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Review

Discovering signaling mechanisms governing metabolism and metabolic diseases with *Drosophila*

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SUMMARY

There has been rapid growth in the use of *Drosophila* and other invertebrate systems to dissect mechanisms governing metabolism. New assays and approaches to physiology have aligned with superlative genetic tools in fruit flies to provide a powerful platform for posing new questions, or dissecting classical problems in metabolism and disease genetics. In multiple examples, these discoveries exploit experimental advantages as-yet unavailable in mammalian systems. Here, we illustrate how fly studies have addressed long-standing questions in three broad areas—inter-organ signaling through hormonal or neural mechanisms governing metabolism, intestinal interoception and feeding, and the cellular and signaling basis of sexually dimorphic metabolism and physiology—and how these findings relate to human (patho)physiology. The imaginative application of integrative physiology and related approaches in flies to questions in metabolism is expanding, and will be an engine of discovery, revealing paradigmatic features of metabolism underlying human diseases and physiological equipoise in health.

> Progress, far from consisting in change, depends on retentiveness.
>
> [W]hen experience is not retained…infancy is perpetual.
>
> —Santayana

INTRODUCTION

Studies with *Drosophila melanogaster* have been a wellspring of biological discoveries for over a century, including pioneering, widely heralded studies revealing general principles of genetics, development, immunity, circadian physiology, neurobiology, and behavior (Bilder and Irvine, 2017). Foundational studies in fruit flies have repeatedly presaged important findings in humans and other vertebrates, making *Drosophila* an indispensable organism for biology. Common elements of animal metabolism include the primacy of glucose for energy generation, the need for mobilizing energy stores like glycogen and lipids in periods of caloric restriction or reproduction, and the mandate to adapt feeding behavior to match nutrient needs. In both *Drosophila* larvae and adults, a high-sugar diet causes hyperglycemia, hyperinsulinemia, and insulin resistance, with adults also demonstrating obesity (Mattila and Hietakangas, 2017; Morris et al., 2012; Musselman et al., 2011; Pasco and Léopold, 2012; van Dam et al., 2020). Similarly, flies fed a high-fat diet also demonstrate insulin resistance, elevated triglycerides, and cardiac dysfunction (Birse et al., 2010). Akin to their effects in humans, adipogenic diets can also promote tumor formation (Hirabayashi, 2016; Hirabayashi et al., 2013; Newton et al., 2020). Based on these and other unifying features, there is growing evidence that integrative studies in flies can reveal important principles of animal metabolism, including the genetic and signaling mechanisms that maintain health and underlie metabolic diseases like obesity and diabetes mellitus. Here, we illustrate findings from recent research in adult flies to expand awareness of this view. An abbreviated selection of studies of larval metabolism and growth is also included, but influential reports or reviews focused on this distinct developmental stage are found elsewhere (Böhni et al., 1999; Brankatschk et al., 2014; Gillette et al., 2021; Grenier and Leulier, 2020; Pasco and Léopold, 2012; Rajan and Perrimon, 2012). Likewise, prior, more focused reports and reviews provide excellent summaries of research on fly lipid metabolism (Heier and Kühnlein, 2018; Kühnlein, 2011; Musselman and Kühnlein, 2018; Palm et al., 2012; Storelli et al., 2019), Warburg effect and tumor growth (Drummond-Barbosa and Tennessen, 2020; Tennessen and Thummel, 2011), hormone biology (Ahmad et al., 2020), circadian regulation of metabolism (King and Sehgal, 2020; Patke et al., 2020), and diabetes modeling (Alfa and Kim, 2016).

The success of metazoans in navigating responses to physiologic and pathologic challenges to metabolism is determined by a combination of intrinsic cellular responses, and adaptations in multiple tissues coordinated by intercellular signaling. The

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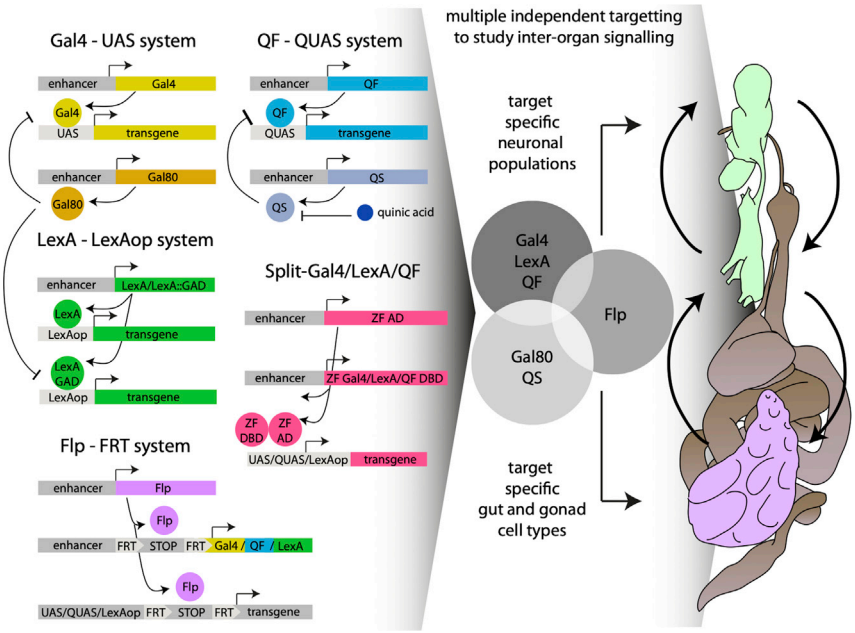

Figure 1. Binary expression systems to study inter-organ signaling in *Drosophila*

The expression of specific genes, reporter genes, guide/siRNAs, or cell activators/silencers can be confined temporally and/or to specific cell types using publicly available binary expression systems. Gal4-driven expression of UAS-fused transgenes (Brand and Perrimon, 1993) can be further refined with a third transgene allowing for tissue-specific and/or temporally controlled expression of a Gal80 protein, which inhibits Gal4 function (McGuire et al., 2003, 2004a). The LexA-LexAop (Lai and Lee, 2006) and QF-QUAS (Potter et al., 2010) systems provide alternative binary systems that, like the Gal4-UAS system, can be further refined with Gal80 or QS/quinic acid, respectively. The cell-type specificity of "driver" (Gal4/LexA/QF) lines can be further increased through intersectional "split" approaches, which use two enhancers with activity in overlapping cell groups to confine Gal4/LexA/QF expression to the intersection of the two groups (Luan et al., 2006; Riabinina et al., 2019; Ting et al., 2011). Finally, the Flp-FRT system (Golic and Lindquist, 1989; Lee and Luo, 2001) can be used to further restrict these binary expression systems to lineage-related subsets of cells, resulting in mosaic expression within a tissue.

experimental toolkit for *Drosophila* is arguably most powerful when applied to the study of inter-organ communication (Droujinine and Perrimon, 2016, 2019). These communication axes are critical for the regulation of hormone or neuropeptide secretion and their signaling. Beyond endocrine signaling of energy status (reviewed below), lipoproteins also communicate information about dietary lipid intake to the brain to regulate insulin secretion (Brankatschk et al., 2014; Palm and Rodenfels, 2020). The transcriptome of each organ is now available, including organ-specific single-cell transcriptomes for the adult brain and intestine (Allen et al., 2020; Croset et al., 2018; Davie et al., 2018; Guo et al., 2019; Hung et al., 2020; Leader et al., 2018; Robinson et al., 2013). Recent descriptive datasets allow identification of candidate systemic signals and their remote targets. Predictions can then be probed functionally thanks to an increasing repertoire of binary systems (Kockel et al., 2019; Lin and Potter, 2016) for gene inhibition, overexpression, and mutation, and an expanding nanobody-based genetic toolkit for protein degradation or re-localization (Aguilar et al., 2019). These tools include the use of the Gal4/UAS system of binary transcriptional activation, and the CRISPR/Cas9 system of genome editing (Bassett and Liu, 2014; Caygill and Brand, 2016; McGuire et al., 2004b; Xu et al., 2019). Binary systems combine the use of tissue-specific promoters to drive the expression of a transcriptional activator (e.g., Gal4), which binds the upstream activating sequence (UAS) to express virtually any DNA sequence of the experimenter's choice. Today, multiple binary systems (e.g., Gal4/UAS, LexA/LexAop, Q systems) can be combined in a single fly to conduct genetic perturbations of multiple tissues simultaneously, and measure the effects of those perturbations (Figure 1). Together, these tools allow exquisite spatial and temporal control of gene expression and protein function, including the ability to genetically target different tissues or organs independently, manipulate the function of a gene in a given tissue or their activity, and determine the interactions among different tissues and organs (Kockel et al., 2019; Lin and Potter, 2016; Wendler et al., 2020) (and references therein). Use of genetic and physiological tools that can unequivocally establish the directionality and significance of signaling across organs is highlighted below. This includes signaling between brain neurons; neuroendocrine and gastrointestinal cells, including enteroendocrine cells; gonads; and the fat body, an organ combining features of mammalian liver and adipose cells (Figures 2A and 2B).

ENDOCRINE REGULATION OF METABOLISM: FOCUS ON *DROSOPHILA* INSULIN, GLUCAGON, AND LEPTINS

To develop, grow, and generate their progeny, all organisms acquire nutrients in order to survive periodic or prolonged nutrient scarcity. This challenge of feeding and fasting embodies one of the *ne plus ultra* selective forces in evolution. The conservation of insulin signaling across metazoa, from insects to mammals (Srivastava et al., 2010), indicates the selective advantage of endocrine systems in the coordination of metabolic responses to feeding and fasting states. Here we review recent progress in understanding the roles and regulation of fly insulin, glucagon, and other hormones in governing metabolism.

