Genetic manipulation of insulin/insulin-like growth factor signaling pathway activity has sex-biased effects on *Drosophila* body size

Jason W. Millington, George P. Brownrigg, Paige J. Basner-Collins, Ziwei Sun, and Elizabeth J. Rideout*

Department of Cellular and Physiological Sciences, Life Sciences Institute, The University of British Columbia, Vancouver, BC V6T 1Z3, Canada

*Corresponding author: Life Sciences Center, 2350 Health Sciences Mall (RM3308), Vancouver, BC V6T 1Z3, Canada. elizabeth.rideout@ubc.ca

Abstract

In *Drosophila* raised in nutrient-rich conditions, female body size is approximately 30% larger than male body size due to an increased rate of growth and differential weight loss during the larval period. While the mechanisms that control this sex difference in body size remain incompletely understood, recent studies suggest that the insulin/insulin-like growth factor signaling pathway (IIS) plays a role in the sex-specific regulation of processes that influence body size during development. In larvae, IIS activity differs between the sexes, and there is evidence of sex-specific regulation of IIS ligands. Yet, we lack knowledge of how changes to IIS activity impact body size in each sex, as the majority of studies on IIS and body size use single- or mixed-sex groups of larvae and/or adult flies. The goal of our current study was to clarify the body size requirement for IIS activity in each sex. To achieve this goal, we used established genetic approaches to enhance, or inhibit, IIS activity, and quantified pupal size in males and females. Overall, genotypes that inhibited IIS activity caused a female-biased decrease in body size, whereas genotypes that augmented IIS activity caused a male-specific increase in body size. These data extend our current understanding of body size regulation by showing that most changes to IIS pathway activity have sex-biased effects, and highlights the importance of analyzing body size data according to sex.

Keywords: *Drosophila*; sex; insulin pathway; body size; genetics

Introduction

Over the past two decades, the *Drosophila* larva has emerged as an important model to study the molecular and developmental processes that contribute to final body size. When nutrients are plentiful, one important factor that affects body size in most *Drosophila* species is whether the animal is male or female: female flies are typically larger than male flies (Alpatov 1930; Pitnick et al. 1995; French et al. 1998; Huey et al. 2006; Okamoto et al. 2013; Testa et al. 2013; Rideout et al. 2015; Sawala and Gould 2017; reviewed in Millington and Rideout 2018). This increased body size is due to an increased rate of larval growth and sexually dimorphic weight loss in wandering larvae, as the duration of the larval growth period does not differ between the sexes in wild-type flies (Okamoto et al. 2013; Testa et al. 2013; Sawala and Gould 2017). While the precise molecular mechanisms underlying the male–female difference in body size remain incompletely understood, recent studies have revealed a key role for the insulin/insulin-like growth factor signaling pathway (IIS) in the sex-specific regulation of developmental processes that influence body size (Shingleton et al. 2005; Gronke et al. 2010; Testa et al. 2013; Rideout et al. 2015; Liao et al. 2020; Millington et al. 2021).

Normally, IIS activity is higher in female larvae than in age-matched males (Rideout et al. 2015; Millington et al. 2021). Given that increased IIS activity is known to promote cell, tissue, and organellar size (Grewal 2009; Teleman, 2010), this suggests that elevated IIS activity is one reason that females have a larger body size. Indeed, the sex difference in body size was abolished between male and female flies carrying a mutation that strongly reduced IIS activity (Testa et al. 2013), and between male and female pupae reared on diets that markedly decrease IIS activity (Rideout et al. 2015). In both cases, the sex difference in body size was eliminated by a female-biased decrease in body size (Testa et al. 2013; Rideout et al. 2015). While these findings suggest that IIS plays a role in sex-specific body size regulation during development, only one genetic combination was used to reduce IIS activity (Testa et al. 2013). Therefore, it remains unclear whether the sex-biased effect of reduced IIS activity on body size is a common feature of genotypes that alter IIS activity.

In the present study, we used multiple genetic approaches to either enhance or inhibit IIS activity, and monitored body size in males and females. While previous studies show that the genetic approaches we employed effectively alter IIS activity, the body size effects in each sex remain unclear due to frequent use of mixed- or single-sex experimental groups, and the fact that statistical tests to detect sex-by-genotype interactions were not applied (Fernandez et al. 1995; Chen et al. 1996; Leevers et al. 1996; Böhni et al. 1999; Broggiolo et al. 2001; Cho et al. 2001; Rintelen et al. 2001; Britton et al. 2002; Ikeya et al. 2002; Rullifson et al. 2002;
Géminard et al. 2009; Zhang et al. 2009; Grönke et al. 2010). Our systematic examination of IIS revealed most genetic manipulations that reduced IIS activity caused a female-biased reduction in body size. In contrast, most genetic manipulations that enhanced IIS activity increased male body size with no effect in females. Together, these findings provide additional genetic support for IIS as one pathway that impacts sex-specific body size regulation in Drosophila.

Materials and methods

Fly husbandry

Drosophila growth medium consisted of: 20.5 g/L sucrose, 70.9 g/L D-glucose, 48.5 g/L cornmeal, 45.3 g/L yeast, 4.55 g/L agar, 0.5 g CaCl₂•2H₂O, 0.5 g MgSO₄•7H₂O, 11.77 mL acid mix (propionic acid/phosphoric acid). Diet data were obtained under “Rideout Lab 2Y diet” in the Drosophila Dietary Composition Calculator (Lesperance and Broderick 2020). Larvae were raised at a density of 50 animals per 10 mL food at 25°C, and sexed by gonad size. Adult flies were maintained at a density of 10 mL of flies per vial in single-sex groups.

Fly strains

The following fly strains from the Bloomington Drosophila Stock Center were used: w¹¹¹8 (#3605), UAS-rpr (#5823), UAS-Imp-L2-RNAi (#55855), Inr¹⁷⁹ (#9646), Inr¹⁷⁹ (#11661), Df(3R)P3K92E¹ (#25900), chico¹ (#10738), foxo²¹ (#80943), foxo²³ (#80944), r4-GAL4 (fat body), and dilp2-GAL4 (insulin-producing cells [IPCs]). Additional fly strains include: UAS-Kir2.1 (Baines et al. 2001), dilp1, dilp3, dilp4, dilp5, dilp6⁺¹, dilp⁷, Df(3L)lp2-3.5, Df(3L)lp1-4.5 (Grönke et al. 2010), Sd¹ (Okamoto et al. 2013), P3K92E²¹ (Halfar et al. 2001), Pdh¹ (Rintelen et al. 2001), Akt¹ (Stocker et al. 2002). All fly strains except dilp6⁺¹ were backcrossed into a w¹¹¹8 background for 6 generations. All strains without a visible marker were crossed six times to a w¹¹¹8 strain carrying a balancer chromosome corresponding to the genomic location of the gene. These crosses were in addition to prior extensive backcrossing of dilp mutant strains (Grönke et al. 2010).

