402–411.

Biteau, B., Karpac, J., Hwangbo, D., and Jasper, H. (2011b). Regulation of Drosophila lifespan by JNK signaling. Exp. Gerontol. *46*, 349–354.

Blüher, M., Kahn, B.B., and Kahn, C.R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. Science *299*, 572–574.

Buchon, N., Broderick, N.A., Chakrabarti, S., and Lemaitre, B. (2009). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. Genes Dev. *23*, 2333–2344.

Calnan, D.R., and Brunet, A. (2008). The FoxO code. Oncogene *27*, 2276–2288.

Chakrabarti, P., and Kandror, K.V. (2009). FoxO1 controls insulin-dependent adipose triglyceride lipase (ATGL) expression and lipolysis in adipocytes. J. Biol. Chem. *284*, 13296–13300.

Choi, N.H., Kim, J.G., Yang, D.J., Kim, Y.S., and Yoo, M.A. (2008). Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. Aging Cell *7*, 318–334.

Demontis, F., and Perrimon, N. (2010). FOXO/4E-BP signaling in Drosophila muscles regulates organism-wide proteostasis during aging. Cell *143*, 813–825.

Deng, X., Zhang, W., O-Sullivan, I., Williams, J.B., Dong, Q., Park, E.A., Raghow, R., Unterman, T.G., and Elam, M.B. (2012). FoxO1 inhibits sterol regulatory element-binding protein-1c (SREBP-1c) gene expression via transcription factors Sp1 and SREBP-1c. J. Biol. Chem. *287*, 20132–20143.

Eijkelenboom, A., and Burgering, B.M. (2013). FOXOs: signalling integrators for homeostasis maintenance. Nat. Rev. Mol. Cell Biol. *14*, 83–97.

Essers, M.A., Weijzen, S., de Vries-Smits, A.M., Saarloos, I., de Ruiter, N.D., Bos, J.L., and Burgering, B.M. (2004). FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. EMBO J. *23*, 4802–4812.

Garofalo, R.S. (2002). Genetic analysis of insulin signaling in Drosophila. Trends Endocrinol. Metab. *13*, 156–162.

Géminard, C., Rulifson, E.J., and Léopold, P. (2009). Remote control of insulin secretion by fat cells in Drosophila. Cell Metab. *10*, 199–207.

Giannakou, M.E., Goss, M., Jünger, M.A., Hafen, E., Leevers, S.J., and Partridge, L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science *305*, 361.

Hirosumi, J., Tuncman, G., Chang, L., Görgün, C.Z., Uysal, K.T., Maeda, K., Karin, M., and Hotamisligil, G.S. (2002). A central role for JNK in obesity and insulin resistance. Nature *420*, 333–336.

Hwangbo, D.S., Gershman, B., Tu, M.P., Palmer, M., and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature *429*, 562–566.

Karpac, J., and Jasper, H. (2009). Insulin and JNK: optimizing metabolic homeostasis and lifespan. Trends Endocrinol. Metab. *20*, 100–106.

Karpac, J., Hull-Thompson, J., Falleur, M., and Jasper, H. (2009). JNK signaling in insulin-producing cells is required for adaptive responses to stress in Drosophila. Aging Cell *8*, 288–295.

Karpac, J., Younger, A., and Jasper, H. (2011). Dynamic coordination of innate immune signaling and insulin signaling regulates systemic responses to localized DNA damage. Dev. Cell *20*, 841–854.

Kido, Y., Burks, D.J., Withers, D., Bruning, J.C., Kahn, C.R., White, M.F., and Accili, D. (2000). Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. J. Clin. Invest. *105*, 199–205.

Kitamura, T., Kahn, C.R., and Accili, D. (2003). Insulin receptor knockout mice. Annu. Rev. Physiol. *65*, 313–332.

Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell *115*, 489–502.

Lin, K., Dorman, J.B., Rodan, A., and Kenyon, C. (1997). daf-16: An HNF-3/forkhead family member that can function to double the life-span of Caenorhabditis elegans. Science *278*, 1319–1322.

