THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DIVISION OF SCIENCE

CHARACTERIZING THE IMPACTS OF ORNITHINE DECARBOXYLASE, SPERMIDINE SYNTHASE, AND SPERMINE SYNTHASE ON POLYAMINE PATHWAY REGULATION AND CELL METABOLISM CHANGES IN DROSOPHILA MELANOGASTER

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SPRING 2021

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry and Molecular Biology
with honors in Biochemistry and Molecular Biology

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ABSTRACT

Polyamines are essential for normal cell growth and development. Altered expression of polyamines has been implicated in numerous physiological anomalies including neoplasia, aging, and cardiac hypertrophy. Genetic mutations in enzymes responsible for polyamine metabolism have also been shown to cause or contribute to conditions such as Snyder-Robinson Syndrome and changes in energy production. The exact mechanisms by which polyamine metabolism contributes to these conditions and ways in which the regulatory enzymes interact is not completely known. Furthermore, previous studies have shown a connection between polyamine regulatory enzymes and glycogen and lipid metabolism, potentially contributing to the development of certain conditions related to obesity and diabetes, yet the manner in which each regulatory gene contributes to the development of conditions involving polyamines has not been fully explored. The existing sexual dimorphisms in polyamine regulation between males and females have also not be fully detailed.

In this study, the manner by which three polyamine pathway genes, ornithine decarboxylase (Odc1), spermidine synthase (SpdS), and spermine synthase (Sms) regulated expression of other pathway genes, intracellular concentration of polyamines, and glycogen and lipid stores was characterized in *Drosophila melanogaster (D. melanogaster)*. Utilizing male and female null and knockdown *D. melanogaster* models, the roles of Odc1, SpdS, and Sms were able to be better detailed, and the variations between males and females were further clarified. A better understanding of the internal regulation of this pathway will aid in identifying potential therapeutic targets for physical anomalies that demonstrate altered expression of polyamine enzymes.

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Drosophila	*Drosophila melanogaster*
Odc1	Ornithine decarboxylase
Oda	Ornithine decarboxylase antizyme
SpdS	Spermidine synthase
Sms	Spermine synthase
Sat1/2	Spermidine/spermine N^1- acetyltransferase
PAO	Polyamine oxidase
SamDC	S-adenosylmethionine decarboxylase
ODC	Mammalian ornithine decarboxylase
SPDS	Mammalian spermidine synthase
SMS	Mammalian spermine synthase
SSAT	Mammalian Spermidine/spermine N^1-acetyltransferase
SMO	Spermine oxidase
dcAdoMet	Decarboxylated adenosylmethionine

ACKNOWLEDGMENTS

I always thought completing my undergraduate thesis was going to be an intense journey, but I could not have imagined how the chaotic circumstances of the past year would really make it unique. That said, I would not have been able to make it to this point in my academic career at Penn State Berks, let alone this past year, without the enormous support from so many mentors, friends, and family members during my time here.

In particular, I want to thank Dr. Sandy Feinstein for much of the motivation to expand my academic horizons. As early as my first day on campus, Dr. Feinstein was encouraging me to reach out and gain experience within the sciences and elsewhere (always with an interdisciplinary focus), and she has not stopped since. That extra push from Dr. Feinstein was exactly what I needed to pursue a few of the most extraordinary academic experiences I have had to date and will serve as a reminder to always reach for excellence and knowledge in and out of my discipline as I progress in my future career.

Next, I must thank the professor, thesis supervisor, and mentor who, over the past few years, has really taught me what it means to be a scientist: Dr. Shannon Nowotarski. Dr. Nowotarski took me into her lab group almost three years ago, and, since then, she has not withheld any time or spared any effort in teaching me various laboratory techniques and ways to analyze data, as well as in helping me hone my communication skills in the sciences. For all of the guidance over the past three years, especially this year, and opportunities you've helped me explore in this field, I just want to say thank you Dr. N.

Lastly, to my family, namely my parents, Lilly and Al Morales, and my brothers, and my close friends, I know I would not be the man I am today without all of the support you've given

and experiences we've shared. I look back at who I was just four years ago and know that I've changed so much for the better, and it is in large part due to you all. While I am unsure what may happen in the years ahead (let alone in a few months), I am confident and glad that WE ARE in it together.

Thank you to everyone at Berks for making these last few years memorable and purposeful, and I wish you all the best as you also continue onward on your Penn State journeys.

Chapter 1

Introduction

1.1 The use of *Drosophila melanogaster* as a model organism to study human genetics

1.1.1 History of Drosophila melanogaster as a model organism

Drosophila melanogaster (*D. melanogaster*), commonly known as the fruit fly, has been used to study both genetics and physiology for over a hundred years. Beginning with T.H. Morgan's heredity experiments at Columbia University in 1910, *D. melanogaster* has been shown to be a valuable model for the study of genetics (Morgan, 1915). In fact, it was while studying genetics in *D. melanogaster* that Morgan first proposed gene linkage, or the tendency of physically close genes to be inherited together. This theory was later used to map gene locations in the early twentieth century (Morgan, 1915).

The *D. melanogaster* genome was one of the first genomes sequenced in the late twentieth century (Fortini et al., 2000). Not only did this mark a significant milestone in the study of genetics and genome assembly, but it also revealed similarities between the genetic codes of *D. melanogaster* and humans. Despite differences in genome size and variations in chromosomal organization, the sequence homology amongst genes in flies and humans highlighted that flies were indeed a valid model for studying mutations and human disease (Figure 1.1).

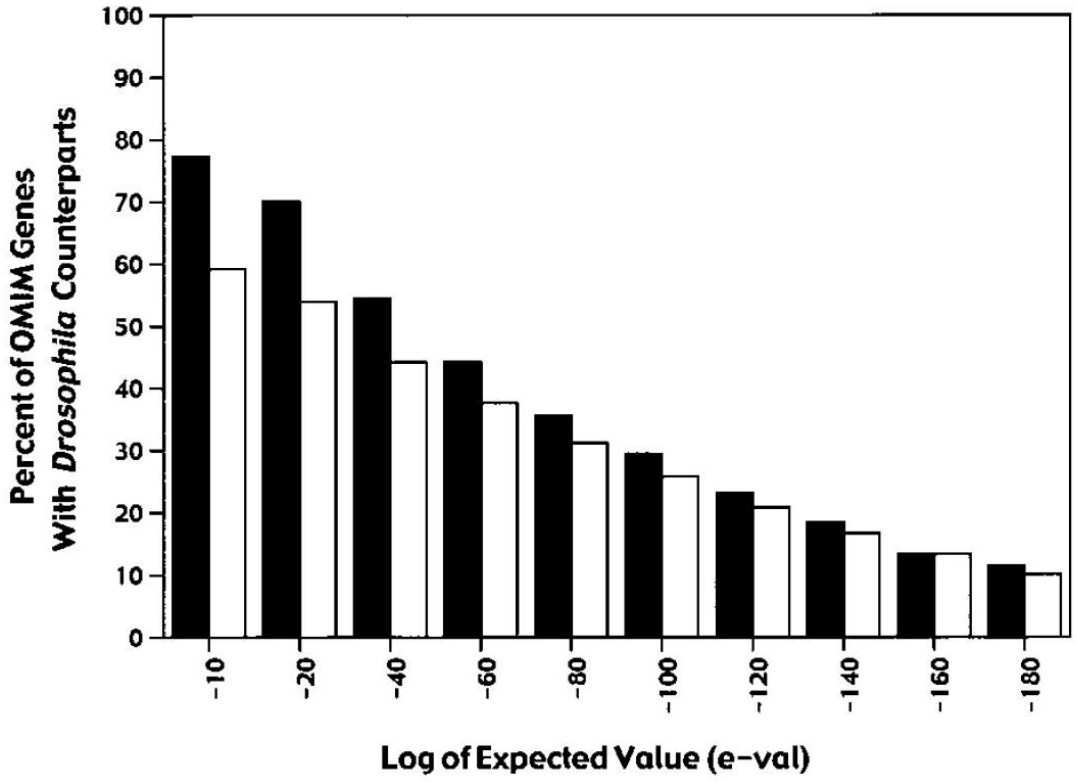

Figure 1. 1 Drosophila melanogaster shares approximately 54% of disease related genes with humans.

A graph of the percent of human disease genes with similar sequences in *D. melanogaster* as a function of *E*-value. Black-filled bars indicate the percent of human Locuslink entries (929 total) with matches to *D. melanogaster* sequences. White-filled bars indicate the percent of unique *Drosophila melanogaster* sequences that match one or more human disease gene sequences. As of 2001, 54% of human disease genes had homologs in *Drosophila melanogaster*.
(Source: Reiter et al., 2001).

In an early analysis of the human and fly genomes, it was found that 62 percent of the 287 human genes known to be involved in human disease had a homolog in the fly (Fortini et al., 2000). However, since the completion of sequencing the human genome in 2001, it has been initially estimated that 54 percent of the studied 929 genes implicated in human diseases have fly homologs, and, after further analysis of distinct disease genes, it was found that approximately 75 percent of the genes had fly counterparts (Reiter et al., 2001). This homology in sequences has been utilized by researchers who study and model human disease. For example, flies have been used as model organisms to study such physiological anomalies like Parkinson's disease, cancer, and diabetes (Aryal and Lee, 2019; Reviewed in [Mirzoyan et al., 2019]; Murillo-Maldonado and Riesgo-Escovar, 2017; Musselman and Kuhnlein, 2018).

1.1.2 Traits that positively contribute to the selection of Drosophila melanogaster as a model organism

Beyond the genetic similarities, flies continue to be utilized as a model organism in science because they are readily available in nature, have a short life cycle, are easy to maintain in a laboratory setting, and are relatively inexpensive to maintain (Figure 1.2) (Griffiths et al., 2020). Additionally, crosses in flies can be performed easily and there are a variety of genetic manipulations available to researchers who utilize flies in their studies, such as target gene knockdowns through RNA interference as well as controlling gene expression using the Gal4/UAS system (Lam and Thummel, 2000; Yamaguchi and Yoshida, 2018).

Since its advent as a model organism, *D. melanogaster* has contributed to the understanding of genetics and development by being used to show the role of the hedgehog (hh*)* protein in limb patterning (Basler and Struhl, 1994). Moreover, tissue specific gene inactivation

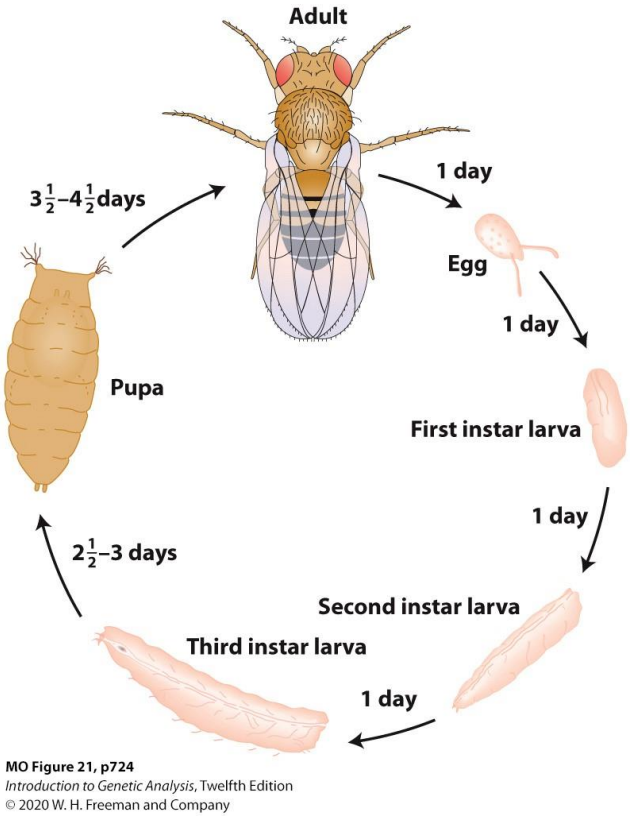

Figure 1. 2 The short life cycle of *Drosophila melanogaster* makes it an ideal model organism

Drosophila melanogaster takes 3.5-4.5 days to mature into fully viable adults, allowing for timely breeding and genetic manipulations. Following fertilization of the egg, the embryonic phase of fly development will take place over the first day. During this time, limb patterning and segmentation will occur. Following the first day, the developing fly moves into the first larval stage and becomes the first instar (intermediate) larva. During this stage the fly will feed and undergo two molts. Parts of the mouth will also start to become more pronounced during these larval stages. Next, the larva proceeds to a second instar stage after completing their first two molts and they continue feeding underneath the surface of the food. The larva continues to molt during the second instar stage. More skin is molted to reveal a more pronounced mouth and cranial features. Following the completion of the second molt and progression to the third instar larva, the larva continue feeding until ready to become pupas 2.5-3 days later As pupas the final stage of fly development will occur with remaining undifferentiated cells held in imaginal discs undergoing differentiation and larval skin and remaining tissue being degraded at 3.5-4.5 days. Afterward the flies are viable and able to undergo breeding.
(Source: Griffiths et al. 2020).

in *D. melanogaster* has been utilized to determine signaling mechanisms involved in limb patterning. For example, the temperature regulated Gal4 -UAS modeling system has been used to reduce JAK/STAT activity, resulting in a correlative decrease in wing size (Recasens-Alvarez et al., 2017). Additionally, *D. melanogaster* has previously been used in studies investigating triglycerides storage and lipid metabolism, supporting its potential as a valuable model organism for studying metabolic changes and diabetes (DiAngelo and Birnbaum, 2009).

1.2 The polyamine pathway

1.2.1 Overview

Initially isolated in human seminal fluid by Antonie van Leeuwenhoek in 1678, the cationic polyamines are small, ubiquitous polycations (Bachrach, 2010). The tetramine spermine, which was first discovered by Leeuwenhoek, triamine spermidine, and diamine putrescine have been detected in prokaryotic and eukaryotic organisms including humans, plants, bacteria, and flies (Bachrach, 2010; Vigne and Frelin, 2008). Despite Leeuwenhoek's initial discovery, the structures of these molecules were not fully understood until 1924, when the chemical formula and molecular weight of spermine was initially determined (Dudley et al., 1924).

Polyamines are necessary for normal cell growth and development and their depletion can lead to cytostasis and apoptosis (Fozard et al.,1980; Casero et al., 2018). Their charge distribution allows polyamines to interact with DNA, RNA, proteins, ion channels, and acidic phospholipids (Schuber, 1989; Pegg, 2016). It is through this binding to numerous macromolecules that the intracellular polyamine pool can influence gene expression (Casero et al., 2018).

Intracellular polyamine levels are highly regulated and are maintained by numerous anabolic steps, catabolic steps, and a poorly understood transport system (Figure 3). In mammalian cells as well as fly cells, the amino acid ornithine is converted to the diamine putrescine through ornithine decarboxylase (Odc1). This decarboxylation can be inhibited through an endogenous inhibitor of Odc1, ornithine decarboxylase antizyme (Oda). *S*-adenosylmethionine decarboxylase (SamDC) removes a carboxyl group from *S*-adenosylmethionine (AdoMet) to form decarboxylated AdoMet (dcAdoMet), which is then used as an alternative substrate for further polyamine synthesis. Spermidine synthase (SpdS) utilizes the aminopropyl group from dcAdoMet and transfers it to putrescine to produce the polyamine spermidine. Spermine synthase (Sms) works in a similar manner, transferring the aminopropyl group donated by dcAdoMet to spermidine to form spermine (Casero et al., 2018, Figure 1.3).

