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REVIEW

Modeling metabolic homeostasis and nutrient sensing in *Drosophila*: implications for aging and metabolic diseases

Edward Owusu-Ansah^{1,*} and Norbert Perrimon^{1,2,*}**ABSTRACT**

Over the past decade, numerous reports have underscored the similarities between the metabolism of *Drosophila* and vertebrates, with the identification of evolutionarily conserved enzymes and analogous organs that regulate carbohydrate and lipid metabolism. It is now well established that the major metabolic, energy-sensing and endocrine signaling networks of vertebrate systems are also conserved in flies. Accordingly, studies in *Drosophila* are beginning to unravel how perturbed energy balance impinges on lifespan and on the ensuing diseases when energy homeostasis goes awry. Here, we highlight several emerging concepts that are at the nexus between obesity, nutrient sensing, metabolic homeostasis and aging. Specifically, we summarize the endocrine mechanisms that regulate carbohydrate and lipid metabolism, and provide an overview of the neuropeptides that regulate feeding behavior. We further describe the various efforts at modeling the effects of high-fat or -sugar diets in *Drosophila* and the signaling mechanisms involved in integrating organ function. Finally, we draw attention to some of the cardinal discoveries made with these disease models and how these could spur new research questions in vertebrate systems.

KEY WORDS: Metabolic homeostasis, Nutrient sensing, *Drosophila***Introduction**

Metabolic syndrome – often considered a harbinger of cardiovascular disease – is a complex clinical disorder characterized primarily by abnormal blood lipid levels (dyslipidemia), central obesity, high blood pressure and elevated fasting glucose levels. Although previously considered a debilitating condition restricted to affluent societies, it has now emerged as an issue of major public health significance worldwide. Attempts to uncover therapeutic strategies for alleviating this global phenomenon have focused largely on vertebrate model systems. However, recent observations in *Drosophila* have given credence to the hypothesis that this simple model organism can provide useful information for elucidating the complexities of mammalian metabolism.

Drosophila have organ systems that perform essentially the same metabolic functions as their vertebrate counterparts (Leopold and Perrimon, 2007). For instance, there are both oxidative and glycolytic muscles (such as flight and leg muscles, respectively) that consume energy during flight or other forms of locomotion. In

addition, the fat body, which stores excess fat as triglycerides (which can be mobilized during times of need using lipases that are orthologous to those found in mammals), functions as the liver and white adipose tissue (Baker and Thummel, 2007; Leopold and Perrimon, 2007). A group of specialized cells, referred to as oenocytes, can function as hepatocytes by mobilizing stored lipids in the fat body during periods of food deprivation (Gutierrez et al., 2007). Moreover, the sophisticated genetic tools available for studies in this organism (del Valle Rodríguez et al., 2011), coupled with its relatively short lifespan, have facilitated the discovery of novel molecules and modes of regulation of multiple aspects of metabolism and aging (Leopold and Perrimon, 2007; Karpac and Jasper, 2009; Alic and Partridge, 2011; Biteau et al., 2011). Here, we highlight recent advances in modeling aspects of metabolic homeostasis in *Drosophila*, especially as it relates to diabetes, obesity and the overall aging process. We begin by highlighting the pathways that regulate normal metabolic homeostasis in *Drosophila*.

Regulating metabolic homeostasis through the *Drosophila* orthologs of glucagon and insulin

Homeostatic regulation of circulating sugar levels is essential for the health of organisms. For example, impaired fasting glucose (elevated blood sugar) is an important risk factor associated with the development of cardiovascular disease in humans (Kannel et al., 1990). In addition, one of the severe metabolic complications of diabetes is ketoacidosis, which can result from exceptionally high circulating glucose levels (Forbes and Cooper, 2013). In mammals, glucagon and insulin are synthesized in pancreatic α - and β -cells, respectively, with the former largely responsible for breaking down glycogen into sugar, whereas insulin regulates the converse process. Similarly, *Drosophila* produce a glucagon-like peptide, referred to as the adipokinetic hormone (AKH), in a group of neurosecretory cells in the ring gland known as the corpora cardiaca (Kim and Rulifson, 2004; Lee and Park, 2004). Forced AKH expression from the fat body increases trehalose levels (trehalose is the major circulating sugar in *Drosophila*); in contrast, flies devoid of the AKH-producing neurons display a precipitous drop in trehalose levels (Lee and Park, 2004). Nevertheless, the AKH signaling cascade in *Drosophila* is poorly characterized. For instance, other than the ligand (AKH) and receptor (AKH receptor), very little is known about the downstream intracellular kinases and phosphatases, and it is unclear whether there are other ligands and receptors for the pathway. Significantly, because no overt developmental effects are associated with aberrant AKH signaling under non-stressed conditions, it is particularly amenable to genetic screens because data interpretation is unencumbered by alterations in rates of development.

In contrast to AKH signaling, the insulin–insulin-growth-factor signaling (IIS) pathway in *Drosophila* has been more thoroughly characterized. Like many aspects of *Drosophila* metabolism, there are remarkable differences between the effects of insulin signaling

¹Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA. ²Howard Hughes Medical Institute, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.

*Authors for correspondence (ekowuans@genetics.med.harvard.edu; perrimon@receptor.med.harvard.edu)

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Table 1. Expression pattern and function of *Drosophila* insulin-like peptides (DILPs)

Insulin-like peptide	Expression pattern	Function	References
DILP1	MNCs of larval and adult brains	Ubiquitous overexpression increases body size, and deletion results in reduced body mass	Ikeya et al., 2002; Rulifson et al., 2002; Gronke et al., 2010; Bai et al., 2012
DILP2	Mesoderm and midgut of embryos, imaginal discs of larvae, and in the MNCs of both larval and adult brains	Overexpression increases cell size and number; loss of DILP2 results in a delay in development, reduced body mass, compensatory activation of DILP3 and DILP5, and increased lifespan. Regulates trehalose levels. Overexpression retards loss of germline stem cells	Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Broughton et al., 2005; Broughton et al., 2008; Hsu and Drummond-Barbosa, 2009; Gronke et al., 2010
DILP3	Mesoderm and midgut of embryos, in the MNCs of both larval and adult brains, and in the visceral muscle of the adult midgut	Regulates growth of intestinal stem cells	Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Broughton et al., 2005; Veenstra et al., 2008; O'Brien et al., 2011
DILP4	Mesoderm and anterior midgut rudiment of embryos, as well as larval midgut	Ubiquitous overexpression is sufficient to promote growth	Brogiolo et al., 2001; Ikeya et al., 2002
DILP5	Mesoderm and midgut of embryos, and the MNCs of both larval and adult brains	Ubiquitous overexpression is sufficient to promote growth	Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Broughton et al., 2005
DILP6	Expressed predominantly in larval and adult fat body; low signal in larval gut, expressed in a population of surface glia that surround neuroblasts	Regulates postfeeding growth; overexpression in adult fat body extends lifespan; required for neuroblast reactivation from quiescence	Brogiolo et al., 2001; Okamoto et al., 2009; Slaidina et al., 2009; Chell and Brand, 2010; Sousa-Nunes et al., 2011; Bai et al., 2012
DILP7	Moderate expression in embryonic midguts; expressed in sections of the larval and adult ventral nerve cord	Regulates egg-laying decisions	Brogiolo et al., 2001; Yang et al., 2008
DILP8	Imaginal discs	Regulates adaptive developmental plasticity in injured imaginal discs	Colombani et al., 2012; Garelli et al., 2012

MNCs, median neurosecretory cells.

during the larval and adult phases. For instance, insulin regulates growth of essentially all tissues during the larval stage but its effect in adults is largely restricted to metabolic homeostasis, resistance to stress, fecundity and lifespan (Broughton et al., 2005; Gronke et al., 2010). There are eight *Drosophila* insulin-like peptides (DILPs) (Table 1), some of which have unique properties and varying tissue and temporal expression patterns (Brogiolo et al., 2001; Ikeya et al., 2002; Rulifson et al., 2002; Broughton et al., 2005; Broughton et al., 2008; Veenstra et al., 2008; Yang et al., 2008; Hsu and Drummond-Barbosa, 2009; Okamoto et al., 2009; Slaidina et al., 2009; Chell and Brand, 2010; O'Brien et al., 2011; Sousa-Nunes et al., 2011; Bai et al., 2012; Colombani et al., 2012; Garelli et al., 2012). Genetic ablation of the insulin-producing cells (IPCs) during early larval stages delays development and results in elevated sugar levels in the larval hemolymph (Rulifson et al., 2002); however, ablation during the adult stage results in reduced fecundity, increased storage of triglycerides and sugars, heightened resistance to starvation and oxidative stress, and prolonged lifespan (Broughton et al., 2005). Nevertheless, because IPCs secrete other peptides as well, it is unclear whether the phenotypes associated with IPC ablation are due solely to the secreted ILPs or other peptides as well.