Lowe, M.E. (2000). Properties and function of pancreatic lipase related protein 2. Biochimie *82*, 997–1004.

Luca, F., Perry, G.H., and Di Rienzo, A. (2010). Evolutionary adaptations to dietary changes. Annu. Rev. Nutr. *30*, 291–314.

Luo, X., Puig, O., Hyun, J., Bohmann, D., and Jasper, H. (2007). Foxo and Fos regulate the decision between cell death and survival in response to UV irradiation. EMBO J. *26*, 380–390.

Mamay, C.L., Mingo-Sion, A.M., Wolf, D.M., Molina, M.D., and Van Den Berg, C.L. (2003). An inhibitory function for JNK in the regulation of IGF-I signaling in breast cancer. Oncogene *22*, 602–614.

Matsumoto, M., and Accili, D. (2005). All roads lead to FoxO. Cell Metab. *1*, 215–216.

Micchelli, C.A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature *439*, 475–479.

Michael, M.D., Kulkarni, R.N., Postic, C., Previs, S.F., Shulman, G.I., Magnuson, M.A., and Kahn, C.R. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol. Cell *6*, 87–97.

Mihaylova, M.M., Vasquez, D.S., Ravnskjaer, K., Denechaud, P.D., Yu, R.T., Alvarez, J.G., Downes, M., Evans, R.M., Montminy, M., and Shaw, R.J.

(2011). Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell *145*, 607–621.

Niedernhofer, L.J., Garinis, G.A., Raams, A., Lalai, A.S., Robinson, A.R., Appeldoorn, E., Odijk, H., Oostendorp, R., Ahmad, A., van Leeuwen, W., et al. (2006). A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature *444*, 1038–1043.

Nielsen, M.D., Luo, X., Biteau, B., Syverson, K., and Jasper, H. (2008). 14-3-3 Epsilon antagonizes FoxO to control growth, apoptosis and longevity in Drosophila. Aging Cell *7*, 688–699.

O'Rourke, E.J., Kuballa, P., Xavier, R., and Ruvkun, G. (2013). ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. Genes Dev. *27*, 429–440.

Odegaard, J.I., and Chawla, A. (2013). Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science *339*, 172–177.

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. Nature *389*, 994–999.

Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., and Tissenbaum, H.A. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. Proc. Natl. Acad. Sci. USA *102*, 4494–4499.

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature *439*, 470–474.

Oldham, S., and Hafen, E. (2003). Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. Trends Cell Biol. *13*, 79–85.

Osterwalder, T., Yoon, K.S., White, B.H., and Keshishian, H. (2001). A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. USA *98*, 12596–12601.

Puig, O., Marr, M.T., Ruhf, M.L., and Tjian, R. (2003). Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. *17*, 2006–2020.

Rera, M., Clark, R.I., and Walker, D.W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. Proc. Natl. Acad. Sci. USA *109*, 21528–21533.

Rera, M., Azizi, M.J., and Walker, D.W. (2013). Organ-specific mediation of lifespan extension: more than a gut feeling? Ageing Res. Rev. *12*, 436–444.

Roberts, S.B., and Rosenberg, I. (2006). Nutrition and aging: changes in the regulation of energy metabolism with aging. Physiol. Rev. *86*, 651–667.

Rulifson, E.J., Kim, S.K., and Nusse, R. (2002). Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science *296*, 1118–1120.

Saltiel, A.R., and Kahn, C.R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. Nature *414*, 799–806.

Samuel, V.T., and Shulman, G.I. (2012). Mechanisms for insulin resistance: common threads and missing links. Cell *148*, 852–871.

Schoenborn, V., Heid, I.M., Vollmert, C., Lingenhel, A., Adams, T.D., Hopkins, P.N., Illig, T., Zimmermann, R., Zechner, R., Hunt, S.C., and Kronenberg, F. (2006). The ATGL gene is associated with free fatty acids, triglycerides, and type 2 diabetes. Diabetes *55*, 1270–1275.