Polyamine levels can also be maintained via a catabolic pathway. Spermidine/spermine-N^1-acetyltransferase (Sat1/2) acetylates either spermine or spermidine, forming N^1-acetylspermine or N^1- acetylspermidine by transferring the acetyl group of acetyl-CoA to either spermine or spermidine. The acetylated polyamines can be excreted from the cell or converted to 3-acetylaminopropanal, hydrogen peroxide (H_2O_2) and spermidine or putrescine by undergoing oxidation through polyamine oxidase (PAO) which forms spermidine from N^1-acetylspermine, and putrescine from N^1-acetylspermidine (Casero et al., 2018). It is the intricate balance of these anabolic and catabolic pathways that aid in the prevention of aberrant polyamine levels.

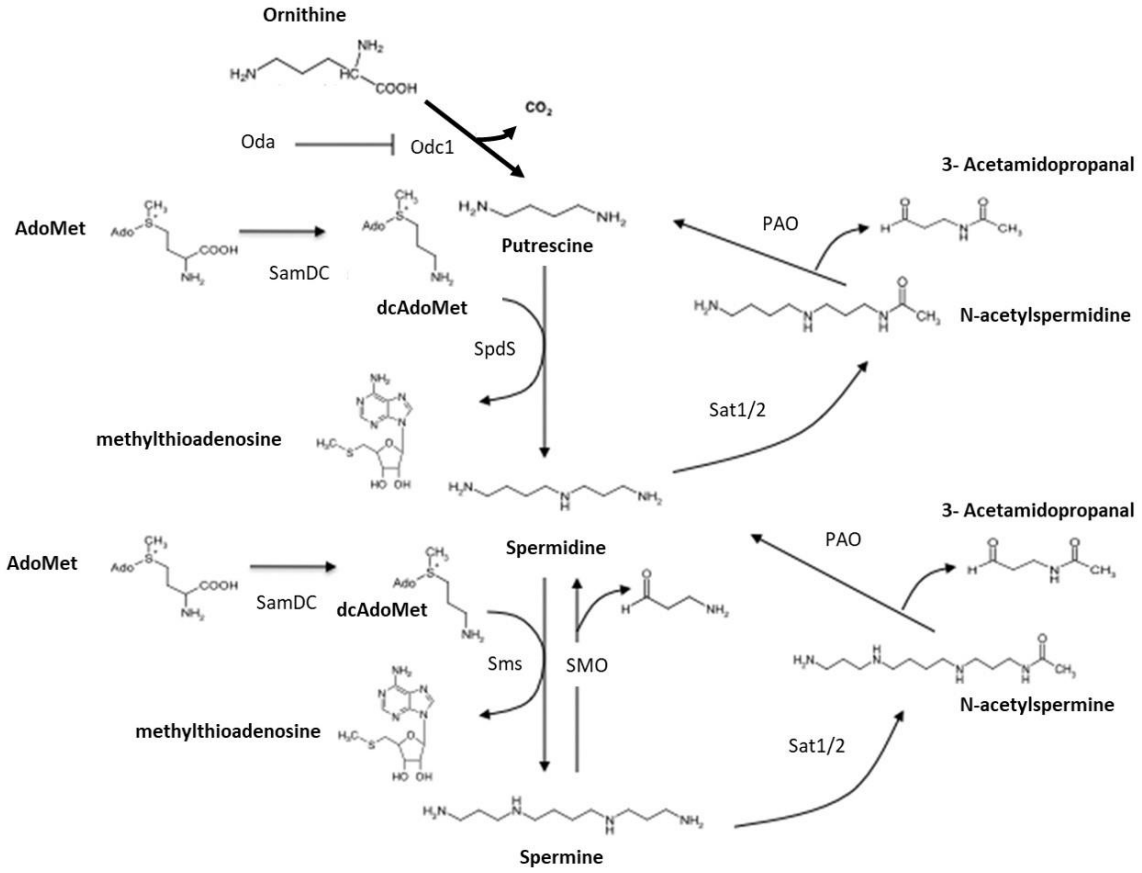

Figure 1. 3 The Polyamine Pathway.

The amino acid ornithine is converted to the diamine putrescine through ornithine decarboxylase (Odc1). This process can be inhibited through ornithine decarboxylase antizyme (Oda). *S*-adenosylmethionine decarboxylase (SamDC) removes a carboxyl group from *S*-adenosylmethionine (AdoMet) to form decarboxylated AdoMet. (dcAdoMet). Spermidine synthase (SpdS) transfers the aminopropyl group from dcAdoMet to putrescine to produce the polyamine spermidine. Spermine synthase (Sms) transfers the aminopropyl group donated by dcAdoMet to spermidine to form spermine. Spermidine/spermine-N^1-acetyltransferase (Sat1/2) transfers the acetyl group from acetyl-CoA to either spermine or spermidine, forming N^1-acetylspermine or N^1-acetylspermidine, respectively. These acetylated polyamines can be excreted from the cell or serve as a substrate for polyamine oxidase (PAO) to form spermidine from acetylated spermine, or putrescine from acetylated spermidine. The transport of polyamines remains poorly understood (not represented in the figure).
(Adapted from (Pegg, 2016)).

1.2.2 Ornithine decarboxylase

Ornithine decarboxylase (Odc1) is a homo-dimeric enzyme and the rate-limiting first step in the synthetic polyamine pathway (Tobias and Kahana, 1993). Odc1 converts the amino acid ornithine, a by-product of the urea cycle, into the diamine precursor, putrescine. Odc1 expression is highly regulated at the levels of transcription, translation and protein degradation (Murakami et al., 1992; Nowotarski and Shantz, 2010; Shantz, 2004). In mammals, Odc1 is endogenously inhibited by the protein antizyme (Oda), which targets Odc1 to the 26S proteasome for degradation (Wu et al., 2015). Odc1 can also be inhibited through the exogenous administration of alpha-difluoromethyl-ornithine (DFMO) (Fozard et al., 1980).

Odc1 has long been associated with increased cell proliferation during development and tissue regeneration (Russell and Snyder, 1968). Odc1 is an essential protein, as complete knockout of mammalian Odc1 in a mouse model results in embryonic lethality following implantation after embryonic day 3.5 (Pendeville et al., 2001). This observation has been recapitulated in *D. melanogaster* as a complete knockout of Odc1 is lethal, with flies containing homozygous deletions in Odc1 not surviving to adulthood (Leon et al., 2020).

1.2.3 Ornithine decarboxylase antizyme

Like its mammalian homolog, fly ornithine decarboxylase antizyme (Oda) is a cytosolic enzyme that inhibits Odc1 dimerization and targets it for proteasomal degradation (Wu et al., 2015)**.** Like Odc1, Oda is tightly regulated. Oda is regulated in a polyamine dependent manner in which high levels of polyamines induce the synthesis of Oda (Nilsson et al., 1997). This

interdependent regulation yet again attests to the importance of maintaining homeostatic levels of the polyamines.

As antizyme can inhibit the function of Odc1, its overexpression has been linked to reproductive complications and sperm defects, which is consistent with the role of Odc1 in normal development **(**Lambertos et al., 2018). Moreover, the overexpression of Oda has been shown to reduce tumor development in animal models where Odc1 is overexpressed. In a study by Feith et al., mice were exposed to 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumor promoting agent, and skin carcinogenesis ensued. Odc1 levels were measured, and, consistent with other highly proliferative tissues, Odc1 levels were upregulated attesting to the role of Odc1in tumorigenesis. The overexpression of Oda in a cohort of these mice reduced tumor burden and Odc1 levels were reduced (Feith et al., 2001). In non-tumorigenic models, high levels of Oda have been shown to prevent putrescine formation and thus inhibit higher polyamine production (Tang et al., 2008).

1.2.4 S-adenosylmethionine decarboxylase

S-adenosylmethionine decarboxylase (SamDC) is a monomeric enzyme that forms decarboxylated S-adenosylmethionine (dcAdoMet), which is later utilized by the aminopropyltransferases spermine synthase and spermidine synthase for making the higher polyamines from putrescine. Similar to the lethality produced by the ablation of Odc1, the knockout of SamDC results in embryonic death **(**Nishimura et al., 2002**)**. Not surprisingly, SamDC downregulation was also found to reduce tumor development (Zabala-Letona et al., 2017). Additionally, SamDC is upregulated when levels of Oda are high. This is due to the

degradation of Odc1 and is an alternative pathway for regulating the levels of the polyamines (Reviewed in [Nowotarski et al., 2013]). The reduction of Odc1 by Oda has been shown to cause an accumulation of dcAdoMet that in turn can act as a competitive inhibitor for methylation reactions and cause a genome-wide hypomethylation of CpG islands (Tsuji et al., 2001).

1.2.5 Spermidine synthase

The supply of dcAdoMet limits the formation of the higher polyamines spermidine and spermine, as dcAdoMet donates a propyl group to create spermidine and spermine (Ikeguchi et ao., 2006; Wahlfors et al., 1990). Spermidine synthase (SpdS) produces the triamine spermidine, from putrescine. The spermidine synthase reaction is the first aminopropyltransferase reaction in the polyamine pathway. Interestingly, SpdS activity is associated with the modification of eIF5A, a translation factor (Park et al., 1981). SpdS is the aminobutyl donor for the post-translational modification of a specific lysine residue on eIF5A. This reaction, mediated by deoxyhypusine synthase creates hypusinated eIF5A, a modification that has been shown to be essential for eIF5A activity (Park et al., 1981;Nishimura et al., 2012; Reviewed in [Pegg, 2016]). Loss of eIF5A activity results in decreased growth in mice highlighting another key function of polyamines in development (Nishimura et al., 2012).

1.2.6 Spermine synthase

Spermine synthase (Sms) is an active cytosolic homodimer (Wu et al., 2008)**.** Sms requires an amine substrate, spermidine, and an aminopropyl group from dcAdoMet in order to form the tetramine spermine (Korhonen et al., 1995). The aminopropyl transfer facilitated by

Sms is irreversible. Sms deficiency has been well characterized in a male mouse model, known as Gy mice, given this name due to their circling behavior (Lyon et al., 1986). These mice exhibited decreased spermine content even when fed a polyamine-rich diet (Mackintosh and Pegg, 2000; Wang et al., 2004). In 2003, Cason et al. discovered that a rare disease, Snyder Robinson Syndrome, was due to a mutation on the human Sms gene (Cason et al., 2003).

1.2.7 Spermidine/spermine-N^1-acetyltransferase and polyamine oxidase

Spermidine/spermine-N^1-acetyltransferase (Sat 1/2), and polyamine oxidase (PAO*)* are catabolic enzymes that aid in the regulation of intracellular polyamine pools. Sat1/2 functions by transferring the acetyl group from acetyl-CoA to either spermine or spermidine when levels of these polyamines are abundant (Hegde et al., 2007). The acetylated polyamines can then be excreted or serve as a substrate for PAO, which will subsequently produce spermidine or putrescine as well as H_2O_2 and 3-acetylaminopropanal. Because polyamine ratios have been shown to impact important biological processes like growth, improper regulation of higher polyamine content could lead to physiological anomalies such as renal damage (Wang et al., 2004; Zahedi et al., 2007). Thus, tight regulation of the polyamines is crucial to maintain physiological homeostasis, as aberrant regulation of polyamines and polyamine enzymes have been implicated in many diseases. For example, the upregulation of PAO has been shown in gastric and colon cancers (Goodwin et al., 2011; Xu et al., 2004).

1.2.8 Polyamine transport

In addition to the production of polyamines through the polyamine pathway, exogenous polyamines are able to be imported and exported in cells via the poorly understood polyamine transport system (PTS).

Polyamines have been shown to enter the cell via a transporter in simple eukaryotes and prokaryotes and these mechanisms have been well-characterized (Belting et al., 2003; Soulet et al., 2004; Uemura et al., 2010). However, to date, the exact mechanisms surrounding polyamine transport in higher organisms are unclear. It is known that in higher eukaryotes polyamine uptake is mediated by an energy-dependent mechanism that is Na+ independent but membrane potential dependent. Moreover, Ca^{2+} or Mg^{2+} is necessary for the activity of the PTS. Based on kinetic data, there is evidence to suggest that the diamine putrescine enters the cell via its own carrier while spermidine and spermine enter via different carrier(s). There are currently three models for the PTS that have been described (Poulin et al., 2012). In the first model, polyamines are translocated into the cell via an unidentified permease (Soulet et al., 2004). These unbound polyamines concentrate in membrane-bound vesicles known as polyamine sequestering vesicles (PSVs) that are part of the late endocytic pathway (i.e., multivesicular bodies, late endosomes and lysosomes). In the second model, Belting et al. described a mechanism of polyamine transport in which the heparan sulfate groups of glycosaminoglycans bind to spermine and translocate spermine into the cell. Spermine is then sequestered in PSVs and through a nitric oxide-dependent mechanism, spermine is released from the PSV (Belting et al., 2003). In the third model, Uemura and colleagues suggest that unidentified polyamine receptors within caveolin-1 enriched regions of the cell membrane import polyamines. The polyamines are released from the endosomes via a nitric oxide-dependent mechanism (Uemura et al., 2010;

Uemura et al., 2008). One common feature amongst all three hypothesized models is that polyamines are sequestered in vesicles upon entry into the cell. Though not completely understood, polyamine transport is an exciting area of study and could be invaluable for understanding the regulation of polyamines.

1.2.9 Polyamines and disease

Aberrant levels of polyamines and polyamine pathway enzymes have been implicated in many physiological anomalies such as Parkinson's disease, cancer, aging, and cardiac hypertrophy (Lewandowski et al., 2010; Minois et al., 2014; Reviewed in [Nowotarski et al., 2013]; Shantz et al., 2001). While increased levels of intracellular polyamines have been linked to proliferation of cancer cells, and decreased levels of intracellular polyamines have also been linked to physiological anomalies, such as neural tube defects (Haghighi et al., 2014; Lima and Shiu, 1985). Studies on the effect of decreased intracellular polyamine levels have shown that polyamine reduction, specifically reductions in spermidine and spermine, lead to apoptosis (Mandal et al., 2015). Interestingly, the apoptosis induced by Odc1 deficiency was rescued by injecting murine cells with exogenous polyamines (Nitta et al., 2002). This study further attests to the importance of maintaining homeostatic levels of polyamines within the cells.

Snyder-Robinson Syndrome (SRS) is an X-linked recessive condition in mammals. The primary cause of SRS is a mutation in the spermine synthase gene located within the X chromosome (Cason et al., 2003). Due to its mode of inheritance, SRS has only been characterized in males (Cason et al., 2003; Murray-Stewart et al., 2018) . In humans, this syndrome is associated with decreased cognitive and speech abilities, seizures, reduced

musculature, and bone abnormalities (Schwartz et al., 2011). In those with SRS, spermidine content is significantly increased while spermine content is almost depleted. To date, Gy mice have been used to study SRS and more recently a fly devoid of Sms has been used to study the effects of depleted spermine levels in the fly (Li et al., 2017; Mackintosh and Pegg, 2000; Wang et al., 2004).

1.2.10 Drosophila melanogaster as model organism for studying polyamines

The polyamine pathway has been studied in the fruit fly for more than 50 years (Herbst and Dion, 1970). The development of polyamine localization techniques and the tracking of polyamine changes throughout *D. melanogaster* development have greatly contributed to the field of polyamines (Herbst and Dion, 1970; Dion and Herbst, 1967). More recently, the effect of spermidine levels on fly cell growth was evaluated and demonstrated their effectiveness at promoting faster cell growth in culture as opposed to media without additional spermidine (Burnette et al., 2014). The fruit fly has also been used to model polyamine deficiencies and related alterations in metabolic processes. In fact, a model displaying a downregulation of Sms resulted in a decrease in overall ATP (Li et al., 2017). These studies strongly support the validity of using *D. melanogaster* as a model organism for studying polyamines.

1.3 Triglyceride and glycogen metabolism

1.3.1 Overview

Energy obtained through diet is often stored in the form of either triglycerides or glycogen. Products and intermediates of glucose metabolism, such as glucose-6-phosphate, glyceraldehyde-3-phosphate, and pyruvate can be shuttled from this pathway into the triglyceride and glycogen synthesis pathways at various points, which are often regulated allosterically or covalently (Appling et al., 2017)**.** These storage forms are useful during times of starvation or physical exertion, as the organism can draw upon stored fat, and readily available glycogen, to continue functioning. Since these metabolic mechanisms are conserved between humans and *D. melanogaster*, examining triglyceride and glycogen metabolism in fruit flies is useful for exploring metabolic changes in humans (DiAngelo and Birnbaum, 2009).

Improper regulation of glycogen and triglyceride storage has been associated with several diseased states such as hypoglycemia and type 2 diabetes (Sever et al., 2012). Millions of individuals are affected by diabetes globally each year. This condition, and related kidney disease, caused 1.3 million deaths in 2013 alone (Ng et al. 2014). Additionally, diabetes and increased glycogen storage has been implicated in retinopathy and neural damage (Gardiner et al., 2015). The healthcare costs associated with diabetes is astronomical. In 2012, it was estimated that treating diabetes in the United States cost $245 billion (American Diabetes, 2013). The upward trend in prevalence of diabetes and its related morbidities supports the need for further investigation into the mechanisms underlying diabetes and abberant triglyercide and glycogen storage (Ng et al. 2014, Gardiner et al., 2015).

1.3.2 Metabolic disorders modeled in Drosophila melanogaster

D. melanogaster has previously been used to study metabolic diseases seen in humans. In one study, the adipose (*adp*) mutant of *D. melanogaster* was utilized as a model of obesity and related phenotypes. Furthermore, this mutant has been used to clarify the role of insulin in the storage of triglycerides in fat bodies (the fly equivalent of human adipose tissue) (Hader et al., 2003).

Investigation of triglyceride and glycogen storage phenotypes has also been conducted in polyamine specific knockdowns in *D. melanogaster*. Specifically, Odc1 heterozygotes were shown to store higher levels of triglycerides, with no significant differences in glycogen storage. Observing the feeding habits of these flies revealed that the Odc1 heterozygotes ate less than wild-type flies but weighed more. The researchers determined this phenotype was due to an increase in the number of fat body cells and storage per fat body cell in the Odc1 heterozygous flies (Leon et al., 2020).

While Leon et al. began to characterize the roles of polyamines in glycogen and triglyceride metabolism, it did not investigate the role that other polyamine enzymes play in fly metabolism (2020). Therefore studies which demonstrate the interplay of polyamine enzymes and overall glycogen and triglyceride metabolism still need to be explored because data from these studies could provide therapeutic targets for combatting metabolic disorders.

Hypothesis and Specific Aims

Hypothesis: Polyamine content is highly regulated and intricately tied to triglyceride and glycogen storage. It is hypothesized that alterations to the polyamine pools by manipulating the expression of key synthetic enzymes will result in dysregulated triglyceride and glycogen storage.

Specific Aim 1: To compare Odc1 wild-type flies and Odc1 heterozygous flies in terms of polyamine content and how the partial knockdown of Odc1 influences the expression of other key enzymes in the polyamine pathway.

Specific Aim 2: To compare SpdS wild-type flies and SpdS knockout flies in terms of polyamine content, size, and triglyceride and glycogen storage, and how the knockout of SpdS influences the expression of other key enzymes in the polyamine pathway.

Specific Aim 3: To compare Sms wild-type flies and Sms knockout flies in terms of polyamine content, size, and triglyceride and glycogen storage, and how the knockout of Sms influences the expression of other key enzymes in the polyamine pathway.

Chapter 2

Materials and Methods

2.1 Fly genetics

The fly lines utilized in these experiments and their Bloomington Stock Center ID (listed in parentheses) are as follows: Odc1 mutant: y[1] w[*]; Miy [+mDint2] = MICOdc1[MI10996]/SM6a (BL #56103), SpdS mutant: y[1] w[*]; Mi{MIC}SpdS[MI00898]/TM3, Sb[1] Ser[1] (BL#34119), SpdS and Odc1 mutant control: y[1]w[1] (BL#1495), Sms mutant: w[*]; P{GSV1}Sms[C909] (BL#43396), Sms mutant control: w^{1118} (BL#5905), UAS-Sms-RNAi: y[1] sc[*] v[1] sev[21]; P{TRiP.HMC03665}attP40 (BL#52924) UAS-SpdS-RNAi: y[1]sc[*]v[1] sev[21]; P{TRiP.HMC04307}attP40 (BL#56011), UAS-EGFP-RNAi: y[1]sc[*]v[1]sev[21]; P{y[+t7.7]v[+t1.8]=VALIUM20-EGFP.shRNA.1}attP40 (BL#41555), Yolk-Gal4 (BL#58814).

For experiments on Odc1 flies, yw flies were crossed with y[1] w[*]; Miy [+mDint2] = MICOdc1[MI10996]/SM6a flies to generate Odc1 heterozygotes (Odc1 +/-). Sms homozygous knockout flies (Sms -/-) were generated by crossing w[*]; P{GSV1}Sms[C909] flies with each other. SpdS homozygous knockouts (SpdS -/-) were generated by crossing y[1] w[*]; Mi{MIC}SpdS[MI00898]/TM3, Sb[1] Ser[1] flies with each other. Flies were grown on a 12 hour to 12 hour light to dark cycle at 25˚C on a standard cornmeal-sucrose medium (100mL Karo Lite Corn Syrup, 65g cornmeal, 40g sucrose, 9g *Drosophila* agar (Genesee Scientific, El Cajon, CA), and 25g whole yeast in 1.25L water).

2.2 Weight assay

One-week old male and female SpdS -/-, Sms -/-, yw, and w^{1118} flies were anesthetized using carbon dioxide (CO_2) gas. The total weight of each sample was measured using an analytical balance prior to calculating average weight per fly (mg).

2.3 Metabolite extraction and protein, triglyceride, DNA, and glycogen assays

One week old male and female Sms -/-, SpdS -/-, yw, and w^{1118} flies , and fat bodies from one week old female yolk-Gal4>SMS-RNAi, yolk-Gal4>-SpdS-RNAi, and yolk-Gal4>EGFP-RNAi flies, were homogenized in lysis buffer (140mM NaCl, 50mM Tris-HCl, pH 7.5, 0.1% Triton-X with 1X protease inhibitor cocktail (Roche, Boston, MA)). Following homogenization, samples were centrifuged at 4ºC for 15 minutes at 16,000g. Proteins were measured in all samples using the Pierce BCA Protein Assay kit (ThermoFisher, Leesport, PA), triglycerides were measured using the Infinity Triglyceride kit (ThermoFisher), and glucose was measured using the Glucose Oxidase kit (ThermoFisher) according to the manufacturer's instructions. Following glucose measurement, samples were incubated in 8mg/mL amyloglucosidase (Sigma, St. Louis, MO), diluted in 0.2M citrate buffer (pH 5.0) and incubated at 37°C for 2 hours. After incubation, free glucose measured was subtracted from total glucose to determine glycogen concentration. All triglyceride and glycogen measures were normalized to total protein content.

In fat body samples, DNA content was measured using the Quant-iT double-stranded DNA high sensitivity kit according to the manufacturer's instructions (ThermoFisher). Triglyceride and glycogen content were normalized to total protein and fat-body DNA content, separately.

2.4 RNA isolation and quantitative PCR

RNA from one-week old male and female flies of each genotype (yw, w^{1118}, Sms -/-, SpdS -/-, and Odc +/-) was isolated using the RiboZol RNA Extraction Reagent according to the manufacturer's instructions (VWR, Radnor, PA). Five µg of total RNA was DNase treated using the TURBO DNA-free kit according to the manufacturer's instructions (ThermoFisher). After treatment, DNase treated RNA was quantitated and 0.5µg DNase treated RNA was utilized to synthesize cDNA using the qScript XLT cDNA Supermix according to the manufacturer's instructions (Quantabio, Beverly, MA). Quantitative PCR (qPCR) was conducted on samples in a 25µl reaction of 1 µl of cDNA, 200 nM primers, and 1X PerfeCTa SYBR Green, according to the manufacturer's instructions (Quantabio). The qPCR conditions were as follows: 95°C for 3 minutes, 40 cycles of 95°C for 30 seconds, 60°C for 1 minute, 72°C for 30 seconds, followed by a melt curve. The primers used in this study were: ornithine decarboxylase (Odc1) Forward, 5' CCTCAACATCTGCGACCTGT 3'; Odc1 Reverse, 5' ACATCGAAGCCCAAGACCAG 3'; ribosomal protein 49 (rp49) Forward, 5' GACGCTTCAAGGGACAGTATCTG 3'; Reverse, 5' AAACGCGGTTCTGCATGAG 3'; ornithine decarboxylase antizyme (Oda), Forward, 5' TGTCACCGAGGACCAGTACA 3'; Reverse, 5' TCCAGAAGCGAGATGAAGGT 3'; S-adenosylmethionine decarboxylase (SamDC), Forward, 5' GCCTGGGTCAGTTGCTTAAG 3'; Reverse, 5' TCTGGATCGGAGTCAACGTT 3'; spermidine synthase (SpdS), Forward, 5' AGGAAGTGCATCAGGTGGAA 3'; Reverse, 5' AGCTGTCGGTGATGATGACA 3'; spermine synthase (Sms), Forward, 5' TCGTTCATGGAAGAGTGGCT 3'; Reverse, 5' GGTTCCCGGTTCCTGACTTA 3'; Spermidine/spermine-N^1-acetyltransferase (Sat1/2), Forward, 5' CGGTACTTCTTCGTCGAGGA 3'; Reverse, 5' CGGCACCAAGACTTTCGTAG 3'; and polyamine oxidase (PAO), Forward, 5' ACAACATGGACGAGGTGGAT 3'; Reverse, 5'

CGGGCATTTGGTCAGTATGG 3'. To quantify the relative gene expression, the comparative cycle threshold (C_t) method was utilized and the C_t values for the gene of interest was normalized to the C_t values of rp49 and were presented in relation to the wild-type (yw or w^{1118}) flies. Analysis was performed on the StepOne Plus real-time PCR software (ThermoFisher).

2.5 High pressure liquid chromatography

Polyamine content was measured using reversed-phase HPLC analysis as described previously (Kabra et al., 1986). The data were normalized to total protein.

2.6 Statistics

The results are expressed as the mean ± standard error (SE). Odc1+/- and SpdS-/- flies were compared to yw control flies and Sms -/- flies were compared to w^{1118} control flies. Yolk-Gal4>SMS-RNAi and yolk-Gal4>-SpdS-RNAi were compared to yolk-Gal4>EGFP-RNAi flies. Statistical analyses were performed using an unpaired Student's t-test. Significance was determined as $p < 0.05$.

Chapter 3

The knockdown of Odc1 alters the expression of the polyamine pathway enzymes and polyamine content in *Drosophila melanogaster*

3.1 Introduction

Since its discovery in 1968, ornithine decarboxylase (Odc1) has been the subject of many studies due to its critical role in numerous cell processes such as cell proliferation and differentiation (Russell and Snyder, 1968). In fact, Odc1 expression is essential, as the ablation of Odc1 is lethal in numerous model organisms, including *Drosophila melanogaster* (*D. melanogaster*) (Pendeville et al., 2001; Leon et al., 2020). Odc1 homozygous knockouts in both mice and fruit flies did not survive into adulthood (Pendeville et al., 2001; Leon et al., 2020). Odc1 is the first rate-limiting enzyme in the polyamine biosynthetic pathway and converts the amino acid ornithine to the diamine putrescine which is further converted to the higher polyamines spermidine and spermine (Reviewed in [Pegg, 2006]).

Polyamine content is tightly regulated by a series of synthetic, catabolic and poorly understood transport mechanisms (Soulet et al., 2004; Uemura et al., 2010)**.** Moreover, the enzymes involved in the polyamine pathway are highly regulated at the level of transcription, translation and degradation (Murakami et al., 1992; Nowotarski and Shantz, 2010, Wu et al. 2015). This intricate level of regulation attests to the importance of polyamines.

Due to their charge, the polyamines are able to bind to DNA, RNA, and proteins and thereby impact gene expression (Schuber, 1989; Casero, 2018). Consequently, polyamines have been implicated in a wide-range of physiological conditions ranging from cancer to neurological defects in humans (Reviewed in [Miller-Fleming et al., 2015]). Recently, studies have sought to

characterize the potential role of Odc1 in triglyceride and glycogen metabolism using the fly model (Leon et al., 2020). Leon et al. observed that in female Odc1 heterozygotes increased triglyceride storage per cell occurred and the number of fat cells was increased (Leon et al., 2020). Moreover, these animals displayed increased expression of two lipid synthesis genes: fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). These studies linked Odc1 expression to triglyceride storage and suggested that the polyamine pathway plays a role in lipid metabolism (Leon et al., 2020). While informative, these studies did not show the effect that knocking down Odc1 had on the expression of other polyamine pathway enzymes. Moreover, the studies did not show the role that polyamine concentration had on the observed phenotype.

In these studies, the effects of knocking down Odc1 in male and female flies were further characterized by determining the gene expression of other polyamine pathway enzymes and by measuring the levels of putrescine, spermidine and spermine. Male Odc1 heterozygous flies displayed a significant reduction in spermidine and an increased spermine content when compared to wild-type flies. Interestingly, these changes to the concentrations of the polyamines occurred without expression changes to other enzymes involved in the synthesis or catabolism of the polyamines. Conversely, female Odc1 heterozygous flies displayed no appreciable changes in polyamine content yet displayed decreased expression of ornithine decarboxylase antizyme (Oda).

3.2 Results

*3.2.1 Odc1 heterozygous male flies display no gene expression changes to enzymes involved in the synthesis and catabolism of the polyamines but display decreased spermidine and increased spermine levels***.**

In order to investigate the impact of Odc1 knockdown on the expression of other enzymes in the polyamine pathway, the model organism *D. melanogaster* was utilized. Male Odc1 heterozygous flies displayed an 80 percent reduction in the expression of Odc1 when compared to wild-type flies. Interestingly, this knockdown in Odc1 expression did not lead to any changes in the expression of other enzymes involved in the polyamine pathway (Figure 3.1). Moreover, the decrease in Odc1 expression led to a decrease in spermidine content and an increase in spermine content (Table 3.1). Taken together these data suggest that the alteration to the levels of spermidine and spermine may be due to the elusive polyamine transport system and not due to changes in the gene expression of polyamine pathway enzymes.

3.2.2 Odc1 heterozygous female flies displayed a decrease in Oda expression but exhibited no changes to the concentrations of the polyamines.

In contrast, female Odc1 heterozygous flies exhibited a 50 percent reduction in Odc1 expression and a 40 percent reduction in Oda (Figure 3.2). There were no changes to the concentration of the polyamines in female Odc1 heterozygous flies suggesting that in females polyamine homeostasis was maintained, despite the changes to Odc1 and Oda expression (Table 3.2).

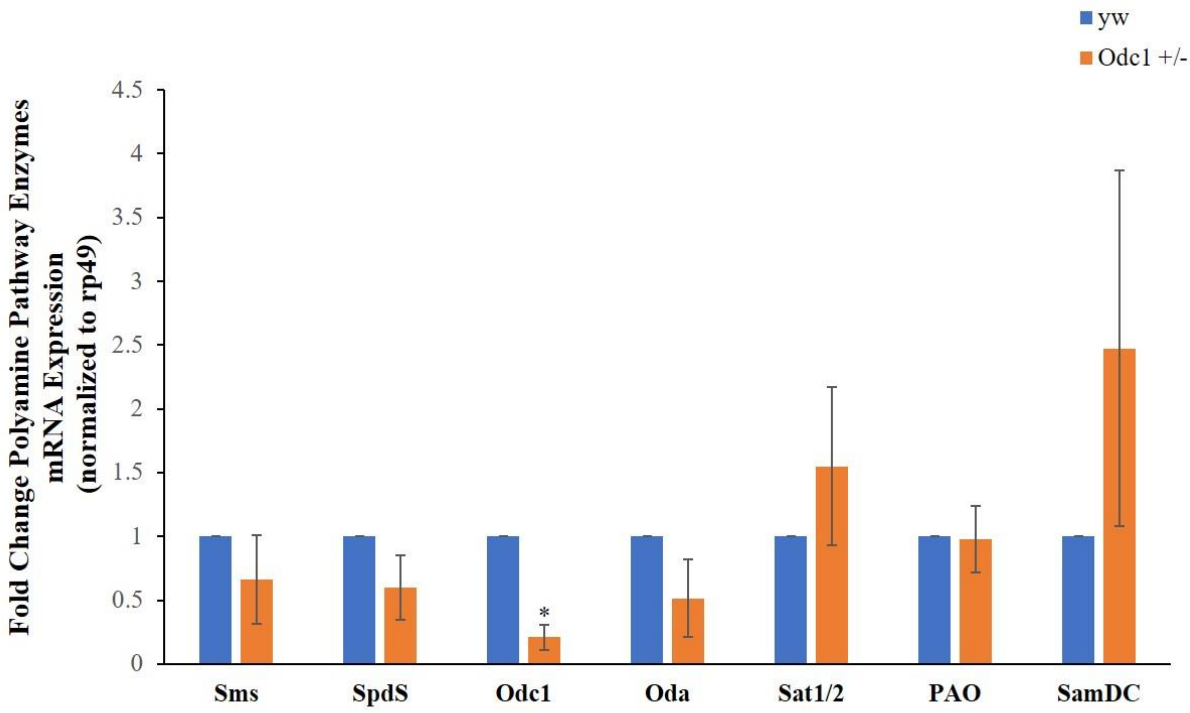

Figure 3. 1 Male Odc1 heterozygous flies exhibit significantly decreased Odc1 expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), polyamine oxidase (*PAO*), and adenosyl-methionine decarboxylase (*SamDC*), was conducted on RNA isolated from one-week old male *OdcMI/+* (*Odc1 +/-*) flies and compared to wild-type *yw* controls. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *yw*. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *Odc1 +/-* to *yw* controls. (n= 5).

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)	Spermidine to spermine ratio
yw	0 + 0.0	6.10 ± 0.50	0.26 ± 0.11	4.97 ± 0.56
Odc1 +/-	0 ± 0.0	3.41 ± 0.69*	1.40 ± 0.17*	2.71 ± 0.60

Table 3. 1 Male Odc1 heterozygous flies display significantly decreased levels of spermidine and significantly increased levels of spermine when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n=14-16).

Data provided by the Casero lab at Johns Hopkins University.

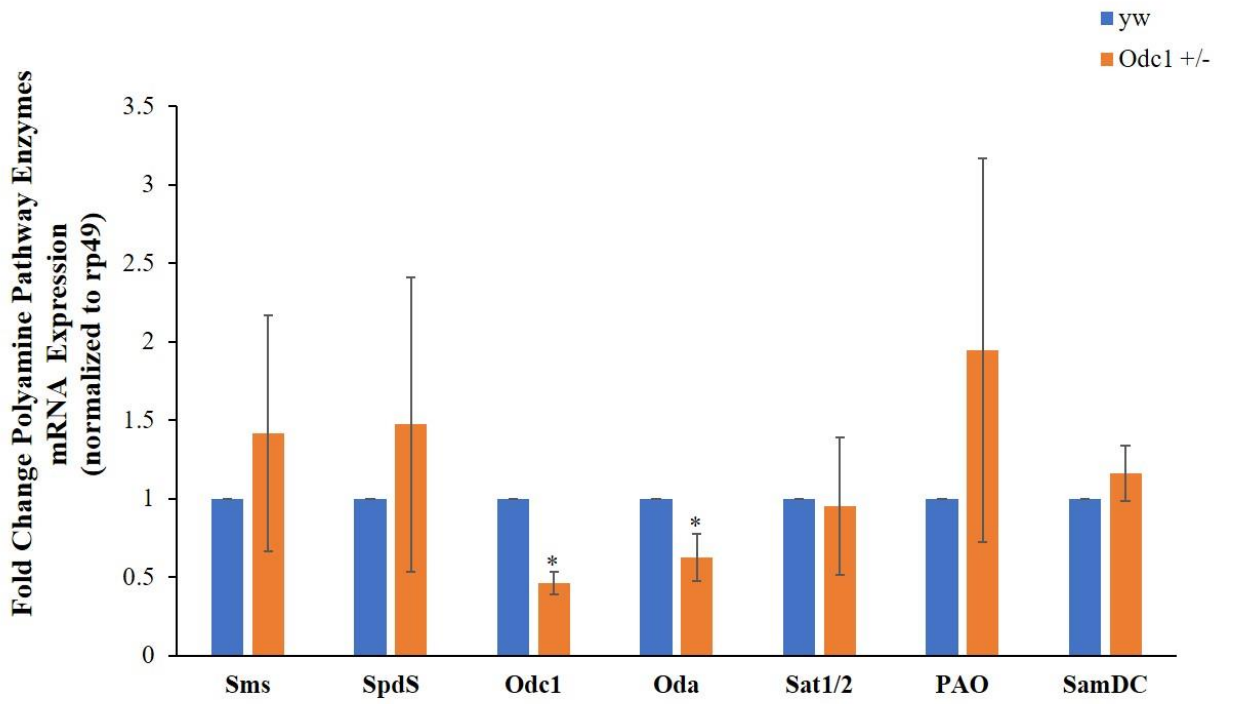

Figure 3. 2 Female Odc1 heterozygous flies exhibit significantly decreased Odc1 and Oda expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), polyamine oxidase (*PAO*), and adenosyl-methionine decarboxylase (*SamDC*), was conducted on RNA isolated from one- week old female *OdcMI/+* (*Odc1 +/-*) flies and compared to wild-type *yw* controls. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *yw*. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Odc1 +/-* to *yw* controls. (n= 5).

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)	Spermidine to spermine ratio
yw	0.01 ± 0.01	9.33 ± 0.50	0.76 ± 0.11	14.69 ± 2.83
Odc1 +/-	0 ± 0.0	8.29 ± 0.34	0.85 ± 0.04	10.44 ± 1.05

Table 3. 2 Female Odc1 heterozygous flies do not display changes in the levels of putrescine, spermidine, or spermine when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n= 14-16).

Data provided by the Casero lab at Johns Hopkins University.

3.3 Discussion

In these studies, it was shown that male Odc heterozygous flies exhibited reduced spermidine and elevated spermine levels, despite a lack of changes to the gene expression of enzymes involved in the polyamine pathway (Figure 3.1 and Table 3.1). Furthermore, female Odc heterozygous flies had decreased Oda gene expression but showed no changes in polyamine content (Figure 3.2 and Table 3.2).

The decrease in Oda expression in the Odc1 heterozygous females is interesting. It is known that Odc1 is negatively regulated by Oda (Murakami and Hayashi, 1985). As seen in mammalian models, Oda expression is regulated, in part, by high levels of polyamines, yet in fly model used in the current study no changes were observed in the polyamine levels (Murakami and Hayashi, 1985). Moreover, it has been shown that decreased spermidine levels correlate with decreased Oda expression; however, in the male Odc1 heterozygotes, there were not any observable changes to Oda expression (Nilsson et al., 1997). Thus, these data suggest that the elusive polyamine transport system may be playing a role in the tight regulation of the polyamines in these Odc1 heterozygous flies.

Odc1 inhibition or mutation have previously been shown to cause the depletion of intracellular polyamines (Nilsson et al., 1997; Wu et al., 2015). However, the marked alterations in polyamine content, namely a decrease in spermidine content and increase in spermine content, were only displayed in male Odc1 heterozygotes. The decrease in spermidine content is consistent with prior studies detailing the reduction in spermidine following Odc1 inhibition (Nilsson et al., 1997). However, the increase in spermine, as well as the maintenance of homeostatic polyamine levels in Odc1 heterozygous females, although not completely defined, could partly be explained as a result of exogenous polyamine transport (Nilsson et al., 1997).

Transport of exogenous polyamines has been previously described as a method for importing polyamines into cells, with various transport models used to describe the potential import mechanisms in eukaryotes (Poulin et al., 2012; Belting et al., 2003). The data presented in the current study support those previous findings and suggest that the polyamine transport system may play a significant role in the changes to polyamine content in male flies and the lack of changes seen in female flies. Investigating the mechanism of polyamine transport would help further the understanding of the regulation of polyamines. However, to date, polyamine transporters have not been identified in flies.

The data also indicate sex may play a role in Oda regulation. Several studies have detailed the differences in mouse ornithine decarboxylase (ODC) and the varying patterns in polyamine production between male and female mice (Goldstone et al., 1982; Bastida 2007). One study in mice described the male and female regulation of ODC in kidney tissue varied due to the testosterone present in males (Goldstone et al., 1982). These data suggest that hormone variations between sexes may alter Oda regulation and potentially the expression of other pathway genes.

A limitation of the analysis in this study is that it measured expression-level changes in the polyamine pathway. It is well known that polyamine enzymes are regulated at the level of transcription, post-transcription and translation (Reviewed in [Nowotarski et al., 2013])**.** Thus, a more robust study in the model system must be conducted in order to determine whether the changes shown at the gene expression level hold true at the protein level.

In summary, the effects of Odc1 knockdown were further characterized in *Drosophila melanogaster*. Novel effects by which the knockdown of Odc1 impacts the polyamine pathway differently in male and female flies were also described. As polyamines are associated with

numerous physiological anomalies any research investigating polyamine regulation could advance the understanding of their contributions to normal and diseased states and present targets for potential therapies and treatments (Murray-Stewart et al., 2018; Xu et al., 2004; Leon et al., 2020).

Chapter 4

The ablation of Spermidine synthase (SpdS) alters metabolite storage, expression of polyamine pathway genes, and polyamine content in *Drosophila melanogaster*

4.1 Introduction

Polyamines are essential for normal cell growth and development (Fozard et al., 1980). The higher polyamines, spermidine and spermine, are synthesized by aminopropyltransferases known in *Drosophila melanogaster* (*D. melanogaster*) as spermidine synthase (SpdS) and spermine synthase (Sms) (Ikeguchi et al., 2006). These enzymes utilize an amine receptor and decarboxylated S-adenosylmethionine (dcAdoMet) as an aminopropyl donor (Wu et al., 2007). However, SpdS uses putrescine as a substrate (Figure 1.3; Ikeguchi et al., 2006).

In many organisms SpdS plays an integral role in growth and development and has been deemed essential, such as in aspergillus (Majumdar et al., 2018). Several studies have previously shown that a reduction in the growth rate of bacteria in mice was linked to the ablation or inhibition of SpdS, emphasizing the importance of understanding its regulation and the role it has in the regulation of other polyamine pathway enzymes (Tholl et al., 1998). Moreover, spermidine has been shown to induce autophagy and has been linked to increased lifespans in nematodes, flies and mice (Madeo et al., 2018).

The deregulation of SpdS and the subsequent alteration to the intracellular levels of spermidine have been studied for decades because they have been implicated in several diseases such as type 2 diabetes, cancer, depression, and renal failure (Madeo et al., 2018; Miao et al., 2016). However, the exact mechanism of how polyamines and their associated enzymes impact fatty acid metabolism is not completely understood. Recently this novel study of ornithine

decarboxylase (Odc1) heterozygous flies revealed metabolic changes which were linked to changes in Odc1 expression (Leon et al., 2020). Yet, whether changes to the expression of SpdS play a role in fly metabolism remain elusive. Moreover, the self-regulation of the polyamine pathway is still being studied because it is evident polyamines are tightly regulated in order to maintain homeostatic cellular levels (Reviewed in [Nowotarski et al., 2013]). By investigating how the ablation of SpdS alters the expression of other polyamine pathway genes and the intracellular polyamine content more insight can be gained as to how the polyamine pathway self-regulates. Moreover, the ablation of SpdS can aid in the understanding of how polyamines affect fatty acid metabolism.

In these studies, the effects of SpdS ablation in male and female flies (denoted herein as SpdS-/-) were further characterized by measuring polyamine content and the expression of key enzymes implicated in polyamine metabolism. The weights of SpdS-/- flies were also measured to determine potential growth-related differences in knockout and wild-type flies. Female SpdS -/- flies underwent further analyses to determine any changes in their whole body and fat body-specific triglyceride and glycogen storage. The number of fat body cells was also measured in female SpdS –/- flies.

Overall SpdS-/- flies displayed increased expression of genes involved in polyamine catabolism. Male SpdS-/- flies showed increased polyamine oxidase (PAO) expression along with unaltered polyamine content, whereas female SpdS -/- flies showed increased spermine/spermidine-N^1- acetyltransferase (Sat1/2) expression. Only females displayed alterations to polyamine concentrations with putrescine increasing and spermine decreasing. Metabolite analyses revealed increased triglyceride storage in the whole body of the organism as

well as in the fat body. This increase was accompanied by an increased number of fat body cells and a decrease in fat body glycogen.

4.2 Results

4.2.1 SpdS knockout flies weighed the same as wild-type flies.

In order to study the effects that the knockout of SpdS had on an entire organism, the fly was utilized due to the ease of creating a knockout animal. Analysis of fly weights revealed no significant changes between knockout flies and wild-type animals (Figures 4.1 and 4.2).

4.2.2 Male SpdS knockout flies displayed increased polyamine oxidase expression, whereas female SpdS knockout flies displayed increased spermidine/spermine N-^{1} acetyl transferase expression and variations in polyamine content.

In order to determine how the polyamine pathway self-regulates, the expression of polyamine pathway enzymes was investigated. Male SpdS-/- flies showed a significant increase in PAO expression when compared to wild-type flies (Figure 4.3). Despite this change in the expression of PAO, there were no marked changes to polyamine content in male SpdS -/- flies (Table 4.1). Interestingly, female SpdS -/- flies experienced an upregulation of Sat1/2 (Figure 4.4). Unlike the male SpdS-/- flies, the female SpdS-/- flies also showed a marked increase in putrescine content and decreased spermine content (Table 4.2). Together these data suggest that polyamine content and gene expression are differentially regulated in male and female flies that are devoid of SpdS activity.

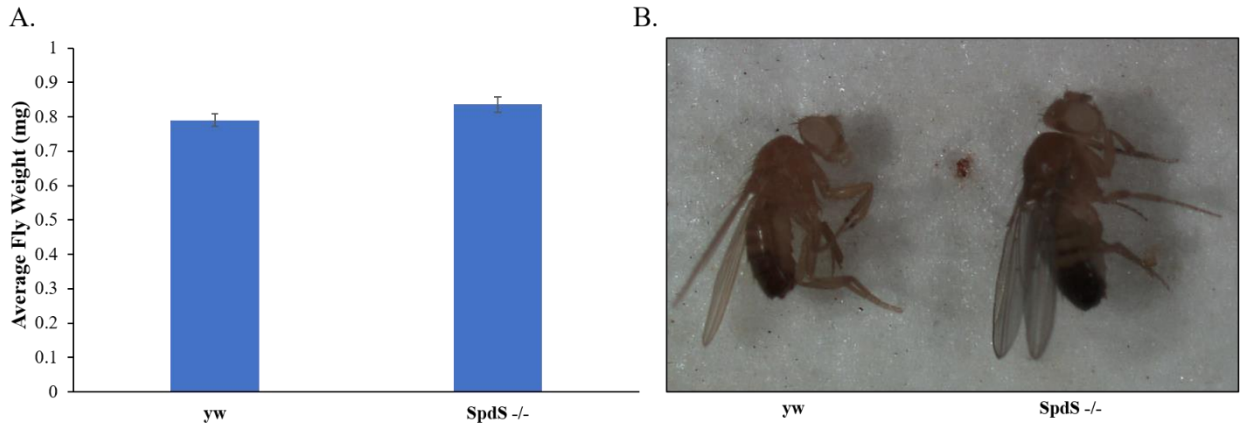

Figure 4. 1 Male SpdS knockout flies weighed the same as control flies.

(A) Weights of one-week old *SpdS* knockout (*SpdS -/-*) males were compared to male wild-type *yw* controls. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *SpdS -/-* to *yw* controls. (n=11-14). (B) One-week old male flies *yw* and *SpdS -/-* flies. A representative image is shown.

Pictures provided by Dr. Justin DiAngelo.

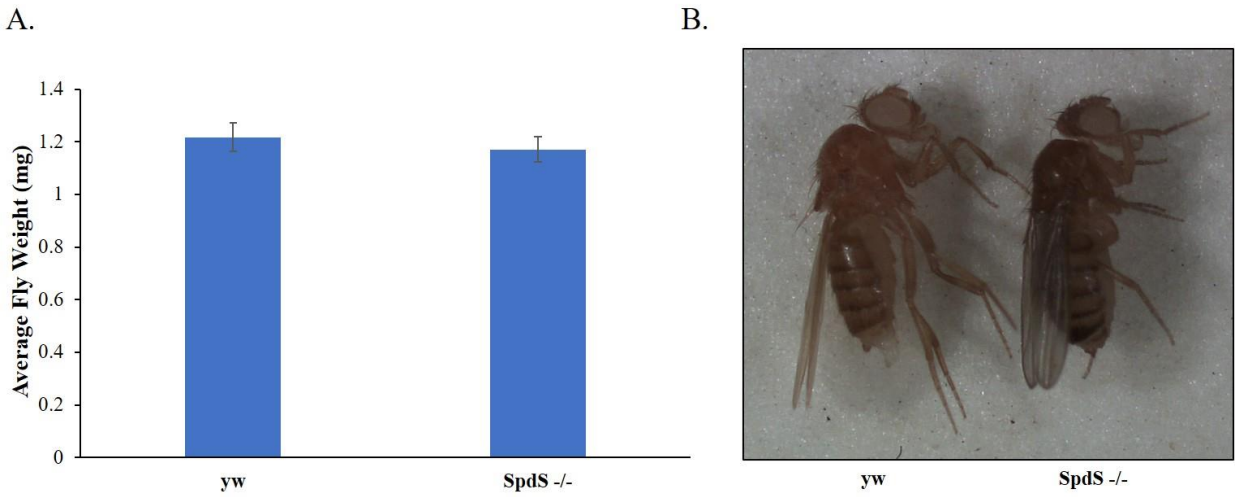

Figure 4. 2 Female SpdS knockout flies weighed the same as control flies.

(A) Weights of one-week old *SpdS* knockout *(SpdS -/-*) females were compared to female wild-type *yw* controls. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *SpdS -/-* to *yw* controls. (n=13-14). (B) One-week old female *yw* and *SpdS-/-* flies. A representative image is shown.

Pictures provided by Dr. Justin DiAngelo.

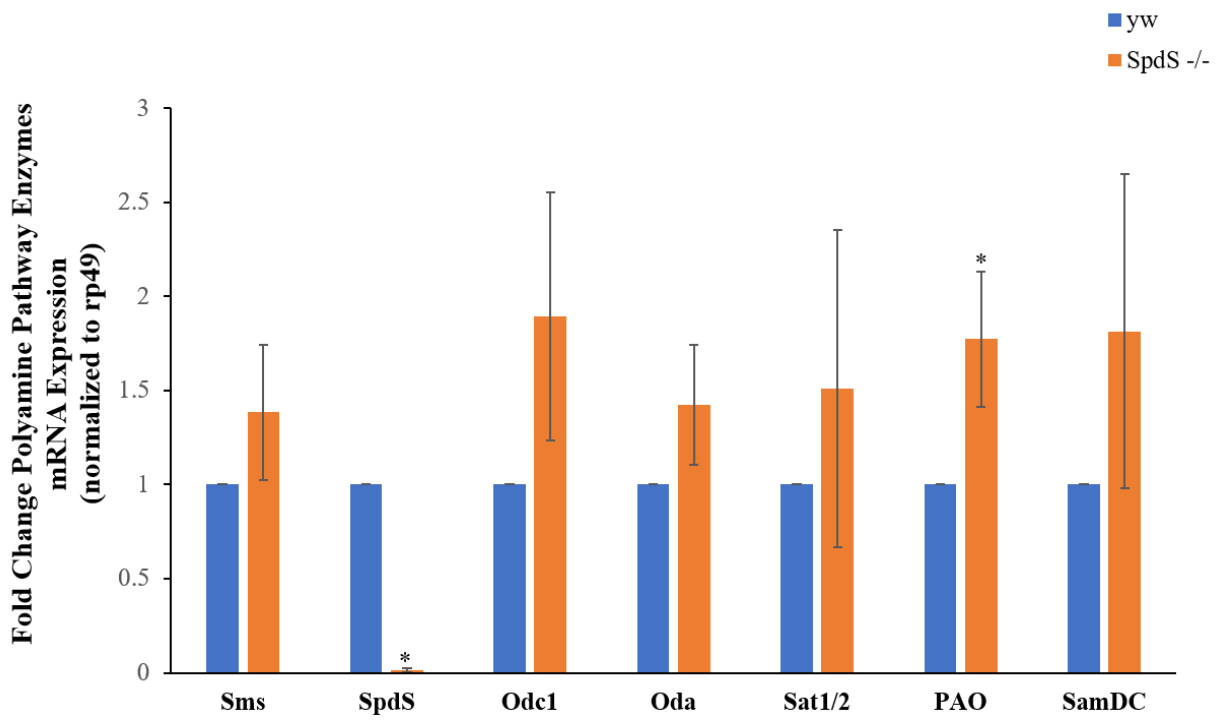

Figure 4. 3 Male SpdS knockout flies exhibit significantly decreased SpdS expression and significantly increased PAO expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), polyamine oxidase (*PAO*), and adenosyl-methionine decarboxylase (*SamDC*), was conducted on RNA isolated from one-week old male $SpdS^{m100898}$ (*SpdS -/-*) flies and *yw* controls. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *yw*. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *SpdS -/-* to *yw* controls. (n= 9).

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)
yw	1.76 ± 0.37	11.89 ± 1.16	0.33 ± 0.18
SpdS -/-	2.80 ± 0.57	9.71 ± 1.53	0 ± 0.0

Table 4. 1 Male SpdS knockout flies do not display changes to the levels of putrescine, spermidine, and spermine when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n= 11-14)
Data provided by the Casero lab at Johns Hopkins University.

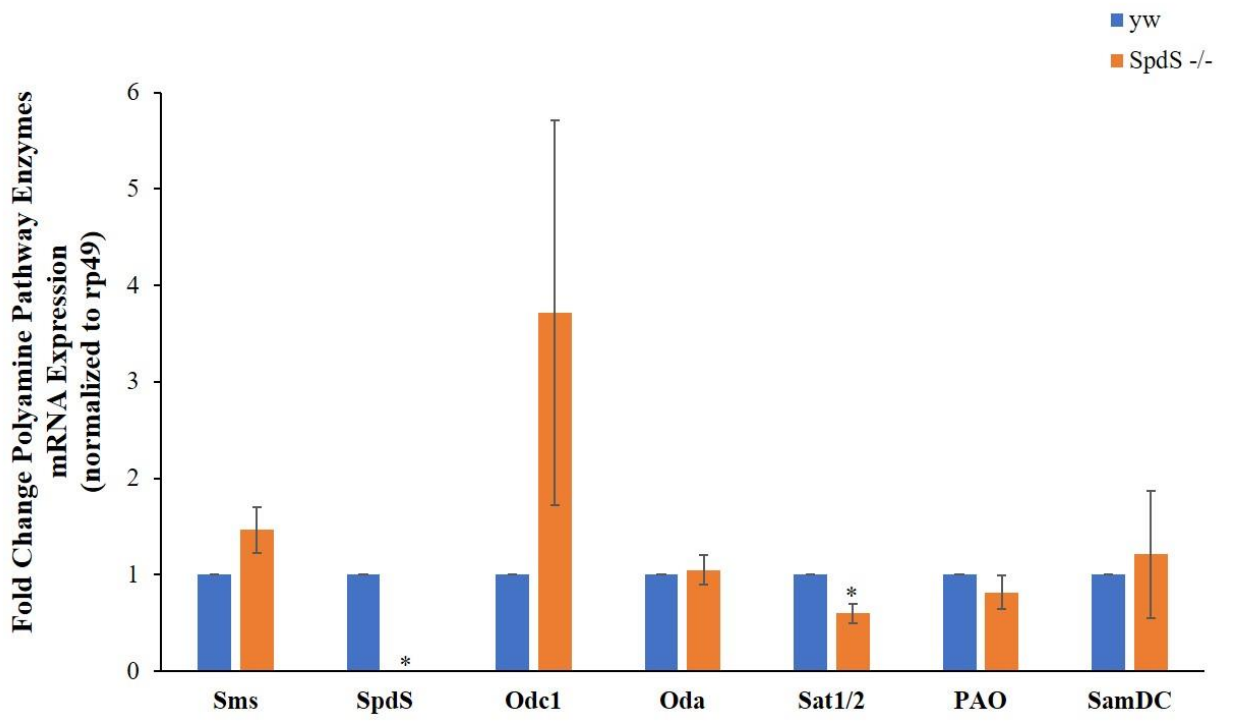

Figure 4. 4 Female SpdS knockout flies exhibit significantly decreased SpdS and Sat1/2 expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), polyamine oxidase (*PAO*), and adenosyl-methionine decarboxylase (*SamDC*), was conducted on RNA isolated from one-week old female $SpdS^{m100898}$ (*SpdS -/-*) flies and *yw* controls. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *yw*. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *SpdS -/-* to *yw* controls. (n= 6).

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)
yw	2.23 ± 0.54	22.09 ± 1.76	1.55 ± 0.38
SpdS -/-	4.88 ± 0.88*	20.71 ± 2.42	0 ± 0.0*

Table 4. 2 Female SpdS knockout flies display significantly increased levels of putrescine and significantly decreased levels of spermine when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n= 13-14).

Data provided by the Casero lab at Johns Hopkins University.

4.2.3 Female SpdS knockout flies displayed increased triglyceride and unaffected glycogen storage, with fat body specific knockdowns showing increased triglyceride storage and an increased number of fat body cells.

To determine whether changes in glycogen and triglyceride storage occurred in female SpdS-/- flies, both glycogen and triglyceride content were measured. Female flies were used in these studies because they are larger and thus provide more material (Table 4.2). Triglyceride content was noticeably increased, and glycogen levels were unchanged when studying the triglyceride and glycogen content in the whole fly (Figures 4.5 and 4.6). RNAi knockdown of SpdS in the fat bodies of flies was used to further characterize the metabolic phenotype in female flies. The fat bodies in which SpdS was decreased showed increased triglyceride storage, a decrease in glycogen storage, and an increase in the overall number of fat body cells (Figures 4.7, 4.8, 4.9). Interestingly, triglyceride and glycogen storage did not vary per fat body cell (Figures 4.10 and 4.11). Together these data suggest a potential role of SpdS in regulating the number of fat storage cells.

4.3 Discussion

In this study both male and female SpdS-/- flies had alterations in polyamine pathway gene expression (Figures 4.3 and 4.4). Male SpdS-/- flies displayed increased PAO activity, while female SpdS-/- flies had increased Sat1/2 expression (Figures 4.3 and 4.4). Additionally, female SpdS-/- flies had increased putrescine and decreased spermine content when compared to control flies (Table 4.2). Male SpdS -/- flies experienced no changes in polyamine content

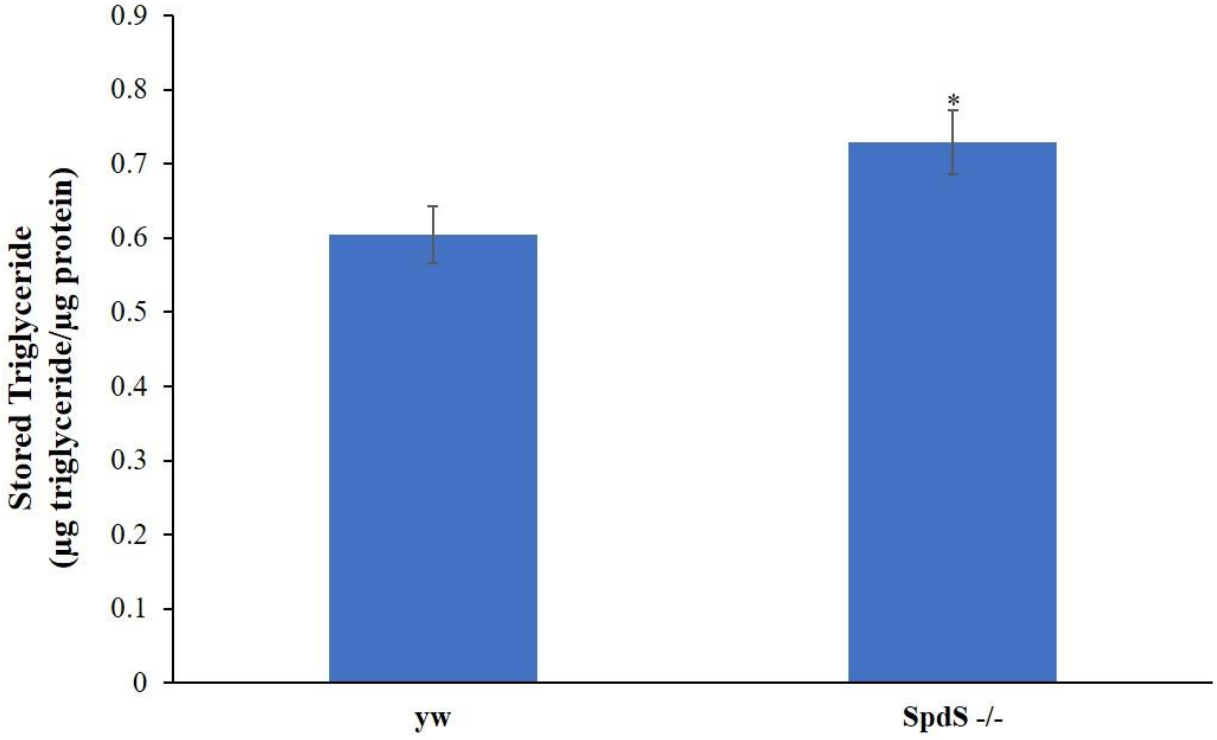

Figure 4. 5 Female SpdS knockout flies store more triglycerides than control flies.

Triglycerides were measured in *SpdS*^{m100898} (*SpdS* -/-) mutants by homogenizing pairs of one-week old adult female flies and *yw* controls. Triglyceride measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *SpdS* -/- to *yw* controls. (n=23-25).

Data collection by Erik Avis and Elise Paskowski.

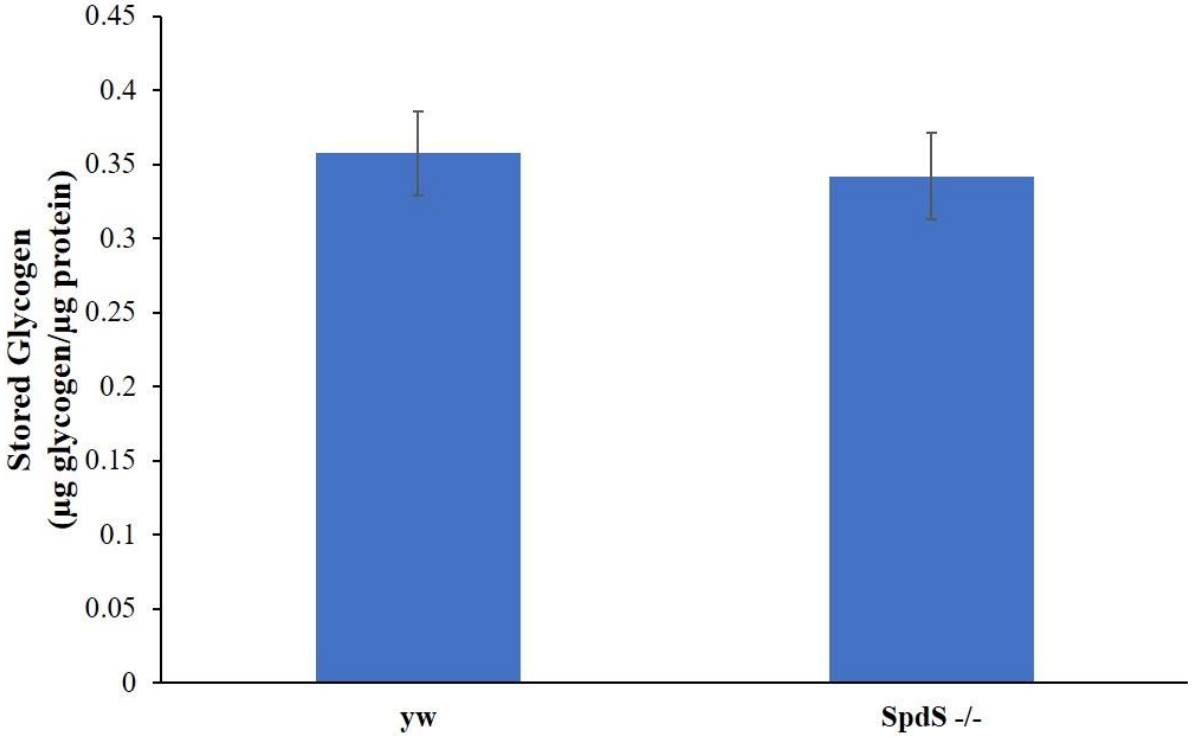

Figure 4. 6 Female SpdS knockout flies store the same amount of glycogen as control flies.

Glycogen was measured in *SpdS*^{m100898} (*SpdS* -/-) mutants by homogenizing pairs of one-week old adult female flies and *yw* controls. Glycogen measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *SpdS* -/- to *yw* controls. (n=23-25).

Data collection by Erik Avis and Elise Paskowski.

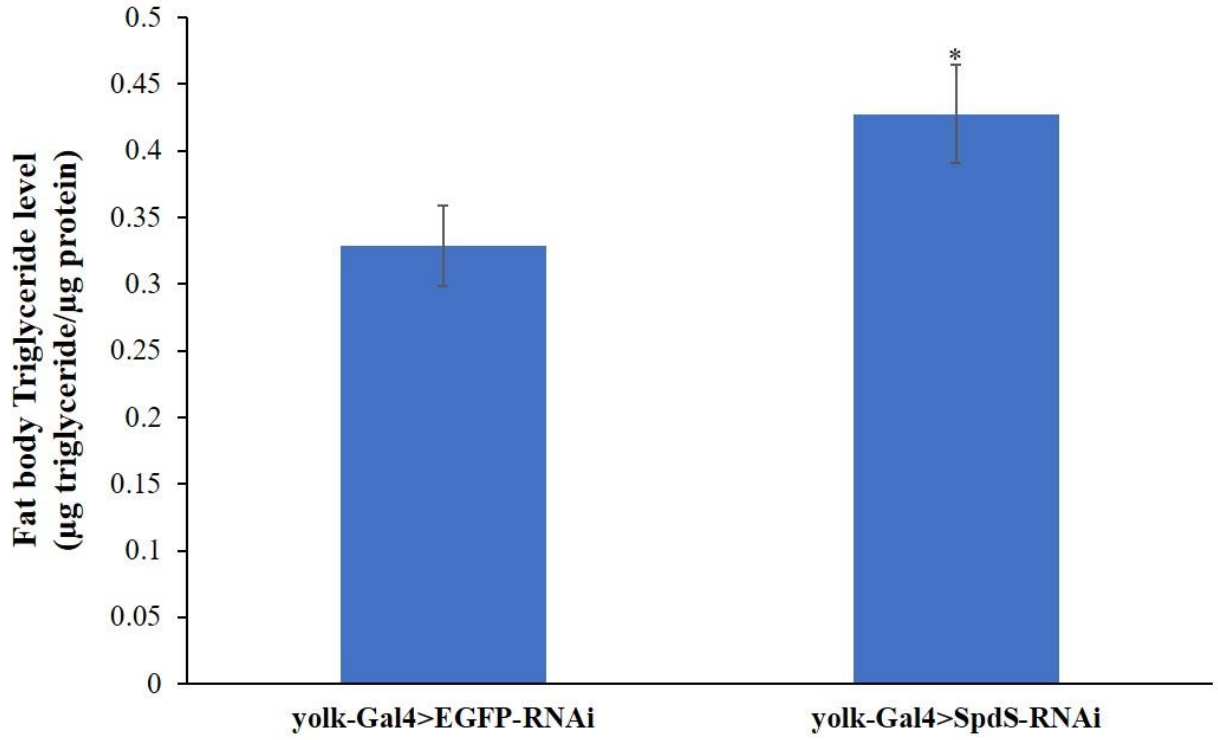

Figure 4. 7 Decreasing SpdS in the fat body increases triglyceride storage in female flies.

Triglycerides were measured in one-week old female *yolk-Gal4>SpdS-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Triglyceride measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>SpdS-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=49-60).

Data collection by Erik Avis and Elise Paskowski.

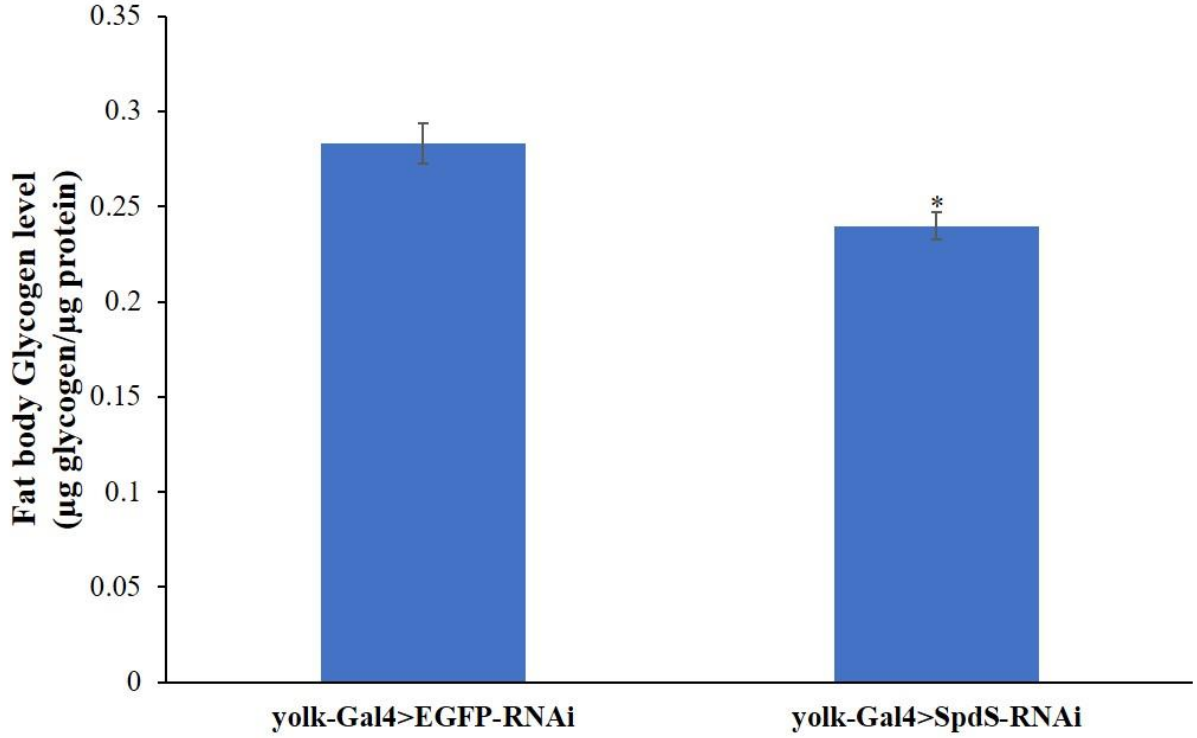

Figure 4. 8 Decreasing SpdS in the fat body results in decreased glycogen storage in female flies.

Glycogen was measured in one-week old female *yolk-Gal4>SpdS-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Glycogen measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *yolk-Gal4>SpdS-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=50-60).

Data collection by Erik Avis and Elise Paskowski.

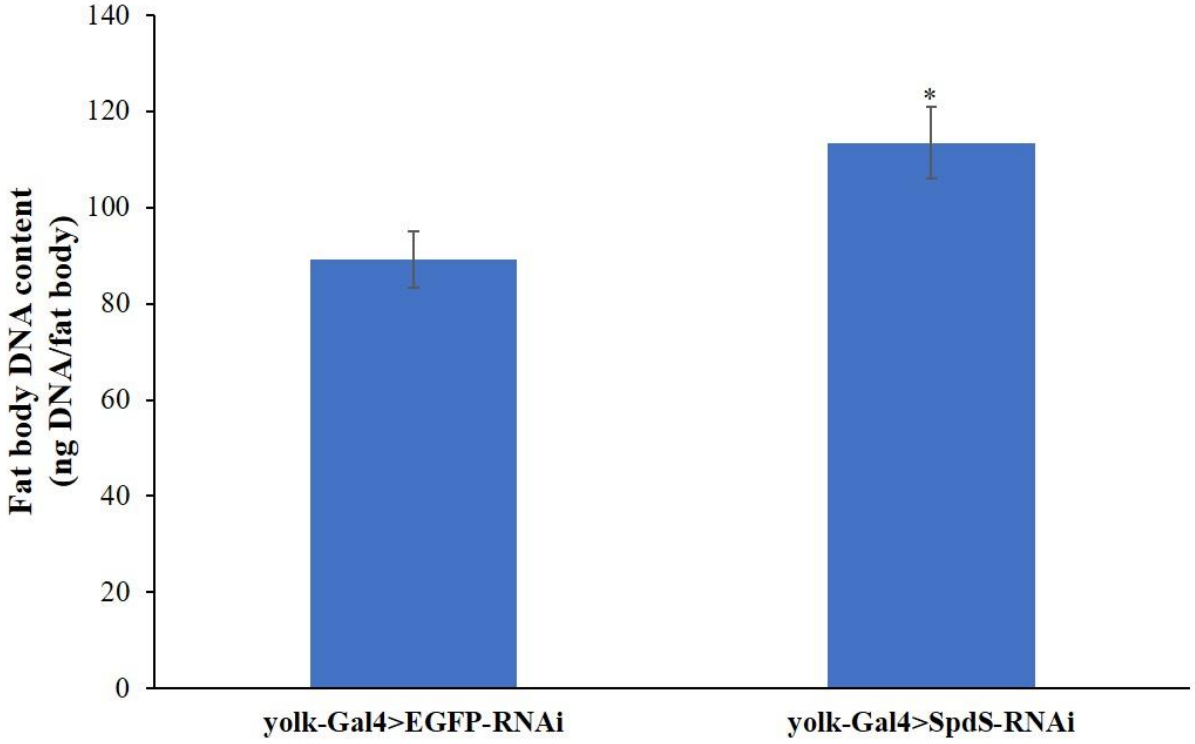

Figure 4. 9 Decreasing SpdS in the fat body increased the number of fat body cells in female flies.

Total DNA content was measured in fat bodies dissected from one-week old female *yolk-Gal4>SpdS-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *yolk-Gal4>SpdS-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=21-26).

Data collection by Erik Avis and Elise Paskowski.

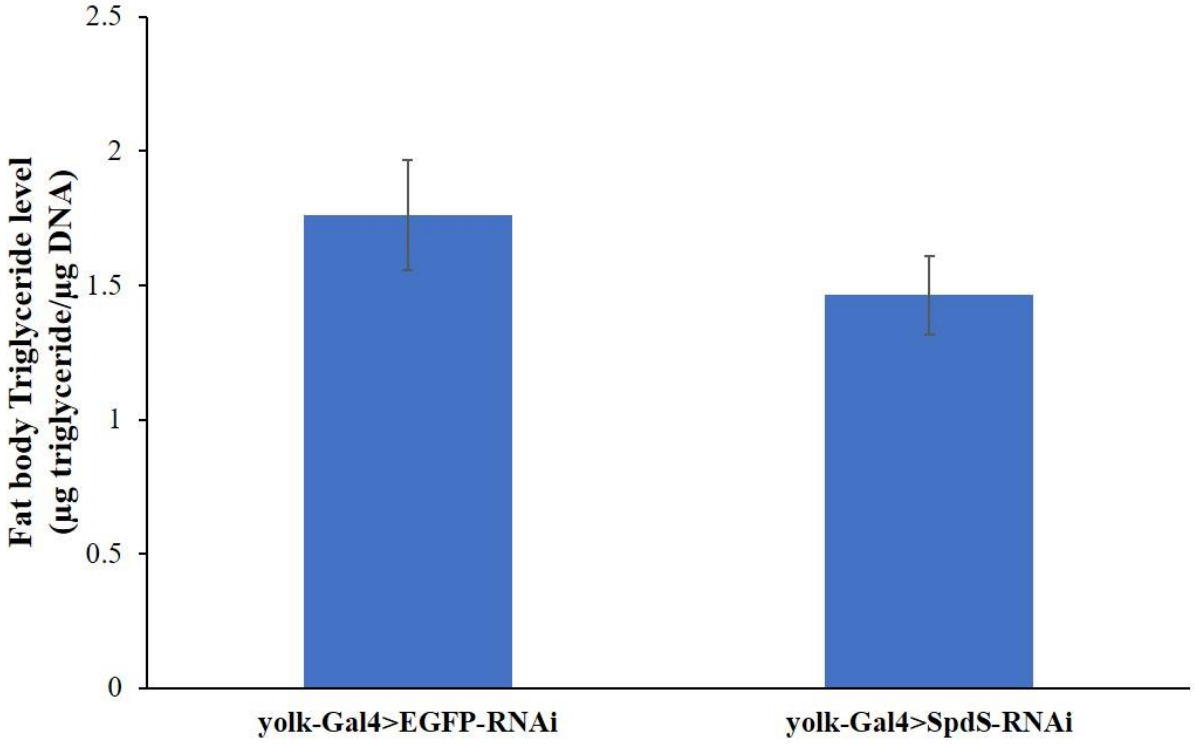

Figure 4. 10 Decreasing SpdS in the fat body of female flies has no effect on the amount of triglyceride stored in each fat body cell.

Triglycerides were measured in fat bodies dissected from one-week old female *yolk-Gal4>SpdS-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Triglyceride measurements were normalized by dividing by total DNA content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>SpdS-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=21-26).

Data collection by Erik Avis and Elise Paskowski.

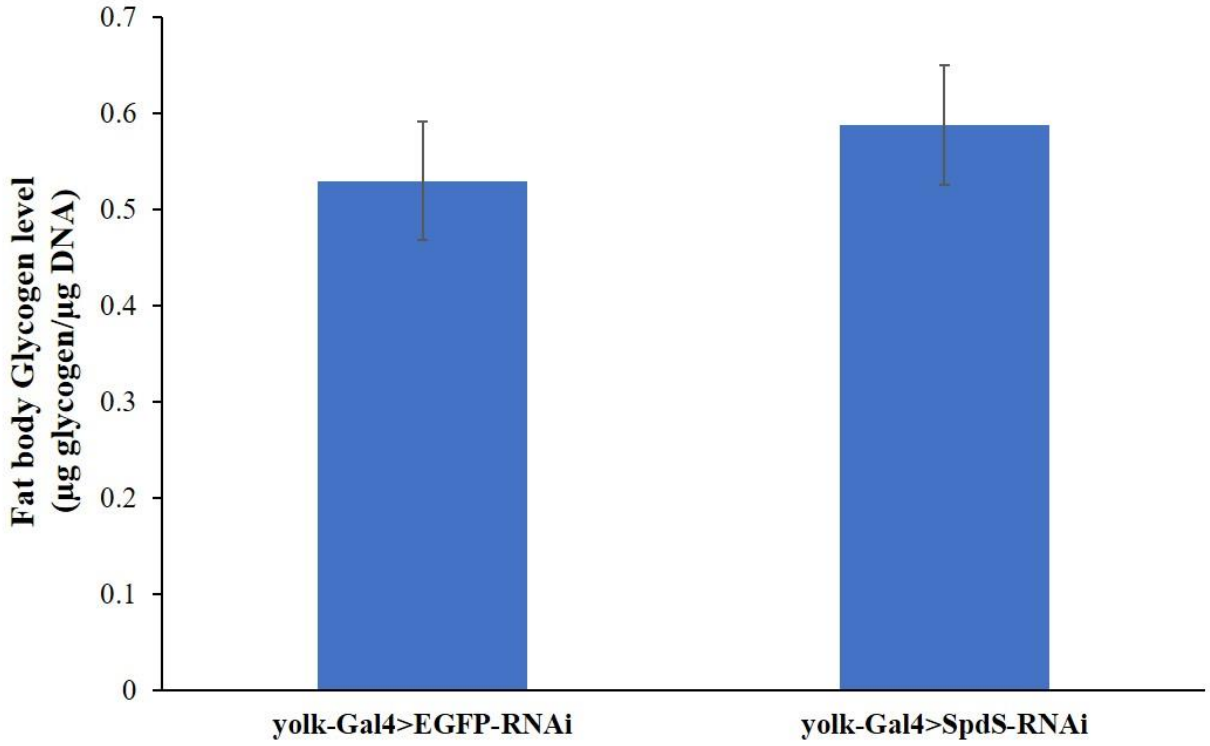

Figure 4. 11 Decreasing SpdS in the fat body of female flies has no effect on the amount of glycogen stored in each fat body cell.

Glycogen was measured in fat bodies dissected from one-week old female *yolk-Gal4>SpdS-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Glycogen measurements were normalized by dividing by total DNA content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>SpdS-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=21-26).

Data collection by Erik Avis and Elise Paskowski.

(Table 4.1). Furthermore, analyses of fly weight revealed that neither male or female SpdS-/- flies had changes in weight when compared to wild-type flies (Figures 4.1 and 4.2). Although no weight changes were detected, studies looking at triglyceride and glycogen storage showed that female SpdS-/- flies had increased triglyceride storage (Figure 4.5). The reduction of SpdS through fat body specific RNAi knockdown resulted in increases in triglyceride storage and the number of fat body cells as well as a decrease in glycogen storage (Figures 4.7, 4.8, and 4.9). However, the storage of triglycerides and glycogen per fat body cell remained unchanged by the SpdS reduction (Figures 4.10 and 4.11).

Studies have shown that the ablation of SpdS in *E.coli* resulted in reduced growth rate, supporting the importance of SpdS in growth and providing rationale for the hypothesis that SpdS -/- flies would be smaller than the controls (Tholl et al., 1998). Thus, the lack of weight variations between SpdS -/- and wild-type flies was surprising given that SpdS has been shown to be integral to the formation of eIF5A, a translation initiation factor implicated in growth in higher organisms (Park et al., 2006; Nishimura et al., 2012; Chen and Chen, 1997). However, when examining the levels of the polyamines themselves, no changes to the polyamines in males were observed, which could explain why there were no changes in the size of the male flies. In contrast, the female SpdS -/- flies displayed higher levels of putrescine and lower levels of spermine but had similar levels of spermidine when compared to the control flies. Given that spermidine acts as the aminobutyl donor for the post-translation modification of translation factor eIF5A and that this modification is essential for eIF5A activity, it could be hypothesized that the lack of growth changes seen in the female flies corresponds to the lack of spermidine changes which in turn aided in the function of eIF5A to maintain growth.

Despite the lack of a weight difference, female SpdS-/- flies displayed a significant increase in putrescine content and a decrease in spermine content (Table 4.2). However, this alteration to polyamine content was only observed in female SpdS knockouts (Tables 4.1 and 4.2). The observed discrepancies in polyamine content between male and female SpdS knockout flies could be partially due to sex differences that regulate the polyamine pathway differently in males and females, potentially via hormones. Previous studies in mice have shown varying polyamine regulation between male and female mice, namely via the activity of ODC, the mammalian analogue to Odc1 (Bastida et al., 2007). The difference in ODC activity was theorized to be due to the increased presence of testosterone in male mice compared to female mice, suggesting the potential role of sex hormones in regulating the polyamine pathway. (Bastida et al., 2007). This theory could thus help to explain the variable expression of polyamine pathway enzymes and the changes in polyamine content seen in the male and female SpdS knockout flies. However, further studies would need to be conducted to prove this hypothesis.

The difference in the regulation of polyamines seen between the two sexes extends beyond the levels of the polyamines themselves. In these studies, it was shown that the ablation of SpdS expression led to increased PAO expression in male flies, whereas SpdS-/- females showed decreased Sat1/2 expression (Figures 4.3 and 4.4). This variation in polyamine pathway gene expression between the sexes, while not fully understood, suggests that males and females may regulate the polyamine pathway differently. These differences in male and female polyamine levels and gene expression changes resulting from the same SpdS mutation establish variations in the regulation of back conversion and other pathway genes, potentially due to sex, as an area of further study in polyamine research.

Macromolecule analyses revealed novel impacts of SpdS ablation in regulating triglyceride and glycogen storage, namely via an increase in overall triglyceride storage in both the whole animal and specifically in the fat body (Figures 4.5, 4.7, 4.9). Polyamines have been previously shown to impact fat metabolism in flies, with the knockdown of Odc1 resulting in increased overall triglyceride storage and fat body specific triglyceride storage (Leon et al., 2020). The Odc1 heterozygotes also showed an increase in the number of fat body cells, an observation that was recapitulated in the SpdS-/- females (Leon et al., 2020).

More precisely determining the role the polyamine pathway plays in lipid metabolism is an area of future study, as polyamines and polyamine pathway enzymes have been linked to metabolic conditions in humans. PAO activity has been previously detected at increased levels in children with *diabetes mellitus* (Bjelakovic et al., 2010). This study and previous data underscores the importance of expanding research in diabetes and lipid metabolism to address the role that the polyamines may play in these physiological processes (Leon et al., 2020).

In summary, the impacts of SpdS deletion on polyamine pathway gene expression, polyamine content, and the storage of triglycerides and glycogen in *D. melanogaster* were further investigated. The lack of clarification surrounding differences in polyamine content and pathway gene expression between male and female SpdS-/- flies warrants further research into possible sex-related factors affecting the regulation of the polyamine pathway. Additionally, the lipid metabolism data suggest that the polyamine pathway plays a role in triglyceride storage and fat cell proliferation. Future studies will have to be conducted to address the intermediate players that integrate the polyamine pathway and lipid metabolism.

Chapter 5

The deletion of Spermine synthase (Sms) changes metabolite storage, expression pf polyamine pathway genes, and polyamine content in *Drosophila melanogaster*

5.1 Introduction

Because polyamines play important roles in growth, development, metabolism, and neurobiology, investigating their regulation and establishing models to study them are of great importance (Wang et al., 2009; Tholl et al., 1998; Monson et al., 2016). For instance, in *E.coli* it has been shown that polyamine deficiencies, namely spermidine, result in a reduction of growth (Tholl et al., 1998). Another study recently showed that the knockdown of ornithine decarboxylase (Odc1) led to altered regulation of lipid metabolism (Leon et al., 2020). One emerging area of study that looks at the physiological role of polyamines involves investigating the role of polyamines in Snyder-Robinson syndrome (SRS).

Originally characterized in humans in 1969, SRS was found to be an X-linked syndrome associated with mental retardation, osteoporosis, low muscle mass, facial asymmetry, seizures and hypotonia (Snyder and Robinson, 1969). SRS has since been linked to a mutation of the spermine synthase (*Sms*) gene which is located, in humans, at chromosome Xp22.11 (Arena et al., 1996). Sms is active as a homodimer and catalyzes the production of spermine from the precursor spermidine (Wu et al., 2008). This reaction involves the transfer of an aminopropyl group from decarboxylated S-adenosylmethionine (dcAdoMet), similar to the formation of spermidine from dcAdoMet and putrescine (Pegg and Williams-Ashman, 1969; Reviewed in [Pegg, 2014]). Due to its mode of inheritance, SRS typically affects males. Males with the most

severe form of this disease lack the functional Sms protein which results in high levels of spermidine and almost completely ablated levels of spermine.

In 1986 a mutated mouse strain was created via X-irradiation (Lyon et al.). The male offspring exhibited rickets, hypophosphatemia, decreased size, sterility, neurological anomalies, shortened lifespan, and a circling behavior which led them to be called Gyro or Gy mice (Lyon et al., 1986). It was not until 1998 that two groups discovered that the Gy mice had deletions in their X-chromosome at the *Sms* gene (Lorenz et al., 1998). In a proof of principle experiment, it was shown that the Gy mice's phenotype was mainly due to the deletion of the *Sms* gene and not due to the partial deletion of an adjacent gene *Phex*. In this experiment, Gy mice were bred with CAG-SpmS mice which overexpress *Sms* constitutively. The offspring with the transgene exhibited normal balance, fertility and lifespan despite the Gy mutation proving that those phenotypic traits were due to the ablation of the *Sms* gene. However, these mice displayed hypophosphatemia and still exhibited lower weight due to bone loss when compared to their control littermates, which was attributed to the partial loss of the *Phex* gene (Lorenz et al., 1998). Thus, it was shown that spermine was needed for normal neurological activity, fertility and viability in male mice (Wang et al., 2004).

In these studies, the impact of Sms deletion on male and female flies (denoted herein as Sms-/-) was described by quantifying polyamine content and expression of polyamine pathway genes. The weights of Sms-/- flies were also measured to determine any changes in growth between Sms-/- and wild-type flies. Moreover, female Sms-/- and control flies were analyzed to better understand the role of Sms in regulating whole-body and fat body-specific storage of triglycerides and glycogen.

Overall it was shown that Sms-/- flies weighed less than the control flies. Male Sms -/- flies showed lower expression of Sms, spermidine synthase (SpdS) and ornithine decarboxylase (Odc1) while also displaying higher polyamine oxidase (PAO) expression levels when compared to control flies. Female flies showed decreased expression of Sms, SpdS, Odc1 and *S*-adenosylmethionine decarboxylase (SamDC) while showing increased spermine/spermidine-N^1-acetyltransferase (Sat1/2) expression. Both male and female Sms-/- flies displayed reduced spermine concentrations and increased spermidine to spermine ratios. Additionally, spermidine levels were decreased in female Sms-/- flies. Metabolite analyses revealed reduced glycogen storage in the whole-body and fat body of female flies. Both the fat body triglyceride levels and number of fat body cells were increased in Sms knockdown flies.

5.2 Results

5.2.1 Male and female Sms knockout flies weighed less than wild-type flies.

To investigate the impact that the ablation of Sms had on an organism, *Drosophila melanogaster* was used as a model system. Both male and female Sms-/- flies had reduced weight when compared to wild-type flies (Figures 5.1 and 5.2).

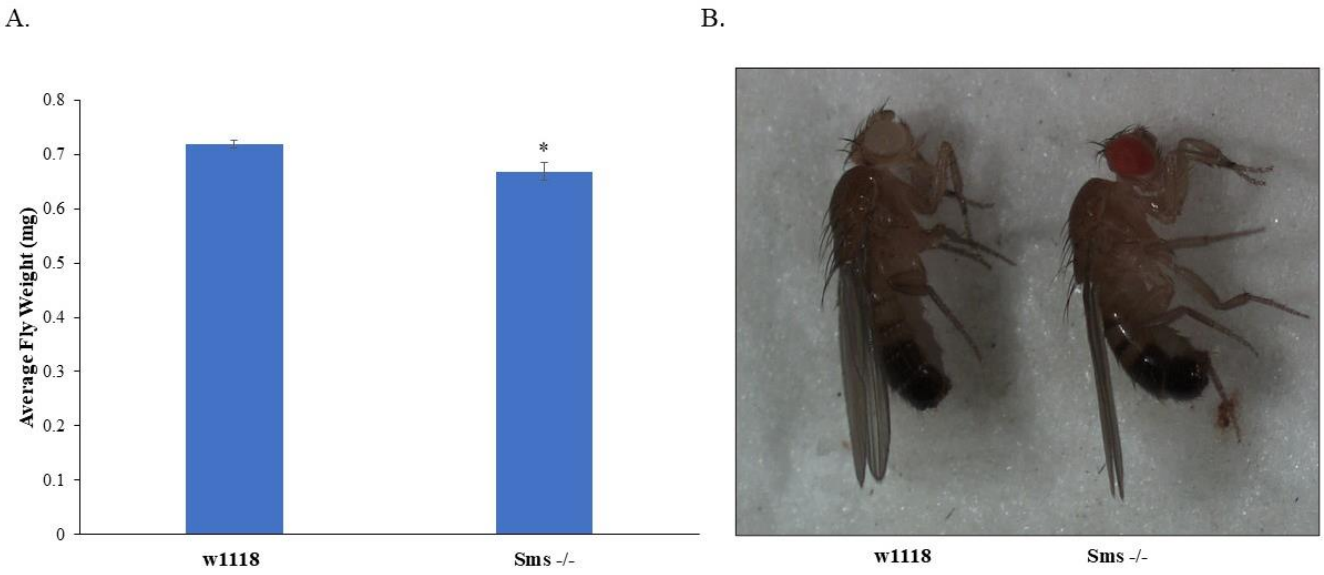

Figure 5. 1 Male Sms knockout flies weighed less than control flies.

(A) Weights of one-week old *Sms*^{c909} (*Sms -/-*) males were compared to male wild-type *w1118* controls. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *Sms -/-* to *w1118* controls. (n=10-14). (B) One-week old male *w1118* and *Sms -/-* flies. A representative image is shown.

Pictures provided by Dr. Justin DiAngelo.

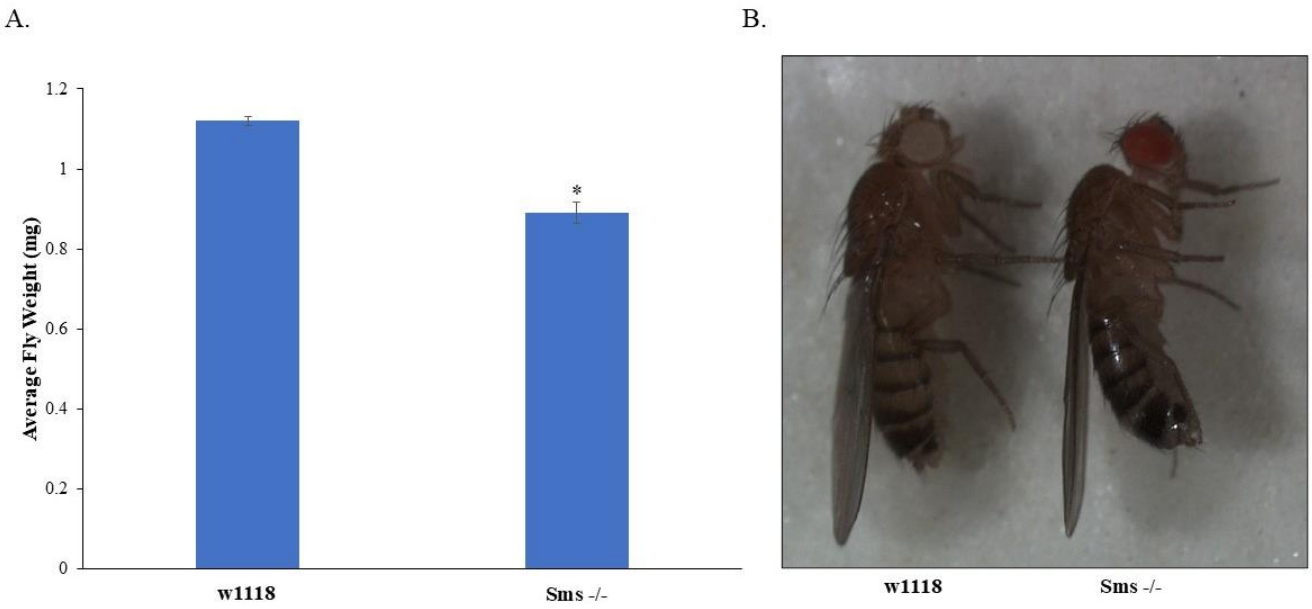

Figure 5. 2 Female Sms knockout flies weighed less than control flies.

(A) Weights of one-week old *Sms*^{c909} (*Sms -/-*) females were compared to female wild-type *w1118* controls. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Sms -/-* to *w1118* controls. (n=11-12). (B) One-week old female *Sms -/-* flies displayed a smaller abdomen compared to *w1118* controls. A representative image is shown.

Pictures provided by Dr. Justin DiAngelo.

5.2.2 Male and female Sms knockout flies displayed expression changes in enzymes that are involved in the polyamine pathway.

In order to determine the effect that Sms knockout had on the expression of other enzymes in the polyamine pathway, RNA expression was measured. Male Sms-/- flies showed significantly decreased expression of Odc1 and SpdS while also displaying a marked increase in PAO expression (Figure 5.3). Female Sms-/- flies also exhibited a downregulation of Odc1and SpdS, along with decreased SamDC expression. Moreover, female Sms-/- flies demonstrated increased Sat1/2 expression (Figure 5.4). Together these data suggest that the knockout of Sms impacts the expression of other enzymes involved in polyamine metabolism.

5.2.3 Both male and female Sms-/- flies displayed reductions in spermine content and increased spermidine to spermine ratios while female knockout flies also showed a decreased spermidine content.

In order to determine the impact of Sms depletion on the polyamine levels, polyamine content was measured. Male Sms-/- flies displayed a substantial decrease in spermine content and an increase in the spermidine to spermine ratio (Table 5.1). Female Sms-/- flies also demonstrated decreased spermine content and an increased spermidine to spermine ratio, but also exhibited a substantial decrease in spermidine content (Table 5.2). These data confirm that knocking out the Sms enzyme can lead to a decrease in the production of spermine. Additionally, they suggest that there could be a sex-linked difference in how flies process polyamines.

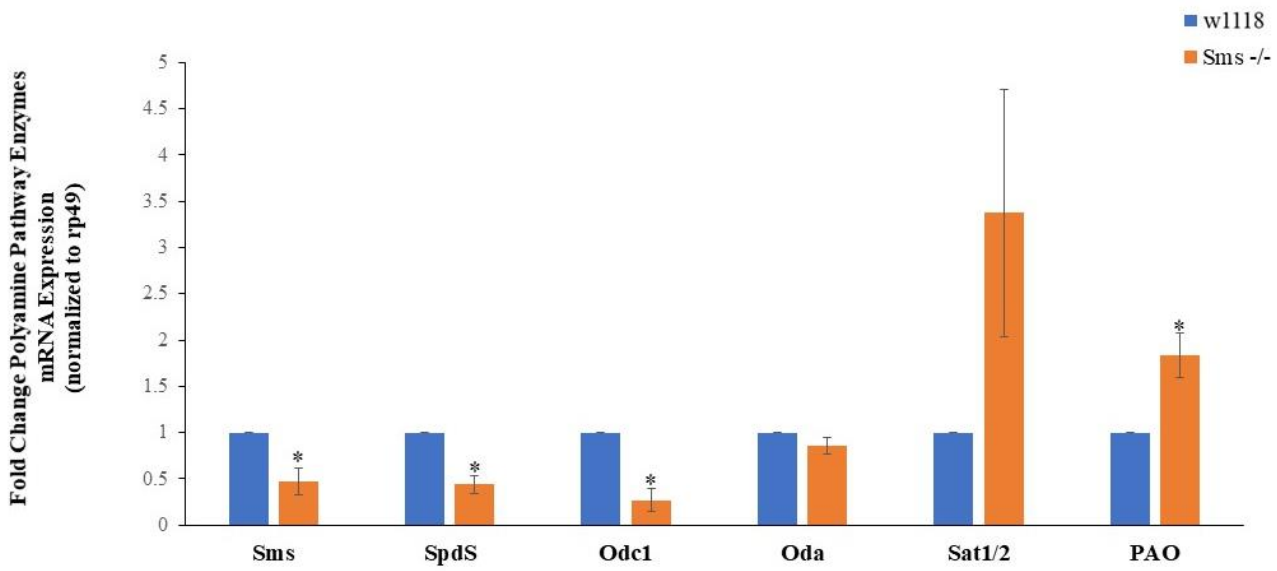

Figure 5. 3 Male Sms knockout flies exhibit significantly decreased Sms, SpdS, and Odc1 expression and significantly increased PAO expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), and polyamine oxidase (*PAO*) was conducted on RNA isolated from one-week old male Sms^{c909} (*Sms -/-*) flies and wild-type *w1118* controls. Adenosyl-methionine decarboxylase (*SamDC*) levels were measured but undetectable in mutants. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *w1118*. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Sms -/-* to *w1118* controls. (n= 4).

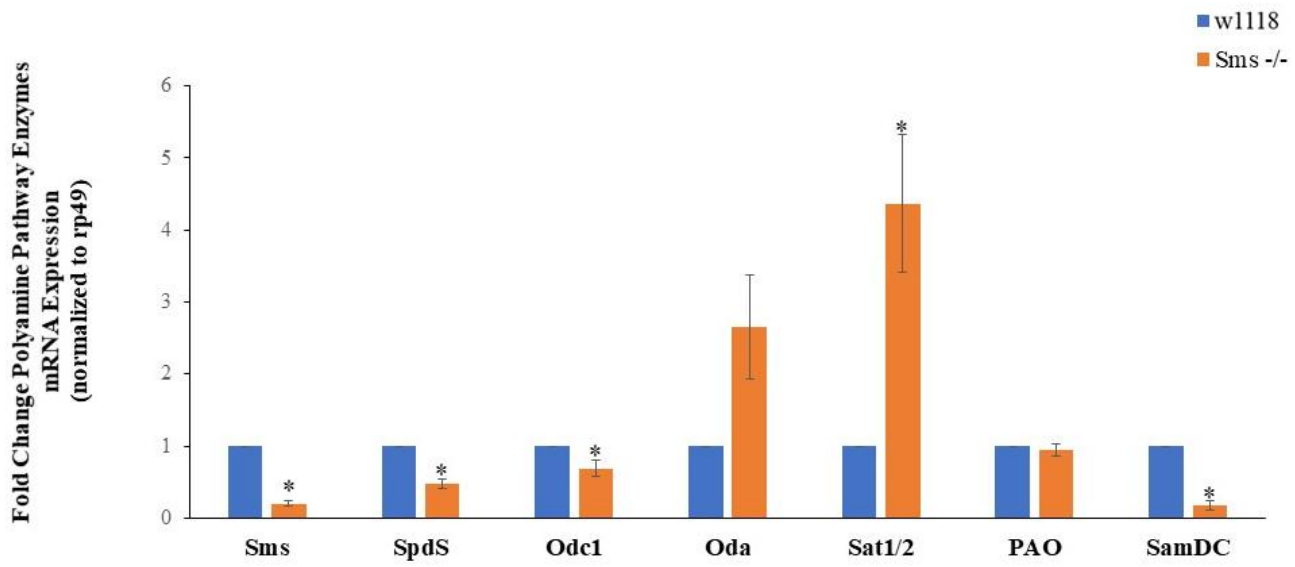

Figure 5. 4 Female Sms knockout flies exhibit significantly decreased Sms, SpdS, Odc1, and SamDC expression and significantly increased Sat1/2 expression.

qPCR analysis for polyamine pathway genes, spermine synthase (*Sms*), spermidine synthase (*SpdS*), ornithine decarboxylase (*Odc1*), ornithine decarboxylase antizyme (*Oda*), spermine/spermidine-N^1- acetyltransferase (*Sat1/2*), polyamine oxidase (*PAO*), and adenosyl-methionine decarboxylase (*SamDC*), was conducted on RNA isolated from one-week old female Sms^{c909} (*Sms -/-*) flies and wild-type *w1118* controls. The expression levels of each gene were normalized to the expression of the ribosomal protein gene, *rp49*. The relative mRNA levels are shown as fold change compared to *w1118*. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Sms -/-* to *w1118* controls. (n= 5).

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)	spermidine to spermine ratio
w1118	0.38 ± 0.05	6.87 ± 0.35	2.09 ± 0.14	3.64 ± 0.54
Sms -/-	0.25 ± 0.09	7.77 ± 0.51	0.86 ± 0.16*	10.70 ± 1.09*

Table 5. 1 Male Sms knockout flies display significantly decreased levels of spermine and a significantly increased spermidine to spermine ratio when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n= 10-14).
Data provided by the Casero lab at Johns Hopkins University

Whole Fly Genotype	Putrescine (nmoles/mg protein)	Spermidine (nmoles/mg protein)	Spermine (nmoles/mg protein)	Spermidine to spermine ratio
w1118	0.66 ± 0.03	13.25 ± 0.48	2.26 ± 0.06	5.84 ± 0.08
Sms -/-	0.60 ± 0.03	10.02 ± 0.24*	0.90 ± 0.04*	11.38 ± 0.52*

Table 5. 2 Female Sms knockout flies display significantly decreased levels of spermidine and spermine and a significantly increased spermidine to spermine ratio when compared to control flies.

Concentrations of putrescine, spermidine, spermine, and spermidine to spermine were measured via HPLC and concentrations are presented as nmol of polyamine per mg of protein. Values are the means ± S.E. *$p<0.05$. (n= 11-12).

Data provided by the Casero lab at Johns Hopkins University.

5.2.4 Female Sms knockout flies showed whole-body decreases in triglycerides and glycogen content, fat body-specific decreases in glycogen levels, and fat body-specific increases in triglycerides and the number of fat body cells.

To characterize the metabolic changes due to the deletion of Sms, triglyceride and glycogen storage were measured in female Sms-/- flies. In the whole-body, the Sms-/- flies demonstrated decreased triglyceride and glycogen content (Figures 5.5 and 5.6). The fat body-specific RNAi knockdowns of Sms revealed increased triglyceride content and decreased glycogen content (Figures 5.7 and 5.8). The number of fat body cells in the Sms knockdowns was also greater when compared to wild-type flies (Figure 5.9). However, the fat body-specific Sms knockdown did not lead to changes in the triglyceride and glycogen content per fat body cell (Figures 5.10 and 5.11). Together these data suggest that Sms plays a role in glycogen and triglyceride storage in the whole animal. The data further suggest that the knockdown of Sms in the fat body can cause an increase in the number of fat body cells.

5.3 Discussion

Both male and female Sms-/- flies displayed reduced weight (Figures 5.1 and 5.2). Additionally, the ablation of Sms was shown to impact the expression of other polyamine pathway genes (Figures 5.3 and 5.4). Male Sms-/- flies displayed decreased expression of Odc1 and SpdS, along with an increased expression of PAO (Figure 5.3). