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REVIEW

Drosophila as a model to study obesity and metabolic disease

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ABSTRACT

Excess adipose fat accumulation, or obesity, is a growing problem worldwide in terms of both the rate of incidence and the severity of obesity-associated metabolic disease. Adipose tissue evolved in animals as a specialized dynamic lipid storage depot: adipose cells synthesize fat (a process called lipogenesis) when energy is plentiful and mobilize stored fat (a process called lipolysis) when energy is needed. When a disruption of lipid homeostasis favors increased fat synthesis and storage with little turnover owing to genetic predisposition, overnutrition or sedentary living, complications such as diabetes and cardiovascular disease are more likely to arise. The vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) is used as a model to better understand the mechanisms governing fat metabolism and distribution. Flies offer a wealth of paradigms with which to study the regulation and physiological effects of fat accumulation. Obese flies accumulate triacylglycerols in the fat body, an organ similar to mammalian adipose tissue, which specializes in lipid storage and catabolism. Discoveries in *Drosophila* have ranged from endocrine hormones that control obesity to subcellular mechanisms that regulate lipogenesis and lipolysis, many of which are evolutionarily conserved. Furthermore, obese flies exhibit pathophysiological complications, including hyperglycemia, reduced longevity and cardiovascular function – similar to those observed in obese humans. Here, we review some of the salient features of the fly that enable researchers to study the contributions of feeding, absorption, distribution and the metabolism of lipids to systemic physiology.

KEY WORDS: Lipid metabolism, Insect, Genetics, Diet, Lipid droplet

Introduction

The regulation of lipid homeostasis is crucial for animals in a wide variety of contexts. Fat reserves are typically tightly regulated to meet energy needs without exceeding a maximum adiposity threshold. In humans, obesity is defined as increased adipose fat accumulation, and typically presents an increased risk to health (WHO fact sheet: Obesity and overweight 2016; www.who.int/mediacentre/factsheets/fs311/en/). Patients with a genetic or environmentally induced excess of fat storage often exhibit hyperglycemia, insulin resistance and cardiovascular disease, hallmarks of metabolic syndrome. Non-alcoholic fatty liver, retinopathy, neuropathy, nephropathy and susceptibility to infection are also increased in obese patients, whereas lifespan is reduced. Even though obesity is an escalating global public health problem, the interactions of genetic predisposition with environmental and lifestyle factors in the etiology of obesity and obesity-related co-morbidities are still poorly understood. Given the inherent genetic heterogeneity of human populations, animal models such as mice and flies are of particular value to disentangle the roles of nurture and nature in fat accumulation and homeostasis.

Researchers began to develop an interest in employing *Drosophila melanogaster* (hereafter called simply *Drosophila*) as a model system for obesity research in the early 1960s after the pioneering work of Dr Winifred Doane, who isolated the first obese fly mutant called *adipose* from a Nigerian wild population. *adipose* mutant flies suffer from excessive fat storage but reduced carbohydrate reserves (Doane, 1961, 1960a,b). However, it was not until 2003 that the affected *adipose* gene was identified using a positional cloning approach (Häder et al., 2003). Excitingly, the Adipose protein proved to be structurally and functionally conserved in mice (Suh et al., 2007), humans (Lai et al., 2009) and even plants (Ducos et al., 2017). A recent study of the mammalian Adipose homolog WDTC1 provided the first mechanistic insight into how Adipose might control body fat accumulation. Groh and colleagues showed that WDTC1 functions as a substrate receptor in the Cullin really interesting new gene (RING) E3 ligase complex (Groh et al., 2016). This complex is proposed to mediate the anti-obesity function of WDTC1 by epigenetic silencing of target genes via monoubiquitylation of histone 2A (Groh et al., 2016). Interestingly, Baumbach et al. had previously identified *Drosophila* Cullin 4 as an anti-obesity gene using a reverse genetic approach (Baumbach et al., 2014a). The finding that the impairment of Cullin 4 in the fly causes an *adipose*-like mutant phenotype suggests that the molecular function of Adipose/WDTC1 might be conserved between *Drosophila* and mammals. Thus, *adipose/WDTC1* is not only the first example of a human obesity-related gene to be discovered in the fly but also serves as a prime example of how *Drosophila* research and mammalian studies complement each other in the discovery and mechanistic understanding of obesity and related metabolic disorders.

Drosophila is a particularly useful model for obesity and metabolic disease for a number of reasons. First, flies contain tissues, organs and systems analogous to all those involved in human obesity and associated metabolic diseases (Fig. 1, Table 1). In addition, *Drosophila* develop obesity and its associated complications during caloric overload, similar to humans. Moreover, most genes and gene families known to function in metabolic disease are conserved between flies and humans (Reiter, 2001). Flies represent an ideal model in which to study the ever-expanding group of complications associated with obesity and metabolic disease owing to their vast range of genetic resources, which can be investigated using cutting-edge approaches. In this Review, we first present organism-level studies of obesity in which flies exhibit a number of phenotypes consistent with a diagnosis of metabolic disease. Then, we discuss the *Drosophila* tissue and organ systems and tools that researchers use to assess the sequelae of

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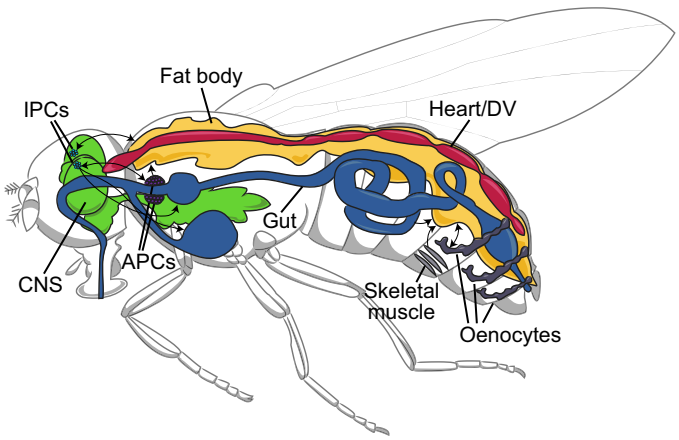

Fig. 1. Systems physiology regulating obesity in *Drosophila*. Food enters through the foregut and is digested and absorbed along the gut depending on enzyme activity, an acidic pH and a healthy microbiome. Neuroendocrine adipokinetic hormone (Akh)-producing cells (APCs) of the corpora cardiaca line the foregut and secrete Akh, which serves as the fly's glucagon, to activate feeding and storage energy mobilization. Counteracting Akh are the insulin-like peptides (Ilps), which are secreted by a different group of neuroendocrine Ilp-producing cells (IPCs) that lie in the anterior of the central nervous system (CNS). Both groups of neurosecretory cells extend axons, which synapse on the heart (among other organs) to release their cargo, enabling their hormones to act systemically on various tissues via the fly blood, which is called hemolymph. The heart or dorsal vessel (DV) pumps and thereby circulates hemolymph, delivering hemocytes to respond to infection or injury, and transporting hormones and nutrients to peripheral tissues. The fat body is the target of many of these molecules and serves as the fly's primary lipid storage organ and – together with the oenocytes – as a liver equivalent. Fat bodies express Ilp and Akh receptors and store fat when nutrients are plentiful or undergo lipolysis during starvation or developmental stages and other conditions where energy is needed. The muscle burns energy when physiology demands, e.g. during foraging, courtship and flight. For simplicity, the compartmentalization of the fat body, muscle and oenocytes into different depots has been omitted. The adult fly is shown; all organs shown have analogous forms in the larva. Examples of endocrine regulation between organs are denoted with arrows and are described with respect to obesity in the text.

obesity and obesity-related metabolic disease. In closing, we highlight emerging strategies that are being used to study the complications of human obesity in *Drosophila*, including endocrinology, reproductive and cancer biology.

Genetic studies of *Drosophila* obesity

Triacylglycerols (TAGs) are the main lipid storage form in the fly, as in humans. Quantification of TAG content has therefore been used to define obesity in flies. TAGs can be measured in extracted lipids or crude fly homogenates using thin layer chromatography (Al-Anzi et al., 2009) or mass spectrometry (Carvalho et al., 2012), or estimated by performing an enzymatic assay that measures glycerol content after lipolysis (Hildebrandt et al., 2011; Williams et al., 2011, after McGowan et al., 1983). At the cellular level, obesity can be characterized via quantification of the fat body lipid droplet (LD) size and number (Grönke et al., 2005; Musselman et al., 2011). Some researchers have taken advantage of the altered ratio of fat-to-lean body mass to identify genotypes with increased fat content using buoyancy-based genetic screens (Reis et al., 2010; Tsuda-Sakurai et al., 2015). Lipogenesis can be further characterized by the incorporation of labeled carbons from the diet into various lipid classes using mass spectrometry (Musselman et al., 2013).

One strength of the fly is its established genetic tool kit. Collections of laboratory-generated and naturally occurring genetic variants isolated from all over the world make *Drosophila* an ideal model in which to test the effects of genes on obesity (Reed et al., 2010; Reis et al., 2010, among others). Genetic screens using these and other resources have identified genes that confer obesity in *Drosophila* via TAG quantification or buoyancy screening (Baumbach et al., 2014a; Lee et al., 2014; Mosher et al., 2015; Reis et al., 2010). Microscopy-based genome-wide *in vitro* screens have identified genes that modulate cellular lipid storage in *Drosophila* tissue culture cells (Beller et al., 2008; Guo et al., 2008). Several conditional *in vivo* gene expression systems, among which the bipartite GAL4-UAS system is the most popular (Brand and Perrimon, 1993), enable overexpression or ribonucleic acid interference (RNAi)-mediated transgenic gene knock down in a spatially or temporarily restricted manner. Conditional strategies have been used to rapidly identify specific regulators of obesity in a single gene or genes using various reverse genetics paradigms (exemplified by Lee et al., 2014; Pospisilik et al., 2010). Finally, knock-in by homologous recombination (Rong et al., 2002) and gene editing technologies such as transcription activator-like effector nucleases (TALENs) (Beumer et al., 2008) and clustered regularly interspaced short palindromic repeats (CRISPR) (Bassett et al., 2013; Gratz et al., 2013) allow *in vivo* genome editing with single base resolution and have been successfully employed in genetic studies of *Drosophila* obesity research (Allen et al., 2017; Gáliková et al., 2015; Sajwan et al., 2015).

Diet-induced obesity in *Drosophila*

Chronic feeding of high-carbohydrate and high-fat diets produces obesity in flies, as in humans, along with a host of pathophysiological complications. Diet-induced obesity can be generated by several strategies in both larvae and adult *Drosophila*. For high-carbohydrate feeding, high-sucrose diets are the most common (Buescher et al., 2013; Garrido et al., 2015; Havula et al., 2013; Navrotskaya et al., 2016; Musselman et al., 2011; Pasco and Léopold, 2012; Reis, 2016; Rovenko et al., 2015a); however, high-glucose and high-fructose diets have also been used (Rovenko et al., 2015b). For high-fat feeding, coconut oil supplementation is the most common way to elicit diet-induced obesity in flies (Birse et al., 2010; Heinrichsen et al., 2014; Hong et al., 2016; Reed et al., 2010), although lard and the hydrogenated soybean and palm oil product known as Crisco have also been used (Lee et al., 2017; Musselman et al., 2011; Woodcock et al., 2015). The formulation of these diets can be tricky and care must be taken to avoid reduced survival owing to unusually sticky or dry food conditions. A small filter paper wick (1 cm×6 cm) can be used to stabilize many diets, providing a water source and a place to rest. Some investigators have recently begun using an obesogenic high-sugar, high-fat diet in *Drosophila* obesity studies that is similar to that used in many rodent studies and is probably closest to the typical 'Western' obesogenic diet (G. Melkani, personal communication). Diet-induced obesity in flies is associated with many of the pathophysiological consequences found in humans, including hyperglycemia, insulin resistance, cardiac arrhythmia and fibrosis, reduced longevity (Birse et al., 2010; Na et al., 2013) and nephrosis (Na et al., 2015). One can also assess the ability to survive on and process high-calorie obesogenic diets, or the degree of 'obesity tolerance'. Like humans, some obese flies can be quite healthy (Musselman et al., 2013), whereas other genotypes develop severe effects with only modest levels of overfeeding (Garrido et al., 2015; Nakagami et al., 2003; Teesalu et al., 2017). A collection of

Table 1. Tissues and organs involved in human obesity and its complications and their functional counterparts in flies