Conserved endocrine mechanisms govern metabolism in *Drosophila* and humans

Drosophila research over the past two decades has demonstrated that glucose, amino acid, and lipid metabolism are regulated by fly orthologs of insulin, glucagon, leptin, and other hormones (Box 1). In *Drosophila* and other insects, insulin-producing cells (IPCs) are specialized neurons that synthesize and secrete insulin to maintain glucose and lipid homeostasis (Grönke et al., 2010; Haselton and Fridell, 2010; Ikeya et al., 2002; Rulifson et al., 2002), through activation of insulin receptor (InR) and InR substrates (IRS1/2) in

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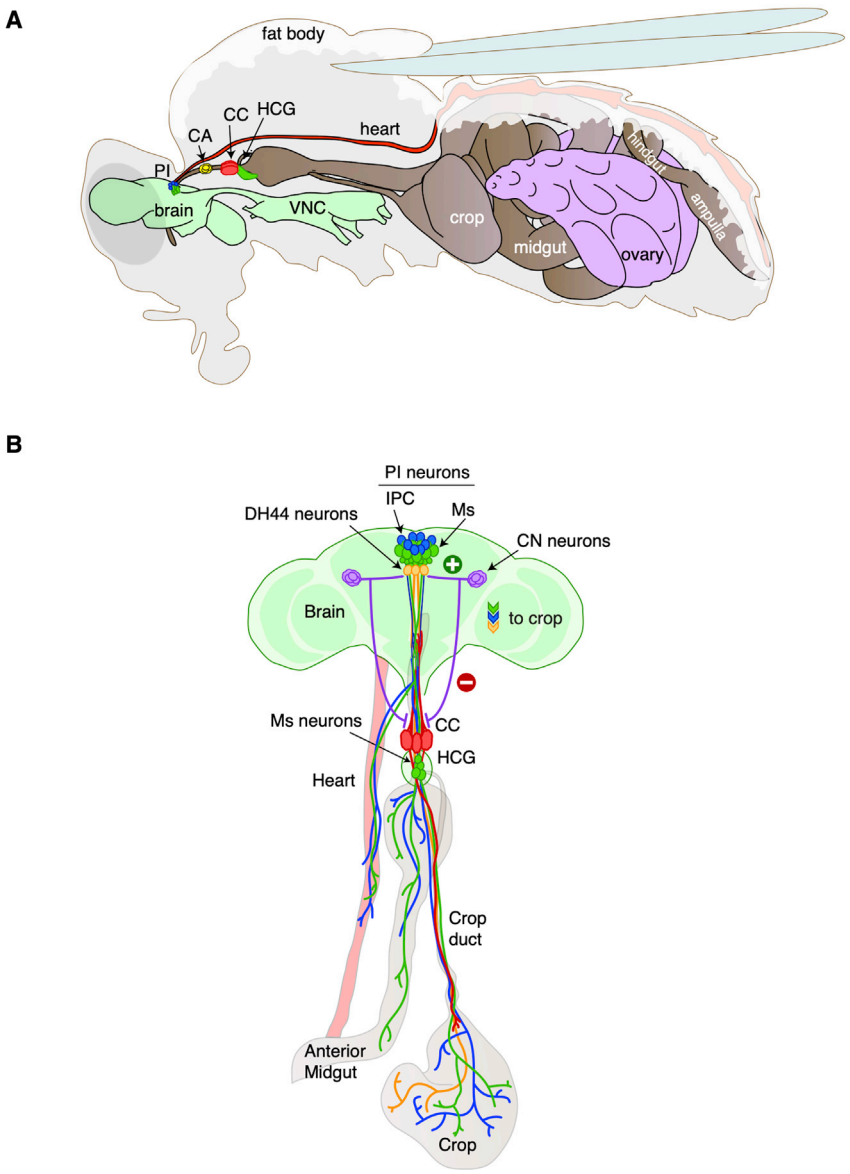

Figure 2. Overview of anatomy and cell interactions in adult flies

(A) Schematic of an adult female *Drosophila* highlighting organs involved in energy homeostasis and metabolism. IPCs and other cells discussed here (not shown) are located in the pars intercerebralis (PI) of the brain, a distinct structure from the abdominal ganglion or ventral nerve cord (VNC). Cells in the corpora cardiaca (CC) secrete AKH, a glucagon-like hormone. Processes emanating from both IPCs and CC cells have direct contact with the heart tube. The corpora allata (CA) produces juvenile hormone to sustain intestinal stem cell proliferation and remodel enterocytes in mated female flies. The fat body, an insulin target tissue analogous to mammalian liver and adipose tissue, lines the body cavity of the abdomen and thorax. Neurons in the PI and hypocerebral ganglion (HCG) innervate the fly crop and gut. Food is stored in the crop where digestion begins, then transits through the midgut, hindgut, and rectal ampulla. The midgut, analogous to the small intestine, is involved in nutrient sensing and interoception. The *Drosophila* ovary is posterior and adjacent to the gastrointestinal tract.

(B) Neurons including IPCs, DH44, Ms, and CN neurons are depicted and discussed in the text. Plus (+) and minus (–) signs indicate activating or inhibitory interactions between CN neurons and IPCs or CC cells, which produce AKH and are adjacent to the HCG. Projections of IPC and Ms neurons to the crop and other GI organs, or the IPCs and CC cells to the heart, are shown.

targets like brain, muscle, and the fat body (Figure 2A). Corpora cardiaca (CC) cells produce and secrete adipokinetic hormone (AKH), the insect ortholog of glucagon (Alfa and Kim, 2016; Isabel et al., 2005; Kim and Rulifson, 2004; Lee and Park, 2004). Elements of IPC and CC developmental genetics and specification resemble those of pancreatic islet β and α cells (Clements et al., 2008; Kim and Rulifson, 2004; Miguel-Aliaga et al., 2008; Park et al., 2011), like transcription factors governing IPC and β cell development and functional maturation (Barry and Thummel, 2016). Moreover, direct contact between fly IPCs and CC cells with the fly heart and other cells described below appears to be homologous to those between β cells, α cells, and vessels in islets (Figure 2B). Fly leptin-like adipokines and other hormones have also been shown to regulate metabolism (Ahmad et al., 2020; Alfa et al., 2015; Beshel et al., 2017; Hentze et al., 2015; Mattila and Hietakangas, 2017; Rajan and Perrimon, 2012). Below, we focus on recent findings involving fly insulin-like peptides; the glucagon ortholog, AKH; and leptins.

Secretion of insulin by pancreatic β cells and glucagon by α cells is governed by nutrient sensing coupled to well-delineated electrophysiological signaling mechanisms (Macdonald, 2016; Rorsman and Braun, 2013). Genetic studies, measures of insulin or AKH secretion, and electrophysiology show that principal features of α cell and β cell "stimulus-secretion coupling" are remarkably conserved in adult fly IPCs and CC cells. For example, AKH secretion by CC cells appears to be suppressed by feeding or glucose, reminiscent of glucagon regulation in islet α cells (Alfa et al., 2015; Kim and Rulifson, 2004; Oh et al., 2019). In IPCs, secretion of insulin-like peptides is regulated by glucose and lipids (Ahmad et al., 2020; Alfa and Kim, 2016). Also like in islet β cells, IPC secretion is governed by mitochondrial metabolism (Barry and Thummel, 2016; Fridell et al., 2009; Storelli et al., 2019); inward rectifying potassium channel-dependent mechanisms and depolarization, resulting in calcium transients (Kréneisz et al., 2010); and release of a minor fraction of pre-synthesized, processed, and stored insulin (Park et al., 2014). After fasting and re-feeding, the degree and tempo of insulin-like peptide 2 (Ilp2) increase and clearance in adult fly hemolymph (Figure 3A) are strikingly similar to serum insulin excursions observed in mice or humans after glucose challenge (Figure 3B).

The multiple homologies of IPCs and islet β cells, coupled with the ability to measure total and circulating insulins (like Ilp2) using

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Box 1. Advantages of *Drosophila* for studies of metabolism

Conserved physiology
- Systemic insulin from insulin-producing cells (IPCs) regulates metabolism
- Systemic glucagon-like hormone (AKH from CC cells) regulates metabolism
- Functional orthologues of leptin (Upd1, Upd2) identified in *Drosophila*
- Insulin, AKH, and other hormone secretion are responsive to fasting and re-feeding
- Fly IPCs and human β cells have similar transcriptomes and stimulus-secretion coupling and nutrient regulation
- In multiple cases (14/14), loss of gene function in IPCs and islet β cells had a similar effect on insulin output: insulin, InR, IRS1/2, AKT1, GLUT1, GLIS3, ZNT8, ABCC8, DGKB, SUR1, ADRA2, BCL11A, SIX2, and PRC1
- Fly AKH-secreting CC cells and human α cells have similar stimulus-secretion coupling and nutrient regulation

Conserved pathophysiology
- Can challenge flies by fasting, re-feeding, diet, and other metabolic stress
- Insulin insufficiency leads to hyperglycemia
- Insulin excess leads to excess adiposity, growth, and hypoglycemia
- Glucagon-like hormone (AKH) deficiency leads to hypoglycemia
- Insulin resistance develops from dietary challenge or mutation, and stimulates adaptive hyperinsulinemia
- Striking concordance of insulin output phenotypes after loss- or gain-of-function studies in IPCs and pancreatic islet β cells

Experimental advantages
- Unbiased screens to identify novel mechanisms governing metabolism
- Genetic toolkit permits targeted loss- or gain-of-function studies in specific cells, including simultaneous targeting in two or more distinct cell types
- Temporal control of gene expression permitting uncoupling of developmental from homeostatic/adult effects
- Quantitative assays to measure glucose, adiposity, weight, insulin, and AKH levels

ELISA assays, have motivated genetic studies to identify intrinsic regulators of insulin production and secretion (Barry and Thummel, 2016; Park et al., 2014; Peiris et al., 2018). For example, using RNAi-based suppression of genes encoding orthologs known to regulate islet β cell insulin production or secretion, it was shown—in 14/14 cases—that targeted loss-of-function studies in fly IPCs led to changes in insulin output similar to those observed after homologous loss-of-function studies in pancreatic islets. This included genes encoding Ilp2, and orthologs of insulin receptor, insulin receptor substrates 1/2 (IRS1/2), GLUT1, GLIS3, ZNT8, ABCC8, DGKB, and ADRA2. Changes of insulin production and output were distinct or not detected after shRNA-mediated gene suppression in the fly fat body, demonstrating specific requirements for these factors in fly IPCs (Park et al., 2014; Peiris et al., 2018)

Discovering regulators of pancreatic islet function with flies

Genetic and physiological homologies between fly IPCs and islet β cells predicted that discovery of IPC regulators could unveil conserved mechanisms governing insulin secretion. Multiple recent studies have supported this heuristic (Bevacqua et al., 2021; Peiris et al., 2018). Peiris et al. (2018) investigated the *in vivo* function of fly genes orthologous to imputed human diabetes risk genes without known roles in β cells (Mahajan et al., 2018). Measures of insulin output after RNAi targeting in IPCs led to identification of three novel IPC regulators, *CG9650*, *fascetto*, and *optix*, the respective orthologs of mammalian genes *BCL11A*, *PRC1*, and *SIX2*. In fly IPCs, RNAi-mediated suppression of *CG9650* (*BCL11A*) or *fascetto* (*PRC1*) led to increased circulating levels of insulin; remarkably, loss of *BCL11A* in primary human β cells or mouse β cells also led to increased insulin output (Park et al., 2014; Peiris et al., 2018), while induction of *CG9650* in IPCs or *BCL11A* in β cells led to reduced insulin output. Targeted suppression of *optix* in IPCs led to reduced insulin secretion, and recent studies show that *SIX2* loss in human β cells also leads to reduced glucose-dependent insulin output (Bevacqua et al., 2021). In these examples, fly studies correctly predicted the *direction* of islet β cell phenotypes arising from genetic loss of function. By contrast, knockdown of *CG9650*, *fascetto*, or *optix* in the adult fat body did not detectably alter circulating Ilp2 levels (Peiris et al., 2018). Thus, integrated genetic, molecular, and physiological approaches using fruit flies, mice, and human tissues provide a powerful new strategy for discovering tissue-specific functions of imputed diabetes risk regulators (Figure 3C; Box 1).

