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Original Research

Glucose Transporter 1 (GLUT1) Functions in the *Drosophila* Intestine to Regulate Feeding and Nutrient Storage

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Abstract

Background: Obesity is a major global health issue driven by an imbalance between energy intake and expenditure, with triglyceride (TAG) storage as a key factor. Recent research has identified the gene *Glut1*, which encodes a glucose transporter in *Drosophila*, as a prospective regulator of obesity and TAG metabolism. However, the tissue-specific role of GLUT1 in coordinating carbohydrate and lipid metabolism remains unknown. Thus, this study focuses on the role of GLUT1 in *Drosophila* intestinal cells, which are central regulators of dietary sugar and lipid uptake. **Methods:** Here, we used RNA interference (RNAi) to specifically target *Glut1* in the intestine and measured glycogen and TAG storage in whole animals and in isolated intestines. **Results:** Intestinal knockdown of *Glut1* resulted in decreased glycogen levels in whole animals while intestinal glycogen levels remained unchanged. In addition, reducing *Glut1* in the fly intestine led to reduced food consumption, which may explain the decreased glycogen storage. We also observed that TAGs accumulated in *Glut1* RNAi intestines, possibly due to reduced expression of the apolipoprotein-like lipid-transport gene *microsomal TAG transfer protein* (*mtp*). **Conclusions:** These results indicate a role for GLUT1 in the fly intestine in regulating metabolic homeostasis and increase our knowledge about tissue-specific regulation of nutrient storage and metabolism.

Keywords: *Drosophila*; intestines; facilitative glucose transport proteins; glycogen; triglycerides; apolipoproteins

1. Introduction

Obesity, a globally recognized chronic disease associated with increased mortality and morbidity, remains a growing worldwide concern and often increases the risk of cardiovascular and metabolic diseases [1]. Approximately 60–70% of individuals diagnosed with obesity develop dyslipidemia, characterized by elevated very-low-density lipoprotein (VLDL) levels, low high-density lipoprotein (HDL) levels, and significantly elevated triglyceride (TAG) levels [2]. In addition, the accumulation of TAGs in adipose tissue contributes to excess weight in obese individuals [3]. Dyslipidemia is associated with a range of other cardiovascular conditions, including an increased risk of coronary artery disease (CAD) and acute coronary syndrome (ACS) [4]. Obesity is a multifaceted disease with a complex, multivariable etiology, including genetics, diet, environmental factors, and more [5,6]. Despite the rapid worldwide rise in obesity rates, decades of research, and therapeutic advances, the mechanisms through which genetic and/or environmental factors influence obesity remain poorly understood.

Studying obesity-related genes in humans is challenging. The fruit fly, *Drosophila melanogaster*, is an excellent model organism for studying the genetic control of fat storage because mammalian and *Drosophila* genomes are well conserved and many genetic tools are available [7]. Additionally, fruit flies have repeatedly been recognized as a useful model organism due to the presence and similarity of organs, tissues, and systems involved in human obesity [8,9]. Prior studies using the *Drosophila* system have identified hundreds of genes that may be involved in obesity. These genes were discovered through a variety of genetic screens performed in cultured cells, larvae, and adult flies, in which individual genes were systematically altered using diverse genetic manipulation tools [10,11,12]. In a single screen, fruit fly larvae with mutations in different genes were submerged in a sucrose solution to determine their buoyancy, which was directly correlated with higher lipid levels [13]. From this screen, 66 genes were identified as potentially important in obesity. Among those genes was *Glut1*, which encodes a transmembrane protein that regulates glucose transport across the plasma membrane. Earlier findings from our group showed that *Glut1* in fly fat and neuronal tissues plays a role in facilitating TAG and glycogen metabolism [14,15,16]. In fat tissue, targeting RNAi to *Glut1* leads to decreased storage of TAG and glycogen in whole flies.

Additionally, expression of *fatty acid synthase 1* (*FASN1*), an important lipid-synthesis enzyme, was blunted in flies with *Glut1* specifically decreased in fat tissue [14]. In neuronal tissue, targeting *Glut1* with RNAi across different neuronal populations likewise reduced organismal glycogen and TAG levels. Furthermore, expression of *Drosophila melanogaster insulin-like peptides* (*Ilps*), specifically *Ilp3* and *Ilp5*, in the brain was decreased in these flies [15]. While the function of GLUT1 in fly fat

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tissue and brain has been characterized, it remains unclear whether GLUT1 functions in other metabolic tissues, such as the *Drosophila* intestine (also referred to as the gut), to regulate TAG and/or glycogen storage. Given that the gut is the entry point for dietary lipids and carbohydrates and a hub of nutrient absorption, this organ is particularly important in the study of obesity.

This study used *Glut1* RNAi to reduce *Glut1* levels in the *Drosophila* gut and examined the effects on carbohydrate and lipid storage in whole animals. In addition, glucose, glycogen, and TAG storage were measured in isolated intestines from flies with gut-specific *Glut1* RNAi, and the expression of key lipid metabolic enzyme genes was assessed in these intestines. Overall, the goal of this study is to improve understanding of the role of glucose transporters in the *Drosophila* intestine in regulating intestinal and organismal glycogen and lipid metabolism.