Drosophila	Humans	Tissue-specific expression
Fat body	Adipose, liver, immune system	*Lpp-GAL4* (1), *FB-GAL4* (2), *Lsp2-GAL4* (3), *r4-GAL4* (4), *ppl-GAL4* (5), *cg-GAL4* (6), *adh-GAL4* (7), *to-GAL4* (8), *yolk-GAL4* (9)
Oenocyte	Liver	*oeno-GAL4* (10), *BO-GAL4* (11), *promE-GAL4* (10)
Heart (dorsal vessel)	Heart	*hand-GAL4* (12), *tincΔ4-GAL4* (13), *GMH5-GAL4* (14)
Alimentary tract (gut)	Gastrointestinal tract	*mex-GAL4* (15), *npc1b-GAL4* (16), *esg-GAL4* (17) (midgut+enteroendocrine), *5053A-GAL4* (18) (smooth muscle), *byn-GAL4* (19) (hindgut)
Nephrocytes, Malpighian tubules	Kidney	*dot-GAL4* (20) (nephrocytes), *c42-GAL4* (21), *c724-GAL4* (22) (tubule cells)
Hemocytes	Blood cells, innate immunity	*He-GAL4* (23), *hml-GAL4* (24), *srp-GAL4* (25)
Muscle	Muscle	*24B-GAL4* (26), *mhc-GAL4* (27), *Mef2-GAL4* (28), others with distinct specificity
Central nervous system	Central nervous system	*elav-GAL4* (29), *D42-GAL4* (30), *1407-GAL4* (29), many neuronal subpopulations (31)
Adipokinetic hormone-producing cells (APCs)	Pancreatic α-cells	*Akh-GAL4* (32)
Insulin-like peptide producing cells (IPCs)	Pancreatic ß-cells	*ILP2-GAL4* (33), *ILP3-GAL4* (5)

Note: Expression of these GAL4 drivers outside the tissue of interest must be carefully addressed, as the stage and tissue specificity of expression often varies, as exemplified by Armstrong et al. (2014) for fat-body-expressed GAL4 lines.
[1]Brankatschk and Eaton, 2010; [2]Gronke et al., 2003; [3]Lazareva et al., 2007; [4]Lee and Park, 2004; [5]Colombani et al., 2003; [6]Asha et al., 2003; [7]Fischer et al., 1988; [8]Dauwalder et al., 2002; [9]Georgel et al., 2001; [10]Billeter et al., 2009; [11]Gutierrez et al., 2007; [12]Han et al., 2006; [13]Lo and Frasch, 2001; [14]Wessells et al., 2004; [15]Phillips and Thomas, 2006; [16]Voght et al., 2007; [17]Biteau and Jasper, 2011; [18]Brand and Perrimon, 1993; [19]Takashima and Murakami, 2001; [20]Kimbrell et al., 2002; [21]Rosay et al., 1997; [22]Sözen et al., 1997; [23]Zettervall et al., 2004; [24]Agaisse et al., 2003; [25]Crozatier et al., 2004; [26]Michelson, 1994; [27]Schuster et al., 1996; [28]Ranganayakulu et al., 1998; [29]Luo et al., 1994; [30]Gustafson and Boulianne, 1996; [31]Jenett et al., 2012; [32]Kim and Rulifson, 2004; [33]Rulifson et al., 2002; [34]Armstrong et al., 2014.

wild-caught *Drosophila* strains exhibited various degrees of diet-dependent obesity, consistent with a model where fat storage represents a balance between optimizing storage depots and overloading the animal (Reed et al., 2014, 2010).

Tissues and organ systems in *Drosophila* obesity and metabolic dysfunction

The gut

The *Drosophila* alimentary system is composed of foregut, midgut and hindgut, which are specialized for various functions in both the larva and adult (reviewed in Lemaitre and Miguel-Aliaga, 2013). Food and water enter the foregut after ingestion and travel toward the posterior of the animal. In both developmental stages, the proventriculus represents the beginning of the midgut and stores a striking amount of TAG. The gastric cecae (and crop in adults) contribute to nutrient digestion along the anterior and middle midgut with most absorption occurring with the help of the microvilli lining the posterior midgut (Buchon and Osman, 2015). Specialized sub-regions of the *Drosophila* alimentary tract exist, as in humans, and have been characterized by biochemical and gene expression studies, including regional TAG accumulation (Buchon and Osman, 2015; Gutierrez et al., 2007; Harrop et al., 2014, and references therein). Kidney-like Malpighian tubules link to the intestinal tract at the midgut/hindgut junction and function in filtration and excretion, whereas the hindgut reabsorbs ions and water. With respect to obesity, the gut lumen is of importance owing to two interconnected functions: as the exclusive route for energy intake and as the interaction site of the intestinal microbiome with the fly host.

The gut absorbs dietary macronutrients, including sugars, proteins and fats. The fly midgut serves as the major site of dietary lipid absorption and also metabolizes both glucose and lipids into metabolic intermediates, which are loaded into the hemolymph for use in other tissues and organs. Therefore, the gut is crucial for peripheral body fat storage, as shown in several studies. Lipoprotein complexes containing highly conserved apolipoproteins (called apolipophorins in *Drosophila*) carry sterols and diacylglycerols from the gut to other tissues (Palm et al., 2012). Fly lipoproteins also contain the signaling molecule Hedgehog, a cholesterol-linked, gut-derived ligand that binds the transmembrane receptor Patched on fat body target cells to promote lipolysis during larval starvation (Palm et al., 2013; Rodenfels et al., 2014). Other gut-expressed factors also contribute to the control of systemic lipid homeostasis. Gut enteroendocrine cells secrete tachykinins, a group of peptide hormones that stimulate gut contraction and lipid catabolism during starvation (Song et al., 2014). The nuclear receptor DHR96 promotes midgut absorption and fat body storage of dietary fat, at least in part via transcriptional activation of the gastric lipase encoded by the *magro* gene (Sieber and Thummel, 2009). This study also demonstrated that the human anti-obesity drug orlistat, a gastric lipase inhibitor, is able to reduce body fat accumulation in adult flies. Supporting a crucial role for lipolysis, midgut lipid accumulation and global fat storage are reduced by the insulin signaling pathway inhibitor Foxo in enterocytes, via reducing the expression of *magro* as flies age (Karpac et al., 2013). Excessive lipid accumulation in the fly gut and fat body is also a feature of 'humanized' flies upon cross-species expression of the human peptide neurotensin in *Drosophila* midgut enteroendocrine cells. Obesity in these flies is triggered by an evolutionarily conserved mechanism acting via the cellular energy sensor 5′ adenosine monophosphate (AMP)-activated protein kinase (Li et al., 2016a). In addition, the acidic pH of the gastric lumen may be important for fly obesity given that both global vacuolar-type H^+-adenosinetriphosphatase (ATPase) mutants and flies treated with pharmacological inhibitors of alimentary acidity accumulate extra fat (Lin et al., 2015b). This effect could be mediated via the gut microbiome, which both shapes and depends upon the acidity of the gut (Overend et al., 2016). Collectively, these data emphasize the importance of gut physiology for fat homeostasis in *Drosophila* and highlight the intricate interaction between the gut epithelium and the gut microbiome.

As in humans, the *Drosophila* gut hosts a complex microbiome enriched in *Lactobacillus* and *Acetobacter* species. Both diet and immunity play a role in controlling the composition of the microbiome, affecting fly health and metabolism (reviewed in Wong et al., 2016). Adult axenic flies overstore fats under various dietary conditions compared with flies with natural gut microbiota

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(Wong et al., 2014). *Lactobacillus* sp. abundance promotes co-colonization by *Acetobacter* sp. in the adult gut, which in turn negatively correlates with the fat storage level of the fly (Newell and Douglas, 2014). The composition of the gut microbiome of adult *Drosophila* corresponds to body fat content and depends on the host genotype (Chaston et al., 2016). The fly diet impacts the composition of the microbiota in the gut because a high-sugar diet shifts the gut microbiome to uracil-producing species, which promote fat storage and growth in *Drosophila* larvae (Whon et al., 2017). The availability of dietary glucose to the adult fly depends on the microbiome because flies with commensal *Acetobacter tropicalis* eat more than axenic flies but store less TAG owing to the consumption of dietary sugar by the bacteria (Huang and Douglas, 2015). Collectively, these data demonstrate that the gut microbiota and its metabolism modulate fat storage in the fly.

A range of tools exists for studies of the *Drosophila* gastrointestinal tract. The fly gut is beautifully suited to whole-mount immunohistochemistry and easy to isolate for quantitative biochemistry techniques. Likewise, the GAL4/UAS binary expression system described in whole organism studies enables overexpression, including RNAi, in specific gut cell subpopulations (Table 1). The fly eubacterial microbiome is often characterized by performing 16S ribosomal RNA sequencing and can be manipulated experimentally using axenic culture or culture with a defined microbial composition (Chaston et al., 2016).

The fat body and oenocytes

The insect fat body represents the central metabolic hub in body fat storage control. The fat body accumulates the vast majority of body fat during development and caloric overload, and executes lipolysis when energy from stored nutrients is needed for survival during metamorphosis, starvation or egg production. The fat body takes up lipids from the fly blood or hemolymph and esterifies them as stored TAGs and cholesterol esters when nutrients are abundant. In addition, the fat body carries out glycolysis and lipogenesis using carbohydrates in the blood (reviewed in Arrese and Soulages, 2010).

Fly obesity research centers on fat body studies at two different developmental stages: late larval stages and the adult fat body. The larval and the adult fat body are composed of two different cell lineages. The larval fat body represents a contiguous organ of a fairly constant number of postmitotic, large endoreplicative cells, which undergo histolysis shortly after the adult fly ecloses (Aguila et al., 2013, 2007). In contrast, the adult fat body is believed to be composed of diploid cells derived from cell clusters in the larval body wall and from adepithelial cells of the imaginal discs (Hoshizaki et al., 1995). The adult fat body cells are allocated to various unconnected adipose tissue depots such as the abdominal subcuticular fat body, the head fat body and the pericardial fat body. The ontogenetic origin and the cellular composition of adult adipose tissue depots are fairly ill-defined; however, evidence is accumulating that both hypertrophy and hyperplasia contribute to the plasticity of adult adipose tissue with respect to fat storage capacity in obese flies (DiAngelo and Birnbaum, 2009). Nonetheless, fat bodies at both stages play the same crucial role in fat storage.

The main cellular characteristics of both the larval and the adult fat bodies are the LDs, the universal organelles of intracellular fat storage, which are routinely stained by lipophilic dyes such as Oil Red O, BODIPY493/503 or Nile Red for microscopic imaging. These dyes are also used, albeit less frequently, to detect neutral lipids in other tissues. Fly fat body cells are multilocular, meaning that they contain LD populations of different sizes that belong to at least two distinct functional classes (Wilfling et al., 2013), which differ in their properties owing to differential association with particular sets of LD-associated proteins. Mass spectrometry-based

