lipidology

MDPI

Article

The Role of the SR Protein 9G8 in the *Drosophila* Intestine to Regulate Lipid Metabolism

Roman Voskoboynikov and Justin R. DiAngelo *

Division of Science, Pennsylvania State University, Berks Campus, Reading, PA 19610, USA
* Correspondence: jrd5671@psu.edu

Abstract: Background/Objectives: Metabolic diseases in humans, such as obesity or type 2 diabetes, arise from defects in the body's ability to take in and store nutrients such as carbohydrates and triglycerides. Previous studies in the fruit fly, *Drosophila melanogaster*, have identified SR proteins, mRNA splicing factors that regulate splice-site selection, as regulating lipid storage in the fly fat body. However, whether SR proteins function in other tissues to regulate nutrient metabolism is not known. **Methods:** We focused on studying the role of SR proteins in intestines by decreasing their levels in the fly gut and measuring the concentrations of lipids and glycogen. **Results:** We further characterized the intestinal functions of *9G8*, an SR protein, which displayed an increase in organismal lipid levels when knocked down in the intestine but had less triglyceride storage in isolated intestines. Interestingly, decreasing *9G8* in the intestine resulted in increased intestinal expression of five fatty acid synthesis/elongation enzyme genes, as well as four triglyceride lipase genes, which may contribute to the triglyceride phenotypes we observed in *9G8-RNAi* flies. **Conclusions:** These data suggest that *9G8* regulates whole body and intestinal lipid homeostasis by altering the expression of lipid metabolic enzyme genes in the fly intestine.

Keywords: *Drosophila*; intestine; triglyceride; 9G8; mRNA splicing

Academic Editor: Nicola Ferri

Received: 5 December 2024
Revised: 28 December 2024
Accepted: 31 December 2024
Published: 2 January 2025

Citation: Voskoboynikov, R.; DiAngelo, J.R. The Role of the SR Protein 9G8 in the *Drosophila* Intestine to Regulate Lipid Metabolism. *Lipidology* **2025**, *2*, 1. https://doi.org/10.3390/lipidology2010001

1. Introduction

Many metabolic diseases, like obesity and type 2 diabetes, have been documented to be a result of excess triglyceride storage [1]. Despite decades of ongoing research, the prevalence of obesity has increased from 30.5% to 42.4% from 2000 to 2018 for an average American [2]. While excess caloric intake and a sedentary lifestyle are appreciated risk factors for developing obesity, genetic factors that regulate lipid storage and transport can also contribute to an individual's predisposition to weight gain [1]. Thus, understanding the genes involved in the synthesis, absorption, and storage of lipids will be helpful to allow us to better understand the pathogenesis of obesity and type 2 diabetes.

Drosophila melanogaster has been shown to be an excellent model system to study the genetic control of metabolism, having a short generation time (10–12 days) and over 60% of their genes being orthologous with humans [3]. A fly's food intake, body composition, locomotor activity, cognition, fertility, aging, and lifespan can be systematically measured with minimal effort and time [3]. *Drosophila* also contains several tissues like those involved in human obesity and associated metabolic diseases, such as the gut (intestines), oenocytes (liver), and the fat body (adipose tissue). *Drosophila* can develop an obesity-like state with its associated complications (hyperglycemia, reduced longevity, etc.) during caloric overload, like humans [4]. Together, the genetic and physiological similarities between

flies and humans make *Drosophila* an ideal system with which to study the genetic control of metabolism.

In previous genome-wide RNAi screens performed in adult flies and *Drosophila* cells, several candidate genes responsible for regulating lipid storage were identified [5–7]. One group of genes of particular interest included those that play a role in regulating RNA processing. One major step in RNA processing is mRNA splicing, a process where non-coding introns are removed from primary transcripts to join expressed sequences called exons to create mature mRNA. Various families of regulatory proteins bind to these sequences and enhance mRNA splicing by recruiting the spliceosome [8]. One important group of mRNA splicing regulatory proteins is the serine–arginine (SR) family of proteins, which has primarily been shown to regulate splice-site selection [9]. Eight SR proteins have been identified to regulate splicing in *Drosophila* [10]. Previous studies have shown that some SR proteins function in fat tissue to regulate lipid storage in flies. Specifically, decreasing *9G8*, *SF2*, and *RBP1* in fly adipose tissue using RNAi results in an increase in overall triglycerides stored in these flies [11,12]. In addition, decreasing *9G8* specifically in fat tissue results in altered expression and splicing of several lipid metabolic genes [13]. For example, the splicing of carnitine palmitoyltransferase 1 (*CPT1*), the gene responsible for producing an enzyme involved in the breakdown of lipids, was altered during *9G8* and *SF2* knockdown in the fat body such that CPT1 enzyme activity was decreased [11,12]. The splicing of the gene Zwischenferment (*Zw*), the fly homolog of G6PD in humans, was also significantly altered during *9G8* knockdown in the fly fat tissue [13]. While their role in regulating lipid storage has been studied in *Drosophila* fat tissue, whether SR proteins function in other metabolic tissues such as the fly intestine is still not known.

The *Drosophila* gut/intestine is the primary site for nutrient absorption, including that of triglycerides. In addition, the fly intestine serves as a site of fatty acid synthesis, working in tandem with the fat body to generate fatty acids and triglycerides that can be stored or used for energy [14,15]. However, the molecular control of lipid absorption, transport, and storage, and specifically the roles of SR proteins in regulating intestinal metabolic functions, are not well understood. Thus, the major goal of this study is to assess the intestine-specific functions of SR proteins to regulate carbohydrate and lipid processing and storage in *Drosophila*. To address this question, we measured the effect of decreasing each SR protein in the intestine on overall triglyceride and glycogen levels. To better understand how the SR protein 9G8 regulates nutrient storage and transport, RNA sequencing was performed on RNA isolated from intestines from flies with decreased *9G8*. Several lipases and genes involved in fatty acid elongation were identified as being upregulated through this analysis, and qPCR was performed to confirm these changes from the RNA sequencing experiments. These results will help us better understand the role of SR proteins in general and 9G8 in particular in regulating lipid storage and metabolism in the *Drosophila* intestine.

2. Materials and Methods

Fly Genetics: The following lines were used in this study (Bloomington (BL) and Vienna *Drosophila* Resource Center (VDRC) stock numbers are included in Table 1): *mex-Gal4*, *UAS-EGFP-RNAi-1*, *UAS-EGFP-RNAi-2*, *UAS-GFP-RNAi*, *UAS-SF2-RNAi*, *UAS-RBP1-RNAi*, *UAS-RBP1 like-RNAi*, *UAS-SRP54-RNAi*, *UAS-RSF1-RNAi*, *UAS-9G8-RNAi*, *UAS-B52-RNAi*, *UAS-SC35-RNAi*. The *UAS-EGFP-RNAi-1* line was used as the genetic background control for the *UAS-RBP1-RNAi* and *UAS-RBP1-like-RNAi* lines; the *UAS-EGFP-RNAi-2* line was used as the genetic background control for the *UAS-SF2-RNAi* and *UAS-SRP54-RNAi* lines; and the *UAS-GFP-RNAi* was used as the genetic background control for the remaining RNAi lines. Flies were grown at 25 °C on a 12 h:12 h light–dark cycle on a standard cornmeal-sugar yeast medium (9 g of Drosophila agar (Genesee Sci-

entific, Morrisville, NC, USA), 100 mL of Karo Lite corn syrup, 65 g of cornmeal, 40 g of sucrose, and 25 g of whole yeast in 1.25 L of water).

Table 1. *Drosophila* strains and respective stock numbers used in this study.

Fly Line	Genotype	Source
UAS-EGFP-RNAi-1	*y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = VALIUM20-EGFP.shRNA.1}attP40*	BL#41555
UAS-EGFP-RNAi-2	*y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.GLV21067}attP2*	BL#35702
UAS-GFP-RNAi	*w [1118]; P{w [+mC] = UAS-GFP.dsRNA.R}142*	BL#9330
UAS-SF2-RNAi	*y [1] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HM05199}attP2*	BL#29522
UAS-RBP1-RNAi	*y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HMC03902}attP40*	BL#55688
UAS-RBP1 like-RNAi	*y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HMS02820}attP40*	BL#44100
UAS-SRP54-RNAi	*y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HM05224}attP2*	BL#30533
UAS-RSF1-RNAi	*w [1118]; UAS-IR:Rsf1/TM3*	VDRC#22186
UAS-9G8-RNAi	*w [1118]; attB:UAS-IR:9G8*	VDRC#100226
UAS-B52-RNAi	*w [1118]; attB:UAS-IR:B52*	VDRC#101740
UAS-SC35-RNAi	*w [1118]; attB:UAS-IR:SC35*	VDRC#104978
Mex-Gal4	*w [1118]; P{w[+mC] = mex1-GAL4.2.1}10-8*	BL#91368

Triglyceride, Glycogen, and Protein Measurements: Triglyceride, protein, and carbohydrate measurements were made as described previously [11,12]. Briefly, pairs of one-week-old females or groups of 15 intestines extracted from approximately one-week-old females were homogenized in lysis buffer (140 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.1% Triton-X, and 1X complete EDTA-free protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA)). Triglycerides were measured using the Infinity Triglyceride Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and protein was measured using the BCA protein assay kit (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. To measure glycogen levels, homogenized samples were treated with 8 mg/mL amyloglucosidase (Sigma–Aldrich, St. Louis, MO, USA) in a 0.2 M citrate buffer, pH 5.0, for 2 h at 37 °C, and then total glucose was measured using the Glucose Oxidase Reagent Set (Pointe Scientific, Inc., Canton, MI, USA). Free glucose concentrations were also measured in samples that were not treated with amyloglucosidase. Free glucose was subtracted from total glucose to determine glycogen levels. Triglyceride and glycogen levels were normalized to total protein content.

Feeding Assay: Food consumption was measured using the CAFÉ assay as previously described [16]. Briefly, groups of three one-week-old adult females were placed in 1% agar vials, and the amount of 5% sucrose consumed over 24 h was measured using 5 µl capillary tubes (ThermoFisher Scientific, Waltham, MA, USA). Vials without flies were used to control for evaporation.

RNA Isolation: Groups of 25–30 intestines dissected from one-week-old female flies were suspended in TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and homogenized briefly, followed by a 5 min incubation at room temperature. Samples were extracted using chloroform, and nucleic acids were precipitated by adding isopropanol. Pellets were washed with 70% ethanol, air-dried for 5 min, and then resuspended in nuclease-free water.

RNA Sequencing and Gene Expression Analysis: RNA sequencing was performed on RNA isolated from intestines dissected from *mex-Gal4 > GFPRNAi* and *mex-Gal4 > 9G8RNAi*

females (n = 3) by GeneWiz from Azenta Life Sciences (South Plainfield, NJ, USA) using procedures described previously [13]. Differentially expressed gene lists were analyzed using The Database for Annotation, Visualization, and Integrated Discovery (DAVID) software v2022q4 [17] to identify any enrichment of genes in specific biological processes.

DNase Treatment and cDNA Synthesis: A total of 5 µg of each RNA sample was DNase treated with the DNA-Free Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. A total of 0.25 µg of DNased RNA samples were reverse transcribed using qScript Ultra cDNA Supermix (QuantaBio, Beverly, MA, USA), according to the manufacturer's instructions.

Quantitative PCR: qPCR reactions were made from 1 µL of cDNA, 2× Perfecta SYBR Green (QuantaBio, Beverly, MA, USA), and 200 nM of the forward and reverse primer for each gene segment. The qPCR cycling conditions were as follows: 3 min at 95 °C; 40 cycles of: 30 sec at 95 °C, 1 min at 60 °C, and 30 sec at 72 °C, with a melt curve. The following genes were amplified as described: *rp49* (*cg7939*), *FASN3* (*cg17374*), *eloF* (*cg16905*), *Fad2* (*cg7923*), *cg16904*, *cg30008*, *cg31089*, *cg10163*, *cg31091*, and *cg13562*. Forward and reverse primer sequences are shown in Table 2. Resulting expression levels for each gene were normalized to *rp49*.

Table 2. Forward and reverse primer sequences used to quantify gene expression.

Primer	Forward Sequence (5′ → 3′)	Reverse Sequence (5′ → 3′)
Rp49	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG
FASN3 (*cg17374*)	TGCTGGTACTGGAGGCATTG	CCCCTTTTCCGTTCGTCTCA
eloF (*cg16905*)	CACCGAAAGCCCTTCCATTTG	GATCCATCGGCAGGCTAACA
FAD2 (*cg7923*)	CAACGGTCGTGCTCTTTTGG	TTGCCCTTCTCCACAACCTC
cg16904	GAAGCCGTACAACCTGAGCT	ATGGAGGAACGTGATCTGGC
cg30008	GTAACGCTGGTCTACGCACT	CACCAAGGTCTTATCCGCCA
cg31089	TTCCTTGGTGCACATGTGGT	TCCATTCCGTATCCGCCATG
cg10163	GATGCCCGACTCCATGTGAT	ACGGAGAGATGATGACGCAC
cg31091	ATACGATGTTTGGCTGGGCA	CTGATCCTGTCCGTTCTCCG
cg13562	CTTCGTCTACTCCTGCCACC	CGAAGTTCTCCTCGTAGCCC

Statistics: Mean values for triglyceride, glycogen, feeding, and qPCR experiments were compared between the appropriate experimental and control conditions using an unpaired t-test in Excel. Analysis of the RNA-Seq data was performed as previously described [13]. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. SR Proteins Affect Lipid Levels in Whole Flies

To characterize the metabolic functions of SR proteins in the fly intestines, we used the intestine-specific *mexGal4* driver [18] to induce RNAi towards *9G8* (also known as *x16*), *B52*, *Rbp1*, *RSF1*, *SC35*, *Rbp1-Like*, *SF2*, and *Srp54* individually to lower their expression, specifically in the fly gut. Next, triglycerides normalized to total protein content were measured in females with the expression of a single SR protein reduced. Decreasing *9G8*, *RSF1*, *SC35*, and *Srp54* resulted in triglyceride accumulation, while knockdown of *Rbp1* resulted in lean flies (Figure 1A). To further analyze the metabolic role of SR proteins in the fly intestines, we measured glycogen in whole flies, with individual SR proteins decreased specifically in the intestine. Decreasing *RSF1* and *Rbp1-Like* increased glycogen levels, while

knockdown of *Rbp1* and *Srp54* diminished it (Figure 1B). Together, these triglyceride and glycogen data suggest that SR proteins have distinct functions in the fly intestine to regulate lipid and carbohydrate storage, and these varying phenotypes may arise from different metabolic splicing targets of each SR protein.

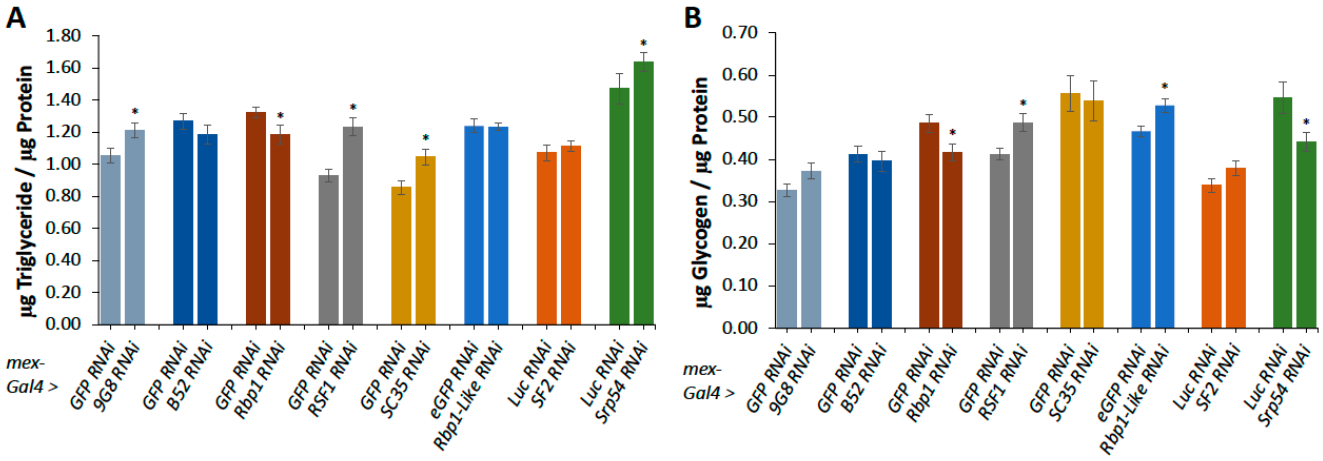

Figure 1. Decreasing SR proteins in the gut alters overall levels of triglycerides and glycogen in female flies. (**A**). Triglycerides and (**B**). glycogen were measured in one-week-old female flies, then normalized to protein content. n = 20–44. Bars indicate average normalized triglyceride concentrations, +/− standard error. * $p < 0.05$ by a t-test comparing control and SR protein gene knockdown conditions.

3.2. 9G8 Regulates Metabolic Gene Expression in the Drosophila Intestine

In previous studies, decreasing *9G8* specifically in adipose tissue yielded a large increase in triglyceride levels [12]. In addition, *9G8* has also been shown to regulate the network of NADPH-producing enzymes, suggesting its potential in controlling cellular metabolism [13]. While the metabolic functions of 9G8 have been characterized in fly fat tissue, how 9G8 acts in the fly intestine to regulate metabolic homeostasis is not understood. Therefore, we decided to investigate further the function of 9G8 in the *Drosophila* intestine. Since triglycerides were higher in flies with intestinal *9G8* knockdown compared to controls (Figure 1A), we wanted to determine whether this increase was due to lipid accumulation in the intestine. To test this, intestines were dissected from *9G8RNAi* females and lipids were measured. Interestingly, intestines from *9G8* knockdown flies had decreased triglyceride levels (Figure 2A), and this finding was not due to any alterations in food consumption (Figure 2B). These data suggest that *9G8* may be acting in the intestine to increase triglyceride storage locally and perhaps limit the amount of lipids transported to other tissues.

To better understand the genes regulated by *9G8* to control lipid absorption and metabolism within the fly gut, we performed RNA-Seq on RNA isolated from *9G8RNAi* fly intestines. Differential expression analysis was used to determine the difference in gene expression caused by the *9G8* knockdown. Of the 104 genes whose differential expression was identified in *9G8RNAi* intestines, 65 were upregulated and 39 were downregulated (Supplementary Table S1). Gene function was then analyzed using gene ontology analysis as described previously [13]. The gene ontology terms resulting from this analysis that were significantly enriched in the upregulated and downregulated genes are shown in Table 3. Of the 65 upregulated genes, 21 independent genes were found to be associated with gene ontology terms that were enriched in the upregulated genes (some genes were found in multiple categories), and 44 genes were not associated with gene ontology terms enriched

in this set of genes. Of the 39 downregulated genes, only three genes were associated with gene ontology terms that were enriched in this set of genes (Table 3).

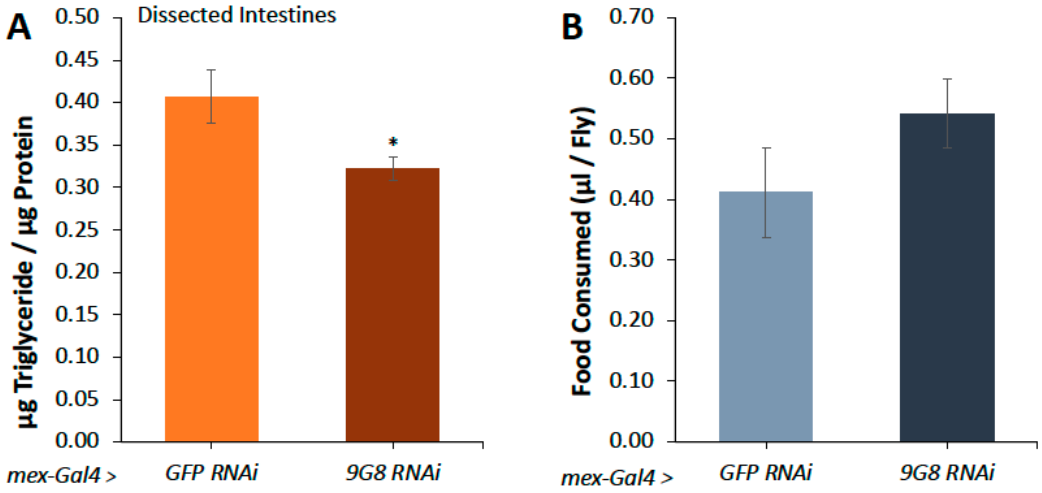

Figure 2. Decreasing *9G8* in the gut decreases gut levels of triglyceride storage in female flies. (**A**). Triglyceride levels of intestines, dissected from one-week-old female *mexGal4* > *GFPRNAi* and *mexGal4* > *9G8RNAi* flies were measured and normalized to protein content. $n = 10$. (**B**). Feeding in approximately one-week-old *mexGal4* > *GFPRNAi* and *mexGal4* > *9G8RNAi* flies was measured over 24 h. $n = 36$. Bars indicate mean, +/− standard error. * $p < 0.05$ by a *t*-test comparing control and *9G8* knockdown conditions.

Table 3. Gene ontology terms identified using RNA-seq during *9G8* knockdown ($n = 3$).

Upregulated		
Term	**# of Genes**	***p* Value**
fatty acid elongation, saturated fatty acid	3	2.60×10^{-3}
fatty acid elongation, monounsaturated fatty acid	3	2.60×10^{-3}
fatty acid elongation, polyunsaturated fatty acid	3	2.60×10^{-3}
lipid catabolic process	4	3.02×10^{-3}
transmembrane transport	6	3.04×10^{-3}
fatty acid elongation	3	3.15×10^{-3}
very long-chain fatty acid biosynthetic process	3	3.44×10^{-3}
sphingolipid biosynthetic process	3	4.39×10^{-3}
pheromone metabolic process	2	1.14×10^{-2}
intracellular cholesterol transport	2	2.65×10^{-2}
lipid metabolic process	3	3.33×10^{-2}
sterol transport	2	4.13×10^{-2}
O-glycan processing, core 1	2	4.13×10^{-2}
response to DDT	2	4.13×10^{-2}
Downregulated		
Term	**# of Genes**	***p* value**
carbohydrate metabolic process	3	1.67×10^{-2}

Most of the gene ontology terms enriched in upregulated genes identified in the *9G8RNAi* fly intestines were involved in lipid metabolism. Specifically, some (*cg16904*, *cg30008*, *Fad2*, and *eloF*) had a role in sphingolipid and fatty acid synthesis and elongation. Others (*cg31089*, *cg10163*, *cg31091*, *cg13562*, and *cg17560*) were predicted to have triglyceride lipase activity or further assist in lipid breakdown. Interestingly, genes responsible for sterol transport (*NPC2F* and *NPC2H*), as well as transmembrane transporter activity (*cg9270*, *cg3168*, *cg12490*, *CG8028*, *CG2187*, and *cg9717*), were also upregulated in *9G8RNAi* guts. Conversely, the only gene ontology term enriched in the downregulated genes functions in carbohydrate (*MAL-A6*, *MAL-A1*, and *CHT4*) metabolism.

Since intestinal triglyceride contents were lower in *9G8RNAi* flies, we decided to focus on many of the lipid metabolic genes that were identified during the RNA-seq analysis. We first focused on genes involved in fatty acid synthesis and elongation, including *FASN3*, *eloF*, *fad2*, *cg16904*, and *cg30008*. All these genes had substantially higher expression in the intestines of *9G8RNAi* flies compared to control flies using qPCR (Figure 3). This suggests that *9G8* acts to limit the synthesis of fatty acids in the fly gut. In addition, we also examined the expression of putative triglyceride lipase genes shown to be upregulated in *9G8RNAi* intestines by the RNA-seq, including *cg31089*, *cg10163*, *cg31091*, and *cg13562*. All these genes had higher levels of expression in the *9G8RNAi* fly guts compared to the control flies via qPCR (Figure 4), suggesting that *9G8* limits the expression of lipases, which may contribute to triglyceride absorption and storage in the fly intestine.

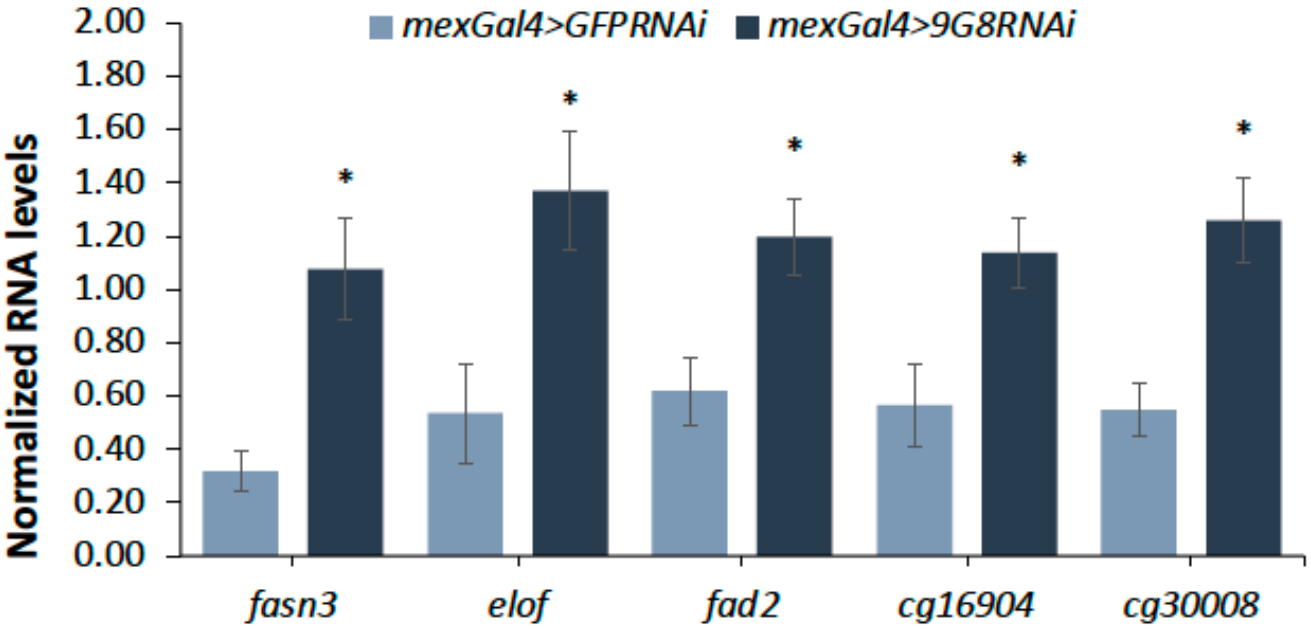

Figure 3. Intestinal knockdown of *9G8* increases the expression of genes involved in fatty acid synthesis and elongation. Levels of gene expression of fatty acid metabolism genes (*fasn3*, *elof*, *fad2*, *cg16904*, *cg30008*) were measured using qPCR on RNA samples from intestines dissected from female *mexGal4* > *GFPRNAi* and *mexGal4* > *9G8RNAi* flies and normalized to *rp49* levels ($n = 8$). Bars indicate average RNA levels, +/− standard error. * $p < 0.05$ by a *t*-test comparing *mexGal4* > *GFPRNAi* and *mexGal4* > *9G8RNAi* conditions.

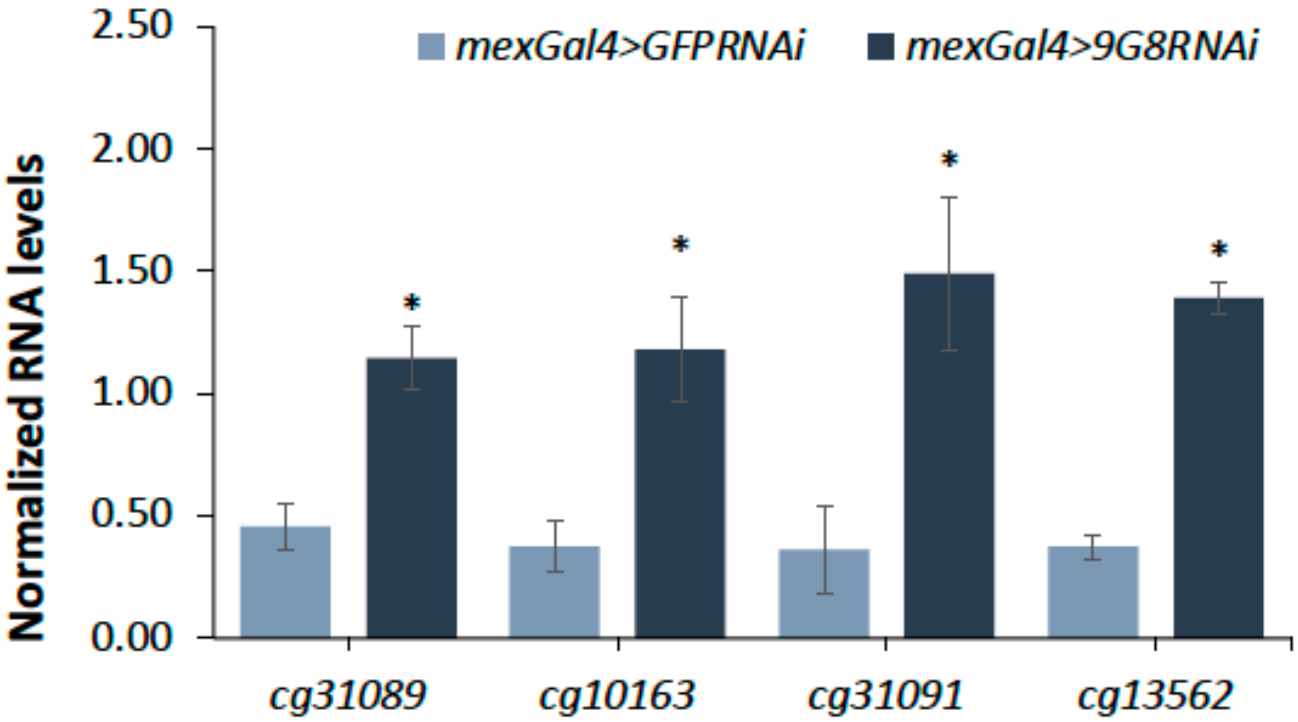

Figure 4. Intestinal knockdown of *9G8* increases the expression of TAG lipase genes. Levels of gene expression of genes with predicted lipase activity (*cg31089*, *cg10163*, *cg31091*, and *cg13562*) were measured using qPCR on RNA samples from intestines dissected from female *mexGal4 > GFPRNAi* and *mexGal4 > 9G8RNAi* flies and normalized to *rp49* levels ($n = 8$). Bars indicate average RNA levels, +/− standard error. * $p < 0.05$ by a *t*-test comparing *mexGal4 > GFPRNAi* and *mexGal4 > 9G8RNAi* conditions.

4. Discussion

In this study, we explored the role various SR proteins play in the intestine to regulate *Drosophila* metabolism. Using the Gal4/UAS system to induce RNAi towards individual SR proteins in the gut, we detected a variety of functions of each SR protein to regulate triglyceride and carbohydrate storage. For example, decreasing intestinal *Rbp1* was shown to blunt the storage of both triglycerides and glycogen in the whole fly. Previous studies of this SR protein revealed that loss of this gene in the fat body increased both whole fly lipid levels and resistance to starvation [11]. This suggests that Rbp1 may play a different metabolic role in the fat body from that of the gut. Conversely, decreasing the SR protein *RSF1* in the gut resulted in an increase in triglyceride and glycogen levels in whole flies. Interestingly, *RSF1* does not function in the fly fat body to control whole fly triglyceride and carbohydrate levels [11], suggesting it has a unique function in the intestine. In addition, loss of *Srp54* and *SC35* in the fly intestine resulted in triglyceride accumulation; however, decreasing *Srp54* resulted in less glycogen, and the loss of *SC35* had no effect on glycogen storage. This suggests that these SR proteins may distinctly affect carbohydrate storage and metabolism in the fly gut. Similarly to *RSF1*, knockdown of *Srp54* and *SC35* in the fat body did not affect organismal nutrient storage [11,12], indicating the intestine-specific function of these genes.

We have also shown that decreasing the SR protein *9G8* in the intestine results in whole fly lipid levels to accumulate. This is consistent with triglyceride accumulation observed in previous experiments where *9G8* was decreased in the fat body [12], indicating that the knockdown in both gut and fat body have a similar whole fly lipid storage phenotypes. However, while triglyceride levels were also higher in isolated fat bodies during fat body-specific *9G8* knockdown [12], decreasing *9G8* in the intestine led to decreased lipid levels in

the gut, indicating 9G8 has varying organ-specific effects. In the fat body, *9G8* knockdown was also shown to downregulate fat body expression of several key NADPH-producing enzyme genes, including Zwischenferment (*Zw*), the homolog of human glucose 6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase, isocitrate dehydrogenase, and malic enzyme [13]. *9G8* was also found to affect lipid breakdown by regulating the splicing of the *CPT1* transcript, producing an enzyme responsible for downstream fatty acid catabolism with less activity [12]. To investigate the effect of *9G8* function on metabolic gene expression in the gut, we performed RNA-seq in *9G8RNAi* intestines. Differential gene expression revealed numerous genes that produce products involved in fatty acid (FA) synthesis, FA elongation, and lipid catabolism as being upregulated. This result is consistent with previous RNA-seq experiments in the *9G8RNAi* fat body, where oxidation-reduction and lipid catabolism genes were found to be upregulated as well [13]. However, the specific intestinal genes regulated by 9G8 are completely distinct from those of the fat body, suggesting *9G8* invokes a similar phenotype by acting on different pathways in both organs. Moreover, since these genes involved in regulating lipid metabolism are upregulated in *9G8RNAi* intestines, it is possible that 9G8 regulates the transcription or mRNA transport of these transcripts, which is consistent with a previous report that *9G8* regulates nuclear mRNA export [19]. Determining whether 9G8 specifically interacts with the upregulated mRNAs identified here would help to determine whether 9G8 is regulating the transcription, transport, or splicing of these transcripts.

Several of the upregulated lipid metabolism genes (i.e., *fasn3*, *eloF*, and *fad2*) in the *9G8RNAi* intestines are involved in the synthesis and elongation of fatty acids. *fasn3*, commonly named fatty acid synthase, is known to synthesize methyl-branched FAs in the presence of methylmalonyl-CoA and malonyl-CoA [20]. *eloF* is known to be primarily expressed in female flies, where it elongates saturated and unsaturated FAs up to 30 carbons [21]. Similarly, fad2, an enzyme primarily active in female flies, has a non-specified role in pheromone diene production [22]. Since these two genes are expressed specifically in females, it is possible that 9G8 plays a sex-specific role in regulating lipid metabolism in flies, and additional experiments designed to study 9G8 function in both male and female intestines are necessary to further address this question. We also identified several FA elongases, which have not been well characterized in the fly. Two genes we identified (cg16904 and cg30008) share homology with ELOVL1 and ELOVL7 in mammals. ELOVL1 is involved in the elongation of saturated and monounsaturated very long FAs (VLCFAs), while ELOVL7 regulates the elongation of polyunsaturated VLCFAs [23,24]. Increased expression of these genes and the decreased intestinal triglycerides in *9G8RNAi* intestines may indicate that free FAs may accumulate in the intestine and these FAs may be very unsaturated. Experiments designed to measure the full lipid profile of *9G8RNAi* intestines would help to confirm this hypothesis.

Additionally, multiple genes identified in the RNA-Seq analysis that were predicted to have triglyceride lipase function were homologous to triglyceride lipases in mammals. Many of these mammalian lipases are known to break down triglycerides and phospholipids, consistent with the low triglyceride phenotype in the fly gut shown here. Therefore, it is possible that these lipases that are altered in *9G8RNAi* intestines function to break down dietary lipids to promote their absorption into the fly intestine. Additionally, we hypothesize that the fatty acids from these dietary lipids may be delivered from the intestine to other organs, where they are utilized for triglyceride synthesis, leading to the increased triglyceride whole fly phenotype observed here. Lipids are transported from the intestine to the rest of the animal in apoB-containing lipoprotein-like molecules called lipophorins [14]. While lipoproteins are made in the *Drosophila* fat body and not the intestine [14], it is possible that intestinal 9G8 could be acting nonautonomously to regulate

lipophorin production in the fat body. Measuring lipophorin levels and fatty acid transport in and out of *9G8RNAi* intestines would help test these hypotheses.

It is interesting that the expression of genes involved in both fatty acid synthesis and elongation as well as lipid catabolism is upregulated in *9G8RNAi* intestines. While it is possible that these genes are coordinately regulated, allowing their products to work together to control lipid metabolism in the fly intestine, it is also possible that some of these genes are directly responsible for the lipid storage phenotypes observed in *9G8RNAi* flies and others could be compensating for these phenotypes. Future study of the functions and regulation of the fatty acid synthesis and elongation genes and lipases discovered here is necessary to better understand the contributions of each gene to regulating intestinal lipid homeostasis in *Drosophila*.

In summary, we have characterized the metabolic phenotypes of loss of SR proteins in the *Drosophila* intestine. These SR proteins are highly conserved in mammals [9], and some have been shown to have metabolic functions. For example, *SRSF3* and *SRSF7*, the mammalian homologs of *Rbp1* and *9G8*, respectively, are decreased in the livers of patients who are obese [25], and this is consistent with the triglyceride accumulation phenotypes when *Rbp1* and *9G8* are decreased in the *Drosophila* intestine and fat body [11,12]. However, whether these SR proteins function in the mammalian intestine to regulate lipid metabolism is not well understood. Together, the work described here advances our understanding of the metabolic roles of SR proteins in the fly intestine specifically and may have implications as potential therapeutic targets for the treatment of obesity and other metabolism-related diseases.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/lipidology2010001/s1, Table S1: Differential expression analysis.

Author Contributions: Conceptualization, J.R.D.; methodology, J.R.D.; validation, J.R.D. and R.V.; formal analysis, R.V.; investigation, J.R.D. and R.V.; writing—original draft preparation, R.V.; writing—review and editing, J.R.D.; visualization, R.V.; supervision, J.R.D.; project administration, J.R.D.; funding acquisition, J.R.D. and R.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by an Undergraduate Research Fellowship from the Cayman Biomedical Research Institute (CABRI) to R.V. and internal funds from Penn State Berks.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: RNA sequencing data were deposited in NCBI GEO, accession number GSE283039 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE283039). The remaining data are available in this article or its online Supplementary Materials.

Acknowledgments: The authors would like to thank Malachi Brought for technical assistance. Stocks obtained from the Vienna Drosophila Resource Center (VDRC) and the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Lin, X.; Li, H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. *Front. Endocrinol.* **2021**, *12*, 706978. [CrossRef] [PubMed]
2. Hales, C.M.; Carroll, M.D.; Fryar, C.D.; Ogden, C.L. Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017–2018. *NCHS Data Brief* **2020**, *360*, 1–8.