Although the core intracellular IIS cascade consists of single genes encoding the insulin-like receptor (InR), Akt (protein kinase B), phosphoinositide 3-kinase (PI3K) and forkhead transcription factor FOXO, regulation of insulin signaling in *Drosophila* is proving to be remarkably complex. In addition to the numerous DILPs that regulate insulin signaling (Table 1), there is a growing list of insulin-antagonizing peptides that have notable impacts on insulin signaling. In mammalian systems, insulin signaling can be modulated by a group of secreted insulin growth factor (IGF)-binding proteins (IGFBPs) and IGF-binding related proteins

(IGFBP-rPs). Notably, binding of these secretory proteins to IGF can either enhance or suppress insulin signaling by altering the bioavailability of insulin, the rate of degradation of insulin or the ability of insulin to bind to its cognate receptor (Hwa et al., 1999). The importance of IGFBPs is underscored by the fact that IGFBP7 can act as a tumor suppressor (Ruan et al., 2007; Chen et al., 2013). The imaginal morphogenesis protein-Late 2 (Imp-L2), which is a neural/ectodermal development factor in *Drosophila*, has been shown to bind to DILP2 and DILP5 to impair insulin signaling (Alic et al., 2011); however, it promotes insulin signaling in a subset of neurons in the larval brain (Bader et al., 2013). Forced expression of Imp-L2 results in induction of 4E-BP (a marker of insulin repression), causes non-autonomous growth inhibition and triggers many of the phenotypes associated with impaired insulin signaling, such as reduced fecundity, increased triglycerides and extended lifespan (Honegger et al., 2008; Alic et al., 2011). Another insulin-binding peptide is acid labile subunit (ALS), which functions in a trimeric complex with Imp-L2 (Arquier et al., 2008). In addition, secreted decoy of insulin receptor (SDR), which is structurally similar to the extracellular domain of the insulin receptor, has been shown to bind to several DILPs *in vitro* (Okamoto et al., 2013) and, when overexpressed, it upregulates several markers of insulin repression. In addition, overexpression of SDR in larvae produces adult flies that are smaller than controls. SDR and Imp-L2 appear to be mutually exclusive with respect to their ability to bind DILPs. It remains to be tested whether SDR overexpression is sufficient to extend lifespan.

Finally, metabolic homeostasis also requires that organs coordinate their activities by means of the secretion of humoral factors or the propagation of nerve impulses between the organs. In mammalian systems, this is a fairly well-established phenomenon, because the brain is known to process signals relating to the

nutritional status of an organism and then elicit appropriate systemic responses. There is mounting evidence that *Drosophila* organs can also ‘communicate’ with each other, and recent studies in flies have unraveled several inter-organ signaling modules, some of which are regulated by DILPs. As a case in point, overexpression of FOXO in flight muscles reduces both feeding behavior and insulin secretion from the IPCs. This in turn delays the accumulation of misfolded protein aggregates not only in muscles, but also non-autonomously in non-muscle tissue (Demontis and Perrimon, 2010).

The fat body has also been shown to remotely control the secretion of DILPs from the IPCs through a mechanism dependent on the ‘target of rapamycin’ (TOR) (Colombani et al., 2003; Géminard et al., 2009). By analyzing a series of elegant *ex vivo* co-cultures of larval brains and fat body tissue, the existence of a humoral signal (or signals) released from the fat body that can impede the secretion of DILPs from the IPCs was proposed (Géminard et al., 2009). Subsequently, unpaired 2 (Upd2), a specific ligand of the *Drosophila* JAK-STAT pathway, was found to be induced in the fat body during the fed state (Rajan and Perrimon, 2012). Interestingly, Upd2 induction in the fat body is also associated with the release of DILPs from the IPCs in the brain (Rajan and Perrimon, 2012). Others have shown that DILP6 was upregulated in the fat body in response to fasting or FOXO overexpression and could also repress secretion of DILP2 from IPCs in the brain (Bai et al., 2012). Importantly, overexpression of DILP6 in head or abdominal fat body caused many of the classic traits associated with downregulation of insulin signaling, such as the presence of increased whole-body triglycerides, stress resistance and lifespan of female flies (Bai et al., 2012). It will be interesting to investigate whether Upd2 and DILP6 act in concert or in parallel to regulate DILP secretion from IPCs.

Regulation of feeding behavior through peptidergic signaling

A major aspect of metabolic homeostasis is the regulation of feeding behavior. Studies in mammalian systems have shown that the presence of food in the gut stimulates a number of endocrine and neuronal signals that act through complex feedback loops to regulate feeding behavior. A growing list of peptides such as ghrelin, cholecystokinin, glucagon-like peptide-1 (GLP-1) and neuropeptide Y are known to regulate food intake and satiety (Steinert et al., 2013). Interestingly, the overwhelming success of Roux-en-Y gastric bypass surgery, in which the stomach is subdivided and reconnected to the small intestine, in causing significant weight loss in morbidly obese individuals has been linked to alterations in gut peptides that regulate feeding and satiety (Kellum et al., 1990; le Roux et al., 2006; Morinigo et al., 2006). Not surprisingly, there is mounting research to explore the possibility of interfering with the action of these factors as a means to control obesity. For instance, GLP-1, produced primarily in the distal intestine, is rapidly released after a meal and suppresses food intake in several organisms (Turton et al., 1996; Donahey et al., 1998; Chelikani et al., 2005). Accordingly, administration of GLP-1 to subjects with type 2 diabetes for 6 weeks resulted in reduced appetite, significant weight loss and a decrease in plasma glucose levels (Zander et al., 2002). In clinical trials, Exenatide (an agonist of the GLP-1 receptor) administered to patients with type 2 diabetes has shown great promise, resulting in a loss of ~2 kg over 4 weeks and improved glycemic control (Poon et al., 2005). By contrast, ghrelin has the opposite effect of GLP-1: it increases food intake in a variety of species (Tschöp et al., 2000; Wren et al., 2001a; Wren et al., 2001b); hence, therapeutic efforts have focused on blocking ghrelin signaling by means of various

receptor antagonists. In another parallel with mammalian systems, there is growing evidence of the regulation of specific food choices and overall feeding behavior by various *Drosophila* peptides (Nässel and Winther, 2010).