2. Materials and Methods

2.1 Drosophila Genetics

The following fly stocks from the Bloomington *Drosophila* Stock Center (BL) were used in this study: *y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS02152}attP40* (BL# 40904, referred to as *UAS-Glut1* RNAi here); *y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=VALIUM20-EGFP.RNAi.1}attP40* (BL# 41555, referred to as *UAS-EGFP* RNAi here); *y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF03060}attP2* (BL# 28645, referred to as *UAS-Glut1* RNAi 2 here); *y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF01355}attP2* (BL# 31603, referred to as *UAS-Luc* RNAi here); and *w[1118]; P{w[+mC]=mex1-Gal4.2.1}10-8* (BL#91368, referred to as *Mex-Gal4* here). Flies were grown on standard cornmeal-yeast medium (9 g *Drosophila* agar (Genesee Scientific, San Diego, CA, USA), 100 mL Karo Lite Corn Syrup, 65 g cornmeal, 40 g sucrose, and 25 g whole yeast in 1.25 L water). *FlyBase* (release FB2026_01) was used to obtain information on phenotypes, function, stocks, gene expression, and sequences [17].

To decrease *Glut1* expression specifically in the fly intestine, a *Drosophila* midgut-specific driver, *Mex-Gal4* [18], was used to drive two distinct RNAi lines targeting *Glut1*. Virgin female Gal4 driver flies were crossed with male UAS responder flies and reared at 25 °C under a 12h:12h light:dark cycle. Approximately 1-week-old mated *Mex-Gal4>Glut1 RNAi* female flies were used in each experiment and compared with the appropriate genetic control flies of the same age (*Mex-Gal4>EGFP* RNAi or *Mex-Gal4>Luc* RNAi). No animals were excluded from this study and confounders were not explicitly controlled. The identities of the different groups were known by all researchers throughout each experiment.

2.2 Metabolite Measurements

Storage of TAGs, glycogen, and glucose was measured in whole flies and in isolated intestines. To measure these metabolites in whole flies, two adult female flies, approximately one week old, were homogenized by sonication using a Q55 Qsonica sonicator (Qsonica, Newtown, CT, USA) in lysis buffer (140 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.1% Triton-X, and 1× EDTA-free protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA)). Total protein concentration was measured using the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. TAG content was quantified with the Infinity Triglyceride Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total glucose concentration was determined by incubating homogenates with 8 mg/mL amyloglucosidase (Sigma-Aldrich, St. Louis, MO, USA) at pH 5.0 and 37 °C for 2 hours, followed by incubation with Glucose Oxidase Reagent (Pointe Scientific, Canton, MI, USA) as per the manufacturer's instructions. Free glucose was measured using the same Glucose Oxidase Reagent (Pointe Scientific, Canton, MI, USA) on homogenates without amyloglucosidase treatment. Glycogen concentration was calculated as the difference between total and free glucose values. TAG, glycogen, and free glucose concentrations were normalized by dividing by the total protein concentration. Each group of two adult female flies was considered a single sample for these experiments.

To measure intestinal TAG, glycogen, and glucose, intestines from approximately 20 female flies were dissected and homogenized by sonication in lysis buffer as described above. TAG, glycogen, and glucose concentrations were measured as described above and normalized to the number of intestines. Each group of approximately 20 intestines was considered a single sample for these experiments.

2.3 Feeding Assay

Food consumption was measured using a capillary feeding (CAFE) assay as previously described [19]. Briefly, groups of three approximately one-week-old adult female flies were placed in *Drosophila* vials containing 1% agar. Drummond capillary tubes (Thermo Fisher Scientific, Waltham, MA, USA) containing approximately 5 µL of 5% sucrose dyed with food coloring were used to measure food consumption at 25 °C for 24 hours. Vials without flies served as evaporation controls.

2.4 RNA Isolation and qPCR

RNA was isolated from intestines dissected from 20–30 females using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) per the manufacturer's instructions. Briefly, nucleic acids were extracted with chloroform, precipitated with isopropanol, pelleted, and washed with 70% ethanol. Pellets were air-dried and resuspended in nuclease-free water. In total, 5 µg of RNA was DNase-treated using the DNA-Free kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Next, 0.25 µg of DNase-treated RNA

Table 1. Primer sequences used in this study for qPCR.

Gene name and Flybase accession number	Forward sequence (5′→ 3′)	Reverse sequence (5′→ 3′)	Primer efficiency (%); product size
Ribosomal protein L32 (*Rp49*); FBgn0002626	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG	90; 141 bp
Glucose transporter 1 (*Glut1*); FBgn0264574	CCAACTTTGTGGTCGGCATTG	GGCCAGGAATACGCTGAACG	101; 85 bp
Fatty acid synthase 1 (*FASN1*); FBgn0283427	CTGGCTGAGCAAGATTGTGTG	TCGCACAACCAGAGCGTAGTA	90; 213 bp
Acetyl-CoA carboxylase (*Acc*); FBgn0033246	AGATGCAGAACGATGTCCGC	CTCTTTGTGAAGCAGCTCCG	85; 490 bp
Withered (*whd*); FBgn0261862	GCAAGTGCAAATTGAGGAAA	AAGTGCTCCTCACCTTCCAC	87; 62 bp
Brummer (*bmm*); FBgn0036449	ACGTGATCATCTCGGAGTTTG	ATGGTGTTCTCGTCCAGAATG	88; 166 bp
Apolipophorin (*apolpp*); FBgn0087002	ATCGGCTCAACACAAAAACC	AGGCAAAAGCGATCTCAAAA	90; 189 bp
Apolipoprotein lipid transfer particle (*apoltp*); FBgn0032136	GTTCGAGGTGAGTGGTTGGT	AGCTGCGTCTCATTGGAGAT	93; 117 bp
Microsomal triacylglycerol transfer protein (*mtp*); FBgn0266369	GTGGGAAGCTTCGTGAAGAG	AAAACGCGATACCATTCGAG	99; 128 bp

was reverse-transcribed into cDNA using the qScript Ultra cDNA Supermix (QuantaBio, Beverly, MA, USA) per the manufacturer's instructions. Gene expression levels were measured by preparing 25 µL qPCR reaction mixes containing 1 µL of cDNA, 2× PerfeCta SYBR Green Master Mix (QuantaBio, Beverly, MA, USA), and gene-specific primers (200–300 nM each, with annealing temperatures of 60 °C for all primer pairs). The qPCR amplification was performed under the following cycling conditions: initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of: 95 °C for 30 seconds, 60 °C for 1 minute, and 72 °C for 30 seconds, with a melt curve. Relative expression of *FASN1*, *whd*, *Acc*, *bmm*, *apolpp*, *apoltp*, and *mtp* was quantified and normalized to *rp49* expression. See Table 1 for a full list of primer sequences, primer efficiencies, and PCR product sizes.