Discovering systemic regulators of insulin and AKH output

Communication between different tissues and cells reflecting fluctuating nutrient availability and energy status enables whole-organism metabolic homeostasis. For example, human β cells are regulated by circulating adipokines and hepatokines (Cantley, 2014; Wente et al., 2006), as well as by intra-islet paracrine signals like somatostatin, glucagon, and the incretin glucagon-like peptide 1 (GLP-1). Fly IPCs and CC cells also receive and integrate long- and short-range signals to regulate insulin or AKH expression and secretion (Ahmad et al., 2020), and exciting work has unveiled new fat body- and brain-based mechanisms for controlling the output of these hormones in adult flies.

Recent studies have identified *Drosophila* neurons that coordinate CC cell and IPC output (Oh et al., 2019). Genetic screens in the brain identified a pair of glucose-sensing neurons, termed *CN*, that project bifurcated axons—one toward CC cells and the other toward IPCs (Figure 2B). *CN* neuronal excitation by feeding and systemic glucose flux led to simultaneous inhibition

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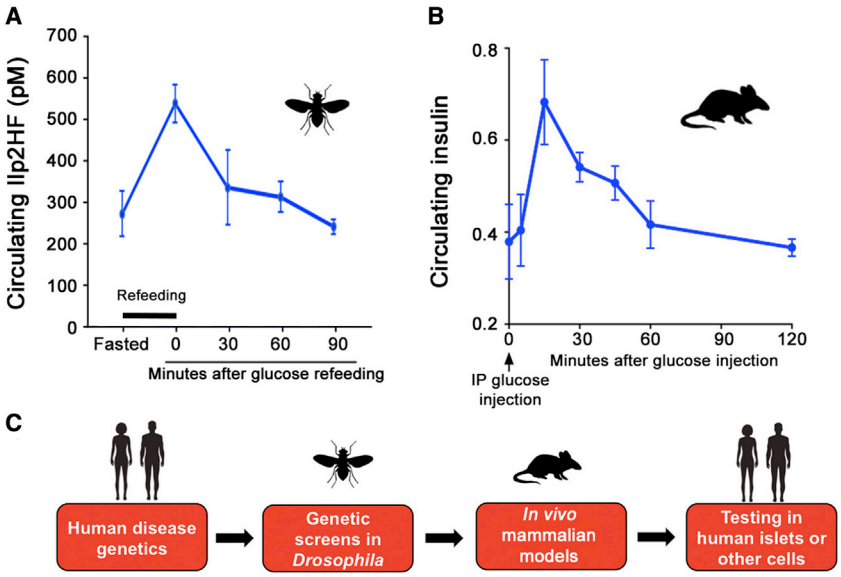

Figure 3. Using *Drosophila* to discover novel regulators of human islet function

(A and B) Glucose-stimulated insulin secretion and clearance in *Drosophila* (A) and mouse (B), measured by insulin ELISA. Data in (A) adapted from Park et al. (2014).

(C) Experimental strategy connecting human studies like genome-wide association studies (GWAS) of disease risk to *in vivo* testing in *Drosophila*, thereby prioritizing secondary and tertiary studies in mammalian systems (mice shown here) and human cells or tissues.

of CC cells and AKH secretion, and stimulation of IPCs and insulin secretion. Fasted flies had a reduction in *CN* neuronal activity, accompanied by a reduction in insulin secretion and an increase in AKH secretion. Electrical silencing of *CN* neurons resulted in elevated glucose levels in circulating hemolymph (analogous to hyperglycemia). Thus, in addition to their intrinsic mechanisms of glucose sensing (Kim and Rulifson, 2004; Park et al., 2014), these studies reveal that IPC and CC cell activity are coordinated by glucose-sensing neurons, whose functions are required to maintain systemic glucose homeostasis.

The fat body also releases factors that regulate insulin-like peptide expression, secretion, and signaling (Colombani et al., 2005; Géminard et al., 2009; Ghosh and O'Connor, 2014; Sousa-Nunes et al., 2011; Koyama and Mirth, 2016; Sano et al., 2015). These include Imp-L2 and dALS, which bind to and inhibit Ilp2 signaling (Arquier et al., 2008; Honegger et al., 2008), and Stunted, which stimulates insulin-like peptide secretion following amino acid ingestion in larvae (Delanoue et al., 2016). Imp-L2 has also been shown to interrupt insulin signaling and mediate cachexia-like wasting in adult flies transplanted with malignant tumors (Figueroa-Clarevega and Bilder, 2015; Kwon et al., 2015). In addition to these secreted factors, the *Drosophila* leptin homolog Unpaired-2 (Upd2) is secreted from fat body following prolonged high-sugar or high-fat feeding. Upd2 elaborates fat body signals that remotely relieve central GABAergic neuronal inhibition of IPCs, leading to increased insulin output (Rajan and Perrimon, 2012). Subsequent work has also revealed how glucagon-leptin-insulin axes are regulated by adipose tissue, demonstrating that AKH signaling in the fat body reduces Upd2 secretion, thereby inhibiting insulin release (Rajan et al., 2017). Like in mammals, recent work demonstrates the essential role of leptin-like molecules called Unpaired-1 (Upd1) for regulating obesity-related traits in adult flies. Disrupting brain-derived Upd1 production leads to phenotypes observed in mammalian obesity, including increased attraction to food cues, hyperphagia, increased weight, and disruption in insulin secretion (Beshel et al., 2017). Thus, Upd1 and Upd2 may regulate distinct central neural circuits governing growth and weight regulation in adult flies.

In adult flies with chronic nutrient excess, Brent and Rajan (2020) recently reported that Upd2-regulated fat body signaling led to synapse reorganization in central GABAergic inhibitory neurons, reducing bouton number and promoting insulin release (Brent and Rajan, 2020). They also found that insulin feeds back on GABAergic neurons to increase their bouton number and re-enforce a negative neural tone for insulin release. Thus, two nutrient surplus-sensing hormonal systems, Upd2 and insulin, signal through a structurally dynamic cellular circuit to regulate insulin output. Intriguingly, pancreatic islet δ cells—which secrete somatostatin to inhibit β cell insulin secretion—were recently demonstrated to have dynamic contacts that regulate β cell secretion, and are regulated by hyperglycemia (Arrojo E Drigo et al., 2019). Moreover, prior studies have shown that mammalian neuronal somatostatin secretion may be inhibited by leptin (Quintela et al., 1997). Further studies are needed to test the possibility that adipokines like leptin could remodel δ cell contacts within pancreatic islets to regulate insulin (or glucagon) secretion.

Investigating the polygenic and multi-organ basis of diabetes in *Drosophila*

Diabetes mellitus is the most common metabolic disease worldwide, and the preponderance of evidence shows that there are both acquired (environmental) and intrinsic (genetic) risks in diabetes, whose *sine qua non* is hyperglycemia. Insulin resistance in specific tissues like fat, liver, and muscle is thought to "drive" the pathogenesis of type 2 diabetes, the most common form in humans (Brown and Goldstein, 2008; Unger and Orci, 2010). *Drosophila* research has made important contributions to elucidating molecular and genetic regulation of insulin signaling in larval and adult organs (Alfa and Kim, 2016), but progress in this area has also been hampered by an overreliance on semiquantitative assays (Britton and Edgar, 1998; Kockel et al., 2010; Puig et al., 2003) to assess insulin signaling. Future advances should be accelerated by the adoption of tissue-specific assays to quantify readouts of insulin receptor activation (like Akt/PKB phosphorylation; S. Park and S.K.K., unpublished data). Likewise, investigations of glucagon resistance, another pathophysiological driver of human diabetes (Unger and Cherrington, 2012), should be advanced by

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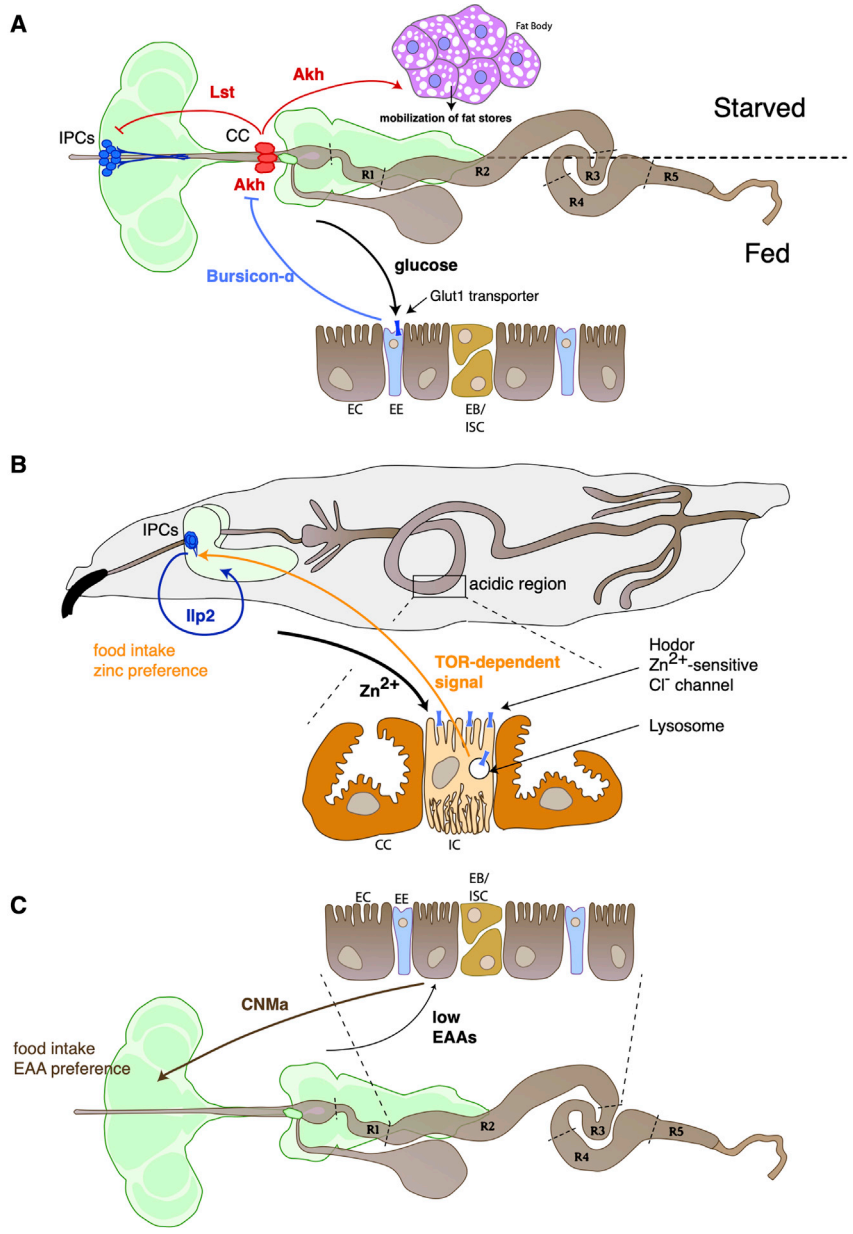

Figure 4. Signaling and cell interactions coordinating fly metabolism

(A) Signaling interactions regulating AKH output by CC cells by enteroendocrine cell-derived Bursicon α signaling. During starvation (top half), glucose entry into enteroendocrine (EE) cells is diminished, Bursicon α is retained in EE cells, and AKH is secreted from CC cells, leading to subsequent catabolism of peripheral fat stores. CC cells also secrete *Limostatin* (Lst), a decretin-like hormone that suppresses insulin-producing cell function. In the fed state (bottom half), Bursicon α is secreted from enteroendocrine cells and suppresses Akh release from the CC cells. The magnified insert depicts enterocytes (EC, brown), enteroendocrine cells (EE, blue), and intestinal progenitors (enteroblasts [EB] or intestinal stem cells [ISC], yellow).

(B) Gut responses to micronutrient consumption. The midgut has a central region with high luminal acidity harboring two types of specialized enterocytes: acid-producing copper cells (CC, dark orange) interspersed between interstitial cells (IC, light orange). In response to zinc ingestion by larval flies, Hodor in the interstitial cells sustains lysosomal acidification and activation of Tor signaling. This signaling increased food intake, dietary zinc preference, and Ilp release from IPCs (dark blue).

(C) Gut responses to amino acid deprivation. In response to essential amino acid (EAA) deprivation, gut enterocytes secrete the neuropeptide CNMamide (CNMa), which may mediate the EAA feeding preference in flies deprived of dietary protein. Importantly, these enterocytes do not secrete CNMa in response to non-essential amino acid (NEAA) deprivation.

quantitative assays of AKH output (Oh et al., 2019) and AKH resistance.

Like in mammals, deficiency for insulin in adult flies elicits an elevation of circulating glucose (Park et al., 2014) as well as impaired regulation of trehalose, a glucose dimer (Broughton et al., 2008; Grönke et al., 2010). While loss of insulin signaling orthologs of IRS1/2, AKT, and insulin receptor affects fertility, size, and lifespan, deficiency of these factors does not reliably produce adult hyperglycemia (Böhni et al., 1999; Park et al., 2014; Ugrankar et al., 2015). Likewise, loss of Upd2 does not cause hyperglycemia in adult flies (Rajan and Perrimon, 2012). This likely reflects compensatory increases of insulin output from IPCs (Park et al., 2014), analogous to human responses to insulin resistance (Hollenbeck and Reaven, 1987). Thus, like multi-organ pathogenesis of diabetes in mammals, hyperglycemia in flies may only manifest with peripheral insulin resistance combined with insulin secretion defects in the IPCs. Reconstituting polygenic and multi-organ mechanisms thought to underlie hyperglycemia in human diabetes is an important unmet goal, but well matched to the experimental paradigms available in flies.

A GUT FEELING: INTESTINAL INTEROCEPTION

The gastrointestinal (GI) tract is recognized as a central regulator of food intake and energy balance (Clemmensen et al., 2017; Soty et al., 2017). It is also a remarkably flexible organ system that can undergo marked adaptations in response to diet and internal state (Beumer and Clevers, 2021). Like its mammalian counterpart, the digestive tract of *Drosophila* is functionally regionalized (Figures 2A and 4A) (Buchon et al., 2013; Marianes and Spradling, 2013; Miguel-Aliaga et al., 2018; O'Brien et al., 2011). It harbors a resident microbiota and consists of different cell types similar to those found in the human GI tract, including digestive/absorptive enterocytes, hormone-secreting enteroendocrine cells, and intestinal stem cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Over the past decade, *Drosophila* has been leveraged to identify molecular and cellular mediators of intestinal plasticity, revealing a central role for adult intestinal stem cells; these have been recently reviewed elsewhere

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(Funk et al., 2020; Miguel-Aliaga et al., 2018). Here we review recent studies revealing other modes of intestinal sensing and adaptation.

Interoception coordinates switching to catabolism

Drosophila is beginning to reveal both conserved and novel mechanisms of intestinal interoception that modulate food intake and choice. For example, reminiscent of the roles of the glucose transporter Glut2 in regulating the post-prandial secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) from enteroendocrine cells (Cani et al., 2007), a *Drosophila* Glut1 homolog gates the release of enteroendocrine hormone Bursα (Scopelliti et al., 2019). Circulating Bursα normally signifies the ''fed'' state, preventing secretion of AKH from CC cells, which are adjacent to the GI tract (Figure 4A). During starvation (or following *Glut1* downregulation), however, Bursα is retained in enteroendocrine (EE) cells, leading to AKH release and the consequent mobilization of peripheral fat stores (Scopelliti et al., 2019). In parallel, starvation also triggers the release of a decretin-like hormone (Limostatin, Lst; Alfa et al., 2015; Figure 4A). Lst suppresses insulin output through its action on a G protein-coupled receptor (LstR) expressed in IPCs (Alfa et al., 2015). Further studies are needed, however, to identify the mammalian islet orthologs of fly IPC Lst/LstR signaling (Alfa et al., 2015; Kuhre et al., 2019). In sum, studies of Bursα and Lst have revealed intestine-associated mechanisms that regulate AKH secretion, mediating the switch to a catabolic state that allows flies to withstand starvation.

Discovery of mechanisms regulating nutrient uptake and food preferences

Sufficient dietary amino acids (AAs), and micronutrients like the trace metals, can profoundly impact health and disease in humans. *Drosophila* studies have revealed unexpected mechanisms of intestinal micronutrient and AA sensing. The novel roles of an intestinal metal sensor in food intake regulation and growth control by enterocytes are a case in point. Redhai et al. identified a novel population of zinc-sensing enterocytes that sustain the voracious feeding of *Drosophila* larvae (Redhai et al., 2020). Within these enterocytes (known as ''interstitial cells''), a zinc-gated chloride channel (Hodor) responds to zinc ingestion by sustaining lysosomal acidification and activating Tor signaling (Figure 4B). Hodor-mediated Tor signaling activity within these enterocytes leads to increased food intake and insulin-like peptide release via an as-yet unidentified systemic signal. Intestinal Hodor also mediates a larval preference for dietary zinc (Redhai et al., 2020). Similar regulation of insulin secretion in response to micronutrient availability is conserved in mammals. In mice, for example, oral zinc administration enhances insulin secretion, likely through gastric inhibitory peptide (GIP) secretion triggered by the zinc-sensing GPR39 receptor on L- and K-cells in the gut (Moran et al., 2019). While Hodor signaling appears specific to insects, an orthologous system or a zinc-gated channel may exist in mammals with roles that might extend beyond regulation of food intake (Fernández-Gallego et al., 2021).

When deprived of dietary protein, *Drosophila* and other animals select a food source that contains a greater amount of dietary protein or essential AAs (EAAs) (Raubenheimer and Jones, 2006; Theall et al., 1984). This suggests that food selection is geared toward acquiring specific macronutrient targets. How this choice is driven has remained a mystery, since known sensors of AAs including taste receptors like T1R1-T1R3 and intracellular factors like GCN2 and TOR do not discriminate between EAAs and non-essential AAs (NEAAs) (Efeyan et al., 2015; Nelson et al., 2002). Recent work shows that protein or EAA deprivation (but not NEAA deprivation) in flies induces production of the neuropeptide CNMamide (CNMa) in a specific population of enterocytes in the gut (Figure 4C). Genetic silencing of the CNMa-CNMa receptor axis blocked the EAA preference in these flies (Kim et al., 2021). This mechanism is reminiscent of how peripheral tissue induction of FGF21 in protein-deprived mammals can signal the brain to regulate feeding behavior (Hill et al., 2017, 2019; Solon-Biet et al., 2016). Moreover, gnotobiotic flies bearing an EAA-producing symbiotic microbiome exhibited reduced compensatory appetite for EAAs. By contrast, gnotobiotic flies carrying a mutant microbiome that failed to produce leucine or other EAAs displayed higher *CNMa* expression and greater compensatory EAA appetite (Kim et al., 2021). These findings reveal that different types of cells in the gut including enterocytes act as a frontline sensor to detect and respond to micro- and macronutrients. It also raises the possibility that these nutrient-sensing cells work together with the gut microbiome to establish nutrient homeostasis.

Finally, beyond canonical signals like peptide hormones, systemic metabolites may also play important roles in the modulation of food intake and choice. Two salient examples are the role of gut-derived citrate in promoting food intake in males, described below (Hudry et al., 2019), and the finding that pentose phosphate pathway activity in the female germline increased an appetite for sugar (Carvalho-Santos et al., 2020). Modulation in sugar appetite by the germline is achieved by regulating the expression of the fat-body-secreted satiety factor, Fit, a sexually dimorphic protein previously shown to suppress protein appetite and promote insulin-like peptide release (Sun et al., 2017). Investigating regulatory roles of metabolites on behavior in the context of inter-organ communication should emerge as an exciting and fruitful area of future research.

Mechanosensory mechanisms of feeding regulation

While a central role for intestinal nutrient sensing is also emerging from studies in mice (Clemmensen et al., 2017; de Araujo et al., 2020; Gribble and Reimann, 2019), two recent *Drosophila* studies remind us that other sensory modalities like mechanosensation may control acute feeding (Clemmensen et al., 2017; de Araujo et al., 2020; Gribble and Reimann, 2019; Min et al., 2021; Wang et al., 2020). The fly homolog of Piezo, a mechanotransduction channel, restrains feeding from at least two independent crop-innervating neuronal populations (IPCs in the brain and a rare population of enteric neurons); silencing or stimulating of either neuronal population results in an increase or a decrease of food consumption, respectively. Other work illustrates that Piezo also inhibits sugar intake through another layer of regulation. Six DH44-expressing neuronal cells, located adjacent to IPCs (Figure 2B), detect the nutritional value of sugar and consumption of sugar macronutrient specifically during food deprivation (Figure 2B) (Dus et al., 2011, 2015). When animals are satiated in the fed state, Piezo suppresses the function of DH44 neurons, thereby suppressing sugar intake (Oh et al., 2021). In

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future studies, it will be interesting to explore whether Piezo expression and/or activity are regulated by the postmating signals recently reported to increase maternal food intake through modulation of Ms neurons, a third population of crop-innervating neurons (Hadjieconomou et al., 2020). The recent finding of vagal mechanosensory neurons with a role in feeding regulation in mice (Bai et al., 2019; Kim et al., 2020) also raises the possibility that Piezo may play similar roles in mammals.

SEXUALLY DIMORPHIC CONTROL OF GUT FUNCTION, METABOLISM, AND REPRODUCTION

There is a growing realization that many aspects of (patho)physiology differ between the sexes (Mauvais-Jarvis et al., 2017; Ober et al., 2008; Tannenbaum et al., 2019; Tramunt et al., 2020). *Drosophila* is no exception: important studies have uncovered sex differences in how IPCs and the fat body communicate to control larval growth (Millington et al., 2021; Rideout et al., 2015; Sawala and Gould, 2017). Unexpectedly, recent studies have revealed the importance of sex differences in intestinal cells and uncovered novel gut-gonad axes in both males and females.

Sex matters: Identifying new signaling axes between the gut and gonads

Most, if not all, organs of the adult fly display sex differences in gene expression (Leader et al., 2018) that impact features of adult physiology such as lipid metabolism (Sieber and Spradling, 2015; Wat et al., 2020). Recent studies have leveraged integrated genetic and physiological approaches to reveal the importance of sex differences in intestinal cells and uncover novel gut-gonad axes in both males and females. An earlier study had indicated that the feces of adult *Drosophila* is unexpectedly predictive of both sex and reproductive status (Cognigni et al., 2011). The subsequent finding that, in the adult midgut (analogous to the mammalian small intestine), approximately 10% of genes are expressed and/or alternatively spliced in a sexually dimorphic manner (Hudry et al., 2016) further suggested sex-specific intestinal physiology. Since then, several studies have illuminated the nature and significance of these sex differences.

One prominent difference lies in the midgut: the adult intestinal stem cells (ISCs) that normally replenish the epithelia divide more often in females than males (Ahmed et al., 2020; Hudry et al., 2016). Increased ISC proliferation maintains the larger size of the midgut in virgin females compared to males, makes females more resistant to acute intestinal challenges such as infection, and allows increases of intestinal size during reproduction (Ahmed et al., 2020; Hudry et al., 2016; Regan et al., 2016; Reiff et al., 2015). This can be good for fly mothers—genetically preventing reproductive intestinal remodeling compromises their fecundity (Ahmed et al., 2020; Reiff et al., 2015). But this advantage comes at a significant cost: increased ISC proliferation renders female flies more susceptible to age-related dysplasia and tumorigenic insults (Ahmed et al., 2020; Hudry et al., 2016; Regan et al., 2016). Several mechanisms account for the sex differences in ISC proliferation. One is the intrinsic sexual identity of ISCs, which explains the basal higher proliferation rate of virgin female versus virgin male guts (Hudry et al., 2016). Second, after mating, a rise in circulating levels of juvenile hormone (JH) and ecdysone (an ovarian steroid hormone) further increases ISC proliferation in females, amplifying this sexual dimorphism (Ahmed et al., 2020; Reiff et al., 2015; Zipper et al., 2020).

A gut-gonad axis that sustains fertility and food intake

ISCs are not the only cells in the intestine that respond to—and subserve—reproduction (Figure 5). In female flies, a postmating rise in JH increases stem cell proliferation to yield a larger organ, but additionally remodels intestinal enterocytes to sustain fecundity (Reiff et al., 2015). Acting through intestinal bHLH-PAS domain proteins methoprene-tolerant (Met) and germ cell-expressed (Gce), JH signals directly to enterocytes to adjust their lipid metabolism by activating sterol regulatory element-binding protein (SREBP) and upregulating expression of genes involved in fatty acid synthesis and activation (Figure 5). Genetically preventing the reproductive, JH-driven metabolic remodeling of enterocytes reduces reproductive output. Mating also leads to an increased number of enteroendocrine cells and increased production of at least two of their peptide hormones. Neuropeptide F (the *Drosophila* homolog of neuropeptide Y) signals back to the ovary to promote germline stem cell proliferation (Ameku et al., 2018). Bursicon α (Bursα, an insect-specific enteroendocrine hormone) signals, together with ecdysone, to a subset of enteric neurons that, through their release of Myosuppressin (Ms) peptide, control the expandability of the crop: a stomach-like organ (Hadjieconomou et al., 2020) (Figures 2B and 5). The post-mating "awakening" of these enteric neurons is significant because, through their effects on the crop, they are responsible for the increased food intake apparent in female flies—like in many mammals—during reproduction. Indeed, preventing the reproductive remodeling of these enteric neurons reduces both reproductive hyperphagia and reproductive fitness (Hadjieconomou et al., 2020).

It could be argued that the reproductive plasticity of all these different intestinal cell types is an insect peculiarity, arguably less relevant to humans and other mammals. By some measures, the nutritional demands of mammalian reproduction are less extreme (flies lay several times their weight in eggs every day), and mammalian adaptations like the placenta or post-partum nursing might have subsumed at least some of these nurturing roles. While descriptive, there are rather extensive data that argue otherwise: increased cell size and proliferation of intestinal epithelial cells have been reported in several mammals including mice and rats during pregnancy and/or lactation (Hammond, 1997; Nilaweera and Speakman, 2018). Similarly, while the reproductive plasticity of GI innervation remains to be investigated, mammalian enteric neurons express sex- and reproductive-hormone receptors (Ameku et al., 2020), and enteroendocrine hormone levels change during reproduction (Johnson et al., 2019). These features suggest it will be productive to explore whether the human digestive system might be similarly modulated by reproductive cues to affect food intake.

Vive la différence

Like in females, singularities of the male gut sustain gametogenesis and fertility (Hudry et al., 2019). However, the mechanisms involved differ from those of females. For example, the male gonad "masculinizes" the enterocytes of a specific region of the intestine by upregulating Jak-Stat signaling, leading to rewiring of enterocyte carbohydrate metabolism and, ultimately, their

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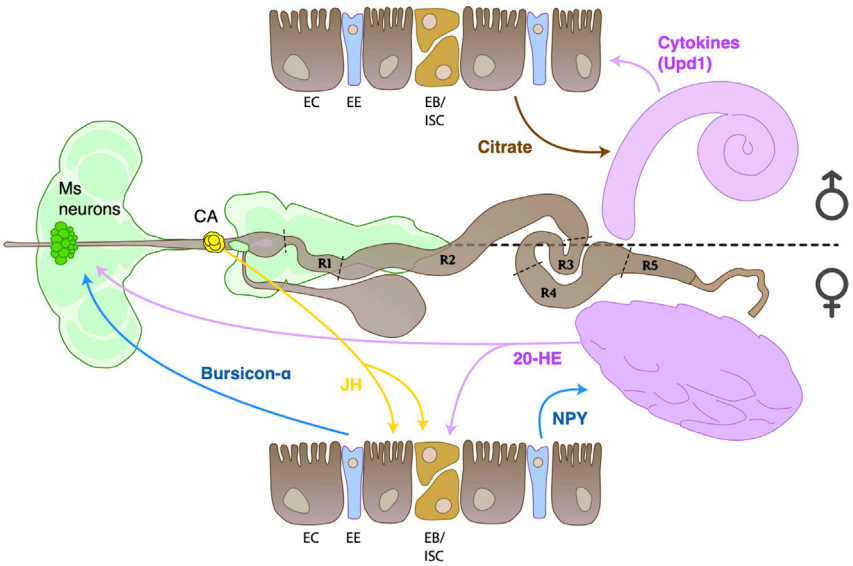

Figure 5. Sexually dimorphic control of gut function, metabolism, and reproduction

In males (upper half), testis-derived cytokines, including Upd1, upregulate Jak-Stat signaling within intestinal enterocytes (brown). This results in enhanced citrate secretion from the enterocyte, which in turn sustains spermatogenesis in the testis and promotes food intake through the action of an unknown neuron. In female flies (lower half), a postmating rise in juvenile hormone (JH) secreted from the corpora allata (CA) and 20-hydroxy ecdysone (20-HE) secreted from the ovary sustains increased intestinal stem cell (ISC, yellow) proliferation, which maintains fecundity. JH also acts directly on enterocytes to adjust their lipid metabolism. After mating, enteroendocrine cells (blue) also increase their production of neuropeptide Y, which promotes germline stem cell proliferation in the ovary. The enteroendocrine cells and the ovary also secrete Bursicon α and 20-HE, respectively, to activate Ms neurons in the pars intercerebralis of the brain. The Ms neurons then increase the expandability of the crop, mediating the postmating rise in food intake seen in female flies.

secretion of citrate (Figure 5). Citrate derived from male enterocytes is then absorbed by the male gonad and used to sustain spermatogenesis. Gut-derived citrate also acts on (as-yet unidentified) neurons to promote food intake in males (Hudry et al., 2019). In mammals including humans, citrate is one of the highest circulating TCA cycle intermediates (Costello and Franklin, 2016; Hui et al., 2017; Mycielska et al., 2009), and recent work in pigs has revealed citrate fluxes across specific tissues (Jang et al., 2019).

Further studies exploring possibly conserved roles of citrate in sex-biased physiology seem warranted. The identification of a fly gonad-to-gut signaling axis also highlights that male fly gonads are adjacent to the gut region they communicate with, indicating previously unappreciated spatial stereotypy in the arrangement of internal organs (Hudry et al., 2019). This organ geometry could facilitate or restrict inter-organ communication, suggesting under-explored dimensions to the study of metabolic disorders and interventions such as obesity and/or gastric bypass.

Mechanistically, these studies have uncovered gut-gonad axes that are sex-specific and govern aspects of whole-body physiology including (but not confined to) reproductive output, and so may have broader implications for human health and diseases. They demonstrate that non-gonadal organs such as the intestine have a sexual identity that is (patho)physiologically significant. Different cell types within an organ acquire their sexual fate through different mechanisms—hormonal and cell-intrinsic. The mechanisms that specify sexual fate are actively maintained in the adult and are therefore genetically reversible. This raises the possibility that they may be plastic in the context of (patho)physiology, motivating and warranting studies to identify internal or environmental cues that modulate the sexual fate of specific intestinal cells.

CONCLUSIONS AND PROSPECTS

Recent findings highlighted here illustrate the formidable experimental advantages of *Drosophila* for investigations of metabolism and inter-organ communication that exploits circulating hormones, short-acting neuropeptides, and neural signaling. These advantages include (1) the ability of investigators to perform high-throughput *in vivo* screens or assess cell interactions in ways less feasible or affordable in mammalian systems; (2) the use of powerful *in vivo* assays to quantify fly hormones, neuropeptides, and metabolites often in a single fly; (3) the availability of behavioral or other physiological assays to discern and measure functional and signaling links between organs like the brain, endocrine cells, intestines, gonads, and fat body; (4) the flexibility of performing complementary gain- or loss-of-function genetics targeted to specific tissues and cell types; (5) the amenability of several binary systems that can be used to manipulate the activities of multiple tissues simultaneously; and (6) the outpouring of new fly strains that enable superlative control of gene and cell function in the GI tract, endocrine cells, and other organ systems (Ariyapala et al., 2020; Kockel et al., 2019; Lim et al., 2021). These findings support the view that *Drosophila* studies will continue to unveil general principles about metabolism and metabolic diseases, serving at the vanguard of modern discoveries in these fields.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Aguilar, G., Matsuda, S., Vigano, M.A., and Affolter, M. (2019). Using nanobodies to study protein function in developing organisms. Antibodies (Basel) *8*, 16.

Ahmad, M., He, L., and Perrimon, N. (2020). Regulation of insulin and adipokinetic hormone/glucagon production in flies. Wiley Interdiscip. Rev. Dev. Biol. *9*, e360.

Ahmed, S.M.H., Maldera, J.A., Krunic, D., Paiva-Silva, G.O., Pénalva, C., Teleman, A.A., and Edgar, B.A. (2020). Fitness trade-offs incurred by ovary-to-gut steroid signalling in Drosophila. Nature *584*, 415–419.

Alfa, R.W., and Kim, S.K. (2016). Using Drosophila to discover mechanisms underlying type 2 diabetes. Dis. Model. Mech. *9*, 365–376.