Body size

Pupal length and width were determined using an automated detection and measurement system. Segmentation of the pupae for automated analysis was carried out using the “Marker-controlled Watershed” function included in the MorphoJ plugin (Klingenberg 2011) in ImageJ. Once length and width were determined using this automated measurement system, pupal volume was calculated as previously described (Delaneau et al. 2010; Marshall et al. 2012; Rideout et al. 2012, 2015; Ghosh et al. 2014). To measure adult weight, 5-day-old virgin male and female flies were collected and weighed in groups of 10 on an analytical balance.

Statistical analysis and data presentation

GraphPad Prism (GraphPad Prism version 8.4.2 for Mac OS X) was used to perform all statistical tests and to prepare all graphs in this manuscript. Statistical tests are indicated in figures and figure legends; all P-values are listed in Supplementary File S1.

Data availability

Original images of pupae are available upon request. Raw values for all data collected and displayed in this manuscript are available in Supplementary File S2. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, tables, and supplementary files. Supplementary material is available at figshare: https://doi.org/10.25387/g3.13191527.

Results

Reduced IPC function causes a female-biased decrease in body size

In Drosophila, the IPCs located in the brain are an important source of IIS ligands called Drosophila insulin-like peptides (Dilps). In larvae, the IPCs synthesize and release Dilp1 (FBgn0044051), Dilp2 (FBgn0036046), Dilp3 (FBgn0044050), and Dilp5 (FBgn0044048) into the hemolymph (Brogioio et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002; Lee et al. 2008; Géminard et al. 2009). When circulating Dilps bind to the Insulin-like Receptor (Inr, FBgn0283499) on the surface of target tissues, an intracellular signaling cascade is initiated which ultimately promotes cell, tissue, and organismal size (Chen et al. 1996; Böhni et al. 1999; Poltilove et al. 2000; Britton et al. 2002; Werz et al. 2009; Almudi et al. 2013). The importance of the IPCs in regulating IIS activity and body size is illustrated by the fact that IPC ablation and silencing both reduce IIS activity and decrease overall body size (Rulifson et al. 2002; Géminard et al. 2009). Yet, the precise requirement for IPCs in body size regulation in each sex remains unclear, as past studies presented data from a mixed-sex population of larvae or reported effects in only a single sex (Rulifson et al. 2002; Géminard et al. 2009). Because recent studies show that the sex of the IPCs contributes to the sex-specific regulation of body size (Sawala and Gould 2017), we asked how the presence and function of the IPCs affected body size in each sex.

First, we ablated the IPCs by overexpressing proapoptotic gene reaper (rpr, FBgn0011706) with the IPC-specific GAL4 driver dilp2-GAL4 (Brogioio et al. 2001; Rulifson et al. 2002). This method eliminates the IPCs during development (Rulifson et al. 2002). To quantify body size, we measured pupal volume to capture developmental processes such as growth and weight loss that occur during the larval period (Delanoue et al. 2010; Testa et al. 2013). In females, pupal volume was significantly lower in dilp2>UAS-rpr pupae compared with dilp2> and +/+UAS-rpr control pupae (Figure 1A). In males, pupal volume was also significantly lower in dilp2>UAS-rpr pupae compared with control dilp2> and +/+UAS-rpr pupae (Figure 1A), however, the magnitude of the decrease in body size was greater in females than in males (sex-genotype interaction P < 0.0001; two-way ANOVA). Next, to determine how reduced IPC function affected body size in each
sex, we overexpressed the inwardly-rectifying potassium channel Kir2.1 (Baines et al. 2001) using dilp2-GAL4. This approach reduces Dilp secretion and lowers IIS activity in a mixed-sex group of larvae (Geminard et al. 2009). We found that pupal volume was significantly reduced in dilp2>UAS-Kir2.1 females compared with dilp2>+ and +>UAS-Kir2.1 control females (Figure 1B). In males, pupal volume was reduced in dilp2>UAS-Kir2.1 pupae compared with dilp2>+ and +>UAS-Kir2.1 control pupae (Figure 1B). Because the magnitude of the decrease in female body size was larger than the reduction in male body size (sex:genotype interaction \( P < 0.0001 \); two-way ANOVA), this result indicates that inhibiting IPC function caused a female-biased reduction in pupal size. Together, these results identify a previously unrecognized sex-biased body size effect caused by manipulating IPC survival and function. Because previous studies show that IPC loss and IPC inhibition affects several developmental processes that impact final body size, these sex-specific body size effects may be due to sex-specific changes in larval growth, growth duration, and larval weight loss (Okamoto et al. 2013; Testa et al. 2013; Rideout et al. 2015; Sawala and Gould 2017).

**Loss of IPC-derived Dilps causes a female-biased reduction in body size**

Given that the larval IPCs produce Dilp1, Dilp2, Dilp3, and Dilp5 (Brogiolo et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002; Lee et al. 2008; Geminard et al. 2009), we tested whether the loss of some (Df(3L)ilp2-3,5) or all (Df(3L)ilp1-4,5) of the IPC-derived Dilps affected pupal size in males and females. While a previous study reported how loss of all IPC-derived dilp genes affected adult weight, data from both sexes was not available for all genotypes (Grönke et al. 2010). In females, pupal volume was significantly smaller in Df(3L)ilp2-3,5 pupae, which lack the coding sequences for dilp2, dilp3, and dilp5 (Grönke et al. 2010), compared with \( w^{1118} \) controls (Figure 1C). In males, body size was also significantly reduced in Df(3L)ilp2-3,5 homozygous pupae compared with \( w^{1118} \) controls (Figure 1C), however, the decrease in body size was significantly greater in females than in males (sex:genotype interaction \( P < 0.0001 \); two-way ANOVA). When we measured body size in males and females lacking all IPC-derived Dilps (Df(3L)ilp1-4,5), which lack the coding sequences for dilp1, dilp2, dilp3, dilp4, and dilp5 (Grönke et al. 2010), we reproduced the female-biased reduction in body size (Figure 1C; sex:genotype interaction \( P < 0.0001 \); two-way ANOVA). This reveals a previously unrecognized sex-biased body size effect arising from loss of most, or all, IPC-derived Dilps. Given that several dilp genes are known to affect developmental processes that impact body size, these sex-specific body size effects may reflect sex-specific changes in larval growth rate and larval weight loss (Okamoto et al. 2013; Testa et al. 2013; Rideout et al. 2015; Sawala and Gould 2017), and possibly sex-specific effects on the duration of the larval growth period.