Neuropeptide F, which is the *Drosophila* ortholog of mammalian neuropeptide Y, was identified based on a radioimmunoassay using a peptide from the corn earworm (Brown et al., 1999). It was subsequently found to regulate feeding behavior and multiple stress responses (Wu et al., 2003; Xu et al., 2010). *Drosophila* also produces a shorter neuropeptide F, referred to as sNPF. Overexpression of sNPF increases both food consumption and overall body size, whereas loss of sNPF decreases food intake (Lee et al., 2004). Later studies revealed that the size increase associated with sNPF overexpression is due largely to its effect on insulin secretion. sNPF regulates the release of DILPs from IPCs. Subsequently, the increased circulating levels of DILPs systemically increase growth and metabolism (Lee et al., 2008b). Importantly, sNPF mutants phenocopy most of the phenotypes associated with dampened insulin signaling, including lifespan extension and elevation of circulating sugar levels, confirming that a major effect of sNPF signaling in *Drosophila* is to augment insulin signaling (Lee et al., 2008b).

Interestingly, although alteration of feeding behavior usually produces a net increase or decrease in total caloric intake, there are instances where there is no net change in total calories consumed. For instance, disruption of the leucokinin pathway at the level of the peptide or receptor in *Drosophila* results in the consumption of large meal portions. However, this is associated with a decreased frequency of meal consumption, resulting in no net change in total caloric intake relative to wild-type flies (Al-Anzi et al., 2010). In addition, activation of neurons expressing allatostatin A suppresses starvation-induced feeding without affecting triglyceride or glucose levels (Hergarden et al., 2012).

There are other relatively less-well-characterized peptides that might also modulate feeding behavior, physiology and metabolism. As a case in point, loss of the brain-secretory polypeptide prothoracicotropic hormone (PTTH) delays larval development, extends the feeding period and leads to a concomitant increase in body size (McBayer et al., 2007). In addition, expression of the cardioactive peptide corazonin decreases during periods of stress, and ablation of corazonin neurons confers tolerance to multiple stresses and alters energy stores (Lee et al., 2008a; Zhao et al., 2010). Intriguingly, sex peptide, a peptide present in seminal fluid introduced into female flies by copulation, increases feeding behavior in females (Carvalho et al., 2006), and diuretic hormone 31 regulates the passage of food along the midgut (LaJeunesse et al., 2010). The neuropeptide hugin regulates feeding behavior and is delivered by axonal projections that reach the pharyngeal muscles, which are required for food uptake (Melcher and Pankratz, 2005). In addition, some DILP7-producing neurons innervate the adult hindgut and regulate feeding behavior in response to nutrient availability (Miguel-Aliaga et al., 2008; Cognigni et al., 2011). Furthermore, it has been shown that, in addition to their established role in DILP secretion, IPCs also secrete drososulfakinins, which act as a satiety signal by regulating both food choice and intake (Söderberg et al., 2012). These observations underscore the complexity of appetite regulation through peptidergic signaling and emphasize the need to study this phenomenon in a combinatorial context. Future studies will clarify the exact molecular mechanisms underpinning the action of these less-characterized peptides, and how their modulation affects lifespan and metabolic homeostasis.

Insights from *Drosophila* models associated with aberrant energy homeostasis

Obesity and high-fat-diet models

Most of the major metabolic enzymes in mammals are conserved in *Drosophila*. For instance, the genes that regulate lipid uptake, transport, storage and mobilization are all well conserved (Oldham and Hafen, 2003; Kim and Rulifson, 2004; Baker and Thummel, 2007; Leopold and Perrimon, 2007; Trinh and Boulianne, 2013). Given the similarities between fly and human metabolism, there have been attempts to unravel as-yet-unknown mechanisms that regulate high-fat-diet (HFD)-induced obesity in *Drosophila* (Birse et al., 2010). In one such study, several parallels between human and fly obesity were observed (Birse et al., 2010). For instance, similar to humans, flies accumulate lipids in a dose-dependent manner, become obese when raised on an HFD and can accumulate excess dietary fat in non-adipose tissue. An interesting observation in flies, which could have etiological implications for cardiovascular diseases in humans, is that flies fed an HFD develop cardiomyopathy. Importantly, systemic inhibition of the TOR pathway disrupts the accumulation of excess fats and prevents the high-fat-induced impairment of cardiac function. In addition, cardiac function was preserved in flies fed an HFD even when downregulation of TOR was restricted only to the heart. Similar observations were made with flies overexpressing FOXO or lipase specifically in the heart. It has therefore been proposed that targeted inhibition of the TOR pathway might be a viable therapeutic approach for ameliorating the effect of obesity on cardiac function (Table 2).

Although the accumulation of excess calories from HFDs is a major environmental factor linked to obesity, there is growing evidence for a strong hereditary component as well. In this regard, genetic screens to uncover the polygenic basis of obesity will hold great promise. Attempts to find unknown genes and pathways that regulate obesity have focused on transgenic and knockout mouse models modeling human obesity, quantitative trait loci (QTL) from animal breeding experiments and linkage analyses (Rankinen et al., 2006). Although such approaches have their merits, a major pitfall is that they preclude large-scale analyses owing to their prohibitive costs. In a particularly illuminating example of how *Drosophila* genetics can be used to bridge this gap, a genome-wide transgenic RNAi screen was performed in *Drosophila* to uncover modulators of fat body formation (Pospisilik et al., 2010). One of the genes identified was *hedgehog* (*hh*), which was subsequently found to play a key role in the determination of brown versus white adipocyte cell

fate in mice (Pospisilik et al., 2010). Importantly, more than 60% of the candidate genes from the screen were conserved from *Drosophila* to humans, and numerous genes previously known to play crucial roles in mammalian lipid metabolism, such as enzymes that regulate glucose or sterol metabolism and membrane lipid biosynthesis, scored positively in the screen. Strikingly, many of the candidate genes for regulating lipid metabolism had no previously ascribed biological functions; thus, further characterization of these candidate genes is likely to reveal additional regulatory strategies for fat metabolism in mammals.

Another promising approach for elucidating conserved novel pathways or genes associated with obesity is to combine studies in *Drosophila* with those in a mammalian system. In one such example, a screen was performed in *Drosophila* to identify genes that either increased or decreased triglyceride levels (Dohrmann, 2004). Out of ~10,000 mutants screened, 200 candidate genes were found to alter total triglyceride content. In parallel experiments to identify chromosomal loci that are susceptible to obesity, the authors analyzed offspring from New Zealand obese (NZO) mice outcrossed to lean mouse strains (Kluge et al., 2000; Plum et al., 2000; Plum et al., 2002). They intensely pursued a specific chromosomal region that contained several genes that were also present in the candidate list obtained from the *Drosophila* screen. Further studies resulted in the characterization of one of these ‘high-confidence’ genes – CG17646 in *Drosophila* – which is the mammalian ortholog of ABCG1 (an ATP-binding cassette transporter). More extensive analyses using knockout mouse models revealed that disruption of ABCG1 expression impeded the rate at which mice gained weight over a 12-week period. This was associated with a reduction in total mass of adipose tissue and a significantly reduced size of the adipocytes (Buchmann et al., 2007). Thus, the combined analyses of data from flies and mice resulted in the elucidation of a previously unrecognized role of ABCG1 in the regulation of metabolic homeostasis.

In summary, because the various models of obesity in *Drosophila* recapitulate the predominant features of obesity in humans, the stage is set for elaborate genetic screens that will help decipher additional causative factors of obesity. In particular, additional studies to further dissect the effect of cardiac lipotoxicity in *Drosophila* could open up novel therapeutic opportunities.