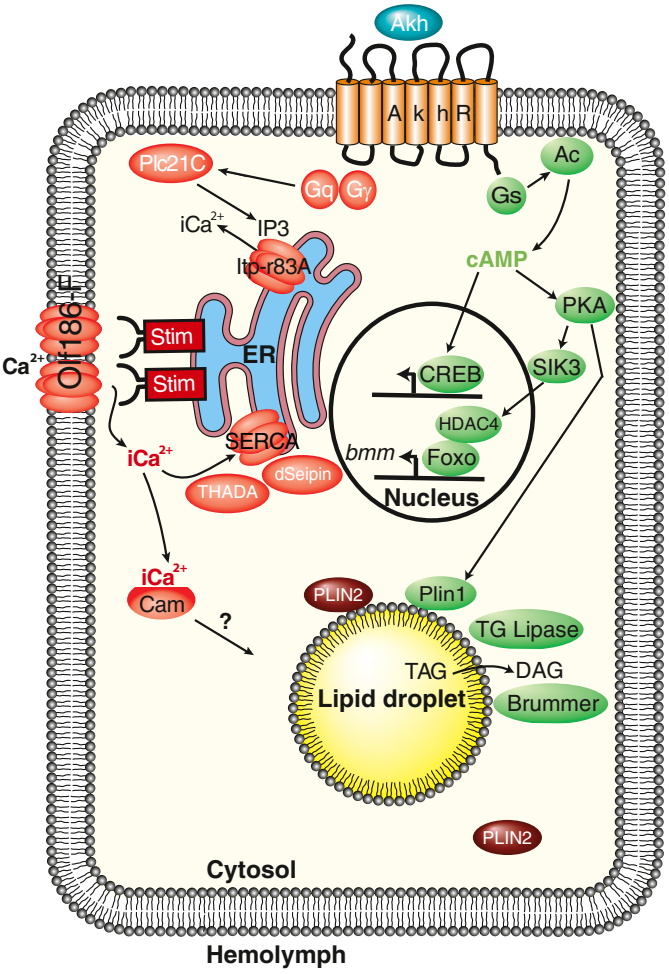

Fig. 2. Model of storage lipid mobilization in an adult *Drosophila* fat body cell. Illustrated are mechanisms of lipocatabolism mediated by cyclic 5′ adenosine monophosphate (cAMP, shown in green on the right-hand side of the fat body) and intracellular calcium influx (iCa^{2+}) (red; left-hand side) second messenger signaling in response to starvation-induced adipokinetic hormone (Akh) binding to its cognate G protein-coupled receptor (AkhR). cAMP is increased by the Gs small G protein subunit and adenylate cyclase (Ac) and acts transcriptionally via activation of cAMP-responsive element binding (CREB) and forkhead box sub-group O (Foxo) by protein kinase A (PKA) and salt-induced kinase 3 (SIK3) kinase-dependent control of histone deacetylase 4 (HDAC4), which increases, e.g. Brummer lipase gene (*bmm*) transcription. Note that Foxo is also activated by starvation-induced low insulin signaling (not shown). Posttranslational cAMP control of fat mobilization is also mediated via PKA-dependent phosphorylation of the perilipin Plin1. iCa^{2+} increases by store-operated calcium entry (SOCE) in response to Akh/AkhR signaling relayed via Gq and Gγ (Gg) small G protein subunits, phospholipase C (Plc21C) and the second messenger inositol trisphosphate (IP3). IP3 binding to its receptor Itp-r83A triggers endoplasmic reticulum (ER) calcium efflux, which initiates the interaction of the ER Ca^{2+}-sensor Stim (Stromal interaction molecule) with the plasma membrane Ca^{2+} channel Olf186-F (dOrai) to allow extracellular Ca^{2+} influx. Increased intracellular Ca^{2+} signals via Ca^{2+}-dependent proteins such as calmodulin (Cam) to promote lipid mobilization by largely unknown mechanisms. The ER inward-directed Ca^{2+} pump Sarco/ER Ca^{2+}-ATPase (SERCA) antagonizes SOCE and is modulated by THADA (encoded by the fly homolog of the *Thyroid Adenoma-Associated* gene) and dSeipin (encoded by the fly homolog of the Bernardelli–Seip congenital lipodystrophytype 2 gene). For more details, see the text. DAG, diacylglycerol; TAG: triacylglycerol. The second perilipin (Plin2) protects lipid droplets from TAG mobilization.

characterization of the fly LD proteome at different developmental stages (Beller et al., 2006; Cermelli et al., 2006; Krahmer et al., 2013) revealed a surprising complexity that is consistent with functional heterogeneity among LDs that varies in relation to the metabolic needs of the animal (reviewed in Farese and Walther, 2009; Walther and Farese, 2012).

Storage lipid mobilization from the LDs depends on the adipokinetic hormone (Akh), which circulates in the hemolymph. The Akh pathway is functionally analogous to mammalian glucagon signaling and represents the most important lipocatabolic pathway required during fasting conditions (Fig. 2). Akh operates via transcriptional and posttranslational mechanisms. The binding of the Akh peptide to its cognate G protein-coupled receptor (Akh receptor; AkhR) (Bharucha et al., 2008; Grönke et al., 2007) on the fat body cell surface triggers canonical cyclic AMP (cAMP)/protein kinase A (PKA) signaling (Fig. 2, green components). Increased cAMP promotes the activity of the cAMP-responsive element binding (CREB) transcription factor (Fig. 2). Consistently, targeted downregulation of CREB specifically in the fat body causes overeating and obesity in flies (Iijima et al., 2009). The cAMP-dependent PKA activation orchestrates at least two different pro-lipolytic responses. On the one hand, PKA-dependent phosphorylation of the LD-associated protein Perilipin 1 (Plin1) is believed to promote the access of TAG lipases to the LD surface (Patel et al., 2006, 2005), thereby allowing storage lipid mobilization (Fig. 2). On the other hand, phosphorylation by PKA inhibits salt-induced kinase 3 (SIK3) (Wang et al., 2011), which results in hypophosphorylation and the consequent nuclear translocation of histone deacetylase 4 (HDAC4) (Wang et al., 2011; Choi et al., 2015). In the nucleus, HDAC4 deacetylates the starvation-induced transcription factor Foxo to boost its transcriptional activity. An important transcriptional target of Foxo is *brummer* (*bmm*) (Grönke et al., 2005), which encodes the fly homolog of adipose triglyceride lipase (ATGL), the major mammalian TAG lipase. Transcriptional regulation of *bmm* adjusts body fat content in a dosage-dependent manner given that *bmm* gain-of-function flies are lean, whereas *bmm* mutants represent a fly obesity model (Fig. 2). Given that the nuclear localization of Foxo is also associated with reduced insulin/insulin-like growth factor signaling (IIS), the activated Akh and the downregulated IIS pathways mechanistically converge under fasting conditions to promote Foxo-dependent transcription and lipocatabolism.

In addition to canonical cAMP/PKA signal transduction, the Akh pathway signals using intracellular calcium (iCa^{2+}) as a second messenger to control fly body fat storage by using a mechanism called store-operated calcium entry (SOCE) (Fig. 2; red components). Akh binding to AkhR triggers an inositol trisphosphate (IP3) second messenger response, which is relayed by small Gg and Gq protein subunits and phospholipase C (Plc21C) (Baumbach et al., 2014b). At the endoplasmic reticulum (ER) membrane, IP3 binding to the IP3 receptor (IP3R; called Itp-r83A in the fly) causes Ca^{2+} efflux, which is sensed by an ER calcium sensor known as the Stromal interaction molecule (Stim, Fig. 2). Stim, in turn, interacts with the plasma membrane calcium channel Orai (called Olf186-F in the fly) to elevate iCa^{2+} levels by extracellular calcium influx. Elevated iCa^{2+} concentrations are translated to a plethora of cellular responses by cytoplasmic Ca^{2+}-dependent proteins such as Calmodulin (Cam). At the ER membrane, the Sarco/ER Ca^{2+}-ATPase (SERCA; also called Ca-P60A in the fly) antagonizes the Ca^{2+} efflux mediated by the action of IP3R and of the related Ryanodine receptor (RyR) to balance iCa^{2+} homeostasis and to aid the termination of G protein-coupled receptor-triggered iCa^{2+} signaling (Fig. 2). Accordingly, genetic manipulation of these factors modulates fat body TAG content in adult flies (Subramanian et al., 2013; Baumbach et al., 2014a,b; Pospisilik et al., 2010). SERCA appears to be a central control element of body fat storage control because impairment of dSeipin, a positive regulator of SERCA encoded by the fly homolog of the human gene, which is affected in Berardinelli–Seip congenital lipodystrophy (Magré et al., 2001), causes lean flies (Bi et al., 2014). By contrast, flies lacking THADA, a SERCA uncoupler encoded by the fly homolog of the human *Thyroid Adenoma Associated* gene, are obese (Moraru et al., 2017). Impairment of iCa^{2+} homeostasis is associated with various tissue-autonomous phenotypes such as reduced lipogenesis and mitochondrial dysfunction. Given the pleiotropic roles of calcium in metabolism (reviewed in Arruda and Hotamisligil, 2015), further research is required to achieve a comprehensive understanding of how iCa^{2+} modulates lipid storage. Remarkably, the role of SOCE in fat storage control has been recently recapitulated in mammals (Maus et al., 2017).

The catabolic Akh pathway is antagonized by the anabolic IIS pathway. Increasing IIS signaling via insulin-like peptides (Ilps) and sugar in the hemolymph promotes fatty acid biosynthesis and esterification by the fat body. Insulin resistance in flies also leads to obesity in some contexts. For example, loss of the insulin receptor substrate Chico or the Chico binding protein dSH2B reduces IIS signaling and increases obesity in flies and mammals (Böhni et al., 1999; Song et al., 2010). Lipogenesis is regulated by a number of factors and substrates other than IIS, including acetyl-CoA and the activated carrier NADPH (Teesalu et al., 2017). The conserved transcription factors sterol regulatory element-binding protein (SREBP) and carbohydrate-responsive element-binding protein (ChREBP; also called Mondo and Mio in the fly) promote the expression of lipogenic enzymes such as fatty acid synthase, stearoyl-CoA desaturase and diacylglycerol O-acyltransferase 1 (DGAT1; also called Midway in flies), which all increase triglyceride storage (Buszczak et al., 2002; Garrido et al., 2015; Havula et al., 2013; Kunte et al., 2006; Musselman et al., 2013; Sassu et al., 2012). Other genes required for lipogenesis in the fat body include those encoding the phosphatidate phosphatase dLipin (Schmitt et al., 2015; Ugrankar et al., 2011), pantothenate kinase and phosphopantothenoylcysteine synthase (Musselman et al., 2016) and Myc (Parisi et al., 2013). These represent potential targets for the development of anti-obesity therapeutic strategies, which can be modeled in the fly.

In addition to fat body-autonomous mechanisms of body fat storage control, the communication between the fat body and other fly organs/tissues is a research area that has seen recent progress (Fig. 1). For example, Unpaired 2 was identified as a leptin-related adipokine, which signals from the fat body to the central brain by modulating Ilp secretion from insulin-like peptide-producing cells (see below) in response to dietary carbohydrate or lipid (Rajan and Perrimon, 2012). Similarly, the Stunted peptide acts as an insulinotropic adipokine in response to dietary amino acids (Delanoue et al., 2016), and the *Drosophila* tumor necrosis factor-α adipokine Eiger represses Ilp production in response to starvation (Agrawal et al., 2016). Yet, adipokine-mediated communication emanating from the fat body is not restricted to the brain. Upon food withdrawal, the adipose secretes Ilp6, which causes neutral lipid accumulation and processing in the so-called oenocytes (Chatterjee et al., 2014).

Oenocytes (‘wine cells’) are goblet-shaped fat body-associated hepatocyte-like cells that are found segmentally alongside both the larval and adult cuticle (Makki et al., 2014). Larval oenocytes control storage lipid turnover and energy homeostasis in peripheral

tissues during fasting (Gutierrez et al., 2007). Adult oenocytes carry out the synthesis of very long chain fatty acids, which fuel the formation of cuticular hydrocarbons that protect the fly from desiccation and act as pheromones (Wicker-Thomas et al., 2015). Interestingly, these authors found that an obesogenic diet decreased cuticular hydrocarbon production whereas fatty acid synthesis and lipophorin receptor expression in the fat body promoted cuticular hydrocarbon synthesis.

Tools to study the fat body by tissue-specific modulation of gene expression are described in Table 1. The larval fat body is also particularly well-suited for clonal analysis, which provides a powerful method to discriminate between cell-autonomous and non-autonomous gene functions with respect to obesity (reviewed in Griffin et al., 2014). *Drosophila* uses the flippase/flippase recognition target (Flp/FRT) recombination system to generate single homozygous mutant clones in otherwise heterozygous, chimeric fat bodies. Clonal analysis has been successfully employed to detect autonomous roles of Plin1 (Beller et al., 2010), dSeipin (Bi et al., 2014) and the lipogenesis enzyme encoded by the *dLipin* gene (Schmitt et al., 2015) in fat body lipid storage droplets. Given the role of the fat body as the central hub in inter-organ communication, *in vivo* monitoring of signaling pathway activities is particularly valuable. Examples are the tGPH sensor (a fusion protein of GFP with the pleckstrin homology domain of the *Drosophila* homolog of the general receptor for phosphoinositides-1 expressed under the control of the *Drosophila* β-tubulin promoter), used to monitor phosphoinositide 3-kinase signaling (Britton et al., 2002), or the calcium-dependent nuclear import of LexA (CaLexA) system, used to monitor iCa^{2+} second messenger signaling (Masuyama et al., 2012). Lipid turnover in larval oenocytes and the fat body has been monitored by coherent anti-Stokes Raman scattering (CARS) microscopy (Chien et al., 2012). This label-free method of LD analysis shows great promise because it is capable of qualitative as well as quantitative lipid composition analyses.

The heart

The fly heart (also called the dorsal vessel, Fig. 1) can exhibit arrhythmic beating, fibrosis and cardiac failure, which is exacerbated during obesity, similar to the diseased human heart (Diop et al., 2015; Hardy et al., 2015; Heinrichsen et al., 2014). Although their ontogeny differs, the *Drosophila* heart is an innervated multichambered tube that circulates the hemolymph via an open circulatory system. Hemolymph, like blood, contains a variety of important hormones and metabolites needed by peripheral tissues and, therefore, the heart plays a key role in metabolic homeostasis. Heart function can be measured by high-speed video analysis of the heart rate *in vivo*, also known as Semi-automated Optical Heartbeat Analysis (SOHA), enabling quantification of systolic and diastolic strength, heart rate, heart period and arrhythmia (Cammarato et al., 2015). Such studies of heart function have been used to identify protective roles for cardiac proliferator-activated receptor-γ coactivator-1 (PGC-1; also called Spargel in the fly) and Bmm (Diop et al., 2015) and a deleterious role for the cardiac target of rapamycin (TOR) pathway (Birse et al., 2010) in flies with diet-induced obesity and cardiovascular disease.

Obese *Drosophila* exhibited increased cardiac steatosis and fibrosis compared with control flies, supporting an analogous burden to the insect and human hearts (Birse et al., 2010; Diop et al., 2015; Hardy et al., 2015; Na et al., 2013). Heart failure rate is highest in obese flies fed high-calorie diets and lowest in lean low-calorie-fed flies (Bazzell et al., 2013). The accumulation of fat in the fly heart is associated with obesity and an increased heart failure rate in fatty acid transport protein mutants (Sujkowski et al., 2012). Finally, the heart itself can control circulating and stored lipids in a non-autonomous manner via the regulation of lipoprotein metabolism. Cardiomyocyte-specific knockdown of the microsomal triglyceride transfer protein or its target lipoprotein, Lpp, prevents fat body TAG accumulation during high-fat diet-induced obesity (Lee et al., 2017).

Other tools to study heart structure and function aside from SOHA and the lipid assays described above have been developed over recent years. Fibrosis can be visualized in whole-mount hearts using confocal microscopy with actin-phalloidin staining or immunohistochemistry for extracellular matrix proteins such as collagens (especially pericardin), perlecan and the ADAMTS (a disintegrin and metalloprotease with thrombospondin repeats) homolog Lonely heart (Chartier et al., 2002; Drechsler et al., 2013). Stimulation-induced heart failure can also be measured in whole flies using external electrical pacing (Wessells et al., 2004).

Muscle

Although *Drosophila* skeletal muscle and flight muscle have both been used extensively to understand muscle physiology, the role of muscles in lipid homeostasis is less well understood. High-calorie obesogenic diets reduced endurance in a repetitive climbing assay (Bazzell et al., 2013), considered to be a measure of both heart and muscle function. Increased muscle activity using a gentle exercise paradigm (approximately 2 h exercise per day over 5 days) led to reduced TAG content, enabling researchers to compare the responses of a range of genotypes to exercise-induced reduction in obesity (Mendez et al., 2016). These so-called ‘gene by environment’ interaction studies (with environment including parameters such as diet and exercise) are a particularly attractive feature of *Drosophila* research.

The fly muscle also plays a functional role in lipid homeostasis and obesity. Interestingly, muscle Foxo is able to promote fat body lipogenesis remotely, inhibiting Akh expression via the secreted myokine Unpaired 2 (Zhao and Karpac, 2017). Similarly, the muscle acts as an endocrine regulator of lipid storage in the fat body by secreting activin, a member of the transforming growth factor beta (TGF-β) cytokine family (Song et al., 2017). Using a muscle-targeted driver to express transgenic RNAi, a genome-wide screen for obesity genes revealed 149 additional putative muscle-specific regulators of systemic TAG content in adult flies, including nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase, pyrroline 5-carboxylate reductase, inositol-requiring enzyme-1, nucleosome remodeling factor-38, the octopamine receptor, ornithine decarboxylase and vascular endothelial growth factor-related factor 1 (Pospisilik et al., 2010). A smaller RNAi screen targeting secreted proteins in the muscle identified Wingless, Ilp4, Angiotensin-converting enzyme, Unpaired 3 and Diptericin B as genes conferring reduced systemic TAG content, possibly by endocrine regulation of the fat body (Lee et al., 2014). Muscle AMP-activated protein kinase promotes adiposity in *Drosophila* larvae by phosphorylation of the myosin regulatory light chain, enabling peristalsis by the gut musculature and, presumably, improved nutrient uptake (Bland et al., 2010).

Tools to study the structure and function of muscle during obesity include immunohistochemistry, exercise endurance, electrophysiology of the flight muscle and the neuromuscular junction, and tissue-specific modulation of gene expression (Table 1).

The nervous and neuroendocrine system

Feeding (and thus energy intake) is a contributor to obesity that depends on the fly nervous system (Branch and Shen, 2017). Larvae feed almost constantly and live immersed in the laboratory diet until they leave the food in preparation for metamorphosis. In contrast, adults feed intermittently, with mated females eating more than males to support oogenesis, and adult feeding cycles under the control of the circadian clock (Xu et al., 2008). The *Drosophila* central nervous system (CNS) executes food intake control using various mechanisms, which can be studied using a wide range of methods. Food labeling by dyes or radioactivity, food uptake quantification by the so-called capillary feeder assay (Ja et al., 2007), proboscis extension reflex assay (Shiraiwa and Carlson, 2007), and automated food intake monitoring using the flyPAD (Itskov et al., 2014) or Fly Liquid-Food Interaction Counter (Ro et al., 2014) can be used to quantify feeding. Food preference assays are used to determine important feeding parameters such as total intake, meal size, meal frequency and feeding motivation (reviewed in Branch and Shen, 2017; Deshpande et al., 2014). Collectively, these parameters determine energy intake, which is crucial for the development of obesity.

A genetic screen approach combined the silencing of various neuronal populations with body fat measurements to locate obesity control centers in the adult fly brain (Al-Anzi et al., 2009). Importantly, this study identified groups of neurons that control body fat levels via feeding and/or metabolic rate control, demonstrating coordinated regulation of energy intake and expenditure by the brain. At the molecular level, a number of signaling molecules act to control feeding in the brain. One such cytokine is Unpaired 1 (Upd1), a leptin-related protein that controls food intake and body weight gain in response to a high fat diet (Beshel et al., 2017). Upd1-expressing neurons suppress the secretion of neuropeptide F (NPF), the fly analog of the mammalian orexigenic neuropeptide Y. A recent study suggests that inhibition of the neuronal orexigenic NPF in the CNS is also mediated by the adipose-derived metabolite tetrahydrobiopterin (Kim et al., 2017). Close communication between the fat body and the CNS in feeding control is also supported by the neuronal upregulation of the orexigenic small NPF, which is associated with the hyperphagia of obese flies caused by reduced iCa^{2+} signaling in the fat body (Baumbach et al., 2014a). In addition to these neuropeptides (reviewed in Schoofs et al., 2017) and metabolites, neurotransmitters/neurohormones such as octopamine (the fly equivalent of norepinephrine) are instrumental for body fat control in the CNS. Lack of octopamine causes a reduced metabolic rate and increased obesity in flies (Li et al., 2016b).

Neurosecretory cells

Two groups of key neurosecretory cells play important roles in the systemic control of fat and carbohydrate storage: corpora cardiaca (CC) cells, which are also known as adipokinetic hormone producing cells (APCs), and median neurosecretory cells (MNCs), known as insulin-like peptide producing cells (IPCs) (Fig. 1). The APCs belong to the *Drosophila* neuroendocrine system and have central catabolic functions in fly physiology similar to those of pancreatic α-cells in human physiology. APCs originate from the embryonic dorsal neuroectoderm (Wang et al., 2007), become part of the ring gland during larval stages, and eventually relocate to the posterior foregut in the adult fly (Gruber et al., 2013). Genetic ablation of APCs by targeted expression of pro-apoptotic genes causes lipid and sugar homeostasis defects in larvae (Kim and Rulifson, 2004; Lee and Park, 2004) and in adult flies (Grönke et al., 2007; Isabel, 2004). This is consistent with APCs being the exclusive source of Akh, a fly glucagon-like neuropeptide that is secreted in response to a drop in circulating sugars (Kim and Rulifson, 2004). Conditional blocking of APC secretion by targeted expression of the tetanus toxin light chain (Sweeney et al., 1995) inhibits the Akh-dependent release of Ilp3 from larval IPCs (Kim and Neufeld, 2015). Conversely, APC hyperpolarization caused by targeted expression of the heat-sensitive TrpA1 channel (Hamada et al., 2008) triggers Akh peptide secretion under permissive temperature conditions, which in turn causes storage fat depletion in flies (Waterson et al., 2014). However, Akh mutant flies are obese and hypoglycemic but lack a pre-adult metabolic phenotype (Gáliková et al., 2015), which suggests some caution is advisable concerning the functional specificity of cell ablation or global manipulation of neurosecretory cells. In line with this, APC cells were reported to also express and secrete Limostatin, which represses Ilp in IPCs (Alfa et al., 2015), illustrating the close interaction between APC and IPC cells in *Drosophila* metabolic control.

IIS signaling is highly conserved between flies and humans. Approximately 14 MNCs are known to be IPCs. IPCs are found in the anterior brain and consist of seven cell bodies in each brain lobe, positioned bilaterally in both the larva and the adult. Ilps, which exhibit structural similarity to human insulin, bind and activate a highly conserved insulin receptor that can also be activated by insulin (Nässel et al., 2015; Sajid et al., 2011; Sekine et al., 2010). Accordingly, IPCs and Ilps regulate metabolic homeostasis and obesity in a manner analogous to that of the pancreatic β-cell; thus, IPC ablation leads to hyperglycemia (Rulifson et al., 2002). IPCs project long axons onto the gut, heart and APCs to deliver their cargo, and receive input from a number of other cells in addition to their primary input of increased circulating nutrient concentration. As is the case for insulin secreted by pancreatic β-cells, feeding promotes Ilp expression and secretion from brain IPCs, although larval IPCs respond to elevated concentrations of protein (in particular the amino acid leucine) rather than sugar (Géminard et al., 2009; Ikeya et al., 2002; Manière et al., 2016). Early genetic ablation studies targeting IPCs by expression of cell death effectors did not reveal major effects on obesity (Broughton et al., 2005; Rulifson et al., 2002). Recently, more nuanced studies have shown that these cells play roles in controlling lipid storage. Conditional ablation of IPCs during adulthood elicits modest increases in stored and circulating TAGs (Haselton et al., 2010). The IPC-expressed gene *nudt3* is a human obesity susceptibility gene that is required in IPCs for lipolysis of stored fat during starvation (Williams et al., 2015). Similarly, the serotonin receptor 5-HT1A is required in IPCs for the catabolism of stored fat to supply energy during starvation (Luo et al., 2012). Reduction of the fly adiponectin receptor in IPCs also led to increased TAG content, consistent with a role for these cells in the control of obesity (Kwak et al., 2013). IPC expression of RNAi targeting the lipogenic transcription factor ChREBP increased the feeding rate, although there was no effect observed on obesity (Docherty et al., 2015). Little is known about whether IPCs produce other peptides, which makes ablation studies difficult to interpret. Drosulfakinin is a cholecystokinin-like satiety hormone that is expressed in IPCs and several other neurons, consistent with a complex role for these cells in the regulation of obesity (Söderberg et al., 2012).

Perspectives

Future studies on fly obesity should greatly benefit from up-and-coming techniques that allow acute and reversible organ-specific genetic manipulation to resolve causality and to limit compensatory regulation underlying energy homeostasis. Particularly promising in

this respect are *in vivo* CRISPR transcriptional activation (CRISPRa) and interference (CRISPRi) approaches (Ghosh et al., 2016; Lin et al., 2015a), which allow tightly regulated and reversible promoter activation and blocking, respectively.

Small molecule screens using whole *Drosophila* (Gasque et al., 2013; Men et al., 2016b) or cells (Tschapalda et al., 2016) to identify anti-obesity agents or anorectic drugs have been successful. Moreover, advances in analytical technology increasingly compensate for the disadvantage of the fly being a small model organism. Some examples of these analytical technologies are direct analysis in real time mass spectrometry in whole flies (Chiang et al., 2016), tissue- and hemolymph-specific mass spectrometry analyses (Chintapalli et al., 2013; Musselman et al., 2016), *in situ* lipid profiling in *Drosophila* sections using matrix-assisted laser desorption/ionization mass spectrometry imaging for comprehensive lipid composition analysis of individual fly organs (Niehoff et al., 2014), and the use of optical coherence tomography for non-invasive monitoring of *Drosophila* heart function (Men et al., 2016a).

Interestingly, an increasing number of obesity-related complications are being modeled in the fly, such as obesity-associated bipolar disorder (Williams et al., 2016) and diet-dependent metastasis of transformed cells (Hirabayashi et al., 2013; Hirabayashi and Cagan, 2015). *Drosophila* is also being used to study the pathophysiological consequences of aberrant lipid accumulation in non-storage tissues. For example, glial cells accumulate an increased number of LDs in response to neuronal mitochondrial dysfunction, which promotes neurodegeneration in both the fly and the mammalian brain (Liu et al., 2015). Reproduction also requires tight control of lipid homeostasis. Although females must increase oocyte and whole-animal lipogenesis to support the production of offspring (Sieber and Spradling, 2015), morbid maternal obesity adversely affects egg size, glycogen and TAG content, adult mass and gene expression in offspring and even in the F2 generation (Buescher et al., 2013; Dew-Budd et al., 2016; Matzkin et al., 2013). Highly obesogenic diets reduce fecundity (Brookheart et al., 2017; Matzkin et al., 2013, 2011) as does genetically induced obesity (Palu et al., 2017). Interestingly, obesity can affect the metabolic state of the offspring through the male *Drosophila* germline via effects on histone H3 methylation, a mechanism that appears to be conserved in humans (Öst et al., 2014).

Collectively, more than 100 years after its first use as a laboratory model organism, *Drosophila* is ready to answer current basic research questions and to help in fighting the pandemic human health threats of the 21st century that comprise obesity and obesity-related metabolic diseases.

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References
Agrawal, N., Delanoue, R., Mauri, A., Basco, D., Pasco, M., Thorens, B. and Léopold, P. (2016). The Drosophila TNF eiger is an adipokine that acts on insulin-producing cells to mediate nutrient response. *Cell Metab.* **23**, 675-684.

Aguila, J. R., Suszko, J., Gibbs, A. G. and Hoshizaki, D. K. (2007). The role of larval fat cells in adult Drosophila melanogaster. *J. Exp. Biol.* **210**, 956-963.

Aguila, J. R., Hoshizaki, D. K. and Gibbs, A. G. (2013). Contribution of larval nutrition to adult reproduction in Drosophila melanogaster. *J. Exp. Biol.* **216**, 399-406.

Agaisse, H., Petersen, U. M., Boutros, M., Mathey-Prevot, B. and Perrimon, N. (2003). Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev. Cell.* **5**, 441-450.

Al-Anzi, B., Sapin, V., Waters, C., Zinn, K., Wyman, R. J. and Benzer, S. (2009). Obesity-blocking neurons in Drosophila. *Neuron* **64**, 290-291.

Alfa, R. W., Park, S., Skelly, K.-R., Poffenberger, G., Jain, N., Gu, X., Kockel, L., Wang, J., Liu, Y., Powers, A. C. et al. (2015). Suppression of insulin production and secretion by a Decretin hormone. *Cell Metab.* **21**, 323-333.

Allen, A. M., Anreiter, I., Neville, M. C. Sokolowski, M. B. (2017). Feeding-related traits are affected by dosage of the foraging gene in Drosophila melanogaster. *Genetics* **205**, 761-773.

Arrese, E. L. and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* **55**, 207-225.

Arruda, A. P. and Hotamisligil, G. S. (2015). Calcium homeostasis and organelle function in the pathogenesis of obesity and diabetes. *Cell Metab.* **22**, 381-397.

Armstrong, A. R., Laws, K. M. and Drummond-Barbosa, D. (2014). Adipocyte amino acid sensing controls adult germline stem cell number via the amino acid response pathway and independently of Target of Rapamycin signaling in Drosophila. *Dev. Camb. Engl.* **141**, 4479-4488.

Asha, H., Nagy, I., Kovacs, G., Stetson, D., Ando, I. and Dearolf, C. R. (2003). Analysis of Ras-induced overproliferation in *Drosophila* hemocytes. *Genetics* **163**, 203-215.

Bassett, A. R., Tibbit, C., Ponting, C. P. and Liu, J.-L. (2013). Highly efficient targeted mutagenesis of Drosophila with the CRISPR/Cas9 system. *Cell Rep.* **4**, 220-228.

Baumbach, J., Hummel, P., Bickmeyer, I., Kowalczyk, K. M., Frank, M., Knorr, K., Hildebrandt, A., Riedel, D., Jäckle, H. and Kühnlein, R. P. (2014a). A Drosophila in vivo screen identifies store-operated calcium entry as a key regulator of adiposity. *Cell Metab.* **19**, 331-343.

Baumbach, J., Xu, Y., Hehlert, P. and Kühnlein, R. P. (2014b). Gαq, Gγ1 and Plc21C control Drosophila body fat storage. *J. Genet. Genomics Yi Chuan Xue Bao* **41**, 283-292.

Bazzell, B., Ginzberg, S., Healy, L. and Wessells, R. J. (2013). Dietary composition regulates Drosophila mobility and cardiac physiology. *J. Exp. Biol.* **216**, 859-868.

Beller, M., Riedel, D., Jänsch, L., Dieterich, G., Wehland, J., Jäckle, H. and Kühnlein, R. P. (2006). Characterization of the Drosophila lipid droplet subproteome. *Mol. Cell. Proteomics* **5**, 1082-1094.

Beller, M., Sztalryd, C., Southall, N., Bell, M., Jäckle, H., Auld, D. S. and Oliver, B. (2008). COPI complex is a regulator of lipid homeostasis. *PLoS Biol.* **6**, e292.

Beller, M., Bulankina, A. V., Hsiao, H.-H., Urlaub, H., Jäckle, H. and Kühnlein, R. P. (2010). PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. *Cell Metab.* **12**, 521-532.

Beshel, J., Dubnau, J. and Zhong, Y. (2017). A Leptin analog locally produced in the brain acts via a conserved neural circuit to modulate obesity-linked behaviors in Drosophila. *Cell Metab.* **25**, 208-217.

Beumer, K. J., Trautman, J. K., Bozas, A., Liu, J.-L., Rutter, J., Gall, J. G. and Carroll, D. (2008). Efficient gene targeting in Drosophila by direct embryo injection with zinc-finger nucleases. *Proc. Natl. Acad. Sci. USA* **105**, 19821-19826.

Bharucha, K. N., Tarr, P. and Zipursky, S. L. (2008). A glucagon-like endocrine pathway in Drosophila modulates both lipid and carbohydrate homeostasis. *J. Exp. Biol.* **211**, 3103-3110.

Bi, J., Wang, W., Liu, Z., Huang, X., Jiang, Q., Liu, G., Wang, Y. and Huang, X. (2014). Seipin promotes adipose tissue fat storage through the ER Ca2+-ATPase SERCA. *Cell Metab.* **19**, 861-871.

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.

Billeter, J.-C., Atallah, J., Krupp, J. J., Millar, J. G. and Levine, J. D. (2009). Specialized cells tag sexual and species identity in *Drosophila melanogaster*. *Nature* **461**, 987-991.

Biteau, B., and Jasper, H. (2011). EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. *Dev. Camb. Engl.* **138**, 1045-1055.

Bland, M. L., Lee, R. J., Magallanes, J. M., Foskett, J. K. and Birnbaum, M. J. (2010). AMPK supports growth in Drosophila by regulating muscle activity and nutrient uptake in the gut. *Dev. Biol.* **344**, 293-303.

Böhni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andruss, B. F., Beckingham, K. and Hafen, E. (1999). Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1-4. *Cell* **97**, 865-875.