**Loss of individual dilp genes causes a female-specific decrease in body size**

While Dilp1, Dilp2, Dilp3, and Dilp5 are all produced by the IPCs, previous studies have uncovered significant differences in

---

**Figure 1** IPC ablation, loss of IPC function, and loss of IPC-derived Dilp ligands all cause a female-biased decrease in growth. (A) Pupal volume in dilp2>UAS-rpr females and males compared to dilp2>+ and +>UAS-rpr controls (\( P < 0.0001 \) for all comparisons; two-way ANOVA followed by Tukey HSD test). \( n = 15–71 \) pupae. (B) Pupal volume in dilp2>UAS-Kir2.1 females and males compared to both dilp2>+ and +>UAS-Kir2.1 controls (\( P < 0.0001 \) for all comparisons; two-way ANOVA). This reveals a previously unrecognized sex-biased body size effect caused by manipulating IPC survival and function. Because previous studies show that IPC loss and IPC inhibition affects several developmental processes that impact final body size, these sex-specific body size effects may reflect sex-specific changes in larval growth, growth duration, and larval weight loss (Okamoto et al. 2013; Testa et al. 2013; Rideout et al. 2015; Sawala and Gould 2017).
regulation, secretion, and phenotypic effects of these IPC-derived Dilps (Brogiolo et al. 2001; Okamoto et al. 2009; Zhang et al. 2009; Grönke et al. 2010; Cognigni et al. 2011; Bai et al. 2012; Stafford et al. 2012; Linneweber et al. 2014; Cong et al. 2015; Liu et al. 2016; Nässel and Vanden Broeck, 2016; Post et al. 2018, 2019; Semaniuk et al. 2018; Ugrankar et al. 2018; Brown et al. 2020). We therefore wanted to determine the individual contributions of IPC-derived Dilps to pupal size in each sex. Furthermore, given that there are non-IPC-derived Dilps that regulate diverse aspects of physiology and behavior (dilp4, FBgn0044049; dilp6, FBgn0044047, and dilp7, FBgn0044046) (Grönke et al. 2010; Castellanos et al. 2013; Garner et al. 2018), we wanted to determine the requirement for these additional Dilps in regulating pupal size in each sex. While a previous study measured adult weight as a read-out for body size in dilp mutants; however, pupal volume was significantly different between y, w, dilp6 mutant pupae and w1118 controls (Figure 2C); however, pupal volume was significantly reduced in y, w, dilp6 pupae compared with y, w controls (Figure 2D). Together, these results extend our current understanding of body size regulation by revealing sex-specific requirements for all individual dilp genes in regulating body size. These sex-specific body size effects may be due to a combination of sex-specific effects on larval growth, weight loss in wandering larvae, or growth duration.

**Loss of Dilp binding factor Imp-L2 causes a male-specific increase in body size**

Once released into the circulation, the Dilps associate with proteins that modulate their growth-promoting effects. For example, Dilp1, Dilp2, Dilp5, and Dilp6 form a high-affinity complex with fat body-derived ecdysone-inducible gene 2 (Imp-L2, FBgn0001257) and Convoluted/Drosophila Acid Labile Subunit (Conv/dALS; FBgn0261269) (Arquier et al. 2008; Honegger et al. 2008; Alic et al. 2011; Okamoto et al. 2013), whereas Dilp3 interacts with Secreted decoy receptor (Sdr) of InR (FBgn0038279) (Okamoto et al. 2013). Binding of the Imp-L2/dALS complex to individual Dilps likely reduces Dilp binding to InR, as reduced fat body levels of either Imp-L2 or dALS augment IIS activity and increase body size (Arquier et al. 2008; Honegger et al. 2008; Alic et al. 2011). Similarly, loss of Sdr in flies carrying an amorphic Sdr allele (Sdr0), increases IIS activity and increases body size (Okamoto et al. 2013). While

---

**Figure 2** Loss of individual dilp genes causes sex-biased effects on growth. (A) In females, pupal volume was significantly reduced compared with w1118 controls in pupae lacking coding sequences for each of the following genes: dilp1, dilp3, dilp4, dilp5, and dilp7 (P < 0.0001, P = 0.0003, P = 0.0136, P < 0.0001, and P < 0.0001, respectively; one-way ANOVA followed by Dunnett’s multiple comparison test). n = 58–74 pupae. (B) Pupal volume was not significantly different between y, w control female pupae and dilp641 mutant females (P = 0.7634, Student’s t test). n = 41–74 pupae. (C) In males, pupal volume was not significantly reduced compared with w1118 controls in pupae lacking coding sequences for each of the following genes: dilp1, dilp3, dilp4, dilp5, and dilp7 (P = 0.7388, P = 0.2779, P = 0.1977, P = 0.9535, and P = 0.4526, respectively; one-way ANOVA followed by Dunnett’s multiple comparison test). n = 66–79 pupae. (D) Pupal volume was significantly reduced in male dilp641 pupae compared with y, w control males (P = 0.0017, Student’s t test). n = 64–70 pupae. * Indicates P < 0.05, ** indicates P < 0.01, *** indicates P < 0.001, **** indicates P < 0.0001, ns indicates not significant, error bars indicate SEM. Panels A and B display female data; panels C and D show male data.
the Sdr study reported that the magnitude of the increase in adult weight was equivalent in both sexes (Okamoto et al. 2013), which we confirm using pupal volume (Figure 3A; sex-genotype interaction P = 0.5261; two-way ANOVA), it remains unclear how the Imp-L2/dALS complex affects pupal size in each sex. Given that one source of secreted Imp-L2 is the fat body (other tissues shown to express Imp-L2 include the corpora cardiaca, insulin-producing cells, and a subset of gut enteroendocrine cells) (Honegger et al. 2008; Sarraf-Zadeh et al. 2013), we overexpressed an RNAi transgene at equivalent levels in each sex (Millington et al. 2021) to reduce Imp-L2 mRNA levels in the fat body. In females, pupal volume was not significantly different between pupae with fat body-specific overexpression of the Imp-L2-RNAi transgene (r4>UAS-Imp-L2-RNAi) and control r4>+. and +>UAS-Imp-L2-RNAi pupae (Figure 3B). In contrast, pupal volume was significantly larger in r4>UAS-Imp-L2-RNAi male pupae compared with r4>+. and +>UAS-Imp-L2-RNAi control males (Figure 3B). This finding aligns with previous studies showing that Imp-L2 loss enhances body size (Honegger et al. 2008). Furthermore, this finding extends our knowledge by identifying a male-specific effect of reduced fat body Imp-L2 on pupal size (sex-genotype interaction P < 0.0001; two-way ANOVA), a sex-biased effect that may arise due to sex-specific changes in larval growth, larval weight loss, or developmental timing.

Altered activity of the intracellular IIS pathway causes sex-biased and non-sex-specific effects on body size

In flies, IIS activity is stimulated by Dilp binding to the InR on the surface of target cells (Fernandez et al. 1995; Chen et al. 1996). This Dilp-InR interaction induces receptor autophosphorylation and recruitment of adapter proteins such as Chico (FBgn0024248), the Drosophila homolog of mammalian insulin receptor substrate (Böhni et al. 1999; Politiove et al. 2000; Werz et al. 2009). The recruitment and subsequent activation of the catalytic subunit of Drosophila phosphatidylinositol 3-kinase (P3K92E; FBgn0015279) increases the production of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the plasma membrane (Levers et al. 1996; Britton et al. 2002), which activates signaling proteins such as Phosphoinositide-dependent kinase 1 (Pdk1; FBgn0020386) and Akt1 (FBgn0010379) (Alessi et al. 1997). Both Pdk1 and Akt1 phosphorylate many downstream effectors to promote body size (Verdu et al. 1999; Cho et al. 2001; Rintelen et al. 2001). The importance of these intracellular IIS components in regulating organism size is illustrated by studies showing that the loss, or reduced function, of most IIS components significantly decreases body size (Chen et al. 1996; Leevers et al. 1996; Böhni et al. 1999; Weinkove et al. 1999; Brogiolo et al. 2002; Rulifson et al. 2002; Gémardin et al. 2009; Zhang et al. 2009; Grönke et al. 2010; Murillo-Maldonado et al. 2011). It is important to note that the effects of intracellular IIS components on body size are due to effects on several developmental processes including larval and pupal growth, larval weight loss, and growth duration (Chen et al. 1996; Böhni et al. 1999; Shingleton et al. 2005; Slaidina et al. 2009; Grönke et al. 2010; Testa et al. 2013). Yet, the majority of studies on the regulation of body size by intracellular IIS components were performed in a single- or mixed-sex population of larvae and/or adult flies, and tests for sex-by-genotype interactions were not applied (Fernandez et al. 1995; Chen et al. 1996; Leevers et al. 1996; Böhni et al. 1999; Brogiolo et al. 2001; Cho et al. 2001; Rintelen et al. 2001; Ikeya et al. 2002; Rulifson et al. 2002; Britton et al. 2002; Gémardin et al. 2009; Zhang et al. 2009; Grönke et al. 2010). Given that recent studies have demonstrated the sex-specific regulation of IIS components such as Akt1 (Rideout et al. 2015), we investigated the requirement for each component in regulating pupal size in males and females. In line with previous results showing a female-biased decrease in adult weight in flies heterozygous for two hypomorphic InR alleles (Testa et al. 2013), we observed a female-biased pupal volume reduction in pupae carrying an additional combination of hypomorphic InR alleles (Figure 4A; sex-genotype interaction P < 0.0001; two-way ANOVA) (Fernandez et al. 1995; Tatar et al. 2001).

To expand these findings beyond InR, we measured pupal volume in males and females with whole-body loss of individual intracellular IIS components. Given that we did not obtain viable pupae homozygous for an amorphic allele of chico (chico0), we measured pupal volume in chico0/+ males and females. In chico0/+ females, pupal volume was significantly reduced compared with control w1118 pupae (Figure 4B). In chico0/+ males, pupal volume was reduced compared with control w1118 pupae (Figure 4B). Given that the magnitude of the reduction in pupal volume was similar in males and females (sex-genotype interaction P = 0.9948 and P < 0.0001 for both sexes; two-way ANOVA followed by Tukey HSD test). P-values for all sex-genotype interactions are indicated on the graphs.

Figure 3 Fat body loss of Dilp binding protein Imp-L2 has sex-biased effects on growth. (A) Pupal volume in Sdr4 mutant females and males compared with w1118 control females and males (P < 0.0001 for both sexes; two-way ANOVA followed by Tukey HSD test). n = 52–88 pupae. (B) In females, pupal volume was not significantly different between pupae with fat body-specific knockdown of Imp-L2 (r4>UAS-Imp-L2-RNAi) compared with r4>+. and +>UAS-Imp-L2-RNAi control pupae (P = 0.9948 and P < 0.0001, respectively; two-way ANOVA followed by Tukey HSD test), whereas r4>UAS-Imp-L2-RNAi males were significantly larger than r4>+. and +>UAS-Imp-L2-RNAi control males (P < 0.0001 for both comparisons; two-way ANOVA followed by Tukey HSD test). n = 70–92 pupae. *** Indicates P < 0.0001; ns indicates not significant, error bars indicate SEM. For all panels, females are shown on the left-hand side of the graph and males are shown on the right-hand side. P-values for all sex-genotype interactions are indicated on the graphs.
Figure 4 Both sex-biased and non-sex-biased effects on growth arise from loss of intracellular IIS pathway components. (A) Pupal volume in females and males heterozygous for two hypomorphic InR alleles (InR<sup>E19</sup>/InRPZ<sup>E2</sup>) compared with sex-matched <sup>w</sup>1118 controls (P < 0.0001 for both sexes; two-way ANOVA followed by Tukey HSD test). n = 32–133 pupae. (B) Pupal volume in females and males heterozygous for an amorphic chico allele (chico<sup>1</sup>/+) compared with sex-matched <sup>w</sup>1118 controls (P < 0.0001 for both females and males; two-way ANOVA followed by Tukey HSD test). n = 93–133 pupae. (C) Pupal volume in females and males heterozygous for a deficiency and loss-of-function allele of Pi3K<sup>92E</sup> (Df(3R)Pi3K92EA/Pi3K92E2H1) compared with sex-matched <sup>w</sup>1118 controls (P < 0.0001 for all comparisons in females and males; two-way ANOVA followed by Tukey HSD test). Note: the Df(3R)Pi3K92EA/PI3K92E2H1 pupae were collected and analyzed in parallel with the InR<sup>E19</sup>/InRPZ<sup>E2</sup> genotype, so the <sup>w</sup>1118 control genotype data is shared between these experiments. n = 52–133 pupae. (D) Pupal volume was not significant different in either females or males homozygous for a loss-of-function Pdk1 allele (Pdk1<sup>4</sup>) compared with <sup>w</sup>1118 controls (P = 0.6739 and P = 0.7847, respectively; two-way ANOVA followed by Tukey HSD test). n = 61–84 pupae. (E) Adult weight in Pdk1<sup>4</sup> females and males compared with <sup>w</sup>1118 controls (P = 0.0017 and P = 0.0491 for females and males respectively; two-way ANOVA followed by Tukey HSD test). n = 5–8 biological replicates of 10 adult flies. (F) Pupal volume in females and males heterozygous for a hypomorphic Akt1 allele (Akt1<sup>3</sup>) compared with sex-matched <sup>w</sup>1118 controls (P < 0.0001 for both sexes; two-way ANOVA followed by Tukey HSD test). n = 44–60 pupae. (G) In females and males heterozygous for two loss-of-function alleles of foxo (foxo<sup>f25</sup>/foxo<sup>f25</sup>), pupal volume was not significantly different compared with sex-matched <sup>w</sup>1118 controls (P = 0.8841 and 0.9646, respectively; two-way ANOVA followed by Tukey HSD test). n = 110–153 pupae. (H) In foxo<sup>f25</sup>/foxo<sup>f25</sup> females, adult weight was not significantly different compared with <sup>w</sup>1118 controls (P = 0.8786; two-way ANOVA followed by Tukey HSD test). In males, adult weight was significantly higher in foxo<sup>f25</sup>/foxo<sup>f25</sup> flies compared with <sup>w</sup>1118 control flies (P < 0.0001; two-way ANOVA followed by Tukey HSD test). n = 5–8 biological replicates of 10 adult flies. * Indicates P < 0.05; ** Indicates P < 0.01; **** Indicates P < 0.0001; ns indicates not significant; error bars indicate SEM. For all panels, females are shown on the left-hand side of the graph and males are shown on the right-hand side. P-values for all sex:genotype interactions are indicated on the graphs.
interaction $P = 0.1399$; two-way ANOVA), reduced chico did not cause a sex-biased effect on pupal size. In females heterozygous for one predicted null and one loss-of-function allele of Pi3K92E, Df(3R)Pi3K92E<sup>A</sup> and Pi3K92E<sup>2H1</sup>, respectively (Weinkove et al. 1999; Halfar et al. 2001), pupal volume was significantly reduced compared with control $w^{1118}$ pupae (Figure 4C). In Df(3R)Pi3K92E<sup>/</sup> Pi3K92E<sup>2H1</sup> males, we observed a significant reduction in pupal volume (Figure 4C), however, the magnitude of the decrease in pupal size was larger in females compared with males (sex-genotype interaction $P < 0.0001$; two-way ANOVA). This indicates that loss of Pi3K92E caused a female-biased decrease in body size. Similarly, a previous study showed that heterozygous loss of Phosphatase and tensin homolog (Pten, FBgn0026379), which antagonizes the lipid kinase activity of Pi3K92E to repress growth, also caused a sex-biased increase in pupal volume (Millington et al. 2021).

Next, we examined pupal size in males and females homozygous for a loss-of-function allele of Pdk1 (Pdk1<sup>3</sup>). We observed no effect on pupal volume in either sex in Pdk1<sup>3</sup> homozygotes (Figure 4D). Given that a previous study showed that adult weight was reduced in Pdk1<sup>3</sup>/Pdk1<sup>17</sup> (Rintelen et al. 2001), we additionally measured adult weight in order to make a direct comparison between our findings and past findings. We found an equivalent body size reduction in Pdk1<sup>3</sup> males and females compared with sex-matched control $w^{1118}$ flies (Figure 4E; sex-genotype interaction $P = 0.5030$; two-way ANOVA). This suggests that reduced Pdk1 did not cause a sex-biased reduction in pupal size. One important target of Pdk1 is the serine/threonine kinase Akt1. In females homozygous for a hypomorphic allele of Akt1 (Akt1<sup>3</sup>), pupal volume was significantly reduced compared with control $w^{1118}$ pupae (Figure 4F). In Akt1<sup>3</sup> males, we observed a significant reduction in pupal size compared with control $w^{1118}$ pupae (Figure 4G). Given that the magnitude of the decrease in pupal size was larger in females than in males (sex-genotype interaction $P < 0.0001$; two-way ANOVA), this indicates that loss of Akt1 caused a female-biased effect on pupal size. Together, these findings identify previously unrecognized sex-biased body size effects of reduced Pi3K92E and Akt1.

One downstream target of IIS that contributes to the regulation of body size is transcription factor forkhead box, sub-group O (foxo, FBgn00383179). When IIS activity is high, Akt1 phosphorylates Foxo to prevent Foxo from translocating to the nucleus (Puig et al. 2003). Given that Foxo positively regulates mRNA levels of many genes that are involved in growth repression and metabolism (Zinke et al. 2002; Jung et al. 2003; Kramer et al. 2003; Alic et al. 2011; Slack et al. 2011), elevated IIS activity enhances body size in part by inhibiting Foxo (Jung et al. 2003; Kramer et al. 2003). Because previous studies show increased Foxo nuclear localization and elevated Foxo target gene expression in males (Rideout et al. 2015; Millington et al. 2021), we examined how Foxo contributes to pupal size in each sex by measuring body size in females and males heterozygous for two different loss-of-function foxo alleles (foxo<sup>21</sup>/foxo<sup>25</sup>). In foxo<sup>21</sup>/foxo<sup>25</sup> females and males, pupal volume was not significantly different from sex-matched $w^{1118}$ control pupae (Figure 4G). To directly compare our findings with prior reports on body size effects of foxo (Kramer et al. 2003; Jung et al. 2003), we also measured adult weight. In adult females, body weight was not significantly different between foxo<sup>21</sup>/foxo<sup>25</sup> mutants and control $w^{1118}$ flies (Figure 4H); however, foxo<sup>21</sup>/foxo<sup>25</sup> adult males were significantly heavier than control $w^{1118}$ males (Figure 4H). Because we observed a male-specific increase in body size (sex-genotype interaction $P = 0.0014$; two-way ANOVA), our data suggest that Foxo function normally contributes to the reduced adult weight of males. This reveals a previously unrecognized sex-specific role for Foxo in regulating body size. Taken together, these results identify sex-biased effects on pupal size arising from reduced function of some intracellular IIS components (e.g., InR, Pi3K92E, Akt1, and foxo). In contrast, other intracellular IIS components have non-sex-specific effects on body size (e.g., chico and Pdk1). It will be important in future studies to address how different developmental mechanisms (e.g., larval growth, larval weight loss, and growth duration) contribute to both sex-biased and non-sex-biased body size effects of individual IIS components.

**Discussion**

An extensive body of work has demonstrated an important role for IIS in promoting cell, tissue, and organismal size in response to nutrient input (Fernandez et al. 1995; Chen et al. 1996; Böhní et al. 1999; Britton et al. 2002; Grewal, 2009; Telemen, 2010). More recently, studies suggest that IIS also plays a role in sex-specific body size regulation (Testa et al. 2013; Rideout et al. 2015; Millington et al. 2021). However, potential links between IIS and the sex-specific regulation of body size were inferred from studies using a limited number of genotypes to modulate IIS activity. The goal of our current study was to determine whether the sex-biased body size effects observed in previous studies represent a common feature of genotypes that affect IIS activity. Overall, we found that the loss of most positive regulators of IIS activity caused a female-biased reduction in body size. On the other hand, loss of genes that normally repress IIS activity caused a male-specific increase in body size. Thus, most changes to IIS activity cause sex-biased, or sex-specific, effects on body size (summarized in Table 1), highlighting the importance of collecting and analyzing data from both sexes separately in studies that manipulate IIS activity and/or examine IIS-responsive phenotypes (e.g., lifespan and immunity).

One important outcome from our study was to provide additional genetic support for IIS as an important regulator of the sex difference in body size. Data implicating IIS in the sex-specific regulation of body size first emerged from a detailed examination of the larval stage of development in wild-type flies of both sexes (Testa et al. 2013). In this study, the authors reported a female-biased body size reduction in flies with decreased InR function (Testa et al. 2013). A subsequent study extended this finding by uncovering a sex difference in IIS activity: late third-instar female larvae had higher IIS activity than age-matched males (Rideout et al. 2015). The reasons for this increased IIS activity remain incompletely understood; however, Dilp2 secretion from the IPCs was higher in female larvae than in males (Rideout et al. 2015). Given that Dilp2 overexpression is known to augment IIS activity and enhance body size (Ikeya et al. 2002; Géminard et al. 2009), these findings suggest a model in which high levels of circulating Dilp2 (and possibly other Dilps) are required in females to achieve and maintain increased IIS activity and a larger body size in nutrient-rich conditions. In males, lower circulating levels of Dilp2 lead to reduced IIS activity and a smaller body size. If this model is accurate, we predict that female body size will be more sensitive to genetic manipulations that reduce Dilp ligands and/or IIS activity. Previous studies provided early support for this model by demonstrating a female-biased reduction in body size due to strong InR inhibition and dilp2 loss (Testa et al. 2013; Liao et al. 2020; Millington et al. 2021). Now, we provide strong genetic support for this model using multiple genetic manipulations to reduce IIS activity, confirming that Drosophila females
Table 1 Summary of sex-biased effects of IIS pathway manipulations on body size

| Genetic manipulation | Female-biased | Male-biased | Non-sex-specific | Percent change body size |
|----------------------|---------------|-------------|------------------|--------------------------|
| Reduced circulating Dilps |                |             |                  |                          |
| IPC ablation      | Yes           | —           | —                | F: −34.5%                |
| IPC silencing     | Yes           | —           | —                | M: −30.5%                |
| dilp2-3,5         | Yes           | —           | —                | F: −39.5%                |
| dilp1-4,5         | Yes           | —           | —                | M: −34.5%                |
| dilp1             | Yes           | —           | —                | F: −41.4%                |
| dilp3             | Yes           | —           | —                | M: −41.5%                |
| dilp4             | Yes           | —           | —                | F: −8.1%                 |
| dilp5             | Yes           | —           | —                | M: ns                     |
| dilp6             | —             | Yes         | —                | F: −3.9%                 |
| dilp7             | Yes           | —           | —                | M: ns                     |
| Increased circulating Dilps |                |             |                  |                          |
| Sdr                | —             | —           | Yes              | F: −27.2%                |
| Fat body imp-L2   | —             | —           | —                | M: ns                     |
| InR                | Yes           | —           | —                | M: +9.0%                 |
| intracellular IIS pathway |             |             |                  |                          |
| chico+/−          | —             | —           | Yes              | F: −54.8%                |
| Pi3K92E           | Yes           | —           | —                | M: −8.1%                 |
| Pdk1              | —             | —           | Yes              | F: −4.2%                 |
| Akt1              | Yes           | —           | —                | M: −40.6%                |
| Foxo              | —             | Yes         | —                | M: −37.0%                |
|                   |               |             |                  |                          |

All data used in this summary table are derived from pupal volume experiments, except for Pdk1 and foxo, where adult weight is shown.

depend on high levels of IIS activity to promote increased body size. One potential reason for this high level of IIS activity in females is to ensure successful reproduction, as IIS activity in females regulates germine stem cell divisions, ovariode number, and egg production (LaFever and Drummond-Barbosa 2005; Hsu et al. 2008; Hsu and Drummond-Barbosa 2009; Grönke et al. 2010; Green and Extavour 2014). Unfortunately, this elevated level of IIS activity shortens lifespan, revealing an important IIS-mediated tradeoff between fecundity and lifespan in females (Broughton et al. 2005).

A second prediction of this model is that augmenting either circulating Dilp levels or IIS activity will enhance male body size. Indeed, we demonstrate that loss of imp-L2, which increases free circulating Dilp levels (Arquier et al. 2008; Honegger et al. 2008; Alic et al. 2011; Okamoto et al. 2013), and loss of foxo, which mediates growth repression associated with low IIS activity (Jünger et al. 2003; Kramer et al. 2003), both cause a male-specific increase in body size. Together, these findings suggest that the smaller body size of male pupae is partly due to low IIS activity. While the reason for lower IIS activity in males remains unclear, studies show that altered IIS activity in either of the two main cell types within the testis compromises male fertility (Ueishi et al. 2009; McLeod et al. 2010; Amoyel et al. 2014, 2016). Future studies will therefore need to determine how males and females each maintain IIS activity within the range that maximizes fertility. In addition, it will be important to determine whether the female-biased phenotypic effects of lower IIS activity that we observe, and which are prevalent in aging and lifespan studies (Clancy et al. 2001; Holzenberger et al. 2003; Magwere et al. 2004; Van Heemst et al. 2005; Selman et al. 2008; Regan et al. 2016; Kane et al. 2018) extend to additional IIS-associated phenotypes (e.g., immunity and sleep) (DiAngelo et al. 2009; Cong et al. 2015; Roth et al. 2018; Suzawa et al. 2019; Brown et al. 2020).

Another important task for future studies will be to gain deeper insight into sex differences in IPC function, as one study identified sex-specific Dilp2 secretion from the IPCs (Rideout et al. 2015). Indeed, recent studies have revealed the sex-specific regulation of one factor (stunted, Fbgn0014391) that influences Dilp secretion from the IPCs (Delanoue et al. 2016; Millington et al. 2021), and female-specific phenotypic effects of another factor that influences IPC-derived Dilp expression (Woodling et al. 2020). Together, these studies suggest that sex differences in IPC function and circulating Dilp levels exist, and may arise from the combined effects of multiple regulatory mechanisms. Given that our knowledge of IPC function has recently expanded in a series of exciting studies (Meschi et al. 2019; Oh et al. 2019), more work will be needed to test whether these newly discovered modes of IPC regulation operate in both sexes. Furthermore, it will be important to ascertain how sex differences in the IPCs are specified. One recent study showed that Sex-lethal (Sxl; Fbgn0264270), a key regulator of female sexual development, acts in the IPCs to regulate the male–female difference in body size (Sawala and Gould 2017). By studying how Sxl function alters IPC gene expression, activity, and connectivity, it will...
be possible to gain mechanistic insight into the sex-specific regulation of body size. Beyond an improved understanding of sex differences in IPC function, it will be essential to study the sex-specific regulation of dilp genes and Dilp proteins, as we show female-specific effects on body size in pupae lacking most individual dilp genes. While two previous studies report female-biased effects of loss of dilp2 (Liao et al. 2020; Millington et al. 2021), this is the first report of a female-specific role for dilp1, dilp3, dilp4, dilp5, and dilp7 in promoting growth. While the female-specific effect of dilp2 loss on pupal size aligns with the fact that female larvae have higher circulating Dilp2 levels (Rideout et al. 2015), much remains to be discovered about the sex-specific regulation of most dilp genes and Dilp proteins. For example, females have an increased number of dilp7-positive cells compared with males (Castellanos et al. 2013; Garner et al. 2018), however, it is unclear whether these additional dilp7-positive cells in females augment circulating Dilp7 levels. A full understanding of the female-specific effects that accompany loss of most individual dilp genes will therefore require more knowledge of sex differences in the regulation of dilp genes and Dilp proteins. In addition to revealing the female-specific effects of many dilp genes on pupal size, we are also the first to report a male-specific body size effect of dilp6. Normally, Dilp6 function sustains growth in nonfeeding conditions, and is upregulated in low-nutrient contexts (Slaidina et al. 2009). Interestingly, male larvae have lower IIS activity than age-matched females (Rideout et al. 2015), where decreased IIS activity phenocopies a low-nutrient environment (Britton et al. 2002). Therefore, one potential explanation for the male-specific effect of dilp6 loss on pupal size is that reduced IIS activity in normal males leads to an increased reliance on Dilp6 to maintain body size. In females, higher levels of potent growth-promoting Dilp2 (Ikeya et al. 2002), and possibly other Dilps, promote IIS activity to minimize the requirement for Dilp6 function. This possibility will be important to test in future studies, alongside experiments to address a potential sex-specific role for other regulators of IIS function, it will be essential to study the sex-specific regulation of body size in pupae lacking most individual dilp genes. While two previous studies report female-biased effects of loss of dilp2 (Liao et al. 2020; Millington et al. 2021), this is the first report of a female-specific role for dilp1, dilp3, dilp4, dilp5, and dilp7 in promoting growth. While the female-specific effect of dilp2 loss on pupal size aligns with the fact that female larvae have higher circulating Dilp2 levels (Rideout et al. 2015), much remains to be discovered about the sex-specific regulation of most dilp genes and Dilp proteins. For example, females have an increased number of dilp7-positive cells compared with males (Castellanos et al. 2013; Garner et al. 2018), however, it is unclear whether these additional dilp7-positive cells in females augment circulating Dilp7 levels. A full understanding of the female-specific effects that accompany loss of most individual dilp genes will therefore require more knowledge of sex differences in the regulation of dilp genes and Dilp proteins. In addition to revealing the female-specific effects of many dilp genes on pupal size, we are also the first to report a male-specific body size effect of dilp6. Normally, Dilp6 function sustains growth in nonfeeding conditions, and is upregulated in low-nutrient contexts (Slaidina et al. 2009). Interestingly, male larvae have lower IIS activity than age-matched females (Rideout et al. 2015), where decreased IIS activity phenocopies a low-nutrient environment (Britton et al. 2002). Therefore, one potential explanation for the male-specific effect of dilp6 loss on pupal size is that reduced IIS activity in normal males leads to an increased reliance on Dilp6 to maintain body size. In females, higher levels of potent growth-promoting Dilp2 (Ikeya et al. 2002), and possibly other Dilps, promote IIS activity to minimize the requirement for Dilp6 function. This possibility will be important to test in future studies, alongside experiments to address a potential sex-specific role for other regulators of dilp6/Dilp6 including steroid hormone ecdyson and the Toll signaling pathway (Slaidina et al. 2009; Suzawa et al. 2019). Furthermore, as our knowledge of how individual dilp genes affect larval development and physiology continues to grow, analyzing data from both sexes will play an important role in extending knowledge of the mechanisms underlying sex differences in body size and other IIS-associated traits.