Diabetes and high-sugar diet models

A number of research groups have established models to study diabetes and the effect of high-sugar diets (HSDs) in *Drosophila*

Table 2. Distinguishing features of *Drosophila* models of aberrant nutrient sensing

Model	Phenotype	Reference
Adults fed an HFD	Accumulate excess fat in both adipose and non-adipose tissues; develop cardiomyopathy. Systemic disruption of the TOR pathway prevents HFD-induced disruption of cardiac function. Expression of TSC1 or 2, FOXO, 4EBP or lipases specifically in the heart ameliorates the HFD-induced cardiomyopathy	Birse et al., 2010
Larvae raised on an HSD	Delayed development, increased fat accumulation, insulin resistance, hyperglycemia, decreased glycogen levels, upregulation of FOXO target genes and genes involved in lipogenesis, gluconeogenesis and β -oxidation	Musselman et al., 2011
Larvae raised on an HSD	Short-term exposure to an HSD caused a rapid increase in circulating glucose (by 2 minutes of feeding) but no effect on circulating trehalose up to 1 hour after feeding; long-term exposure leads to both hyperglycemia and hypertrehalosemia, developmental delay, accumulation of excess fat, and upregulation of acetyl CoA carboxylase. Peripheral insulin resistance ameliorated by forced expression of DILPs from IPCs. Potent induction of JNK target genes, as well as the lipocalin neural lazarus (NLaz), which suppresses HSD-induced insulin resistance	Pasco and Leopold, 2012
Adults fed an HSD	Reduced lifespan, increased fat accumulation, insulin resistance, hyperglycemia, progressive deterioration of heart function, genetic interaction with the p38 and insulin pathways. Disruption of the hexosamine pathway improved cardiac function of flies fed an HSD	Na et al., 2013

HFD, high-fat diet; HSD, high-sugar diet.

(Musselman et al., 2011; Pasco and Léopold, 2012; Na et al., 2013). For instance, larvae fed a high-calorie diet develop hyperglycemia, a hallmark of diabetes in humans, which is typically scored in fruit flies as an increase in both hemolymph glucose and trehalose, the primary circulating sugar in this organism (Musselman et al., 2011). It is noteworthy that the hyperglycemia was more severe when flies were fed an HSD, with the extent of hyperglycemia similar to what is observed in insulin-resistant flies or flies with their IPCs ablated (Rulifson et al., 2002; Song et al., 2010). Additional experiments revealed that larvae fed an HSD also displayed peripheral insulin resistance (Musselman et al., 2011). Thus, all the major features of diabetes were recapitulated in this *Drosophila* larval model (Table 2). In a remarkable parallel with mammalian systems, insulin-resistant animals have higher amounts of stored fat as detected by an increase in total triglyceride levels and size of lipid droplets in their adipocytes (Musselman et al., 2011).

A second *Drosophila* larval diabetic model also revealed that an HSD causes peripheral insulin resistance (Table 2) and that forced secretion of DILPs can overcome the effect of the HSD (Pasco and Léopold, 2012). Unquestionably, the outstanding feature of this report was that the peripheral insulin resistance triggered by the HSD was mediated by the lipocalin NLaz (neural lazarlillo). NLaz is an ortholog of the vertebrate lipocalins – lipocalin 2 and retinol binding protein 4 (RBP4) – which modulate peripheral insulin resistance and have been associated with metabolic homeostasis (Yang et al., 2005; Graham et al., 2006). However, the precise roles of these mammalian lipocalins had been controversial. For instance, although RBP4 levels correlated with insulin resistance in some type-II diabetes patients (Gavi et al., 2007; Kim et al., 2012), other reports have shown the opposite (von Eynatten et al., 2007; Al-Daghri et al., 2009). Thus, the authors sought to clarify the role of NLaz by using their model of HSD-induced insulin resistance. They found that, among the three fly orthologs of lipocalin, only one (i.e. NLaz) was robustly induced in HSD-fed larvae. Subsequently, they observed that a mutant *NLaz* allele, or fat-body-restricted knockdown of *NLaz*, could rescue the metabolic defects of the HSD-fed larvae. Thus, in addition to showing a correlation between HSD-induced insulin resistance and *NLaz* expression, the therapeutic potential of disrupting lipocalin function in type-II diabetes patients was established.

Adult flies fed an HSD develop severe structural and functional alterations of the heart (Table 2), as well as essentially all the phenotypes observed in the larval model (Musselman et al., 2011; Na et al., 2013). Defects in fly heart function were first manifest as arrhythmias, which then progressed to fibrillations and asystolic periods (Na et al., 2013). Previous reports in mice had shown that the hexosamine biosynthetic pathway might influence the extent of pathogenesis of type-II diabetes (Liu et al., 2000; Kaneto et al., 2001; Brownlee, 2005). Accelerating the rate of flux of hexosamine in cardiomyocytes promotes hyperglycemia-induced apoptosis (Frustaci et al., 2000). Accordingly, activation of the hexosamine synthetic pathway in *Drosophila* hearts resulted in aberrant heart function (Na et al., 2013). Interestingly, disrupting the activities of two enzymes that regulate hexosamine biosynthesis suppressed the sugar-induced cardiac dysfunction, raising the possibility that disrupting hexosamine biosynthesis might be a plausible therapeutic option for curtailing diet-induced cardiomyopathy (Na et al., 2013).

Altogether, the HSD diabetic models in *Drosophila* faithfully capture the salient features of type-II diabetes. Given that perturbations of specific biosynthetic or signaling pathways that had previously been associated with type-II diabetes (such as hexosamine biosynthesis and NLaz) notably impact disease

progression, the future is ripe for the identification of druggable targets that can impede the progression of this debilitating condition using well-designed genetic screens in *Drosophila*.

Intersection between nutrient sensing, metabolic homeostasis and aging

Nutrient-sensing pathways have been linked to aging in multiple organisms. It is known that dietary restriction extends lifespan in multiple organisms and at least delays the age-dependent decline in function of primates such as monkeys (Fontana et al., 2010; McKiernan et al., 2011; Anderson and Weindruch, 2012). In humans, it delays the age of onset of diseases such as cancer, diabetes and heart disease (Anderson and Weindruch, 2012). Additionally, reduced insulin or TOR signaling extends lifespan in yeast, the roundworm *Caenorhabditis elegans*, *Drosophila* and mice (Fontana et al., 2010; Kenyon, 2010; Evans et al., 2011; Johnson et al., 2013).

Another nutrient-responsive kinase activated under conditions of energy deprivation is the AMP-activated protein kinase (AMPK) (Hardie et al., 2003). When ATP levels fall, AMP levels rise, resulting in the activation of AMPK, which in turn orchestrates a complex signaling network that restores cellular energy homeostasis (Hardie et al., 2012) (Fig. 1). Evidence of the beneficial effects of AMPK activation in mammalian systems is based on the fact that metformin (an AMPK activator) is a potent anti-diabetic agent (Zhou et al., 2001). In addition, studies in *C. elegans* have highlighted the fact that the ratio of AMP:ATP levels is predictive of lifespan and that AMPK activation promotes longevity (Apfeld et al., 2004; Curtis et al., 2006; Greer et al., 2007; Mair et al., 2011). It has recently been shown that AMPK promotes the secretion of AKH when flies are subjected to metabolic stress. Disruption of AMPK function specifically in AKH-secreting cells recapitulates many of the phenotypes associated with AKH ablation and results in increased lifespan (Braco et al., 2012). Moreover, targeted expression of AMPK in either adult flight muscles or adipose tissue extends lifespan (Stenesen et al., 2013).

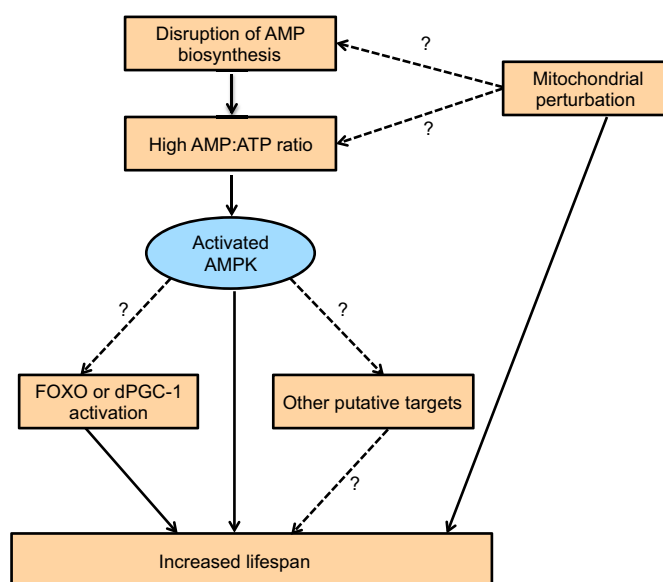


Fig. 1. AMPK-mediated lifespan extension and putative interactors.