Branch, A. and Shen, P. (2017). Central and peripheral regulation of appetite and food intake in *Drosophila*. In *Appetite and Food Intake: Central Control* (ed. R. B. S. Harris), pp. 17-38. Boca Raton, FL: CRC Press/Taylor & Francis.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Britton, J. S., Lockwood, W. K., Li, L., Cohen, S. M. and Edgar, B. A. (2002). Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev. Cell* **2**, 239-249.
Brankatschk, M. and Eaton, S. (2010). Lipoprotein particles cross the blood-brain barrier in *Drosophila*. *J. Neurosci.* **30**, 10441-10447.
Brookheart, R. T., Swearingen, A. R., Collins, C. A., Cline, L. M. and Duncan, J. G. (2017). High-sucrose-induced maternal obesity disrupts ovarian function and decreases fertility in Drosophila melanogaster. *Biochim. Biophys. Acta*.
Broughton, S. J., Piper, M. D. W., Ikeya, T., Bass, T. M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D. J., Leevers, 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.
Buchon, N. and Osman, D. (2015). All for one and one for all: regionalization of the Drosophila intestine. *Insect Biochem. Mol. Biol.* **67**, 2-8.
Buescher, J. L., Musselman, L. P., Wilson, C. A., Lang, T., Keleher, M., Baranski, T. J. and Duncan, J. G. (2013). Evidence for transgenerational metabolic programming in Drosophila. *Dis. Model. Mech.* **6**, 1123-1132.
Buszczak, M., Lu, X., Segraves, W. A., Chang, T. Y. and Cooley, L. (2002). Mutations in the midway gene disrupt a Drosophila acyl coenzyme A: diacylglycerol acyltransferase. *Genetics* **160**, 1511-1518.
Cammarato, A., Ocorr, S. and Ocorr, K. (2015). Enhanced assessment of contractile dynamics in Drosophila hearts. *BioTechniques* **58**, 77-80.
Carvalho, M., Sampaio, J. L., Palm, W., Brankatschk, M., Eaton, S. and Shevchenko, A. (2012). Effects of diet and development on the Drosophila lipidome. *Mol. Syst. Biol.* **8**, 600.
Cermelli, S., Guo, Y., Gross, S. P. and Welte, M. A. (2006). The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr. Biol.* **16**, 1783-1795.
Chartier, A., Zaffran, S., Astier, M., Sémériva, M. and Gratecos, D. (2002). Pericardin, a Drosophila type IV collagen-like protein is involved in the morphogenesis and maintenance of the heart epithelium during dorsal ectoderm closure. *Development* **129**, 3241-3253.
Chaston, J. M., Dobson, A. J., Newell, P. D. and Douglas, A. E. (2016). Host genetic control of the microbiota mediates the Drosophila nutritional phenotype. *Appl. Environ. Microbiol.* **82**, 671-679.
Chatterjee, D., Katewa, S. D., Qi, Y., Jackson, S. A., Kapahi, P. and Jasper, H. (2014). Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in Drosophila oenocytes. *Proc. Natl. Acad. Sci. USA* **111**, 17959-17964.
Chiang, Y. N., Tan, K. J., Chung, H., Lavrynenko, O., Shevchenko, A. and Yew, J. Y. (2016). Steroid hormone signaling is essential for pheromone production and oenocyte survival. *PLoS Genet.* **12**, e1006126.
Chien, C.-H., Chen, W.-W., Wu, J.-T. and Chang, T.-C. (2012). Investigation of lipid homeostasis in living Drosophila by coherent anti-Stokes Raman scattering microscopy. *J. Biomed. Opt.* **17**, 126001-8.
Chintapalli, V. R., Al Bratty, M., Korzekwa, D., Watson, D. G. and Dow, J. A. T. (2013). Mapping an atlas of tissue-specific Drosophila melanogaster metabolomes by high resolution mass spectrometry. *PLoS ONE* **8**, e78066.
Choi, S., Lim, D.-S. and Chung, J. (2015). Feeding and fasting signals converge on the LKB1-SIK3 pathway to regulate lipid metabolism in Drosophila. *PLoS Genet.* **11**, e1005263.
Crozatier, M., Ubeda, J.-M., Vincent, A. and Meister, M. (2004). Cellular immune response to parasitization in *Drosophila* requires the EBF orthologue collier. *PLoS Biol.* **2**, E196.
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.
Dauwalder, B., Tsujimoto, S., Moss, J. and Mattox, W. (2002). The *Drosophila* takeout gene is regulated by the somatic sex-determination pathway and affects male courtship behavior. *Genes Dev.* **16**, 2879-2892.
Delanoue, R., Meschi, E., Agrawal, N., Mauri, A., Tsatskis, Y., McNeill, H. and Léopold, P. (2016). Drosophila insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor. *Science* **353**, 1553-1556.
Deshpande, S. A., Carvalho, G. B., Amador, A., Phillips, A. M., Hoxha, S., Lizotte, K. J. and Ja, W. W. (2014). Quantifying Drosophila food intake: comparative analysis of current methodology. *Nat. Methods* **11**, 535-540.
Dew-Budd, K., Jarnigan, J. and Reed, L. K. (2016). Genetic and sex-specific transgenerational effects of a high fat diet in Drosophila melanogaster. *PLoS ONE* **11**, e0160857.
DiAngelo, J. R. and Birnbaum, M. J. (2009). Regulation of fat cell mass by insulin in Drosophila melanogaster. *Mol. Cell. Biol.* **29**, 6341-6352.
Diop, S. B., Bisharat-Kernizan, J., Birse, R. T., Oldham, S., Ocorr, K. and Bodmer, R. (2015). PGC-1/Spargel counteracts high-fat-diet-induced obesity and cardiac Lipotoxicity downstream of TOR and Brummer ATGL lipase. *Cell Rep.*
Doane, W. W. (1960a). Developmental physiology of the mutant female sterile(2)adipose of Drosophila melanogaster. I. Adult morphology, longevity, egg production, and egg lethality. *J. Exp. Zool. Part Ecol. Genet. Physiol.* **145**, 1-21.
Doane, W. W. (1960b). Developmental physiology of the mutant female sterile(2)adipose of Drosophila melanogaster. II. Effects of altered environment and residual genome on its expression. *J. Exp. Zool. Part Ecol. Genet. Physiol.* **145**, 23-41.
Doane, W. W. (1961). Developmental physiology of the mutant female sterile(2)adipose of Drosophila melanogaster. III. Corpus allatum-complex and ovarian transplantations. *J. Exp. Zool. Part Ecol. Genet. Physiol.* **146**, 275-298.
Docherty, J. E. B., Manno, J. E., McDermott, J. E. and DiAngelo, J. R. (2015). Mio acts in the Drosophila brain to control nutrient storage and feeding. *Gene* **568**, 190-195.
Drechsler, M., Schmidt, A. C., Meyer, H. and Paululat, A. (2013). The conserved ADAMTS-like protein lonely heart mediates matrix formation and cardiac tissue integrity. *PLoS Genet.* **9**, e1003616.
Ducos, E., Vergès, V., Dugé de Bernonville, T., Blanc, N., Giglioli-Guivarc'h, N. and Dutilleul, C. (2017). Remarkable evolutionary conservation of antiobesity ADIPOSE/WDTC1 homologs in animals and plants. *Genetics* **207**, 153-162.
Farese, R. V., Jr and Walther, T. C. (2009). Lipid droplets finally get a little R-E-S-P-E-C-T. *Cell* **139**, 855-860.
Fischer, J. A., Giniger, E., Maniatis, T. and Ptashne, M. (1988). GAL4 activates transcription in *Drosophila*. *Nature* **332**, 853-856.
Gáliková, M., Diesner, M., Klepsatel, P., Hehlert, P., Xu, Y., Bickmeyer, I., Predel, R. and Kühnlein, R. P. (2015). Energy homeostasis control in Drosophila adipokinetic hormone mutants. *Genetics* **201**, 665-683.
Garrido, D., Rubin, T., Poidevin, M., Maroni, B., Le Rouzic, A., Parvy, J.-P. and Montagne, J. (2015). Fatty acid synthase cooperates with glyoxalase 1 to protect against sugar toxicity. *PLoS Genet.* **11**, e1004995.
Gasque, G., Conway, S., Huang, J., Rao, Y. and Vosshall, L. B. (2013). Small molecule drug screening in Drosophila identifies the 5HT2A receptor as a feeding modulation target. *Sci. Rep.* **3**, srep02120.
Géminard, C., Rulifson, E. J. and Léopold, P. (2009). Remote control of insulin secretion by fat cells in Drosophila. *Cell Metab.* **10**, 199-207.
Georgel, P., Naitza, S., Kappler, C., Ferrandon, D., Zachary, D., Swimmer, C., Kopczynski, C., Duyk, G., Reichhart, J. M. and Hoffmann, J. A. (2001). *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. *Dev. Cell.* **1**, 503-514.
Ghosh, S., Tibbit, C. and Liu, J.-L. (2016). Effective knockdown of Drosophila long non-coding RNAs by CRISPR interference. *Nucleic Acids Res.* **44**, e84-e84.
Gratz, S. J., Cummings, A. M., Nguyen, J. N., Hamm, D. C., Donohue, L. K., Harrison, M. M., Wildonger, J. and O'Connor-Giles, K. M. (2013). Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease. *Genetics* **194**, 1029-1035.
Griffin, R., Binari, R. and Perrimon, N. (2014). Genetic odyssey to generate marked clones in Drosophila mosaics. *Proc. Natl. Acad. Sci. USA* **111**, 4756-4763.
Groh, B. S., Yan, F., Smith, M. D., Yu, Y., Chen, X. and Xiong, Y. (2016). The antiobesity factor WDTC1 suppresses adipogenesis via the CRL4WDTC1 E3 ligase. *EMBO Rep.* **17**, 638-647.
Grönke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jäckle, H. and Kühnlein, R. P. (2003). Control of fat storage by a *Drosophila* PAT domain protein. *Curr. Biol.* **13**, 603-606.
Grönke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Müller, G., Jäckle, H. and Kühnlein, R. P. (2005). Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. *Cell Metab.* **1**, 323-330.
Grönke, S., Müller, G., Hirsch, J., Fellert, S., Andreou, A., Haase, T., Jäckle, H. and Kühnlein, R. P. (2007). Dual lipolytic control of body fat storage and mobilization in Drosophila. *PLoS Biol.* **5**, e137.
Gruber, F., Knapek, S., Fujita, M., Matsuo, K., Bräcker, L., Shinzato, N., Siwanowicz, I., Tanimura, T. and Tanimoto, H. (2013). Suppression of conditioned odor approach by feeding is independent of taste and nutritional value in Drosophila. *Curr. Biol.* **23**, 507-514.
Guo, Y., Walther, T. C., Rao, M., Stuurman, N., Goshima, G., Terayama, K., Wong, J. S., Vale, R. D., Walter, P. and Farese, R. V. (2008). Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* **453**, 657-661.
Gutierrez, E., Wiggins, D., Fielding, B. and Gould, A. P. (2007). Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. *Nature* **445**, 275-280.
Gustafson, K., and Boulianne, G. L. (1996). Distinct expression patterns detected within individual tissues by the GAL4 enhancer trap technique. *Genome*. **39**, 174-182.
Häder, T., Müller, S., Aguilera, M., Eulenberg, K. G., Steuernagel, A., Ciossek, T., Kühnlein, R. P., Lemaire, L., Fritsch, R., Dohrmann, C. et al. (2003). Control of triglyceride storage by a WD40/TPR-domain protein. *EMBO Rep.* **4**, 511-516.
Hamada, F. N., Rosenzweig, M., Kang, K., Pulver, S. R., Ghezzi, A., Jegla, T. J. and Garrity, P. A. (2008). An internal thermal sensor controlling temperature preference in Drosophila. *Nature* **454**, 217-220.
Hardy, C. M., Birse, R. T., Wolf, M. J., Yu, L., Bodmer, R. and Gibbs, A. G. (2015). Obesity-associated cardiac dysfunction in starvation-selected Drosophila melanogaster. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **309**, R658-R667.
Harrop, T. W. R., Pearce, S. L., Daborn, P. J. and Batterham, P. (2014). Whole-genome expression analysis in the third instar larval midgut of Drosophila melanogaster. *G3* **4**, 2197-2205.

Haselton, A., Sharmin, E., Schrader, J., Sah, M., Poon, P. and Fridell, Y.-W. C. (2010). Partial ablation of adult Drosophila insulin-producing neurons modulates glucose homeostasis and extends life span without insulin resistance. *Cell Cycle Georget. Tex.* **9**, 3135–33143.

Havula, E., Teesalu, M., Hyötyläinen, T., Seppälä, H., Hasygar, K., Auvinen, P., Orešič, M., Sandmann, T. and Hietakangas, V. (2013). Mondo/ChREBP-Mlx-regulated transcriptional network is essential for dietary sugar tolerance in Drosophila. *PLoS Genet.* **9**, e1003438.

Han, Z., Yi, P., Li, X. and Olson, E. N. (2006). Hand, an evolutionarily conserved bHLH transcription factor required for *Drosophila* cardiogenesis and hematopoiesis. *Development* **133**, 1175-1182.

Heinrichsen, E. T., Zhang, H., Robinson, J. E., Ngo, J., Diop, S., Bodmer, R., Joiner, W. J., Metallo, C. M. and Haddad, G. G. (2014). Metabolic and transcriptional response to a high-fat diet in Drosophila melanogaster. *Mol. Metab.* **3**, 42-54.

Hildebrandt, A., Bickmeyer, I. and Kühnlein, R. P. (2011). Reliable Drosophila body fat quantification by a coupled colorimetric assay. *PLoS ONE* **6**, e23796.

Hirabayashi, S. and Cagan, R. L. (2015). Salt-inducible kinases mediate nutrient-sensing to link dietary sugar and tumorigenesis in Drosophila. *Elife* **4**, e08501.

Hirabayashi, S., Baranski, T. J. and Cagan, R. L. (2013). Transformed Drosophila cells evade diet-mediated insulin resistance through wingless signaling. *Cell* **154**, 664-675.

Hong, S.-H., Kang, M., Lee, K.-S. and Yu, K. (2016). High fat diet-induced TGF-β/Gbb signaling provokes insulin resistance through the tribbles expression. *Sci. Rep.* **6**, 30265.

Hoshizaki, D. K., Lunz, R., Ghosh, M. and Johnson, W. (1995). Identification of fat-cell enhancer activity in Drosophila melanogaster using P-element enhancer traps. *Genome* **38**, 497-506.

Huang, J.-H. and Douglas, A. E. (2015). Consumption of dietary sugar by gut bacteria determines Drosophila lipid content. *Biol. Lett.* **11**, 20150469.

Iijima, K., Zhao, L. J., Shenton, C. and Iijima-Ando, K. (2009). Regulation of energy stores and feeding by neuronal and peripheral CREB activity in Drosophila. *PLoS ONE* **4**, e8498-e8497.

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.

Isabel, G. (2004). AKH-producing neuroendocrine cell ablation decreases trehalose and induces behavioral changes in Drosophila. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R531-R538.

Itskov, P. M., Moreira, J.-M., Vinnik, E., Lopes, G., Safarik, S., Dickinson, M. H. and Ribeiro, C. (2014). Automated monitoring and quantitative analysis of feeding behaviour in Drosophila. *Nat. Commun.* **5**, 4560.

Ja, W. W., Carvalho, G. B., Mak, E. M., de la Rosa, N. N., Fang, A. Y., Liong, J. C., Brummel, T. and Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. *Proc. Natl. Acad. Sci. USA* **104**, 8253-8256.

Jenett, A., Rubin, G. M., Ngo, T.-T. B., Shepherd, D., Murphy, C., Dionne, H., Pfeiffer, B. D., Cavallaro, A., Hall, D., Jeter, J., Iyer, N., Fetter, D., Hausenfluck, J. H., Peng, H., Trautman, E. T., Svirskas, R. R., Myers, E. W., Iwinski, Z. R., Aso, Y., DePasquale, G. M., Enos, A., Hulamm, P., Lam, S. C. B., Li, H.-H., Laverty, T. R., Long, F., Qu, L., Murphy, S. D., Rokicki, K., Safford, T., Shaw, K., Simpson, J. H., Sowell, A., Tae, S., Yu, Y. and Zugates, C. T. (2012). A GAL4-driver line resource for Drosophila neurobiology. *Cell Rep.* **2**, 991-1001.

Karpac, J., Biteau, B. and Jasper, H. (2013). Misregulation of an adaptive metabolic response contributes to the age-related disruption of lipid homeostasis in Drosophila. *Cell Rep.* **4**, 1250-1261.

Kim, J. and Neufeld, T. P. (2015). Dietary sugar promotes systemic TOR activation in Drosophila through AKH-dependent selective secretion of Dilp3. *Nat. Commun.* **6**, 6846.

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, D.-H., Shin, M., Jung, S.-H., Kim, Y.-J. and Jones, W. D. (2017). A fat-derived metabolite regulates a peptidergic feeding circuit in Drosophila. *PLoS Biol.* **15**, e2000532.

Kimbrell, D. A., Hice, C., Bolduc, C., Kleinhesselink, K. and Beckingham, K. (2002). The Dorothy enhancer has Tinman binding sites and drives hopscotch-induced tumor formation. *Genesis* **34**, 23-28.

Krahmer, N., Hilger, M., Kory, N., Wilfling, F., Stoehr, G., Mann, M., Farese, R. V. and Walther, T. C. (2013). Protein correlation profiles identify lipid droplet proteins with high confidence. *Mol. Cell. Proteomics* **12**, 1115-1126.