2.5 Statistics

In all experiments, means were compared between the *Mex-Gal4>EGFP* RNAi controls and *Mex-Gal4>Glut1* RNAi experimental group or the *Mex-Gal4>Luc* RNAi control group and the *Mex-Gal4>Glut1* RNAi 2 experimental group using a two-tailed *t*-test in Excel. A p-value < 0.05 was considered statistically significant and an * over the experimental group for each comparison was used to depict statistical significance.

3. Results

To determine the metabolic effects of intestinal GLUT1 on the whole organism, glycogen, TAGs, and free glucose, all normalized to total protein, were measured in two *Mex-Gal4>Glut1* RNAi female fly lines and compared with appropriate genetic-background controls. Notably, organismal glycogen levels were decreased in both *Glut1* RNAi lines tested (Fig. 1A), while organismal TAG levels were decreased only in one line (Fig. 1B), and organismal free glucose was unchanged in both lines (Fig. 1C). Interestingly, flies with decreased *Glut1* levels from both RNAi lines consumed less food (Fig. 1D), which may contribute to reduced glycogen stores. Since the first *Glut1* RNAi line exhibited a stronger nutrient storage phenotype, we assessed the ability of each RNAi line to reduce *Glut1* levels in the fly intestine. To assess knockdown efficiency, *Glut1* mRNA levels were measured in RNA isolated from dissected whole intestines of each *Glut1* RNAi line crossed to the *Mex-Gal4* driver and compared with the appropriate genetic-background control. Consistent with the stronger nutrient-storage phenotype, the first *Glut1* RNAi line led to a substantial decrease in *Glut1* mRNA levels. In contrast, the second *Glut1* RNAi line showed a much smaller decrease in *Glut1* mRNA that did not reach statistical significance (Fig. 1E). This result is consistent with the first *Glut1* RNAi line targeting more *Glut1* gene isoforms than the second (data not shown). Since nutrient storage and *Glut1* knockdown efficiency were higher in the first *Glut1* RNAi line, we continued our analysis with those flies.

To further understand the observed reduction in nutrient storage in *Glut1* RNAi flies, intestinal levels of glycogen, TAGs, and glucose, normalized by the number of intestines in each sample, were measured in female *Mex-Gal4>Glut1* RNAi flies and compared with those in control flies (Fig. 2). Intestinal TAG levels were increased in *Mex-Gal4>Glut1* RNAi flies, whereas intestinal glycogen storage and glucose levels were unchanged. This finding suggests that decreasing *Glut1* in the intestine leads to TAG accumulation in that tissue.

To better understand this TAG accumulation phenotype in the intestines of *Glut1* RNAi flies, the expression

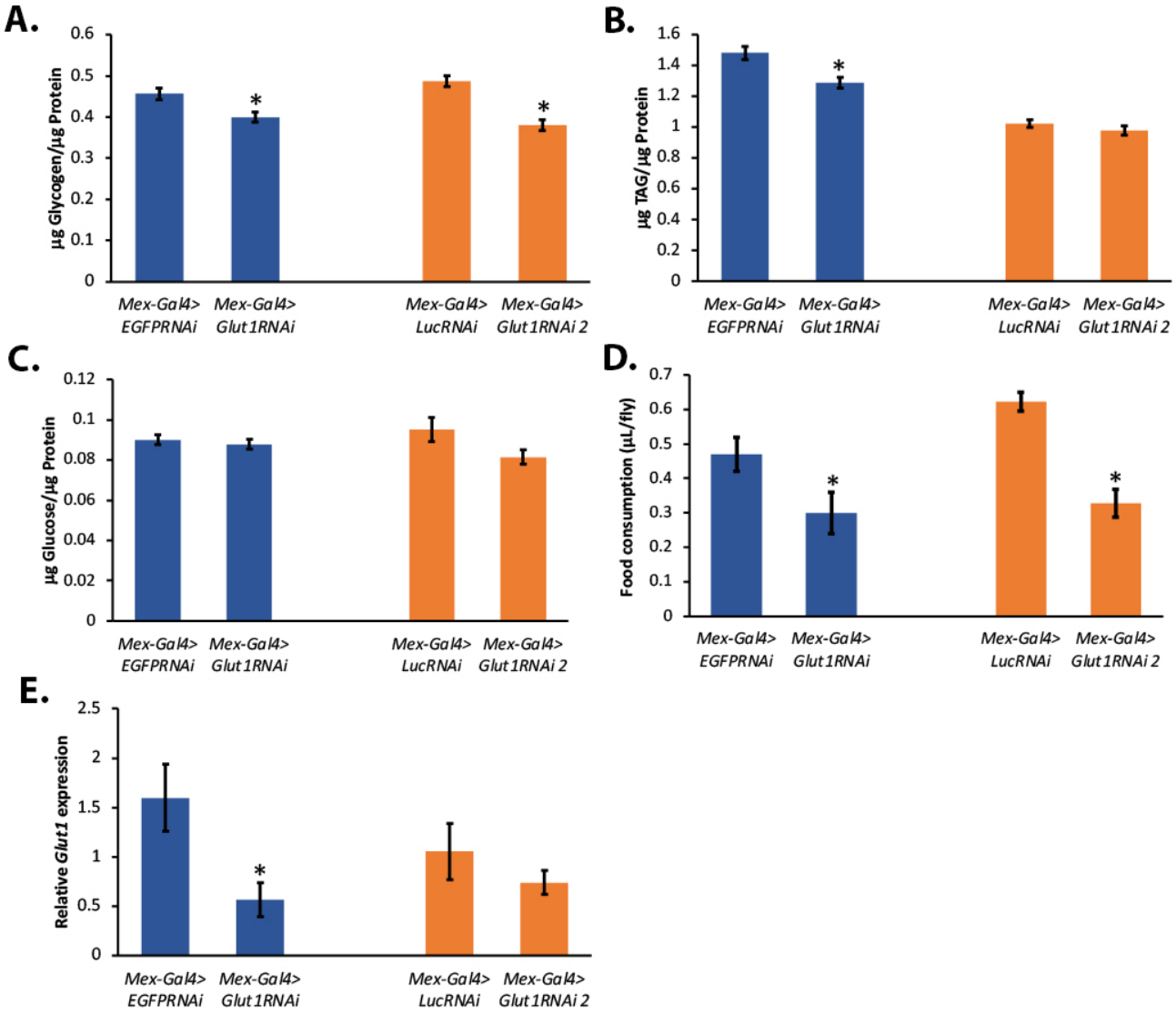


Fig. 1. RNA interference (RNAi) targeting the *Glut1* gene in *Drosophila* intestines reduces whole-organism glycogen levels in female flies. (A) Glycogen normalized to protein content was measured in whole-body samples from one-week-old female *Mex-Gal4>Glut1* RNAi (n = 30) and *Mex-Gal4>Glut1* RNAi 2 (n = 42) flies and compared to *Mex-Gal4>EGFP* RNAi (n = 36) and *Mex-Gal4>Luc* RNAi (n = 27) controls, respectively. Bars indicate average normalized glycogen/protein concentration, +/− standard error. (B) Triglyceride (TAG) normalized to protein content was measured in whole-body samples from one-week-old female *Mex-Gal4>Glut1* RNAi (n = 30) and *Mex-Gal4>Glut1* RNAi 2 (n = 42) flies and compared to *Mex-Gal4>EGFP* RNAi (n = 36) and *Mex-Gal4>Luc* RNAi (n = 27) controls, respectively. Bars indicate average normalized TAG/protein concentration, +/− standard error. (C) Glucose normalized to protein content was measured in whole-body samples from one-week-old female *Mex-Gal4>Glut1* RNAi (n = 30) and *Mex-Gal4>Glut1* RNAi 2 (n = 42) flies and compared to *Mex-Gal4>EGFP* RNAi (n = 36) and *Mex-Gal4>Luc* RNAi (n = 27) controls, respectively. Bars indicate average normalized glucose/protein concentration, +/− standard error. (D) Relative feeding was measured in *Mex-Gal4>Glut1* RNAi (n = 19 vials) and *Mex-Gal4>Glut1* RNAi 2 (n = 30 vials) female flies and compared to *Mex-Gal4>EGFP* RNAi (n = 23 vials) and *Mex-Gal4>Luc* RNAi (n = 17 vials) controls, respectively. Bars indicate average normalized food consumed per fly, +/− standard error. (E) Intestinal *Glut1* normalized to *rp49* was measured in RNA from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) and *Mex-Gal4>Glut1* RNAi 2 (n = 8) female flies and compared to *Mex-Gal4>EGFP* RNAi (n = 8) and *Mex-Gal4>Luc* RNAi (n = 4) controls, respectively. Bars indicate average normalized relative expression, +/− standard error. **p*-value < 0.05 as determined by a Student's *t*-test comparing *Mex-Gal4>Glut1* RNAi to *Mex-Gal4>EGFP* RNAi controls and *Mex-Gal4>Glut1* RNAi 2 to *Mex-Gal4>Luc* RNAi controls.