Alfa, R.W., Park, S., Skelly, K.R., Poffenberger, G., Jain, N., Gu, X., Kockel, L., Wang, J., Liu, Y., Powers, A.C., and Kim, S.K. (2015). Suppression of insulin production and secretion by a decretin hormone. Cell Metab. *21*, 323–334.

Allen, A.M., Neville, M.C., Birtles, S., Croset, V., Treiber, C.D., Waddell, S., and Goodwin, S.F. (2020). A single-cell transcriptomic atlas of the adult *Drosophila* ventral nerve cord. eLife *9*, e54074.

Ameku, T., Yoshinari, Y., Texada, M.J., Kondo, S., Amezawa, K., Yoshizaki, G., Shimada-Niwa, Y., and Niwa, R. (2018). Midgut-derived neuropeptide F controls germline stem cell proliferation in a mating-dependent manner. PLoS Biol. *16*, e2005004.

Ameku, T., Beckwith, H., Blackie, L., and Miguel-Aliaga, I. (2020). Food, microbes, sex and old age: on the plasticity of gastrointestinal innervation. Curr. Opin. Neurobiol. *62*, 83–91.

Ariyapala, I.S., Holsopple, J.M., Popodi, E.M., Hartwick, D.G., Kahsai, L., Cook, K.R., and Sokol, N.S. (2020). Identification of split-GAL4 drivers and enhancers that allow regional cell type manipulations of the *Drosophila melanogaster* intestine. Genetics *216*, 891–903.

Arquier, N., Géminard, C., Bourouis, M., Jarretou, G., Honegger, B., Paix, A., and Léopold, P. (2008). Drosophila ALS regulates growth and metabolism through functional interaction with insulin-like peptides. Cell Metab. *7*, 333–338.

Arrojo E Drigo, R., Jacob, S., García-Prieto, C.F., Zheng, X., Fukuda, M., Nhu, H.T.T., Stelmashenko, O., Peçanha, F.L.M., Rodriguez-Diaz, R., Bushong, E., et al. (2019). Structural basis for delta cell paracrine regulation in pancreatic islets. Nat. Commun. *10*, 3700.

Bai, L., Mesgarzadeh, S., Ramesh, K.S., Huey, E.L., Liu, Y., Gray, L.A., Aitken, T.J., Chen, Y., Beutler, L.R., Ahn, J.S., et al. (2019). Genetic identification of vagal sensory neurons that control feeding. Cell *179*, 1129–1143.e23.

Barry, W.E., and Thummel, C.S. (2016). The Drosophila HNF4 nuclear receptor promotes glucose-stimulated insulin secretion and mitochondrial function in adults. eLife *5*, e11183.

Bassett, A.R., and Liu, J.L. (2014). CRISPR/Cas9 and genome editing in Drosophila. J. Genet. Genomics *41*, 7–19.

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, J., and Clevers, H. (2021). Cell fate specification and differentiation in the adult mammalian intestine. Nat. Rev. Mol. Cell Biol. *22*, 39–53.

Bevacqua, R.J., Lam, J.Y., Peiris, H., Whitener, R.L., Kim, S., Gu, X., Friedlander, M.S.H., and Kim, S.K. (2021). SIX2 and SIX3 coordinately regulate functional maturity and fate of human pancreatic β cells. Genes Dev. *35*, 234–249.

Bilder, D., and Irvine, K.D. (2017). Taking stock of the *Drosophila* research ecosystem. Genetics *206*, 1227–1236.

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.

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.

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.

Brankatschk, M., Dunst, S., Nemetschke, L., and Eaton, S. (2014). Delivery of circulating lipoproteins to specific neurons in the Drosophila brain regulates systemic insulin signaling. eLife *3*, e02862.

Brent, A.E., and Rajan, A. (2020). Insulin and leptin/Upd2 exert opposing influences on synapse number in fat-sensing neurons. Cell Metab. *32*, 786–800.e7.

Britton, J.S., and Edgar, B.A. (1998). Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development *125*, 2149–2158.

Broughton, S., Alic, N., Slack, C., Bass, T., Ikeya, T., Vinti, G., Tommasi, A.M., Driege, Y., Hafen, E., and Partridge, L. (2008). Reduction of DILP2 in Drosophila triages a metabolic phenotype from lifespan revealing redundancy and compensation among DILPs. PLoS ONE *3*, e3721.

Brown, M.S., and Goldstein, J.L. (2008). Selective versus total insulin resistance: a pathogenic paradox. Cell Metab. *7*, 95–96.

Buchon, N., Osman, D., David, F.P., Fang, H.Y., Boquete, J.P., Deplancke, B., and Lemaitre, B. (2013). Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. Cell Rep. *3*, 1725–1738.

Cani, P.D., Holst, J.J., Drucker, D.J., Delzenne, N.M., Thorens, B., Burcelin, R., and Knauf, C. (2007). GLUT2 and the incretin receptors are involved in glucose-induced incretin secretion. Mol. Cell. Endocrinol. *276*, 18–23.

Cantley, J. (2014). The control of insulin secretion by adipokines: current evidence for adipocyte-beta cell endocrine signalling in metabolic homeostasis. Mamm. Genome *25*, 442–454.

Carvalho-Santos, Z., Cardoso-Figueiredo, R., Elias, A.P., Tastekin, I., Baltazar, C., and Ribeiro, C. (2020). Cellular metabolic reprogramming controls sugar appetite in Drosophila. Nat Metab *2*, 958–973.

Caygill, E.E., and Brand, A.H. (2016). The GAL4 system: a versatile system for the manipulation and analysis of gene expression. Methods Mol. Biol. *1478*, 33–52.

Clements, J., Hens, K., Francis, C., Schellens, A., and Callaerts, P. (2008). Conserved role for the Drosophila Pax6 homolog Eyeless in differentiation and function of insulin-producing neurons. Proc. Natl. Acad. Sci. USA *105*, 16183–16188.

Clemmensen, C., Müller, T.D., Woods, S.C., Berthoud, H.R., Seeley, R.J., and Tschöp, M.H. (2017). Gut-brain cross-talk in metabolic control. Cell *168*, 758–774.

Cognigni, P., Bailey, A.P., and Miguel-Aliaga, I. (2011). Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. Cell Metab. *13*, 92–104.

Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., Carré, C., Noselli, S., and Léopold, P. (2005). Antagonistic actions of ecdysone and insulins determine final size in Drosophila. Science *310*, 667–670.

Costello, L.C., and Franklin, R.B. (2016). Plasma citrate homeostasis: how it is regulated; and its physiological and clinical implications. an important, but neglected, relationship in medicine. HSOA J. Hum. Endocrinol. *1*, 005.

Croset, V., Treiber, C.D., and Waddell, S. (2018). Cellular diversity in the *Drosophila* midbrain revealed by single-cell transcriptomics. eLife *7*, e34550.

Davie, K., Janssens, J., Koldere, D., De Waegeneer, M., Pech, U., Kreft, Ł., Aibar, S., Makhzami, S., Christiaens, V., Bravo González-Blas, C., et al. (2018). A

single-cell transcriptome atlas of the aging Drosophila brain. Cell *174*, 982–998.e20.

de Araujo, I.E., Schatzker, M., and Small, D.M. (2020). Rethinking food reward. Annu. Rev. Psychol. *71*, 139–164.

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.

Droujinine, I.A., and Perrimon, N. (2016). Interorgan communication pathways in physiology: focus on Drosophila. Annu. Rev. Genet. *50*, 539–570.

Droujinine, I.A., and Perrimon, N. (2019). The multidimensional organization of interorgan communication networks. Dev. Cell *50*, 395–396.

Drummond-Barbosa, D., and Tennessen, J.M. (2020). Reclaiming Warburg: using developmental biology to gain insight into human metabolic diseases. Development *147*, dev189340.

Dus, M., Min, S., Keene, A.C., Lee, G.Y., and Suh, G.S. (2011). Taste-independent detection of the caloric content of sugar in Drosophila. Proc. Natl. Acad. Sci. USA *108*, 11644–11649.

Dus, M., Lai, J.S., Gunapala, K.M., Min, S., Tayler, T.D., Hergarden, A.C., Geraud, E., Joseph, C.M., and Suh, G.S. (2015). Nutrient sensor in the brain directs the action of the brain-gut axis in Drosophila. Neuron *87*, 139–151.

Efeyan, A., Comb, W.C., and Sabatini, D.M. (2015). Nutrient-sensing mechanisms and pathways. Nature *517*, 302–310.

Fernández-Gallego, N., Sánchez-Madrid, F., and Jiménez-Saiz, R. (2021). Thinking small: zinc sensing by the gut epithelium. Allergy *76*, 411–413.

Figueroa-Clarevega, A., and Bilder, D. (2015). Malignant Drosophila tumors interrupt insulin signaling to induce cachexia-like wasting. Dev. Cell *33*, 47–55.

Fridell, Y.W., Hoh, M., Kréneisz, O., Hosier, S., Chang, C., Scantling, D., Mulkey, D.K., and Helfand, S.L. (2009). Increased uncoupling protein (UCP) activity in Drosophila insulin-producing neurons attenuates insulin signaling and extends lifespan. Aging (Albany N.Y.) *1*, 699–713.

Funk, M.C., Zhou, J., and Boutros, M. (2020). Ageing, metabolism and the intestine. EMBO Rep. *21*, e50047.

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

Ghosh, A.C., and O'Connor, M.B. (2014). Systemic Activin signaling independently regulates sugar homeostasis, cellular metabolism, and pH balance in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *111*, 5729–5734.

Gillette, C.M., Tennessen, J.M., and Reis, T. (2021). Balancing energy expenditure and storage with growth and biosynthesis during Drosophila development. Dev. Biol. *475*, 234–244.

Golic, K.G., and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell *59*, 499–509.

Grenier, T., and Leulier, F. (2020). How commensal microbes shape the physiology of Drosophila melanogaster. Curr. Opin. Insect Sci. *41*, 92–99.

Gribble, F.M., and Reimann, F. (2019). Function and mechanisms of enteroendocrine cells and gut hormones in metabolism. Nat. Rev. Endocrinol. *15*, 226–237.

Grönke, S., Clarke, D.F., Broughton, S., Andrews, T.D., and Partridge, L. (2010). Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet. *6*, e1000857.

Guo, X., Yin, C., Yang, F., Zhang, Y., Huang, H., Wang, J., Deng, B., Cai, T., Rao, Y., and Xi, R. (2019). The cellular diversity and transcription factor code of Drosophila enteroendocrine cells. Cell Rep. *29*, 4172–4185.e5.

Hadjieconomou, D., King, G., Gaspar, P., Mineo, A., Blackie, L., Ameku, T., Studd, C., de Mendoza, A., Diao, F., White, B.H., et al. (2020). Enteric neurons increase maternal food intake during reproduction. Nature *587*, 455–459.

Hammond, K.A. (1997). Adaptation of the maternal intestine during lactation. J. Mammary Gland Biol. Neoplasia *2*, 243–252.

Haselton, A.T., and Fridell, Y.W. (2010). Adult Drosophila melanogaster as a model for the study of glucose homeostasis. Aging (Albany N.Y.) *2*, 523–526.

Heier, C., and Kühnlein, R.P. (2018). Triacylglycerol metabolism in *Drosophila melanogaster*. Genetics *210*, 1163–1184.

Hentze, J.L., Carlsson, M.A., Kondo, S., Nässel, D.R., and Rewitz, K.F. (2015). The neuropeptide allatostatin A regulates metabolism and feeding decisions in Drosophila. Sci. Rep. *5*, 11680.

Hill, C.M., Laeger, T., Albarado, D.C., McDougal, D.H., Berthoud, H.R., Münzberg, H., and Morrison, C.D. (2017). Low protein-induced increases in FGF21 drive UCP1-dependent metabolic but not thermoregulatory endpoints. Sci. Rep. *7*, 8209.

Hill, C.M., Laeger, T., Dehner, M., Albarado, D.C., Clarke, B., Wanders, D., Burke, S.J., Collier, J.J., Qualls-Creekmore, E., Solon-Biet, S.M., et al. (2019). FGF21 signals protein status to the brain and adaptively regulates food choice and metabolism. Cell Rep. *27*, 2934–2947.e3.

Hirabayashi, S. (2016). The interplay between obesity and cancer: a fly view. Dis. Model. Mech. *9*, 917–926.

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.

Hollenbeck, C., and Reaven, G.M. (1987). Variations in insulin-stimulated glucose uptake in healthy individuals with normal glucose tolerance. J. Clin. Endocrinol. Metab. *64*, 1169–1173.

Honegger, B., Galic, M., Köhler, K., Wittwer, F., Brogiolo, W., Hafen, E., and Stocker, H. (2008). Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in Drosophila and is essential for starvation resistance. J. Biol. *7*, 10.

Hudry, B., Khadayate, S., and Miguel-Aliaga, I. (2016). The sexual identity of adult intestinal stem cells controls organ size and plasticity. Nature *530*, 344–348.

Hudry, B., de Goeij, E., Mineo, A., Gaspar, P., Hadjieconomou, D., Studd, C., Mokochinski, J.B., Kramer, H.B., Plaçais, P.Y., Preat, T., and Miguel-Aliaga, I. (2019). Sex differences in intestinal carbohydrate metabolism promote food intake and sperm maturation. Cell *178*, 901–918.e16.

Hui, S., Ghergurovich, J.M., Morscher, R.J., Jang, C., Teng, X., Lu, W., Esparza, L.A., Reya, T., Le Zhan, Yanxiang Guo, J., et al. (2017). Glucose feeds the TCA cycle via circulating lactate. Nature *551*, 115–118.

Hung, R.J., Hu, Y., Kirchner, R., Liu, Y., Xu, C., Comjean, A., Tattikota, S.G., Li, F., Song, W., Ho Sui, S., and Perrimon, N. (2020). A cell atlas of the adult *Drosophila* midgut. Proc. Natl. Acad. Sci. USA *117*, 1514–1523.

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., Martin, J.R., Chidami, S., Veenstra, J.A., and Rosay, P. (2005). AKH-producing neuroendocrine cell ablation decreases trehalose and induces behavioral changes in Drosophila. Am. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R531–R538.

Jang, C., Hui, S., Zeng, X., Cowan, A.J., Wang, L., Chen, L., Morscher, R.J., Reyes, J., Frezza, C., Hwang, H.Y., et al. (2019). Metabolite exchange between mammalian organs quantified in pigs. Cell Metab. *30*, 594–606.e3.

Johnson, M.L., Saffrey, M.J., and Taylor, V.J. (2019). Gastrointestinal capacity, gut hormones and appetite change during rat pregnancy and lactation. Reproduction *157*, 431–443.

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.Y., Heo, G., Kim, M., Kim, H., Jin, J.A., Kim, H.K., Jung, S., An, M., Ahn, B.H., Park, J.H., et al. (2020). A neural circuit mechanism for mechanosensory feedback control of ingestion. Nature *580*, 376–380.

Kim, B., Kanai, M.I., Oh, Y., Kyung, M., Kim, E.K., Jang, I.H., Lee, J.H., Kim, S.G., Suh, G.S.B., and Lee, W.J. (2021). Response of the microbiome-gut-brain axis in *Drosophila* to amino acid deficit. Nature *593*, 570–574.

King, A.N., and Sehgal, A. (2020). Molecular and circuit mechanisms mediating circadian clock output in the Drosophila brain. Eur. J. Neurosci. *51*, 268–281.

Kockel, L., Kerr, K.S., Melnick, M., Brückner, K., Hebrok, M., and Perrimon, N. (2010). Dynamic switch of negative feedback regulation in Drosophila Akt-TOR signaling. PLoS Genet. *6*, e1000990.

Kockel, L., Griffin, C., Ahmed, Y., Fidelak, L., Rajan, A., Gould, E.P., Haigney, M., Ralston, B., Tercek, R.J., Galligani, L., et al. (2019). An interscholastic network to generate LexA enhancer trap lines in Drosophila. G3 (Bethesda) *9*, 2097–2106.

Koyama, T., and Mirth, C.K. (2016). Growth-blocking peptides as nutrition-sensitive signals for insulin secretion and body size regulation. PLoS Biol. *14*, e1002392.

Kréneisz, O., Chen, X., Fridell, Y.W., and Mulkey, D.K. (2010). Glucose increases activity and Ca2+ in insulin-producing cells of adult Drosophila. Neuroreport *21*, 1116–1120.

Kühnlein, R.P. (2011). The contribution of the Drosophila model to lipid droplet research. Prog. Lipid Res. *50*, 348–356.

Kuhre, R.E., Ghiasi, S.M., Adriaenssens, A.E., Wewer Albrechtsen, N.J., Andersen, D.B., Aivazidis, A., Chen, L., Mandrup-Poulsen, T., Ørskov, C., Gribble, F.M., et al. (2019). No direct effect of SGLT2 activity on glucagon secretion. Diabetologia *62*, 1011–1023.

Kwon, Y., Song, W., Droujinine, I.A., Hu, Y., Asara, J.M., and Perrimon, N. (2015). Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist ImpL2. Dev. Cell *33*, 36–46.

Lai, S.-L., and Lee, T. (2006). Genetic mosaic with dual binary transcriptional systems in Drosophila. Nat. Neurosci. *9*, 703–709.

Leader, D.P., Krause, S.A., Pandit, A., Davies, S.A., and Dow, J.A.T. (2018). FlyAtlas 2: a new version of the Drosophila melanogaster expression atlas with RNA-seq, miRNA-seq and sex-specific data. Nucleic Acids Res. *46* (D1), D809–D815.

Lee, T., and Luo, L. (2001). Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci. *24*, 251–254.

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.

Lim, S.Y., You, H., Lee, J., Lee, J., Lee, Y., Lee, K.-A., Kim, B., Lee, J.-H., Jeong, J., Jang, S., et al. (2021). Identification and characterization of GAL4 drivers that mark distinct cell types and regions in the Drosophila adult gut. J. Neurogenet. *35*, 33–44.

Lin, C.C., and Potter, C.J. (2016). Editing transgenic DNA components by inducible gene replacement in Drosophila melanogaster. Genetics *203*, 1613–1628.

Luan, H., Peabody, N.C., Vinson, C.R., and White, B.H. (2006). Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. Neuron *52*, 425–436.

Macdonald, I.A. (2016). A review of recent evidence relating to sugars, insulin resistance and diabetes. Eur. J. Nutr. *55* (*Suppl 2*), 17–23.

Mahajan, A., Taliun, D., Thurner, M., Robertson, N.R., Torres, J.M., Rayner, N.W., Payne, A.J., Steinthorsdottir, V., Scott, R.A., Grarup, N., et al. (2018). Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat. Genet. *50*, 1505–1513.

Marianes, A., and Spradling, A.C. (2013). Physiological and stem cell compartmentalization within the Drosophila midgut. eLife *2*, e00886.

Mattila, J., and Hietakangas, V. (2017). Regulation of carbohydrate energy metabolism in *Drosophila melanogaster*. Genetics *207*, 1231–1253.

Mauvais-Jarvis, F., Arnold, A.P., and Reue, K. (2017). A guide for the design of pre-clinical studies on sex differences in metabolism. Cell Metab. *25*, 1216–1230.

McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. Science *302*, 1765–1768.

McGuire, S.E., Mao, Z., and Davis, R.L. (2004a). Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. Sci. STKE *2004*, pl6.

McGuire, S.E., Roman, G., and Davis, R.L. (2004b). Gene expression systems in Drosophila: a synthesis of time and space. Trends Genet. *20*, 384–391.

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

Miguel-Aliaga, I., Thor, S., and Gould, A.P. (2008). Postmitotic specification of Drosophila insulinergic neurons from pioneer neurons. PLoS Biol. *6*, e58.

Miguel-Aliaga, I., Jasper, H., and Lemaitre, B. (2018). Anatomy and physiology of the digestive tract of *Drosophila melanogaster*. Genetics *210*, 357–396.

Millington, J.W., Brownrigg, G.P., Chao, C., Sun, Z., Basner-Collins, P.J., Wat, L.W., Hudry, B., Miguel-Aliaga, I., and Rideout, E.J. (2021). Female-biased upregulation of insulin pathway activity mediates the sex difference in *Drosophila* body size plasticity. eLife *10*, e58341.

Min, S., Oh, Y., Verma, P., Whitehead, S.C., Yapici, N., Van Vactor, D., Suh, G.S., and Liberles, S. (2021). Control of feeding by Piezo-mediated gut mechanosensation in *Drosophila*. eLife *10*, e63049.

Moran, B.M., Miskelly, M.G., Abdel-Wahab, Y.H.A., Flatt, P.R., and McKillop, A.M. (2019). Zinc-induced activation of GPR39 regulates glucose homeostasis through glucose-dependent insulinotropic polypeptide secretion from enteroendocrine K-cells. Biol. Chem. Published online June 20, 2019. https://doi.org/10.1515/hsz-2018-0393.

Morris, S.N., Coogan, C., Chamseddin, K., Fernandez-Kim, S.O., Kolli, S., Keller, J.N., and Bauer, J.H. (2012). Development of diet-induced insulin resistance in adult Drosophila melanogaster. Biochim. Biophys. Acta *1822*, 1230–1237.

Musselman, L.P., and Kühnlein, R.P. (2018). *Drosophila* as a model to study obesity and metabolic disease. J. Exp. Biol. *221* (*Suppl 1*), jeb163881.

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.

Mycielska, M.E., Patel, A., Rizaner, N., Mazurek, M.P., Keun, H., Patel, A., Ganapathy, V., and Djamgoz, M.B. (2009). Citrate transport and metabolism in mammalian cells: prostate epithelial cells and prostate cancer. BioEssays *31*, 10–20.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L., Zhao, G., Ryba, N.J.P., and Zuker, C.S. (2002). An amino-acid taste receptor. Nature *416*, 199–202.

Newton, H., Wang, Y.F., Camplese, L., Mokochinski, J.B., Kramer, H.B., Brown, A.E.X., Fets, L., and Hirabayashi, S. (2020). Systemic muscle wasting and coordinated tumour response drive tumourigenesis. Nat. Commun. *11*, 4653.

Nilaweera, K.N., and Speakman, J.R. (2018). Regulation of intestinal growth in response to variations in energy supply and demand. Obes. Rev. *19* (*Suppl 1*), 61–72.

O'Brien, L.E., Soliman, S.S., Li, X., and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. Cell *147*, 603–614.

Ober, C., Loisel, D.A., and Gilad, Y. (2008). Sex-specific genetic architecture of human disease. Nat. Rev. Genet. *9*, 911–922.

Oh, Y., Lai, J.S., Mills, H.J., Erdjument-Bromage, H., Giammarinaro, B., Saadipour, K., Wang, J.G., Abu, F., Neubert, T.A., and Suh, G.S.B. (2019). A glucose-sensing neuron pair regulates insulin and glucagon in Drosophila. Nature *574*, 559–564.

Oh, Y., Lai, J.S., Min, S., Huang, H.W., Liberles, S.D., Ryoo, H.D., and Suh, G.S.B. (2021). Periphery signals generated by Piezo-mediated stomach stretch and Neuromedin-mediated glucose load regulate the Drosophila brain nutrient sensor. Neuron. Published online May 13, 2021. https://doi.org/10.1016/j.neuron.2021.04.028.

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

Palm, W., and Rodenfels, J. (2020). Understanding the role of lipids and lipoproteins in development. Development *147*, dev186411.

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.

Park, S., Bustamante, E.L., Antonova, J., McLean, G.W., and Kim, S.K. (2011). Specification of Drosophila corpora cardiaca neuroendocrine cells from mesoderm is regulated by Notch signaling. PLoS Genet. *7*, e1002241.

Park, S., Alfa, R.W., Topper, S.M., Kim, G.E., Kockel, L., and Kim, S.K. (2014). A genetic strategy to measure circulating Drosophila insulin reveals genes regulating insulin production and secretion. PLoS Genet. *10*, e1004555.

Pasco, M.Y., and Léopold, P. (2012). High sugar-induced insulin resistance in Drosophila relies on the lipocalin Neural Lazarillo. PLoS ONE *7*, e36583.

Patke, A., Young, M.W., and Axelrod, S. (2020). Molecular mechanisms and physiological importance of circadian rhythms. Nat. Rev. Mol. Cell Biol. *21*, 67–84.

Peiris, H., Park, S., Louis, S., Gu, X., Lam, J.Y., Asplund, O., Ippolito, G.C., Bottino, R., Groop, L., Tucker, H., and Kim, S.K. (2018). Discovering human diabetes-risk gene function with genetics and physiological assays. Nat. Commun. *9*, 3855.

Potter, C.J., Tasic, B., Russler, E.V., Liang, L., and Luo, L. (2010). The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. Cell *141*, 536–548.

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

Quintela, M., Señaris, R., Heiman, M.L., Casanueva, F.F., and Dieguez, C. (1997). Leptin inhibits in vitro hypothalamic somatostatin secretion and somatostatin mRNA levels. Endocrinology *138*, 5641–5644.

Rajan, A., and Perrimon, N. (2012). Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. Cell *151*, 123–137.

Rajan, A., Housden, B.E., Wirtz-Peitz, F., Holderbaum, L., and Perrimon, N. (2017). A mechanism coupling systemic energy sensing to adipokine secretion. Dev. Cell *43*, 83–98.e6.

Raubenheimer, D., and Jones, S.A. (2006). Nutritional imbalance in an extreme generalist omnivore: tolerance and recovery through complementary food selection. Anim. Behav. *71*, 1253–1262.

Redhai, S., Pilgrim, C., Gaspar, P., Giesen, L.V., Lopes, T., Riabinina, O., Grenier, T., Milona, A., Chanana, B., Swadling, J.B., et al. (2020). An intestinal zinc sensor regulates food intake and developmental growth. Nature *580*, 263–268.

Regan, J.C., Khericha, M., Dobson, A.J., Bolukbasi, E., Rattanavirotkul, N., and Partridge, L. (2016). Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. eLife *5*, e10956.

Reiff, T., Jacobson, J., Cognigni, P., Antonello, Z., Ballesta, E., Tan, K.J., Yew, J.Y., Dominguez, M., and Miguel-Aliaga, I. (2015). Endocrine remodelling of the adult intestine sustains reproduction in Drosophila. eLife *4*, e06930.

Riabinina, O., Vernon, S.W., Dickson, B.J., and Baines, R.A. (2019). Split-QF system for fine-tuned transgene expression in *Drosophila*. Genetics *212*, 53–63.

Rideout, E.J., Narsaiya, M.S., and Grewal, S.S. (2015). The sex determination gene transformer regulates male-female differences in Drosophila body size. PLoS Genet. *11*, e1005683.

Robinson, S.W., Herzyk, P., Dow, J.A., and Leader, D.P. (2013). FlyAtlas: database of gene expression in the tissues of Drosophila melanogaster. Nucleic Acids Res. *41*, D744–D750.

Rorsman, P., and Braun, M. (2013). Regulation of insulin secretion in human pancreatic islets. Annu. Rev. Physiol. *75*, 155–179.

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.

Sano, H., Nakamura, A., Texada, M.J., Truman, J.W., Ishimoto, H., Kamikouchi, A., Nibu, Y., Kume, K., Ida, T., and Kojima, M. (2015). The nutrient-responsive hormone CCHamide-2 controls growth by regulating insulin-like peptides in the brain of Drosophila melanogaster. PLoS Genet. *11*, e1005209.

Sawala, A., and Gould, A.P. (2017). The sex of specific neurons controls female body growth in Drosophila. PLoS Biol. *15*, e2002252.

Scopelliti, A., Bauer, C., Yu, Y., Zhang, T., Kruspig, B., Murphy, D.J., Vidal, M., Maddocks, O.D.K., and Cordero, J.B. (2019). A neuronal relay mediates a nutrient responsive gut/fat body axis regulating energy homeostasis in adult Drosophila. Cell Metab. *29*, 269–284.e10.

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.

Solon-Biet, S.M., Cogger, V.C., Pulpitel, T., Heblinski, M., Wahl, D., McMahon, A.C., Warren, A., Durrant-Whyte, J., Walters, K.A., Krycer, J.R., et al. (2016). Defining the nutritional and metabolic context of FGF21 using the geometric framework. Cell Metab. *24*, 555–565.

Soty, M., Gautier-Stein, A., Rajas, F., and Mithieux, G. (2017). Gut-brain glucose signaling in energy homeostasis. Cell Metab. *25*, 1231–1242.

Sousa-Nunes, R., Yee, L.L., and Gould, A.P. (2011). Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. Nature *471*, 508–512.

Srivastava, M., Simakov, O., Chapman, J., Fahey, B., Gauthier, M.E., Mitros, T., Richards, G.S., Conaco, C., Dacre, M., Hellsten, U., et al. (2010). The Amphimedon queenslandica genome and the evolution of animal complexity. Nature *466*, 720–726.

Storelli, G., Nam, H.J., Simcox, J., Villanueva, C.J., and Thummel, C.S. (2019). Drosophila HNF4 directs a switch in lipid metabolism that supports the transition to adulthood. Dev. Cell *48*, 200–214.e6.

Sun, J., Liu, C., Bai, X., Li, X., Li, J., Zhang, Z., Zhang, Y., Guo, J., and Li, Y. (2017). Drosophila FIT is a protein-specific satiety hormone essential for feeding control. Nat. Commun. *8*, 14161.

Tannenbaum, C., Ellis, R.P., Eyssel, F., Zou, J., and Schiebinger, L. (2019). Sex and gender analysis improves science and engineering. Nature *575*, 137–146.

Tennessen, J.M., and Thummel, C.S. (2011). Coordinating growth and maturation - insights from Drosophila. Curr. Biol. *21*, R750–R757.

Theall, C.L., Wurtman, J.J., and Wurtman, R.J. (1984). Self-selection and regulation of protein: carbohydrate ratio in foods adult rats eat. J. Nutr. *114*, 711–718.

Ting, C.-Y., Gu, S., Guttikonda, S., Lin, T.-Y., White, B.H., and Lee, C.-H. (2011). Focusing transgene expression in Drosophila by coupling Gal4 with a novel split-LexA expression system. Genetics *188*, 229–233.

Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.F., Montagner, A., and Gourdy, P. (2020). Sex differences in metabolic regulation and diabetes susceptibility. Diabetologia *63*, 453–461.

Ugrankar, R., Berglund, E., Akdemir, F., Tran, C., Kim, M.S., Noh, J., Schneider, R., Ebert, B., and Graff, J.M. (2015). Drosophila glucome screening identifies Ck1alpha as a regulator of mammalian glucose metabolism. Nat. Commun. *6*, 7102.

Unger, R.H., and Cherrington, A.D. (2012). Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J. Clin. Invest. *122*, 4–12.

Unger, R.H., and Orci, L. (2010). Paracrinology of islets and the paracrinopathy of diabetes. Proc. Natl. Acad. Sci. USA *107*, 16009–16012.

van Dam, E., van Leeuwen, L.A.G., Dos Santos, E., James, J., Best, L., Lennicke, C., Vincent, A.J., Marinos, G., Foley, A., Buricova, M., et al. (2020). Sugar-induced obesity and insulin resistance are uncoupled from shortened survival in Drosophila. Cell Metab. *31*, 710–725.e7.

Wang, P., Jia, Y., Liu, T., Jan, Y.N., and Zhang, W. (2020). Visceral mechanosensing neurons control Drosophila feeding by using Piezo as a sensor. Neuron *108*, 640–650.e4.

Wat, L.W., Chao, C., Bartlett, R., Buchanan, J.L., Millington, J.W., Chih, H.J., Chowdhury, Z.S., Biswas, P., Huang, V., Shin, L.J., et al. (2020). A role for triglyceride lipase brummer in the regulation of sex differences in Drosophila fat storage and breakdown. PLoS Biol. *18*, e3000595.

Wendler, F., Park, S., Hill, C., Galasso, A., Chang, K.R., Awan, I., Sudarikova, Y., Bustamante, M., Liu, S., Sung, E., et al. (2020). A toolkit to generate inducible and interconvertible Drosophila transgenes. bioRxiv. https://doi.org/10.1101/2020.08.18.256461.

Wente, W., Efanov, A.M., Brenner, M., Kharitonenkov, A., Köster, A., Sandusky, G.E., Sewing, S., Treinies, I., Zitzer, H., and Gromada, J. (2006). Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes *55*, 2470–2478.

Xu, R.G., Wang, X., Shen, D., Sun, J., Qiao, H.H., Wang, F., Liu, L.P., and Ni, J.Q. (2019). Perspectives on gene expression regulation techniques in Drosophila. J. Genet. Genomics *46*, 213–220.

Zipper, L., Jassmann, D., Burgmer, S., Görlich, B., and Reiff, T. (2020). Ecdysone steroid hormone remote controls intestinal stem cell fate decisions via the *PPARγ*-homolog *Eip75B* in *Drosophila*. eLife *9*, e55795.