Kunte, A. S., Matthews, K. A. and Rawson, R. B. (2006). Fatty acid auxotrophy in Drosophila larvae lacking SREBP. *Cell Metab.* **3**, 439-448.

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.

Lai, C.-Q., Parnell, L. D., Arnett, D. K., García-Bailo, B., Tsai, M. Y., Kabagambe, E. K., Straka, R. J., Province, M. A., An, P., Borecki, I. B. et al. (2009). WDTC1, the ortholog of Drosophila adipose gene, associates with human obesity, modulated by MUFA intake. *Obesity* **17**, 593-600.

Lazareva, A. A., Roman, G., Mattox, W., Hardin, P. E. and Dauwalder, B. (2007). A role for the adult fat body in *Drosophila* male courtship behavior. *PLoS Genet.* **3**, e16.

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, J.-H., Bassel-Duby, R. and Olson, E. N. (2014). Heart- and muscle-derived signaling system dependent on MED13 and Wingless controls obesity in Drosophila. *Proc. Natl. Acad. Sci. USA* **111**, 9491-9496.

Lee, S., Bao, H., Ishikawa, Z., Wang, W. and Lim, H.-Y. (2017). Cardiomyocyte regulation of systemic lipid metabolism by the Apolipoprotein B-containing lipoproteins in Drosophila. *PLoS Genet.* **13**, e1006555.

Lemaitre, B. and Miguel-Aliaga, I. (2013). The digestive tract of Drosophila melanogaster. *Annu. Rev. Genet.* **47**, 377-404.

Li, J., Song, J., Zaytseva, Y. Y., Liu, Y., Rychahou, P., Jiang, K., Starr, M. E., Kim, J. T., Harris, J. W., Yiannikouris, F. B. et al. (2016a). An obligatory role for neurotensin in high-fat-diet-induced obesity. *Nature* **533**, 411-415.

Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C. and Roeder, T. (2016b). Octopamine controls starvation resistance, life span and metabolic traits in Drosophila. *Sci. Rep.* **6**, 35359.

Lin, S., Ewen-Campen, B., Ni, X., Housden, B. E. and Perrimon, N. (2015a). In vivo transcriptional activation using CRISPR/Cas9 in Drosophila. *Genetics* **201**, 433-442.

Lin, W.-S., Huang, C.-W., Song, Y.-S., Yen, J.-H., Kuo, P.-C., Yeh, S.-R., Lin, H.-Y., Fu, T.-F., Wu, M.-S., Wang, H.-D. et al. (2015b). Reduced Gut acidity induces an obese-like phenotype in Drosophila melanogaster and in mice. *PLoS ONE* **10**, e0139722.

Lo, P. C., and Frasch, M. (2001). A role for the COUP-TF-related gene seven-up in the diversification of cardioblast identities in the dorsal vessel of *Drosophila*. *Mech. Dev.* **104**, 49-60.

Liu, L., Zhang, K., Sandoval, H., Yamamoto, S., Jaiswal, M., Sanz, E., Li, Z., Hui, J., Graham, B. H., Quintana, A. et al. (2015). Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. *Cell* **160**, 177-190.

Luo, L., Liao, Y. J., Jan, L. Y. and Jan, Y. N. (1994). Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.* **8**, 1787-1802.

Luo, J., Becnel, J., Nichols, C. D. and Nässel, D. R. (2012). Insulin-producing cells in the brain of adult Drosophila are regulated by the serotonin 5-HT1A receptor. *Cell. Mol. Life Sci. CMLS* **69**, 471-484.

Magré, J., Delépine, M., Khallouf, E., Gedde-Dahl, T., Van Maldergem, L., Sobel, E., Papp, J., Meier, M., Mégarbané, A., Lathrop, M. et al. (2001). Identification of the gene altered in Berardinelli–Seip congenital lipodystrophy on chromosome 11q13. *Nat. Genet.* **28**, 365-370.

Makki, R., Cinnamon, E. and Gould, A. P. (2014). The development and functions of Oenocytes. *Annu. Rev. Entomol.* **59**, 405-425.

Manière, G., Ziegler, A. B., Geillon, F., Featherstone, D. E. and Grosjean, Y. (2016). Direct sensing of nutrients via a LAT1-like transporter in Drosophila insulin-producing cells. *Cell Rep.* **17**, 137-148.

Masuyama, K., Zhang, Y., Rao, Y. and Wang, J. W. (2012). Mapping neural circuits with activity-dependent nuclear import of a transcription factor. *J. Neurogenet.* **26**, 89-102.

Matzkin, L. M., Johnson, S., Paight, C., Bozinovic, G. and Markow, T. A. (2011). Dietary protein and sugar differentially affect development and metabolic pools in ecologically diverse Drosophila. *J. Nutr.* **141**, 1127-1133.

Matzkin, L. M., Johnson, S., Paight, C. and Markow, T. A. (2013). Preadult parental diet affects offspring development and metabolism in Drosophila melanogaster. *PLoS ONE* **8**, e59530.

Maus, M., Cuk, M., Patel, B., Lian, J., Ouimet, M., Kaufmann, U., Yang, J., Horvath, R., Hornig-Do, H.-T., Chrzanowska-Lightowlers, Z. M. et al. (2017). Store-operated Ca2+ entry controls induction of lipolysis and the transcriptional reprogramming to lipid metabolism. *Cell Metab.* **25**, 698-712.

McGowan, M. W., Artiss, J. D., Strandbergh, D. R. and Zak, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29**, 538-542.

Men, J., Jerwick, J., Wu, P., Chen, M., Alex, A., Ma, Y., Tanzi, R. E., Li, A. and Zhou, C. (2016a). Drosophila preparation and longitudinal imaging of heart function in vivo using Optical Coherence Microscopy (OCM). *J. Vis. Exp.*, e55002.

Men, T. T., Thanh, D. N. V., Yamaguchi, M., Suzuki, T., Hattori, G., Arii, M., Huy, N. T. and Kamei, K. (2016b). A Drosophila model for screening antiobesity agents. *BioMed Res. Int.* **2016**, 6293163.

Mendez, S., Watanabe, L., Hill, R., Owens, M., Moraczewski, J., Rowe, G. C., Riddle, N. C. and Reed, L. K. (2016). The TreadWheel: a novel apparatus to measure genetic variation in response to gently induced exercise for Drosophila. *PLoS ONE* **11**, e0164706.

Michelson, A. M. (1994). Muscle pattern diversification in *Drosophila* is determined by the autonomous function of homeotic genes in the embryonic mesoderm. *Dev. Camb. Engl.* **120**, 755-768.

Moraru, A., Cakan-Akdogan, G., Strassburger, K., Males, M., Mueller, S., Jabs, M., Muelleder, M., Frejno, M., Braeckman, B. P., Ralser, M. et al. (2017).

THADA regulates the organismal balance between energy storage and heat production. *Dev. Cell* **41**, 72-81.e6.
Mosher, J., Zhang, W., Blumhagen, R. Z., D'Alessandro, A., Nemkov, T., Hansen, K. C., Hesselberth, J. R. and Reis, T. (2015). Coordination between Drosophila Arc1 and a specific population of brain neurons regulates organismal fat. *Dev. Biol.* **405**, 280-290.
Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, 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.
Musselman, L. P., Fink, J. L., Ramachandran, P. V., Patterson, B. W., Okunade, A. L., Maier, E., Brent, M. R., Turk, J. and Baranski, T. J. (2013). Role of fat body lipogenesis in protection against the effects of caloric overload in Drosophila. *J. Biol. Chem.* **288**, 8028-8042.
Musselman, L. P., Fink, J. L. and Baranski, T. J. (2016). CoA protects against the deleterious effects of caloric overload in Drosophila. *J. Lipid Res.* **57**, 380-387.
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.
Na, J., Sweetwyne, M. T., Park, A. S. D., Susztak, K. and Cagan, R. L. (2015). Diet-induced podocyte dysfunction in drosophila and mammals. *Cell Rep.* **12**, 636-647.
Nakagami, T., Qiao, Q., Carstensen, B., Nhr-Hansen, C., Hu, G., Tuomilehto, J., Balkau, B. and Borch-Johnsen, K. ; **DECODE-DECODA Study Group**. (2003). Age, body mass index and Type 2 diabetes-associations modified by ethnicity. *Diabetologia* **46**, 1063-1070.
Nässel, D. R., Liu, Y. and Luo, J. (2015). Insulin/IGF signaling and its regulation in Drosophila. *Gen. Comp. Endocrinol.* **221**, 255-266.
Navrotskaya, V., Oxenkrug, G., Vorobyova, L. and Summergrad, P. (2016). Attenuation of high sucrose diet-induced insulin resistance in ABC transporter deficient white mutant of Drosophila melanogaster. *Integr. Obes. Diabetes* **2**, 187-190.
Newell, P. D. and Douglas, A. E. (2014). Interspecies interactions determine the impact of the Gut Microbiota on nutrient allocation in Drosophila melanogaster. *Appl. Environ. Microbiol.* **80**, 788-796.
Niehoff, A.-C., Kettling, H., Pirkl, A., Chiang, Y. N., Dreisewerd, K. and Yew, J. Y. (2014). Analysis of Drosophila lipids by matrix-assisted laser desorption/ionization mass spectrometric imaging. *Anal. Chem.* **86**, 11086-11092.
Öst, A., Lempradl, A., Casas, E., Weigert, M., Tiko, T., Deniz, M., Pantano, L., Boenisch, U., Itskov, P. M., Stoeckius, M. et al. (2014). Paternal diet defines offspring chromatin state and intergenerational obesity. *Cell* **159**, 1352-1364.
Overend, G., Luo, Y., Henderson, L., Douglas, A. E., Davies, S. A. and Dow, J. A. T. (2016). Molecular mechanism and functional significance of acid generation in the Drosophila midgut. *Sci. Rep.* **6**, 27242.
Palm, W., Sampaio, J. L., Brankatschk, M., Carvalho, M., Mahmoud, A., Shevchenko, A. and Eaton, S. (2012). Lipoproteins in Drosophila melanogaster–assembly, function, and influence on tissue lipid composition. *PLoS Genet.* **8**, e1002828.
Palm, W., Swierczynska, M. M., Kumari, V., Ehrhart-Bornstein, M., Bornstein, S. R. and Eaton, S. (2013). Secretion and signaling activities of lipoprotein-associated hedgehog and non-sterol-modified hedgehog in flies and mammals. *PLoS Biol.* **11**, e1001505.
Palu, R. A. S., Praggastis, S. A. and Thummel, C. S. (2017). Parental obesity leads to metabolic changes in the F2 generation in Drosophila. *Mol. Metab.* **6**, 631-639.
Parisi, F., Riccardo, S., Zola, S., Lora, C., Grifoni, D., Brown, L. M. and Bellosta, P. (2013). dMyc expression in the fat body affects DILP2 release and increases the expression of the fat desaturase Desat1 resulting in organismal growth. *Dev. Biol.* **379**, 64-75.
Pasco, M. Y. and Léopold, P. (2012). High sugar-induced insulin resistance in Drosophila relies on the lipocalin Neural Lazarillo. *PLoS ONE* **7**, e36583.
Patel, R. T., Soulages, J. L., Hariharasundaram, B. and Arrese, E. L. (2005). Activation of the lipid droplet controls the rate of lipolysis of triglycerides in the insect fat body. *J. Biol. Chem.* **280**, 22624-22631.
Patel, R. T., Soulages, J. L. and Arrese, E. L. (2006). Adipokinetic hormone-induced mobilization of fat body triglyceride stores in Manduca sexta: Role of TG-lipase and lipid droplets. *Arch. Insect Biochem. Physiol.* **63**, 73-81.
Pospisilik, J. A., Schramek, D., Schnidar, H., Cronin, S. J. F., 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.
Phillips, M. D., and Thomas, G. H. (2006). Brush border spectrin is required for early endosome recycling in *Drosophila*. *J. Cell Sci.* **119**, 1361-1370.
Rajan, A. and Perrimon, N. (2012). Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* **151**, 123-137.
Ranganayakulu, G., Elliott, D. A., Harvey, R. P. and Olson, E. N. (1998). Divergent roles for NK-2 class homeobox genes in cardiogenesis in flies and mice. *Dev. Camb. Engl.* **125**, 3037-3048.
Reed, L. K., Williams, S., Springston, M., Brown, J., Freeman, K., DesRoches, C. E., Sokolowski, M. B. and Gibson, G. (2010). Genotype-by-diet interactions drive metabolic phenotype variation in Drosophila melanogaster. *Genetics* **185**, 1009-1019.
Reed, L. K., Lee, K., Zhang, Z., Rashid, L., Poe, A., Hsieh, B., Deighton, N., Glassbrook, N., Bodmer, R. and Gibson, G. (2014). Systems genomics of metabolic phenotypes in wild-type Drosophila melanogaster. *Genetics* **197**, 781-793.
Reis, T. (2016). Effects of synthetic diets enriched in specific nutrients on Drosophila development, body fat, and lifespan. *PLoS ONE* **11**, e0146758.
Reis, T., Van Gilst, M. R. and Hariharan, I. K. (2010). A buoyancy-based screen of Drosophila larvae for fat-storage mutants reveals a role for Sir2 in coupling fat storage to nutrient availability. *PLoS Genet.* **6**, e1001206.
Reiter, L. T. (2001). A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome Res.* **11**, 1114-1125.
Ro, J., Harvanek, Z. M. and Pletcher, S. D. (2014). FLIC: high-throughput, continuous analysis of feeding behaviors in Drosophila. *PLoS ONE* **9**, e101107.
Rodenfels, J., Lavrynenko, O., Ayciriex, S., Sampaio, J. L., Carvalho, M., Shevchenko, A. and Eaton, S. (2014). Production of systemically circulating Hedgehog by the intestine couples nutrition to growth and development. *Genes Dev.* **28**, 2636-2651.
Rong, Y. S., Titen, S. W., Xie, H. B., Golic, M. M., Bastiani, M., Bandyopadhyay, P., Olivera, B. M., Brodsky, M., Rubin, G. M. and Golic, K. G. (2002). Targeted mutagenesis by homologous recombination in D. melanogaster. *Genes Dev.* **16**, 1568-1581.
Rovenko, B. M., Kubrak, O. I., Gospodaryov, D. V., Perkhulyn, N. V., Yurkevych, I. S., Sanz, A., Lushchak, O. V. and Lushchak, V. I. (2015a). High sucrose consumption promotes obesity whereas its low consumption induces oxidative stress in Drosophila melanogaster. *J. Insect Physiol.* **79**, 42-54.
Rovenko, B. M., Perkhulyn, N. V., Gospodaryov, D. V., Sanz, A., Lushchak, O. V. and Lushchak, V. I. (2015b). High consumption of fructose rather than glucose promotes a diet-induced obese phenotype in Drosophila melanogaster. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **180**, 75-85.
Rosay, P., Davies, S. A., Yu, Y., Sözen, M. A., Kaiser, K. and Dow, J. A. (1997). Cell-type specific calcium signalling in a *Drosophila* epithelium. *J. Cell Sci.* **110**, 1683-1692.
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.
Sajid, W., Kulahin, N., Schluckebier, G., Ribel, U., Henderson, H. R., Tatar, M., Hansen, B. F., Svendsen, A. M., Kiselyov, V. V., Nørgaard, P. et al. (2011). Structural and biological properties of the Drosophila insulin-like peptide 5 show evolutionary conservation. *J. Biol. Chem.* **286**, 661-673.
Sajwan, S., Sidorov, R., Stašková, T., Žaloudíková, A., Takasu, Y., Kodrík, D. and Zurovec, M. (2015). Targeted mutagenesis and functional analysis of adipokinetic hormone-encoding gene in Drosophila. *Insect Biochem. Mol. Biol.* **61**, 79-86.
Sassu, E. D., McDermott, J. E., Keys, B. J., Esmaeili, M., Keene, A. C., Birnbaum, M. J. and DiAngelo, J. R. (2012). Mio/dChREBP coordinately increases fat mass by regulating lipid synthesis and feeding behavior in Drosophila. *Biochem. Biophys. Res. Commun.* **426**, 43-48.
Schmitt, S., Ugrankar, R., Greene, S. E., Prajapati, M. and Lehmann, M. (2015). Drosophila Lipin interacts with insulin and TOR signaling pathways in the control of growth and lipid metabolism. *J. Cell Sci.* **128**, 4395-4406.
Schoofs, L., De Loof, A. and Van Hiel, M. B. (2017). Neuropeptides as Regulators of Behavior in Insects. *Annu. Rev. Entomol.* **62**, 35-52.
Schuster, C. M., Davis, G. W., Fetter, R. D. and Goodman, C. S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity. *Neuron*. **17**, 655-667.
Sekine, O., Love, D. C., Rubenstein, D. S. and Hanover, J. A. (2010). Blocking O-linked GlcNAc cycling in Drosophila insulin-producing cells perturbs glucose-insulin homeostasis. *J. Biol. Chem.* **285**, 38684-38691.
Shiraiwa, T. and Carlson, J. R. (2007). Proboscis extension response (PER) assay in Drosophila. *J. Vis. Exp.*, e193.
Sieber, M. H. and Spradling, A. C. (2015). Steroid signaling establishes a female metabolic state and regulates SREBP to control oocyte lipid accumulation. *Curr. Biol.* **25**, 993-1004.
Sieber, M. H. and Thummel, C. S. (2009). The DHR96 nuclear receptor controls triacylglycerol homeostasis in Drosophila. *Cell Metab.* **10**, 481-490.
Söderberg, J. A. E., 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.
Song, W., Veenstra, J. A. and Perrimon, N. (2014). Control of lipid metabolism by tachykinin in Drosophila. *Cell Rep.* **9**, 40-47.
Song, W., Cheng, D., Hong, S., Sappe, B., Hu, Y., Wei, N., Zhu, C., O'Connor, M. B., Pissios, P. and Perrimon, N. (2017). Midgut-derived activin regulates glucagon-like action in the fat body and glycemic control. *Cell Metab.* **25**, 386-399.
Sözen, M. A., Armstrong, J. D., Yang, M., Kaiser, K. and Dow, J. A. (1997). Functional domains are specified to single-cell resolution in a *Drosophila* epithelium. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 5207-5212.