Solid arrows denote established connections, whereas broken arrows with question marks highlight possible interactors. Rising AMP levels lead to activation of AMPK, which ultimately promotes an extension to lifespan; the targets of AMPK are not fully known but might include FOXO and dPGC-1 (see text for details).

Surprisingly, mutations in several genes controlling AMP biosynthesis, which intuitively should reduce AMP levels and consequently the ratio of AMP:ATP, also increased lifespan. The authors resolved this apparent paradox by demonstrating that mutations in genes for AMP biosynthesis actually increased AMP:ATP ratios and activated AMPK (Stenesen et al., 2013). However, in an additional study where flies were fed metformin, an increase in lifespan was not observed (Slack et al., 2012). Further studies are required to resolve whether the disparity is due to nonspecific effects of metformin or a requirement for AMPK activation in specific tissues instead of globally. Additionally, although it remains to be seen how AMPK activation mechanistically engages a pro-longevity cue, studies in other organisms might provide some valuable hints. For instance, the lifespan-promoting effect of AMPK in *C. elegans* is partially dependent on the well-established anti-aging transcription factor FOXO (Greer et al., 2007). Furthermore, the growing list of factors activated by AMPK in mammalian systems includes several pro-longevity proteins such as mTOR and PGC-1 α (Hardie et al., 2012). In this regard, it is interesting to note that overexpression of the *Drosophila* ortholog of PGC-1 (dPGC-1 or Spargel) in stem and progenitor cells of the adult fly gut extends lifespan. In addition, forced expression of dPGC-1 in either larvae or adults is sufficient to increase mitochondrial activity (Rera et al., 2011). It is unclear whether dPGC-1 mediates some of the longevity-enhancing effects of AMPK (Fig. 1); nevertheless, further studies in *Drosophila*, focusing on how disruption of various putative downstream targets of AMPK affect AMPK-mediated lifespan extension, should help resolve the AMPK targets responsible for lifespan extension.

Given that AMPK activation increases lifespan, it is worth speculating whether reduced ATP levels and subsequent AMPK activation could account for the pro-longevity effect of mild mitochondrial perturbation – a phenomenon that has been consistently observed in both *C. elegans* and *Drosophila* (Lee et al., 2003; Copeland et al., 2009). However, long-lived flies with perturbed mitochondrial function do not consistently display reduced ATP levels (Copeland et al., 2009). This raises the possibility that other metabolites that become elevated in response to mitochondrial perturbation should be investigated for their possible lifespan-promoting effects. Interestingly, some *Drosophila* mitochondrial mutants increase reactive oxygen species (ROS) production, which in turn activates signaling cascades to elicit specific developmental or cell cycle responses (Owusu-Ansah et al., 2008; Owusu-Ansah and Banerjee, 2009). Nevertheless, whether elevated levels of ROS are required for lifespan extension in flies is still an open question.

Concluding remarks

Here, we have highlighted some of the advances made in modeling nutrient sensing and metabolic homeostasis in *Drosophila*. Interestingly, a recent biochemical resource uncovered more than 400 different lipids that vary in expression during the life cycle of *Drosophila* (Guan et al., 2013), and a narrowly tuned fructose receptor was shown to function as a nutrient sensor in the brain (Miyamoto et al., 2012). In addition, it was recently shown that a *Drosophila* adiponectin receptor in IPCs regulates circulating trehalose levels (Kwak et al., 2013); however, the precise ligand (the *Drosophila* functional ortholog of adiponectin) that signals through the receptor remains to be identified. Thus, it appears that much remains to be uncovered in this highly burgeoning field. An important limitation of metabolic studies in *Drosophila* is that the

main circulating sugar is trehalose, instead of glucose as in humans. Interestingly, trehalose has potent antioxidant activity (Alvarez-Peral et al., 2002) – hence, its elevation in response to various signaling pathways might impact an organism's response to stress, thereby producing phenotypes some of which might not extrapolate to those of humans. Similar concerns arise over the fact that flies are unable to synthesize cholesterol (Gilbert et al., 2002). In addition, future studies will have to more thoroughly dissect the neural circuits that regulate feeding behavior, especially determining how such circuits are related to others that regulate alternative behaviors. Some recent reports have broached this subject: specific interneurons acting downstream of metabolic cues have been shown to control the decision to feed (Flood et al., 2013), and a pair of interneurons in the ventral nerve cord regulates the choice between locomotion and feeding (Mann et al., 2013). An additional drawback at the moment is that most *Drosophila* models of metabolism have only served to recapitulate phenotypes that are well-established in mammalian systems. Although this admittedly has served to validate the use of *Drosophila* to study mammalian metabolism, future studies will have to 'set the pace' by uncovering novel signaling modules or therapeutic strategies for countering diseases associated with aberrant metabolic homeostasis in humans.

Competing interests

The authors declare no competing financial interests.

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References

- Al-Anzi, B., Armand, E., Nagamei, P., Olszewski, M., Sapin, V., Waters, C., Zinn, K., Wyman, R. J. and Benzer, S. (2010). The leucokinin pathway and its neurons regulate meal size in *Drosophila*. *Curr. Biol.* **20**, 969-978.
- Al-Daghri, N. M., Al-Attas, O. S., Alokail, M., Draz, H. M., Bamakhramah, A. and Sabico, S. (2009). Retinol binding protein-4 is associated with TNF-alpha and not insulin resistance in subjects with type 2 diabetes mellitus and coronary heart disease. *Dis. Markers* **26**, 135-140.
- Alic, N. and Partridge, L. (2011). Death and dessert: nutrient signalling pathways and ageing. *Curr. Opin. Cell Biol.* **23**, 738-743.
- Alic, N., Hoddinott, M. P., Vinti, G. and Partridge, L. (2011). Lifespan extension by increased expression of the *Drosophila* homologue of the IGF1R tumour suppressor. *Aging Cell* **10**, 137-147.
- Alvarez-Peral, F. J., Zaragoza, O., Pedreno, Y. and Argüelles, J. C. (2002). Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. *Microbiology* **148**, 2599-2606.
- Anderson, R. M. and Weindruch, R. (2012). The caloric restriction paradigm: implications for healthy human aging. *Am. J. Hum. Biol.* **24**, 101-106.
- Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P. S. and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev.* **18**, 3004-3009.
- Arquier, N., Géminard, C., Bourouis, M., Jarretou, G., Honegger, B., Paix, A. and Léopold, P. (2008). *Drosophila* ALS regulates growth and metabolism through functional interaction with insulin-like peptides. *Cell Metab.* **7**, 333-338.
- Bader, R., Sarraf-Zadeh, L., Peters, M., Moderau, N., Stocker, H., Köhler, K., Pankratz, M. J. and Hafen, E. (2013). The IGF1R homolog Imp-L2 promotes insulin signaling in distinct neurons of the *Drosophila* brain. *J. Cell Sci.* **126**, 2571-2576.
- Bai, H., Kang, P. and Tatar, M. (2012). *Drosophila* insulin-like peptide-6 (dIlp6) expression from fat body extends lifespan and represses secretion of *Drosophila* insulin-like peptide-2 from the brain. *Aging Cell* **11**, 978-985.
- Baker, K. D. and Thummel, C. S. (2007). Diabetic larvae and obese flies-emerging studies of metabolism in *Drosophila*. *Cell Metab.* **6**, 257-266.
- Birse, R. T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., Ocorr, K., Bodmer, R. and Oldham, S. (2010). High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in *Drosophila*. *Cell Metab.* **12**, 533-544.
- Biteau, B., Karpac, J., Hwangbo, D. and Jasper, H. (2011). Regulation of *Drosophila* lifespan by JNK signaling. *Exp. Gerontol.* **46**, 349-354.