3. Staats, S.; Luersen, K.; Wagner, A.E.; Rimbach, G. Drosophila melanogaster as a Versatile Model Organism in Food and Nutrition Research. *J. Agric. Food Chem.* **2018**, *66*, 3737–3753. [CrossRef] [PubMed]
4. Musselman, L.P.; Kuhnlein, R.P. Drosophila as a model to study obesity and metabolic disease. *J. Exp. Biol.* **2018**, *221*, jeb163881. [CrossRef]
5. Beller, M.; Sztalryd, C.; Southall, N.; Bell, M.; Jackle, H.; Auld, D.S.; Oliver, B. COPI complex is a regulator of lipid homeostasis. *PLoS Biol.* **2008**, *6*, e292. [CrossRef]
6. Guo, Y.; Walther, T.C.; Rao, M.; Stuurman, N.; Goshima, G.; Terayama, K.; Wong, J.S.; Vale, R.D.; Walter, P.; Farese, R.V. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature* **2008**, *453*, 657–661. [CrossRef]
7. Pospisilik, J.A.; Schramek, D.; Schnidar, H.; Cronin, S.J.; Nehme, N.T.; Zhang, X.; Knauf, C.; Cani, P.D.; Aumayr, K.; Todoric, J.; et al. Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell* **2010**, *140*, 148–160. [CrossRef]
8. Matera, A.G.; Wang, Z. A day in the life of the spliceosome. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 108–121. [CrossRef]
9. Shepard, P.J.; Hertel, K.J. The SR protein family. *Genome Biol.* **2009**, *10*, 242. [CrossRef]
10. Bradley, T.; Cook, M.E.; Blanchette, M. SR proteins control a complex network of RNA-processing events. *RNA* **2015**, *21*, 75–92. [CrossRef]
11. Bennick, R.A.; Nagengast, A.A.; DiAngelo, J.R. The SR proteins SF2 and RBP1 regulate triglyceride storage in the fat body of Drosophila. *Biochem. Biophys. Res. Commun.* **2019**, *516*, 928–933. [CrossRef] [PubMed]
12. Gingras, R.M.; Warren, M.E.; Nagengast, A.A.; Diangelo, J.R. The control of lipid metabolism by mRNA splicing in Drosophila. *Biochem. Biophys. Res. Commun.* **2014**, *443*, 672–676. [CrossRef] [PubMed]
13. Weidman, T.; Nagengast, A.A.; DiAngelo, J.R. The splicing factor 9G8 regulates the expression of NADPH-producing enzyme genes in Drosophila. *Biochem. Biophys. Res. Commun.* **2022**, *620*, 92–97. [CrossRef] [PubMed]
14. Palm, W.; Sampaio, J.L.; Brankatschk, M.; Carvalho, M.; Mahmoud, A.; Shevchenko, A.; Eaton, S. Lipoproteins in Drosophila melanogaster--assembly, function, and influence on tissue lipid composition. *PLoS Genet.* **2012**, *8*, e1002828. [CrossRef] [PubMed]
15. Parvy, J.P.; Napal, L.; Rubin, T.; Poidevin, M.; Perrin, L.; Wicker-Thomas, C.; Montagne, J. Drosophila melanogaster Acetyl-CoA-carboxylase sustains a fatty acid-dependent remote signal to waterproof the respiratory system. *PLoS Genet.* **2012**, *8*, e1002925. [CrossRef]
16. Ja, W.W.; Carvalho, G.B.; Mak, E.M.; de la Rosa, N.N.; Fang, A.Y.; Liong, J.C.; Brummel, T.; Benzer, S. Prandiology of Drosophila and the CAFE assay. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8253–8256. [CrossRef]
17. Dennis, G., Jr.; Sherman, B.T.; Hosack, D.A.; Yang, J.; Gao, W.; Lane, H.C.; Lempicki, R.A. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* **2003**, *4*, P3. [CrossRef]
18. Phillips, M.D.; Thomas, C.M. Brush border spectrin is required for early endosome recycling in Drosophila. *J. Cell Sci.* **2006**, *119*, 1361–1370. [CrossRef]
19. Sanford, J.R.; Ellis, J.; Caceres, J.F. Multiple roles of arginine/serine-rich splicing factors in RNA processing. *Biochem. Soc. Trans.* **2005**, *33*, 443–446. [CrossRef]
20. de Renobales, M.; Blomquist, G.J. Biosynthesis of medium chain fatty acids in Drosophila melanogaster. *Arch. Biochem. Biophys.* **1984**, *228*, 407–414. [CrossRef]
21. Chertemps, T.; Duportets, L.; Labeur, C.; Ueda, R.; Takahashi, K.; Saigo, K.; Wicker-Thomas, C. A female-biased expressed elongase involved in long-chain hydrocarbon biosynthesis and courtship behavior in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4273–4278. [CrossRef] [PubMed]
22. Chertemps, T.; Duportets, L.; Labeur, C.; Ueyama, M.; Wicker-Thomas, C. A female-specific desaturase gene responsible for diene hydrocarbon biosynthesis and courtship behaviour in Drosophila melanogaster. *Insect Mol. Biol.* **2006**, *15*, 465–473. [CrossRef] [PubMed]
23. Jakobsson, A.; Westerberg, R.; Jacobsson, A. Fatty acid elongases in mammals: Their regulation and roles in metabolism. *Prog. Lipid Res.* **2006**, *45*, 237–249. [CrossRef] [PubMed]
24. Leonard, A.E.; Pereira, S.L.; Sprecher, H.; Huang, Y.S. Elongation of long-chain fatty acids. *Prog. Lipid Res.* **2004**, *43*, 36–54. [CrossRef]
25. Pihlajamaki, J.; Lerin, C.; Itkonen, P.; Boes, T.; Floss, T.; Schroeder, J.; Dearie, F.; Crunkhorn, S.; Burak, F.; Jimenez-Chillaron, J.C.; et al. Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. *Cell Metab.* **2011**, *14*, 208–218. [CrossRef]