Subramanian, M., Metya, S. K., Sadaf, S., Kumar, S., Schwudke, D. and Hasan, G. (2013). Altered lipid homeostasis in Drosophila InsP3 receptor mutants leads to obesity and hyperphagia. *Dis. Model. Mech.* **6**, 734-744.

Suh, J. M., Zeve, D., McKay, R., Seo, J., Salo, Z., Li, R., Wang, M. and Graff, J. M. (2007). Adipose is a conserved dosage-sensitive antiobesity gene. *Cell Metab.* **6**, 195-207.

Sujkowski, A., Saunders, S., Tinkerhess, M., Piazza, N., Jennens, J., Healy, L., Zheng, L. and Wessells, R. (2012). dFatp regulates nutrient distribution and long-term physiology in Drosophila. *Aging Cell* **11**, 921-932.

Sweeney, S. T., Broadie, K., Keane, J., Niemann, H. and O'Kane, C. J. (1995). Targeted expression of tetanus toxin light chain in Drosophila specifically eliminates synaptic transmission and causes behavioral defects. *Neuron* **14**, 341-351.

Takashima, S., and Murakami, R. (2001). Regulation of pattern formation in the *Drosophila* hindgut by wg, hh, dpp, and en. *Mech. Dev.* **101**, 79-90.

Teesalu, M., Rovenko, B. M. and Hietakangas, V. (2017). Salt-inducible Kinase 3 provides sugar tolerance by regulating NADPH/NADP(+) Redox balance. *Curr. Biol.* **27**, 458-464.

Tschapalda, K., Zhang, Y.-Q., Liu, L., Golovnina, K., Schlemper, T., Eichmann, T. O., Lal-Nag, M., Sreenivasan, U., McLenithan, J., Ziegler, S. et al. (2016). A class of Diacylglycerol Acyltransferase 1 inhibitors identified by a combination of phenotypic high-throughput screening, genomics, and genetics. *EBIOM* **8**, 49-59.

Tsuda-Sakurai, K., Seong, K.-H., Horiuchi, J., Aigaki, T. and Tsuda, M. (2015). Identification of a novel role for Drosophila MESR4 in lipid metabolism. *Genes Cells Devoted Mol. Cell. Mech.* **20**, 358-365.

Ugrankar, R., Liu, Y., Provaznik, J., Schmitt, S. and Lehmann, M. (2011). Lipin is a central regulator of adipose tissue development and function in Drosophila melanogaster. *Mol. Cell. Biol.* **31**, 1646-1656.

Voght, S. P., Fluegel, M. L., Andrews, L. A. and Pallanck, L. J. (2007). *Drosophila* NPC1b promotes an early step in sterol absorption from the midgut epithelium. *Cell Metab.* **5**, 195-205.

Walther, T. C. and Farese, R. V.Jr. (2012). Lipid droplets and cellular lipid metabolism. *Annu. Rev. Biochem.* **81**, 687-714.

Wang, S., Tulina, N., Carlin, D. L. and Rulifson, E. J. (2007). The origin of islet-like cells in Drosophila identifies parallels to the vertebrate endocrine axis. *Proc. Natl. Acad. Sci. USA* **104**, 19873-19878.

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

Waterson, M. J., Chung, B. Y., Harvanek, Z. M., Ostojic, I., Alcedo, J. and Pletcher, S. D. (2014). Water sensor ppk28 modulates Drosophila lifespan and physiology through AKH signaling. *Proc. Natl. Acad. Sci. USA* **111**, 8137-8142.

Wessells, R. J., Fitzgerald, E., Cypser, J. R., Tatar, M. and Bodmer, R. (2004). Insulin regulation of heart function in aging fruit flies. *Nat. Genet.* **36**, 1275-1281.

Whon, T. W., Shin, N.-R., Jung, M.-J., Hyun, D.-W., Kim, H. S., Kim, P. S. and Bae, J.-W. (2017). Conditionally pathogenic gut microbes promote larval growth by increasing redox-dependent fat storage in high-sugar diet-fed Drosophila. *Antioxid. Redox Signal.* **27**, 1361-1380.

Wicker-Thomas, C., Garrido, D., Bontonou, G., Napal, L., Mazuras, N., Denis, B., Rubin, T., Parvy, J.-P. and Montagne, J. (2015). Flexible origin of hydrocarbon/pheromone precursors in Drosophila melanogaster. *J. Lipid Res.* **56**, 2094-2101.

Wilfling, F., Wang, H., Haas, J. T., Krahmer, N., Gould, T. J., Uchida, A., Cheng, J.-X., Graham, M., Christiano, R., Fröhlich, F. et al. (2013). Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Dev. Cell* **24**, 1-16.

Williams, C. M., Thomas, R. H., MacMillan, H. A., Marshall, K. E. and Sinclair, B. J. (2011). Triacylglyceride measurement in small quantities of homogenised insect tissue: comparisons and caveats. *J. Insect Physiol.* **57**, 1602-1613.

Williams, M. J., Eriksson, A., Shaik, M., Voisin, S., Yamskova, O., Paulsson, J., Thombare, K., Fredriksson, R. and Schiöth, H. B. (2015). The obesity-linked gene Nudt3 Drosophila homolog aps is associated with insulin signaling. *Mol. Endocrinol. Baltim. Md* **29**, 1303-1319.

Williams, M. J., Klockars, A., Eriksson, A., Voisin, S., Dnyansagar, R., Wiemerslage, L., Kasagiannis, A., Akram, M., Kheder, S., Ambrosi, V. et al. (2016). The Drosophila ETV5 homologue Ets96B: molecular link between obesity and bipolar disorder. *PLoS Genet.* **12**, e1006104.

Wong, A. C.-N., Dobson, A. J. and Douglas, A. E. (2014). Gut microbiota dictates the metabolic response of Drosophila to diet. *J. Exp. Biol.* **217**, 1894-1901.

Wong, A. C. N., Vanhove, A. S. and Watnick, P. I. (2016). The interplay between intestinal bacteria and host metabolism in health and disease: lessons from Drosophila melanogaster. *Dis. Model. Mech.* **9**, 271-281.

Woodcock, K. J., Kierdorf, K., Pouchelon, C. A., Vivancos, V., Dionne, M. S. and Geissmann, F. (2015). Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in Drosophila fed a lipid-rich diet. *Immunity* **42**, 133-144.

Xu, K., Zheng, X. and Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. *Cell Metab.* **8**, 289-300.

Zettervall, C.-J., Anderl, I., Williams, M. J., Palmer, R., Kurucz, E., Ando, I. and Hultmark, D. (2004). A directed screen for genes involved in *Drosophila* blood cell activation. *Proc. Natl. Acad. Sci. USA* **101**, 14192-14197.

Zhao, X. and Karpac, J. (2017). Muscle directs diurnal energy homeostasis through a myokine-dependent hormone module in Drosophila. *Curr. Biol.* **27**, 1941-1955.e6.