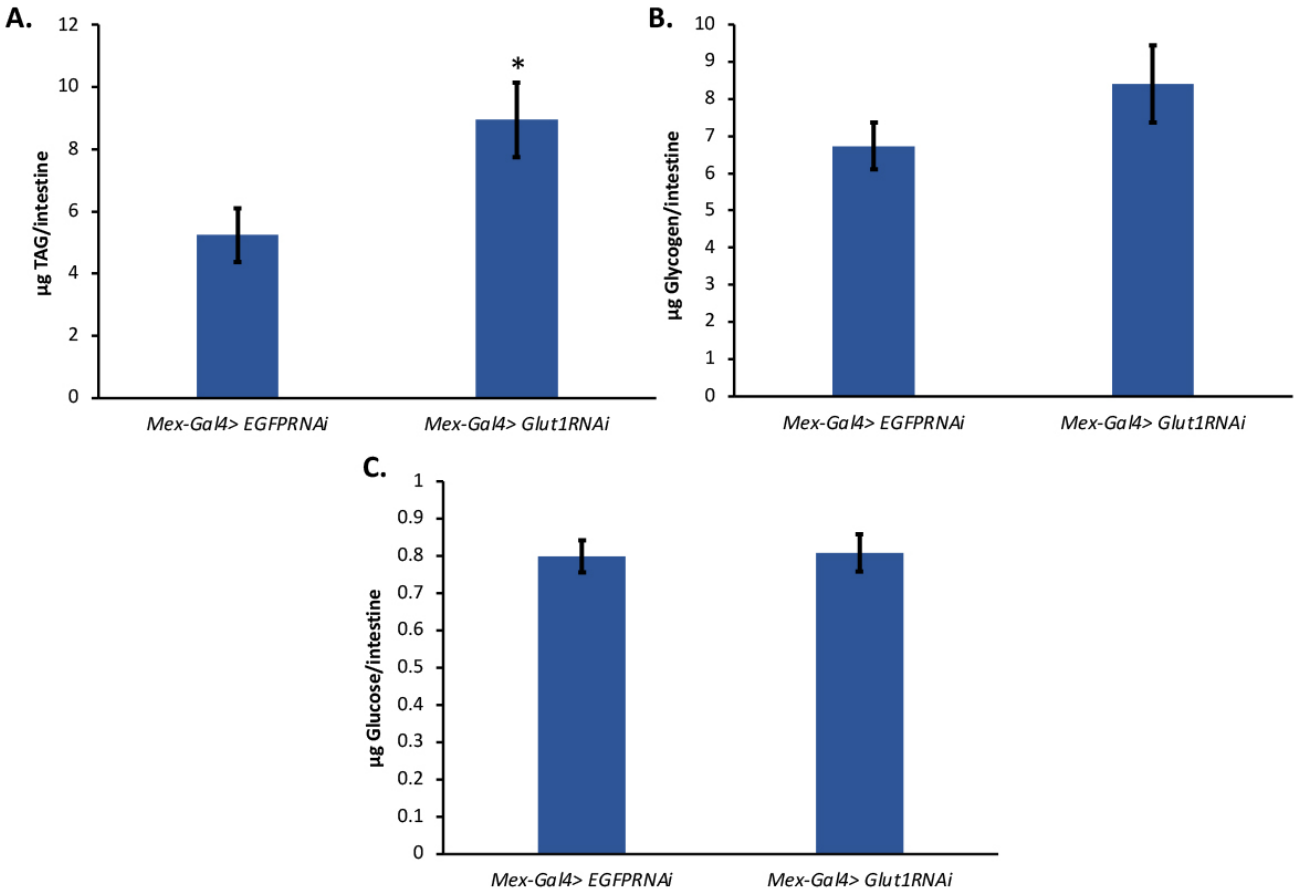


Fig. 2. Decreasing *Glut1* in the *Drosophila* intestine leads to accumulation of intestinal TAG but does not affect glycogen or free glucose levels. (A) Intestinal TAG concentrations were normalized to the number of intestines in *Mex-Gal4>Glut1* RNAi (n = 20) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 23) controls. Bars indicate the average normalized intestinal TAG concentration per intestine, +/− standard error. (B) Intestinal glycogen concentrations were normalized to the number of intestines in *Mex-Gal4>Glut1* RNAi (n = 20) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 23) controls. Bars indicate the average normalized intestinal glycogen concentration per intestine, +/− standard error. (C) Intestinal glucose concentrations were normalized to the number of intestines in *Mex-Gal4>Glut1* RNAi (n = 20) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 23) controls. Bars indicate the average normalized intestinal glucose concentration per intestine, +/− standard error. **p*-value < 0.05, determined by a Student's *t*-test comparing *Mex-Gal4>Glut1* RNAi to *Mex-Gal4>EGFP* RNAi controls.

of several major lipid metabolism enzymes (*FASN1*, *Acc*, *whd*, and *bmm*) was assessed. *FASN1* is a multi-domain enzyme responsible for nearly all fatty acid synthesis; *Acc* encodes a lipid synthesis enzyme that converts acetyl-CoA to malonyl-CoA, a substrate for fatty acid synthesis. *whd* is the fly homolog of *CPT1*, which encodes the rate-limiting enzyme of beta-oxidation, carnitine palmitoyltransferase 1, and *bmm* is the fly homolog of adipose TAG lipase, an important lipolysis enzyme responsible for converting TAGs into diglycerides [8]. Interestingly, while intestinal *FASN1* and *whd* levels were unchanged (Fig. 3A,D), intestinal *Acc* levels were decreased (Fig. 3B), and *bmm* lipase gene levels were increased (Fig. 3C).

Since the increased storage of TAGs in intestines subjected to *Glut1* RNAi did not appear to result from increased fatty acid synthesis or decreased TAG breakdown, we hypothesized that the intestinal accumulation of TAGs in *Glut1* RNAi flies is due to altered transport of TAGs out of enterocytes. In *Drosophila*, dietary lipids are packaged and exported from enterocytes to other tissues and cells via a series of ApoB family proteins, helping maintain lipid homeostasis throughout the organism [20]. To test this hypothesis, we measured the mRNA levels of apolipophorin lipid transfer particle (LTP) (*apoltp*), apolipophorin (*apolpp*), and microsomal TAG transfer protein (*mtp*), three genes involved in lipoprotein-mediated lipid export from intestinal cells. The product of the *apoltp* gene facilitates the transfer of lipids between the gut, circulating lipophorin, and peripheral tissues. In contrast, the product of *apolpp* is the major lipoprotein responsible for lipid transport to the hemolymph [20]. *mtp* is required for lipoprotein assembly and lipid loading in enterocytes [20,21]. In *Glut1* RNAi

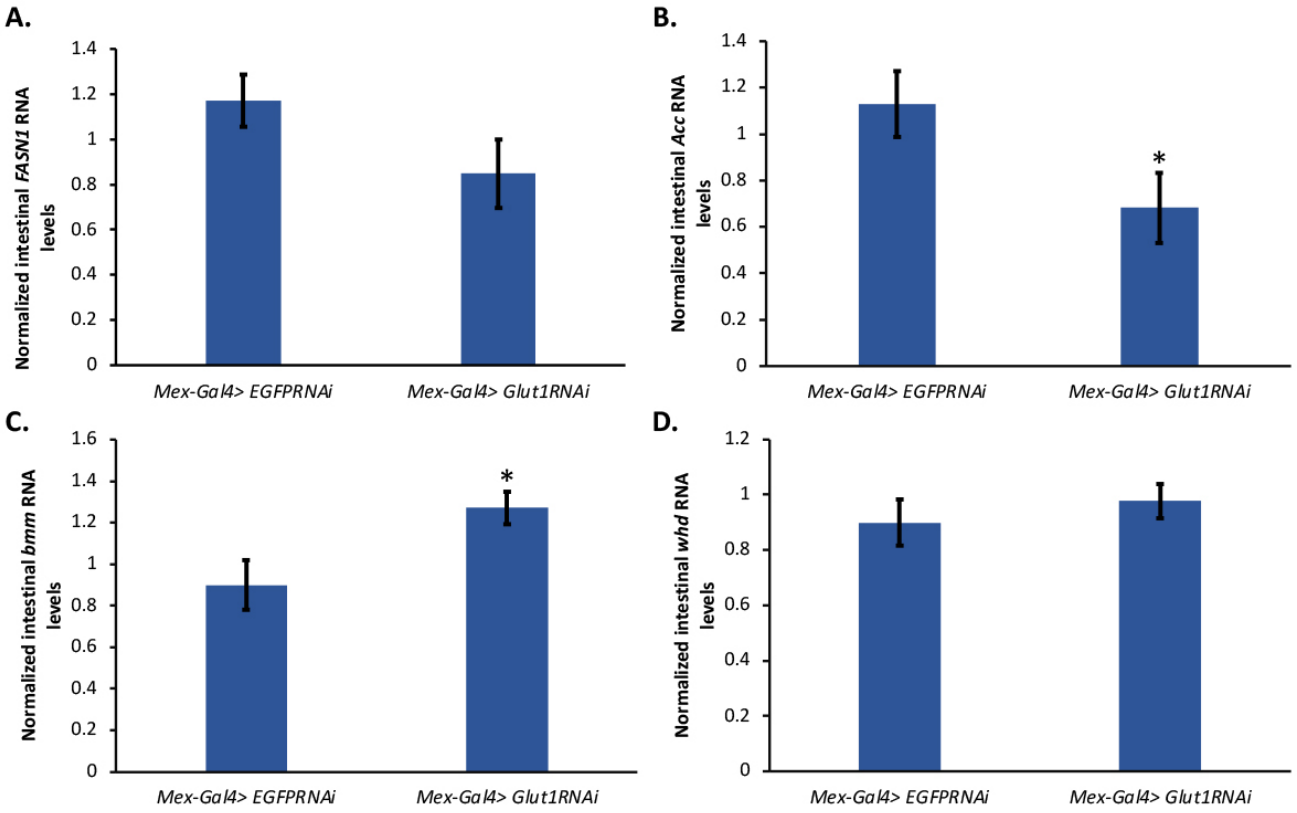


Fig. 3. Targeting RNAi against the *Glut1* gene in *Drosophila* intestines leads to decreased intestinal *Acc* levels and increased intestinal *bmm* levels. (A) *FASN1*, normalized to *rp49*, was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate the mean normalized relative *FASN1* expression, +/− standard error. (B) *Acc*, normalized to *rp49*, was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate the mean normalized relative *Acc* expression, +/− standard error. (C) *bmm*, normalized to *rp49*, was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate the mean normalized relative *whd* expression, +/− standard error. (D) *whd*, normalized to *rp49*, was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate the mean normalized relative *bmm* expression, +/− standard error. **p*-value < 0.05, determined by a Student's *t*-test comparing *Mex-Gal4>Glut1* RNAi to *Mex-Gal4>EGFP* RNAi controls.

flies, all three genes showed a trend toward reduced expression; however, only *mtp* showed *a* statistically significant decrease (Fig. 4).

4. Discussion

This study explored the role of intestinal *Glut1* in nutrient storage in *Drosophila*. Organismal glycogen levels were reduced in intestine-specific *Glut1* RNAi flies, whereas TAG and glucose levels showed no consistent changes. These findings suggest that intestinal glucose uptake via glucose transporters may play an important role in regulating systemic nutrient storage. In animals, two major types of glucose transporters exist in the intestine: the GLUT family and the SGLT family. At least two GLUT family genes have been identified in *Drosophila melanogaster*: *Glut1* and *Glut3* [22]. Recent findings have also identified *dSLC5A5*, a member of the SGLT family, in *Drosophila melanogaster*. The *dSLC5A5* gene is the putative ortholog of human SLC5A1, a sodium-dependent symporter in mammalian cells [23,24]. In mammals, glucose is absorbed into intestinal cells through a transporter on the apical side and then transported out of the cell into the bloodstream through another transporter on the opposite (basolateral) side [25]. While the function of intestinal glucose transporters is well characterized in mammalian cells, little is known about their precise localization and/or function in *Drosophila* intestinal cells, warranting further investigation [26]. If the function of SGLT/GLUT transporters in *Drosophila* is similar to that of mammalian cells, we would expect that when *Glut1* RNAi decreases *Glut1* levels, less glucose would be absorbed into enterocytes and ultimately the hemolymph, providing less substrate for glycogen synthesis in fat and muscle cells, which would explain the decrease in organismal glycogen observed in intestine-

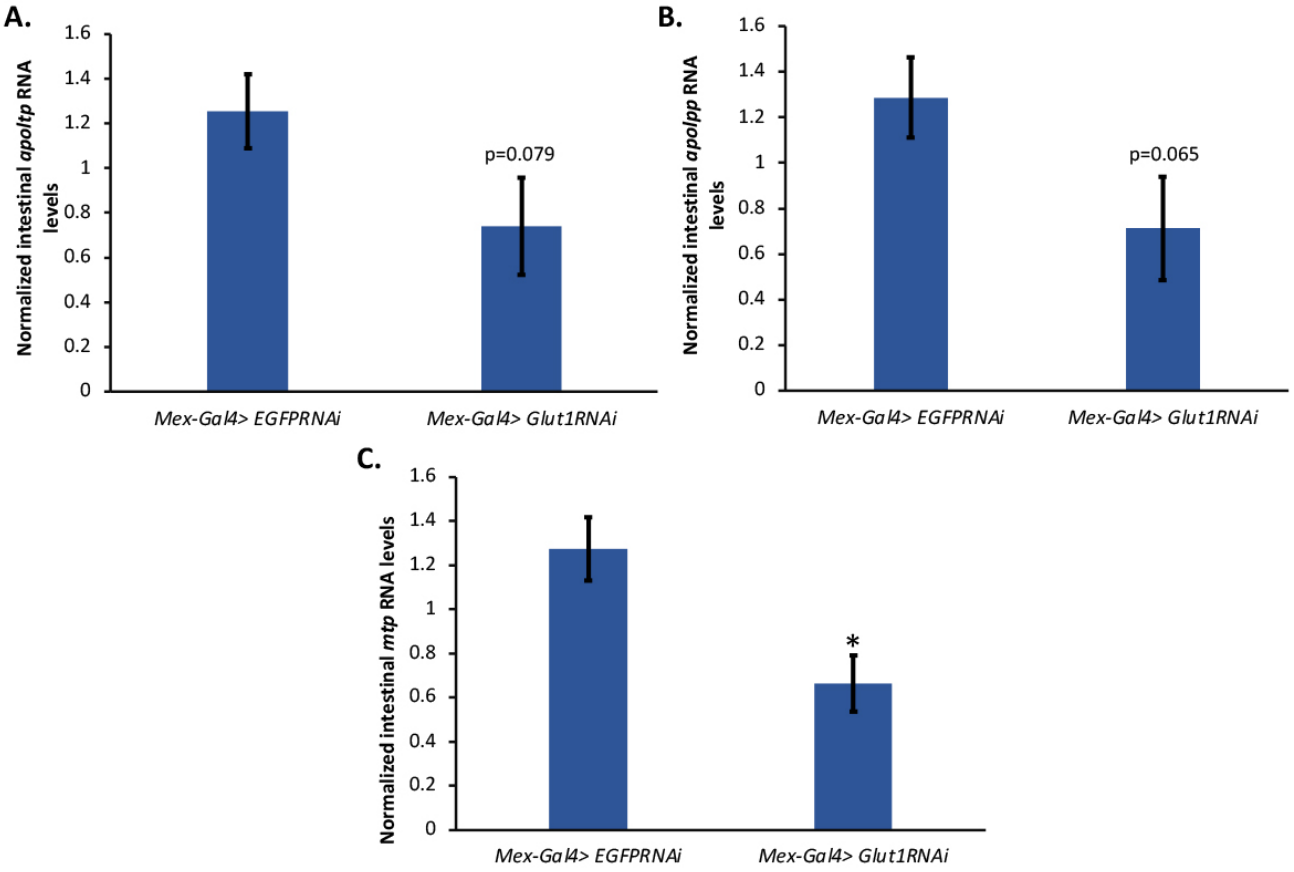


Fig. 4. Targeting RNAi against the *Glut1* gene in *Drosophila* intestines leads to decreased intestinal *mtp* levels and a trend toward reduced expression of *apoltp* and *apolpp*. (A) *apoltp* normalized to *rp49* was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate average normalized relative *apoltp* expression, +/− standard error. (B) *apolpp* normalized to *rp49* was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate average normalized relative *apolpp* expression, +/− standard error. (C) *mtp* normalized to *rp49* was measured in RNA purified from the intestines of *Mex-Gal4>Glut1* RNAi (n = 8) female flies and compared with *Mex-Gal4>EGFP* RNAi (n = 8) controls. Bars indicate average normalized relative *mtp* expression, +/− standard error. **p*-value < 0.05, determined by a Student's *t*-test comparing *Mex-Gal4>Glut1* RNAi to *Mex-Gal4>EGFP* RNAi controls. For *apoltp* and *apolpp*, *p*-values are included in the figure.

specific *Glut1* RNAi flies. If the basolateral exit of glucose to the hemolymph is severely diminished in *Glut1* RNAi flies, glucose would accumulate in the enterocytes, and less would be available in the hemolymph, resulting in less glucose available for glycogen production. To test whether defects in glucose absorption could cause the low-glycogen phenotype, *dSLC5A5* levels can be specifically reduced in the fly intestine to block glucose absorption, and then glycogen levels measured. If glucose cannot be absorbed by intestinal cells, we would expect glycogen levels throughout the organism to decrease, as we observed in flies administered *Glut1* RNAi.

Another potential cause of the decrease in glycogen storage observed in *Glut1* RNAi flies is reduced food consumption, a finding of this study. Flies eating less would provide less substrate for glycogen synthesis in fat and muscle cells, potentially explaining the decrease in organismal glycogen observed in intestine-specific *Glut1* RNAi flies. Interestingly, decreasing neuronal *Glut1* in *Drosophila* does not affect food consumption [15]; however, we show here that *Glut1* in *Drosophila* intestines plays a role in regulating food intake, suggesting tissue-specific functions of GLUT1 in feeding regulation. Intestinal cells use a variety of molecules to regulate food intake. Hormones secreted by enteroendocrine cells of the intestine have emerged as important mediators of food consumption regulation in both mammals and *Drosophila* [27]. While significant advances have been made in the classification of enteroendocrine cells and associated hormones in the *Drosophila* gut in recent years, many of the secretory mechanisms and the specific proteins involved in hormone sensing and secretion remain poorly understood. Since the *Mex-Gal4* driver used in this study expresses specifically in intestinal enterocytes and not enteroendocrine cells [18], GLUT1 may control nu-

trient sensing in enterocytes, which could indirectly regulate enteroendocrine hormones to control food intake and ultimately glycogen storage; however, additional experimentation is necessary to test this hypothesis.

We also observed TAG accumulation in the intestines of flies that underwent *Glut1* RNAi, but no effect on glycogen and glucose levels in these intestines. The *Drosophila* intestine is a major organ where food is digested, nutrients are absorbed, and energy substrates are stored [26]. However, in nature, fruit flies rarely consume diets rich in TAG and other fat molecules. Instead, the diets of these flies are rich in complex carbohydrates, and as a result, fruit flies convert sugars into TAGs in the fly midgut [20]. However, we observed a decrease in the expression of the gene encoding the fatty acid synthesis enzyme, *Acc*, and an increase in the expression of the gene encoding the TAG lipase *bmm*, suggesting that fatty acid synthesis may be diminished. Lipolysis increased in flies treated with *Glut1* RNAi, which is not consistent with the TAG accumulation phenotype in the intestines of these flies. Another possible explanation for the excess intestinal TAG storage phenotype is that *Glut1* RNAi flies may have a defect in lipid transport out of the intestine. In *Drosophila*, lipids are packaged into lipophorin proteins, a process mediated by the apolipoprotein LTP and MTP [20]. Decreased expression of each of these apoB-containing protein genes results in lipid droplet accumulation in the fly gut [20]. Consistent with this finding, we observed that *apoltp* and *apolpp* expression decreased and approached statistical significance, whereas a decrease in *mtp* expression was statistically significant in *Glut1* RNAi flies. This finding may suggest that reduced intestinal *Glut1* expression promotes TAG accumulation in the intestine, potentially through impaired lipoprotein assembly, thereby decreasing lipid export from enterocytes. However, how loss of *Glut1* in the fly intestine results in changes in the expression of these apoB-containing lipoproteins is unknown. Perhaps altered glucose transport in the intestine might signal a glucose-responsive transcription factor to block transcription of these lipid transport genes; further experimentation will be necessary to test this hypothesis.

5. Limitations

A limitation of this study is that we have shown that the expression of genes encoding proteins important for lipid metabolism and transport is altered by measuring mRNA levels of these genes. While mRNA levels of a given gene often correlate with protein levels or even protein activity, that is not always the case. Overall lipid metabolic flux and/or transport may be unchanged in *Glut1* RNAi intestines despite alterations in mRNA expression of genes encoding key lipid metabolic enzymes. Measuring the protein levels and/or activity of the specific enzymes important for lipid metabolism and transport that were shown to be differentially expressed in this study, such as *Acc*, *bmm*, and *mtp*, could help test this hypothesis. Another limitation of this study is that the second *Glut1* RNAi line was less effective at reducing *Glut1* mRNA levels than the first. Since the *Mex-Gal4* driver is expressed only in intestinal enterocytes [18], other cell types may dilute an otherwise mild *Glut1* knockdown in whole-intestine samples. However, since glycogen levels and feeding were decreased when both RNAi lines were used to target *Glut1*, this suggests that *Glut1* levels in enterocytes are being reduced by the second line, even though this decrease cannot be detected by qPCR of whole intestines. Further characterization of this second *Glut1* RNAi line will help us better understand the *Glut1* threshold in the intestine necessary to regulate nutrient storage in the intestine and throughout the fly.

6. Conclusions

In summary, this study focused on better characterizing the metabolic effects observed in *Drosophila* with intestine-specific reductions in the glucose transporter *Glut1*. Together, the data presented here identify a role for glucose transport in a single tissue in controlling organismal metabolism and behavior, thereby promoting physiological homeostasis. Glucose transporters in *Drosophila* and humans are highly conserved (in fruit flies, the GLUT1 transporter is homologous to human GLUT1–GLUT5 [28]), suggesting that what we learn here may also hold in higher organisms. For example, *Glut2* is expressed in several human cell types, including intestinal epithelial cells, where it functions as a glucose transporter, allowing glucose to move both into and out of enterocytes [29,30]. In addition, mice with a homozygous deletion of *Glut2* are hyperglycemic and have impaired insulin secretion, hallmarks of diabetes [31]. Another mammalian glucose transporter, *Glut4*, is expressed in the insulin-sensitive tissues of the heart, skeletal muscle, and adipose tissue and translocates to the plasma membrane in response to insulin, thereby regulating glucose uptake rates across different metabolic states [32]. Moreover, some human populations with type 2 diabetes had decreased *Glut4* gene expression or *Glut4* gene variants [33,34], suggesting a role for this glucose transporter in the pathogenesis of the disease. Therefore, it is possible that glucose transporters could act in the mammalian intestine to control hepatic and/or muscle glycogen storage as well as food consumption to regulate metabolic homeostasis, as observed here in flies. Expanding our knowledge of the tissue-specific control of energy storage and feeding behavior may deepen our understanding of the pathogenesis of metabolic diseases and potentially lead to the development of additional treatments.

Availability of Data and Materials

The datasets used and analyzed during this study are available from the corresponding author by request.

Author Contributions

Conceptualization, JRD; methodology, JRD; validation, VD, MRK; formal analysis, VD, MRK; investigation, JRD, VD and MRK; resources, JRD; writing—original draft preparation, VD; writing—review and editing, JRD, VD and MRK; visualization, VD; supervision, JRD; project administration, JRD; funding acquisition, JRD. All authors have read and agreed to the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study complied with ARRIVE 2.0 guidelines. Penn State does not require ethical approval for the use of invertebrates like *Drosophila*.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBS55008.

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