In contrast to the female-biased effects of most genetic manipulations that reduced Dilp availability, we observed both sex-biased and non-sex-biased effects on body size in pupae with reduced function of key intracellular IIS components. For example, reduced InR, P3K92E, and Akt1 function caused a female-biased reduction in body size, whereas there was an equivalent reduction in male and female body size due to lower chico and Pdk1 function. While more information on larval growth, developmental timing, and larval weight loss are needed to fully understand why different IIS components have sex-biased or non-sex-biased body size effects, one recent study showed that heterozygous loss of chico caused insulin hypersecretion (Sanaki et al. 2020). Given that hyperinsulinemia contributes to insulin resistance, and that insulin resistance decreases Drosophila body size (Musselman et al. 2011; 2017; Pasco and Léopold 2012), more studies will be needed to determine whether the smaller body size of chico+/- male and female pupae, and possibly Pdk1 mutant flies, can be attributed to insulin resistance. In fact, more knowledge of sex-specific tissue responses to insulin is urgently needed in male and female flies, as studies in mice and humans have identified sex differences in insulin sensitivity (Geer and Shen 2009; Macotela et al. 2009). Because Drosophila is an emerging model to understand the mechanisms underlying the development of insulin resistance (Musselman et al. 2011), this knowledge would help determine whether flies are a good model to investigate the sex-biased incidence of diseases associated with insulin resistance, such as the metabolic syndrome and type 2 diabetes (Mauvais-Jarvis 2015).

Acknowledgments

We would like to thank Charlotte Chao for developing the automated pupal volume analysis method. We would like to thank Dr. Linda Partridge for sharing the dilp mutant strains used in this study, Dr. Takashi Nishimura for sharing Sdr” flies, and Dr. Ernst Hafen for providing Akt1Δ, Pdk1Δ, and P3K92E mutant strains. Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. We thank the TRiP at Harvard Medical School (NIH/NIGMS R01-GM084947) for providing transgenic RNAi fly stocks and/or plasmid vectors used in this study. We acknowledge critical resources and information provided by FlyBase (Thurmond, J. 2019, NAR; FlyBase is supported by a grant from the National Human Genome Research Institute at the U.S. National Institutes of Health (U41 HG000739) and by the British Medical Research Council (MR/N030117/1). Funding

Funding for this study was provided by grants to EJR from the Canadian Institutes for Health Research (PJF-153072), Natural Sciences and Engineering Research Council of Canada (NSERC, RGPIN-2016-04249), Michael Smith Foundation for Health Research (16876), and the Canadian Foundation for Innovation (JELF-34879). J.W.M. was supported by a 4-year CELL Fellowship from UBC, and Z.S. was supported by an NSERC Undergraduate Student Research Award. We would like to acknowledge that our research takes place on the traditional, ancestral, and unceded territory of the Musqueam people; a privilege for which we are grateful.

Conflicts of interest: None declared.

Literature cited

Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, et al. 1997. 3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. Curr Biol. 7:776–789.

Alic N, Hoddinott MP, Vinti G, Partridge L. 2011. Lifespan extension by increased expression of the Drosophila homologue of the IGFBP7 tumour suppressor. Aging Cell 10:137–147.

Almudi I, Poernbacher I, Hafen E, Stocker H. 2013. The Lnk/SH2B adaptor provides a fail-safe mechanism to establish the Insulin receptor-Chico interaction. Cell Commun Signal. 11:26.

Alpatov WW. 1930. Phenotypical variation in body and cell size of Drosophila melanogaster. Biol Bull. 58:85–103.

Amoyle M, Hillion KH, Margolis SR, Bach EA. 2016. Somatic stem cell differentiation is regulated by P3K/Tor signaling in response to local cues. Development. 143:3914–3925.

Amoyle M, Simons BD, Bach EA. 2014. Neutral competition of stem cells is skewed by proliferative changes downstream of Hh and Hpo. EMBO J. 33:2295–2313.
Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD. 1996. The Drosophila phosphoinositide 3-kinase Dp110 promotes cell growth. EMBO J. 15:6584–6594.

LaFever L, Drummond-Barbosa D. 2005. Direct control of germline stem cell division and cyst growth by neural insulin in Drosophila. Science. 309:1071–1073.

Lesperance DNA, Broderick NA. 2020. Meta-analysis of diets used in Drosophila microbiome research and introduction of the Drosophila Dietary Composition Calculator (DDCC). G3 (Bethesda). 10:2207–2211.

Liao S, Post S, Lehmann P, Veenstra JA, Tatar M, et al. 2020. Regulatory roles of Drosophila Insulin-Like Peptide 1 (DILP1) in metabolism differ in pupal and adult stages. Front Endocrinol. 11:180.

Linneweber GA, Jacobson J, Busch KE, Hudry B, Christov CP, et al. 2012. Nutrient/TOR-dependent regulation of RNA polymerase III controls tissue and organismal growth in Drosophila. EMBO J. 31:1916–1930.

Macotela Y, Boucher J, Tran TT, Kahn CR. 2009. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. Diabetes. 58:803–812.

Magwere T, Chapman T, Partridge L. 2004. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male Drosophila melanogaster. J Gerontol A Biol Sci Med Sci. 59:3–9.