- Braco, J. T., Gillespie, E. L., Alberto, G. E., Brenman, J. E. and Johnson, E. C. (2012). Energy-dependent modulation of glucagon-like signaling in *Drosophila* via the AMP-activated protein kinase. *Genetics* **192**, 457-466.
- Brogio, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R. and Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr. Biol.* **11**, 213-221.
- Broughton, S. J., Piper, M. D., Ikeya, T., Bass, T. M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D. J., Leivers, S. J. et al. (2005). Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl. Acad. Sci. USA* **102**, 3105-3110.
- Broughton, S., Alic, N., Slack, C., Bass, T., Ikeya, T., Vinti, G., Tommasi, A. M., Driege, Y., Hafen, E. and Partridge, L. (2008). Reduction of DILP2 in *Drosophila* triages a metabolic phenotype from lifespan revealing redundancy and compensation among DILPs. *PLoS ONE* **3**, e3721.
- Brown, M. R., Crim, J. W., Arata, R. C., Cai, H. N., Chun, C. and Shen, P. (1999). Identification of a *Drosophila* brain-gut peptide related to the neuropeptide Y family. *Peptides* **20**, 1035-1042.
- Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* **54**, 1615-1625.
- Buchmann, J., Meyer, C., Neschen, S., Augustin, R., Schmolz, K., Kluge, R., Al-Hasani, H., Jürgens, H., Eulenberg, K., Wehr, R. et al. (2007). Ablation of the cholesterol transporter adenosine triphosphate-binding cassette transporter G1 reduces adipose cell size and protects against diet-induced obesity. *Endocrinology* **148**, 1561-1573.
- Carvalho, G. B., Kapahi, P., Anderson, D. J. and Benzer, S. (2006). Allosteric modulation of feeding behavior by the Sex Peptide of *Drosophila*. *Curr. Biol.* **16**, 692-696.
- Chelikani, P. K., Haver, A. C. and Reidelberger, R. D. (2005). Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am. J. Physiol.* **288**, R1695-R1706.
- Chell, J. M. and Brand, A. H. (2010). Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell* **143**, 1161-1173.
- Chen, D., Siddiq, A., Emdad, L., Rajasekaran, D., Gredler, R., Shen, X. N., Santhekadur, P. K., Srivastava, J., Robertson, C. L., Dmitriev, I. et al. (2013). Insulin-like growth factor-binding protein-7 (IGFBP7): a promising gene therapeutic for hepatocellular carcinoma (HCC). *Mol. Ther.* **21**, 758-766.
- Cognigni, P., Bailey, A. P. and Miguel-Aliaga, I. (2011). Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. *Cell Metab.* **13**, 92-104.
- Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J. and Léopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell* **114**, 739-749.
- Colombani, J., Andersen, D. S. and Léopold, P. (2012). Secreted peptide Dilp8 coordinates *Drosophila* tissue growth with developmental timing. *Science* **336**, 582-585.
- Copeland, J. M., Cho, J., Lo, T., Jr, Hur, J. H., Bahadorani, S., Arabyan, T., Rabie, J., Soh, J. and Walker, D. W. (2009). Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. *Curr. Biol.* **19**, 1591-1598.
- Curtis, R., O'Connor, G. and DiStefano, P. S. (2006). Aging networks in *Caenorhabditis elegans*: AMP-activated protein kinase (aak-2) links multiple aging and metabolism pathways. *Aging Cell* **5**, 119-126.
- del Valle Rodríguez, A., Didiano, D. and Desplan, C. (2011). Power tools for gene expression and clonal analysis in *Drosophila*. *Nat. Methods* **9**, 47-55.
- Demontis, F. and Perrimon, N. (2010). FOXO/4E-BP signaling in *Drosophila* muscles regulates organism-wide proteostasis during aging. *Cell* **143**, 813-825.
- Dohrmann, C. E. (2004). Target discovery in metabolic disease. *Drug Discov. Today* **9**, 785-794.
- Donahay, J. C., van Dijk, G., Woods, S. C. and Seeley, R. J. (1998). Intraventricular GLP-1 reduces short- but not long-term food intake or body weight in lean and obese rats. *Brain Res.* **779**, 75-83.
- Evans, D. S., Kapahi, P., Hsueh, W. C. and Kockel, L. (2011). TOR signaling never gets old: aging, longevity and TORC1 activity. *Ageing Res. Rev.* **10**, 225-237.
- Flood, T. F., Iguchi, S., Gorczyca, M., White, B., Ito, K. and Yoshihara, M. (2013). A single pair of interneurons commands the *Drosophila* feeding motor program. *Nature* **499**, 83-87.
- Fontana, L., Partridge, L. and Longo, V. D. (2010). Extending healthy life span – from yeast to humans. *Science* **328**, 321-326.
- Forbes, J. M. and Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiol. Rev.* **93**, 137-188.
- Frustaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., Nadal-Ginard, B. and Anversa, P. (2000). Myocardial cell death in human diabetes. *Circ. Res.* **87**, 1123-1132.
- Garelli, A., Gontijo, A. M., Miguela, V., Caparros, E. and Dominguez, M. (2012). Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. *Science* **336**, 579-582.
- Gavi, S., Stuart, L. M., Kelly, P., Melendez, M. M., Mynarcik, D. C., Gelato, M. C. and McNurlan, M. A. (2007). Retinol-binding protein 4 is associated with insulin resistance and body fat distribution in nonobese subjects without type 2 diabetes. *J. Clin. Endocrinol. Metab.* **92**, 1886-1890.
- Géminard, C., Rulifson, E. J. and Léopold, P. (2009). Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metab.* **10**, 199-207.
- Gilbert, L. I., Rybczynski, R. and Warren, J. T. (2002). Control and biochemical nature of the ecdysteroidogenic pathway. *Annu. Rev. Entomol.* **47**, 883-916.
- Graham, T. E., Yang, Q., Blüher, M., Hammarstedt, A., Ciaraldi, T. P., Henry, R. R., Wason, C. J., Oberbach, A., Jansson, P. A., Smith, U. et al. (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med.* **354**, 2552-2563.
- Greer, E. L., Dowlatabadi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., Gygi, S. P. and Brunet, A. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr. Biol.* **17**, 1646-1656.
- Grönke, S., Clarke, D. F., Broughton, S., Andrews, T. D. and Partridge, L. (2010). Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. *PLoS Genet.* **6**, e1000857.
- Guan, X. L., Cestra, G., Shui, G., Kuhrs, A., Schittenhelm, R. B., Hafen, E., van der Goot, F. G., Robinett, C. C., Gatti, M., Gonzalez-Gaitan, M. et al. (2013). Biochemical membrane lipidomics during *Drosophila* development. *Dev. Cell* **24**, 98-111.
- Gutierrez, E., Wiggins, D., Fielding, B. and Gould, A. P. (2007). Specialized hepatocyte-like cells regulate *Drosophila* lipid metabolism. *Nature* **445**, 275-280.
- Hardie, D. G., Scott, J. W., Pan, D. A. and Hudson, E. R. (2003). Management of cellular energy by the AMP-activated protein kinase system. *FEBS Lett.* **546**, 113-120.
- Hardie, D. G., Ross, F. A. and Hawley, S. A. (2012). AMP-activated protein kinase: a target for drugs both ancient and modern. *Chem. Biol.* **19**, 1222-1236.
- Hergarden, A. C., Tayler, T. D. and Anderson, D. J. (2012). Allatostatin-A neurons inhibit feeding behavior in adult *Drosophila*. *Proc. Natl. Acad. Sci. USA* **109**, 3967-3972.
- Honegger, B., Galic, M., Köhler, K., Wittwer, F., Brogiolo, W., Hafen, E. and Stocker, H. (2008). Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in *Drosophila* and is essential for starvation resistance. *J. Biol.* **7**, 10.
- Hsu, H. J. and Drummond-Barbosa, D. (2009). Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **106**, 1117-1121.
- Hwa, V., Oh, Y. and Rosenfeld, R. G. (1999). The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr. Rev.* **20**, 761-787.
- Ikeya, T., Galic, M., Belawat, P., Nairz, K. and Hafen, E. (2002). Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr. Biol.* **12**, 1293-1300.
- Johnson, S. C., Rabinovitch, P. S. and Kaeblerlein, M. (2013). mTOR is a key modulator of ageing and age-related disease. *Nature* **493**, 338-345.
- Kaneto, H., Xu, G., Song, K. H., Suzuma, K., Bonner-Weir, S., Sharma, A. and Weir, G. C. (2001). Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J. Biol. Chem.* **276**, 31099-31104.
- Kannel, W. B., D'Agostino, R. B., Wilson, P. W., Belanger, A. J. and Gagnon, D. R. (1990). Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. *Am. Heart J.* **120**, 672-676.
- Karpac, J. and Jasper, H. (2009). Insulin and JNK: optimizing metabolic homeostasis and lifespan. *Trends Endocrinol. Metab.* **20**, 100-106.
- Kellum, J. M., Kuemmerle, J. F., O'Dorisio, T. M., Rayford, P., Martin, D., Engle, K., Wolf, L. and Summer, H. J. (1990). Gastrointestinal hormone responses to meals before and after gastric bypass and vertical banded gastroplasty. *Ann. Surg.* **211**, 763-771.
- Kenyon, C. J. (2010). The genetics of ageing. *Nature* **464**, 504-512.
- Kim, S. K. and Rulifson, E. J. (2004). Conserved mechanisms of glucose sensing and regulation by *Drosophila* corpora cardiaca cells. *Nature* **431**, 316-320.
- Kim, Y. L., Kim, T. K., Cheong, E. S., Shin, D. G., Choi, G. S., Jung, J., Han, K. A. and Min, K. W. (2012). Relation of absolute or relative adiposity to insulin resistance, retinol binding protein-4, leptin, and adiponectin in type 2 diabetes. *Diabetes Metab. J.* **36**, 415-421.
- Kluge, R., Giesen, K., Bahrenberg, G., Plum, L., Ortlepp, J. R. and Joost, H. G. (2000). Quantitative trait loci for obesity and insulin resistance (Nob1, Nob2) and their interaction with the leptin receptor allele (LeprA720T/T1044I) in New Zealand obese mice. *Diabetologia* **43**, 1565-1572.
- Kwak, S. J., Hong, S. H., Bajracharya, R., Yang, S. Y., Lee, K. S. and Yu, K. (2013). *Drosophila* adiponectin receptor in insulin producing cells regulates glucose and lipid metabolism by controlling insulin secretion. *PLoS ONE* **8**, e68641.
- LaJeunesse, D. R., Johnson, B., Presnell, J. S., Catignas, K. K. and Zapotocny, G. (2010). Peristalsis in the junction region of the *Drosophila* larval midgut is modulated by DH31 expressing enteroendocrine cells. *BMC Physiol.* **10**, 14.
- le Roux, C. W., Aylwin, S. J., Batterham, R. L., Borg, C. M., Coyle, F., Prasad, V., Shurey, S., Ghatei, M. A., Patel, A. G. and Bloom, S. R. (2006). Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann. Surg.* **243**, 108-114.
- Lee, G. and Park, J. H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics* **167**, 311-323.
- Lee, S. S., Lee, R. Y., Fraser, A. G., Kamath, R. S., Ahlinger, J. and Ruvkun, G. (2003). A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**, 40-48.
- Lee, K. S., You, K. H., Choo, J. K., Han, Y. M. and Yu, K. (2004). *Drosophila* short neuropeptide F regulates food intake and body size. *J. Biol. Chem.* **279**, 50781-50789.
- Lee, G., Kim, K. M., Kikuno, K., Wang, Z., Choi, Y. J. and Park, J. H. (2008a). Developmental regulation and functions of the expression of the neuropeptide corazonin in *Drosophila melanogaster*. *Cell Tissue Res.* **331**, 659-673.
- Lee, K. S., Kwon, O. Y., Lee, J. H., Kwon, K., Min, K. J., Jung, S. A., Kim, A. K., You, K. H., Tatar, M. and Yu, K. (2008b). *Drosophila* short neuropeptide F

- signalling regulates growth by ERK-mediated insulin signalling. *Nat. Cell Biol.* **10**, 468-475.
- Leopold, P. and Perrimon, N. (2007). *Drosophila* and the genetics of the internal milieu. *Nature* **450**, 186-188.
- Liu, K., Paterson, A. J., Chin, E. and Kudlow, J. E. (2000). Glucose stimulates protein modification by O-linked GlcNAc in pancreatic beta cells: linkage of O-linked GlcNAc to beta cell death. *Proc. Natl. Acad. Sci. USA* **97**, 2820-2825.
- Mair, W., Morante, I., Rodrigues, A. P., Manning, G., Montminy, M., Shaw, R. J. and Dillin, A. (2011). Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. *Nature* **470**, 404-408.
- Mann, K., Gordon, M. D. and Scott, K. (2013). A pair of interneurons influences the choice between feeding and locomotion in *Drosophila*. *Neuron* **79**, 754-765.
- McBrayer, Z., Ono, H., Shimell, M., Parvy, J. P., Beckstead, R. B., Warren, J. T., Thummel, C. S., Dauphin-Villemant, C., Gilbert, L. I. and O'Connor, M. B. (2007). Prothoracicotropic hormone regulates developmental timing and body size in *Drosophila*. *Dev. Cell* **13**, 857-871.
- McKiernan, S. H., Colman, R. J., Lopez, M., Beasley, T. M., Aiken, J. M., Anderson, R. M. and Weindruch, R. (2011). Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. *Exp. Gerontol.* **46**, 23-29.
- Melcher, C. and Pankratz, M. J. (2005). Candidate gustatory interneurons modulating feeding behavior in the *Drosophila* brain. *PLoS Biol.* **3**, e305.
- Miguel-Aliaga, I., Thor, S. and Gould, A. P. (2008). Postmitotic specification of *Drosophila* insulinergic neurons from pioneer neurons. *PLoS Biol.* **6**, e58.
- Miyamoto, T., Slone, J., Song, X. and Amrein, H. (2012). A fructose receptor functions as a nutrient sensor in the *Drosophila* brain. *Cell* **151**, 1113-1125.
- Morinigo, R., Moizé, V., Musri, M., Lacy, A. M., Navarro, S., Marin, J. L., Delgado, S., Casamitjana, R. and Vidal, J. (2006). Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. *J. Clin. Endocrinol. Metab.* **91**, 1735-1740.
- Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiraman, S. S., Cagan, R. L. and Baranski, T. J. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Dis. Model. Mech.* **4**, 842-849.
- Na, J., Musselman, L. P., Pendse, J., Baranski, T. J., Bodmer, R., Ocorr, K. and Cagan, R. (2013). A *Drosophila* model of high sugar diet-induced cardiomyopathy. *PLoS Genet.* **9**, e1003175.
- Nässel, D. R. and Winther, A. M. (2010). *Drosophila* neuropeptides in regulation of physiology and behavior. *Prog. Neurobiol.* **92**, 42-104.
- O'Brien, L. E., Soliman, S. S., Li, X. and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. *Cell* **147**, 603-614.
- Okamoto, N., Yamanaka, N., Yagi, Y., Nishida, Y., Kataoka, H., O'Connor, M. B. and Mizoguchi, A. (2009). A fat body-derived IGF-like peptide regulates postfeeding growth in *Drosophila*. *Dev. Cell* **17**, 885-891.
- Okamoto, N., Nakamori, R., Murai, T., Yamauchi, Y., Masuda, A. and Nishimura, T. (2013). A secreted decoy of InR antagonizes insulin/IGF signaling to restrict body growth in *Drosophila*. *Genes Dev.* **27**, 87-97.
- 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.
- Owusu-Ansah, E. and Banerjee, U. (2009). Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature* **461**, 537-541.
- Owusu-Ansah, E., Yavari, A., Mandal, S. and Banerjee, U. (2008). Distinct mitochondrial retrograde signals control the G1-S cell cycle checkpoint. *Nat. Genet.* **40**, 356-361.
- Pasco, M. Y. and Léopold, P. (2012). High sugar-induced insulin resistance in *Drosophila* relies on the lipocalin Neural Lazarillo. *PLoS ONE* **7**, e36583.
- Plum, L., Kluge, R., Giesen, K., Altmüller, J., Ortlepp, J. R. and Joost, H. G. (2000). Type 2 diabetes-like hyperglycemia in a backcross model of NZO and SJL mice: characterization of a susceptibility locus on chromosome 4 and its relation with obesity. *Diabetes* **49**, 1590-1596.
- Plum, L., Giesen, K., Kluge, R., Junger, E., Linnartz, K., Schürmann, A., Becker, W. and Joost, H. G. (2002). Characterisation of the mouse diabetes susceptibility locus Nidd/SJL: islet cell destruction, interaction with the obesity QTL Nob1, and effect of dietary fat. *Diabetologia* **45**, 823-830.
- Poon, T., Nelson, P., Shen, L., Mihm, M., Taylor, K., Fineman, M. and Kim, D. (2005). Exenatide improves glycemic control and reduces body weight in subjects with type 2 diabetes: a dose-ranging study. *Diabetes Technol. Ther.* **7**, 467-477.
- Pospisilik, J. A., Schramek, D., Schnidar, H., Cronin, S. J., Nehme, N. T., Zhang, X., Knauf, C., Cani, P. D., Aumayr, K., Todoric, J. et al. (2010). *Drosophila* genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell* **140**, 148-160.
- Rajan, A. and Perrimon, N. (2012). *Drosophila* cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* **151**, 123-137.
- Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B., Pérusse, L. and Bouchard, C. (2006). The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* **14**, 529-644.
- Rera, M., Bahadorani, S., Cho, J., Koehler, C. L., Ugheirai, M., Hur, J. H., Ansari, W. S., Lo, T., Jr, Jones, D. L. and Walker, D. W. (2011). Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog. *Cell Metab.* **14**, 623-634.
- Ruan, W., Xu, E., Xu, F., Ma, Y., Deng, H., Huang, Q., Lv, B., Hu, H., Lin, J., Cui, J. et al. (2007). IGFBP7 plays a potential tumor suppressor role in colorectal carcinogenesis. *Cancer Biol. Ther.* **6**, 354-359.
- Rulifson, E. J., Kim, S. K. and Nüsse, R. (2002). Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* **296**, 1118-1120.
- Slack, C., Foley, A. and Partridge, L. (2012). Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PLoS ONE* **7**, e47699.
- Slaïdina, M., Delanoue, R., Gronke, S., Partridge, L. and Léopold, P. (2009). A *Drosophila* insulin-like peptide promotes growth during nonfeeding states. *Dev. Cell* **17**, 874-884.
- Söderberg, J. A., Carlsson, M. A. and Nässel, D. R. (2012). Insulin-producing cells in the *Drosophila* brain also express satiety-inducing cholecystokinin-like peptide, drosulfakinin. *Front. Endocrinol.* **3**, 109.
- Song, W., Ren, D., Li, W., Jiang, L., Cho, K. W., Huang, P., Fan, C., Song, Y., Liu, Y. and Rui, L. (2010). SH2B regulation of growth, metabolism, and longevity in both insects and mammals. *Cell Metab.* **11**, 427-437.
- Sousa-Nunes, R., Yee, L. L. and Gould, A. P. (2011). Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. *Nature* **471**, 508-512.
- Steinert, R. E., Feinle-Bisset, C., Geary, N. and Beglinger, C. (2013). Digestive physiology of the pig symposium: secretion of gastrointestinal hormones and eating control. *J. Anim. Sci.* **91**, 1963-1973.
- Stenesen, D., Suh, J. M., Seo, J., Yu, K., Lee, K. S., Kim, J. S., Min, K. J. and Graff, J. M. (2013). Adenosine nucleotide biosynthesis and AMPK regulate adult life span and mediate the longevity benefit of caloric restriction in flies. *Cell Metab.* **17**, 101-112.
- Trinh, I. and Boulianne, G. L. (2013). Modeling obesity and its associated disorders in *Drosophila*. *Physiology (Bethesda)* **28**, 117-124.
- Tschöp, M., Smiley, D. L. and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* **407**, 908-913.
- Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M., Meeran, K., Choi, S. J., Taylor, G. M., Heath, M. M., Lambert, P. D. et al. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **379**, 69-72.
- Veenstra, J. A., Agricola, H. J. and Sellami, A. (2008). Regulatory peptides in fruit fly midgut. *Cell Tissue Res.* **334**, 499-516.
- von Eynatten, M., Lepper, P. M., Liu, D., Lang, K., Baumann, M., Nawroth, P. P., Bierhaus, A., Dugi, K. A., Heemann, U., Allolio, B. et al. (2007). Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. *Diabetologia* **50**, 1930-1937.
- Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., Dhillo, W. S., Ghatei, M. A. and Bloom, S. R. (2001a). Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* **86**, 5992.
- Wren, A. M., Small, C. J., Abbott, C. R., Dhillo, W. S., Seal, L. J., Cohen, M. A., Batterham, R. L., Taheri, S., Stanley, S. A., Ghatei, M. A. et al. (2001b). Ghrelin causes hyperphagia and obesity in rats. *Diabetes* **50**, 2540-2547.
- Wu, Q., Wen, T., Lee, G., Park, J. H., Cai, H. N. and Shen, P. (2003). Developmental control of foraging and social behavior by the *Drosophila* neuropeptide Y-like system. *Neuron* **39**, 147-161.
- Xu, J., Li, M. and Shen, P. (2010). A G-protein-coupled neuropeptide Y-like receptor suppresses behavioral and sensory response to multiple stressful stimuli in *Drosophila*. *J. Neurosci.* **30**, 2504-2512.
- Yang, Q., Graham, T. E., Mody, N., Preitner, F., Peroni, O. D., Zabolotny, J. M., Kotani, K., Quadro, L. and Kahn, B. B. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* **436**, 356-362.
- Yang, C. H., Belawat, P., Hafen, E., Jan, L. Y. and Jan, Y. N. (2008). *Drosophila* egg-laying site selection as a system to study simple decision-making processes. *Science* **319**, 1679-1683.
- Zander, M., Madsbad, S., Madsen, J. L. and Holst, J. J. (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* **359**, 824-830.
- Zhao, Y., Bretz, C. A., Hawksworth, S. A., Hirsh, J. and Johnson, E. C. (2010). Corazonin neurons function in sexually dimorphic circuitry that shape behavioral responses to stress in *Drosophila*. *PLoS ONE* **5**, e9141.
- Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyl-Melody, J., Wu, M., Ventre, J., Doeber, T., Fujii, N. et al. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **108**, 1167-1174.