Shimomura, I., Matsuda, M., Hammer, R.E., Bashmakov, Y., Brown, M.S., and Goldstein, J.L. (2000). Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. Mol. Cell *6*, 77–86.

Sieber, M.H., and Thummel, C.S. (2009). The DHR96 nuclear receptor controls triacylglycerol homeostasis in Drosophila. Cell Metab. *10*, 481–490.

Sieber, M.H., and Thummel, C.S. (2012). Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. Cell Metab. *15*, 122–127.

Skorupa, D.A., Dervisefendic, A., Zwiener, J., and Pletcher, S.D. (2008). Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. Aging Cell *7*, 478–490.

Slack, C., Giannakou, M.E., Foley, A., Goss, M., and Partridge, L. (2011). dFOXO-independent effects of reduced insulin-like signaling in Drosophila. Aging Cell *10*, 735–748.

Sunayama, J., Tsuruta, F., Masuyama, N., and Gotoh, Y. (2005). JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3. J. Cell Biol. *170*, 295–304.

Suzuki, R., Lee, K., Jing, E., Biddinger, S.B., McDonald, J.G., Montine, T.J., Craft, S., and Kahn, C.R. (2010). Diabetes and insulin in regulation of brain cholesterol metabolism. Cell Metab. *12*, 567–579.

Taguchi, A., and White, M.F. (2008). Insulin-like signaling, nutrient homeostasis, and life span. Annu. Rev. Physiol. *70*, 191–212.

Tatar, M., Bartke, A., and Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. Science *299*, 1346–1351.

Teleman, A.A. (2010). Molecular mechanisms of metabolic regulation by insulin in Drosophila. Biochem. J. *425*, 13–26.

van der Pluijm, I., Garinis, G.A., Brandt, R.M., Gorgels, T.G., Wijnhoven, S.W., Diderich, K.E., de Wit, J., Mitchell, J.R., van Oostrom, C., Beems, R., et al. (2007). Impaired genome maintenance suppresses the growth hormone—insulin-like growth factor 1 axis in mice with Cockayne syndrome. PLoS Biol. *5*, e2.

Vihervaara, T., and Puig, O. (2008). dFOXO regulates transcription of a Drosophila acid lipase. J. Mol. Biol. *376*, 1215–1223.

Wang, M.C., Bohmann, D., and Jasper, H. (2005). JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. Cell *121*, 115–125.

Wang, M.C., O'Rourke, E.J., and Ruvkun, G. (2008). Fat metabolism links germline stem cells and longevity in C. elegans. Science *322*, 957–960.

Wang, B., Moya, N., Niessen, S., Hoover, H., Mihaylova, M.M., Shaw, R.J., Yates, J.R., 3rd, Fischer, W.H., Thomas, J.B., and Montminy, M. (2011). A hormone-dependent module regulating energy balance. Cell *145*, 596–606.

Wong, R., Piper, M.D., Wertheim, B., and Partridge, L. (2009). Quantification of food intake in Drosophila. PLoS ONE *4*, e6063.

Xu, X., Gopalacharyulu, P., Seppänen-Laakso, T., Ruskeepää, A.L., Aye, C.C., Carson, B.P., Mora, S., Orešič, M., and Teleman, A.A. (2012). Insulin signaling regulates fatty acid catabolism at the level of CoA activation. PLoS Genet. *8*, e1002478.

Yoshida, K., Yamaguchi, T., Natsume, T., Kufe, D., and Miki, Y. (2005). JNK phosphorylation of 14-3-3 proteins regulates nuclear targeting of c-Abl in the apoptotic response to DNA damage. Nat. Cell Biol. *7*, 278–285.

Zhang, P., Judy, M., Lee, S.J., and Kenyon, C. (2013). Direct and indirect gene regulation by a life-extending FOXO protein in C. elegans: roles for GATA factors and lipid gene regulators. Cell Metab. *17*, 85–100.