Marshall L, Rideout EJ, Grewal SS. 2012. Nutrient/TOR-dependent regulation of RNA polymerase III controls tissue and organismal growth in Drosophila. EMBO J. 31:1916–1930.

Mauvais-Jarvis F. 2015. Sex differences in metabolic homeostasis, diabetes, and obesity. Biol Sex Differ. 6:14.

McLeod CJ, Wang L, Wong C, Jones DL. 2010. Stem cell dynamics in response to nutrient availability. Curr Biol. 20:2100–2105.

Meschi E, Léopold P, Delanoue R. 2019. An EGF-responsive neural circuit couples insulin secretion with nutrition in Drosophila. Dev Cell 48:76–86.e5

Millington JW, Chao C, Sun Z, Basner-Collins PJ, Brownrigg GP, et al. 2021. Female-specific upregulation of insulin pathway activity mediates the sex difference in Drosophila body size plasticity. eLife. 10: e58341.

Millington JW, Rideout EJ. 2018. Sex differences in Drosophila development and physiology. Curr Opin Physiol. 6:46–56.

Murillo-Maldonado JM, Sanchez-Chavez G, Salgado LM, Salceda R, Riesgo-Escovar JR. 2011. Drosophila insulin pathway mutants affect visual physiology and brain function besides growth, lipid, and carbohydrate metabolism. Diabetes. 60:1632–1636.

Musselman LP, Fink JL, Grant AR, Gatto JA, Tuthill BF, et al. 2017. A complex relationship between immunity and metabolism in Drosophila diet-induced insulin resistance. Mol Cell Biol. 38: e00259–17.

Musselman LP, Fink JL, Narzinski K, Ramachandran PV, Hathiramani SS, et al. 2011. A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. Dis Models Mech. 4:842–849.

Nässel DR, Vanden Broeck J. 2016. Insulin/IGF signaling in Drosophila and other insects: factors that regulate production, release and post-release action of the insulin-like peptides. Cell Mol Life Sci. 73:271–290.

Oh Y, Sih Lai JY, Mills HJ, Erdjument-Bromage H, Giammarinaro B, et al. 2019. A glucose-sensing neuron pair regulates insulin and glucagon in Drosophila. Nature. 574:559–564.

Okamoto N, Nakamori R, Muri T, Yamauchi Y, Masuda A, et al. 2013. A secreted decoy of InR antagonizes insulin/IGF signaling to restrict body growth in Drosophila. Genes Dev. 27:87–97.

Okamoto N, Yamanaka N, Yagi Y, Nishida Y, Kataoka H, et al. 2009. A fat body-derived IGF-like peptide regulates postfeeding growth in Drosophila. Dev Cell 17:885–891.

Pasco MY, Léopold P. 2012. High sugar-induced insulin resistance in Drosophila relies on the lipocalin neural lazzarilla. PLoS One. 7: e36583.

Pitnick S, Markow TA, Spicer GS. 1995. Delayed male maturity is a cost of producing large sperm in Drosophila. Proc Natl Acad Sci USA. 92:10614–10618.

Politoove RM, Jacobs AR, Haft CR, Xu P, Taylor SI. 2000. Characterization of Drosophila insulin receptor substrate. J Biol Chem. 275:23346–23354.

Post S, Karashchuk G, Wade JD, Sajid W, Meysts PD, et al. 2018. Insulin-like peptides DILP2 and DILP5 differentially stimulate cell signaling and glycogen phosphorylase to regulate longevity. Front Endocrinol. 9:245.

Post S, Liao S, Yamamoto R, Veenstra JA, Nässel DR, et al. 2019. Drosophila insulin-like peptide Dilp1 increases lifespan and glucagon-like Akh expression epistatic to Dilp2. Aging Cell. 18: e12863.

Puig O, Marr MT, Ruft M, Tjian R. 2003. Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. 17:2006–2020.

Regan JC, Khericha M, Dobson AJ, Bolukbasi E, Rattanavirotkul N, et al. 2016. Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. eLife. 5:e10956.

Rideout EJ, Marshall L, Grewal SS. 2012. Drosophila RNA Polymerase III repressor Maf1 controls body size and developmental timing by modulating trNA Met synthesis and systemic insulin signaling. Proc Natl Acad Sci USA. 109:1139–1144.

Rideout EJ, Narisaiya MS, Grewal SS. 2015. The sex determination gene transformer regulates male-female differences in Drosophila body size. PLoS Genet. 11:e1005683.

Rintelen F, Stocker H, Thomas G, Hafen E. 2001. PDK1 regulates growth through Akt and S6K in Drosophila. Proc Natl Acad Sci USA. 98:15020–15025.

Roth SW, Bitterman MD, Birnbaum MJ, Bland ML. 2018. Innate immune signaling in Drosophila blocks insulin signaling by uncoupling PI(3,4,5)P3 production and Akt activation. Cell Rep. 22: 2550–2556.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, et al. 2017. ImageJ2. ImageJ for the next generation of scientific image data. BMC Bioinformatics. 18:529.

Rulifson EJ, Kim SK, Nusse R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science. 296: 1118–1120.

Sanaki Y, Nagata R, Kizawa D, Léopold P, Igaki T. 2020. Hyperinsulinemia drives epithelial tumorigenesis by abrogating cell competition. Dev Cell. 53:379–389 e5.

Sarraf-Zadeh L, Christen S, Sauer U, Cognigni P, Miguel-Aliaga I, et al. 2013. Local requirement of the Drosophila insulin binding protein imp-L2 in coordinating developmental progression with nutritional conditions. Dev Biol. 381:97–106.

Sawala A, Gould AP. 2017. The sex of specific neurons controls female body growth in Drosophila. PLoS Biol. 15:e2002252.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, et al. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682.
Selman C, Lingard S, Choudhury AI, Batterham RL, Claret M, et al. 2008. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. FASEB J. 22: 807–818.

Semaniuk UV, Gospodaryov DV, Feden’ko KM, Yurkevych IS, Vaiserman AM, et al. 2018. Insulin-like peptides regulate feeding preference and metabolism in Drosophila. Front Physiol. 9:1083.

Singleton AW, Das J, Vinicius L, Stern DL. 2005. The temporal requirements for insulin signaling during development in Drosophila. PLoS Biol. 3:e289.

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

Slaidina M, Delanoue R, Grönke S, Partridge L, Léopold P. 2009. A Drosophila insulin-like peptide promotes growth during non-feeding states. Dev Cell. 17:874–884.

Stafford JW, Lynd KM, Jung AY, Gordon MD. 2012. Integration of taste and calorie sensing in Drosophila. J Neurosci. 32:14767–14774.

Stocker H, Andjelkovic M, Oldham S, Laffargue M, Wymann MP, et al. 2002. Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. Science. 295: 2088–2091.

Suzawa M, Muhammad NM, Joseph BS, Bland ML. 2019. The toll signaling pathway targets the insulin-like peptide Dilp6 to inhibit growth in Drosophila. Cell Rep. 28:1439–1446.e5.

Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, et al. 2001. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science. 292:107–110.

Telegman AA. 2010. Molecular mechanisms of metabolic regulation by insulin in Drosophila. Biochem J. 425:13–26.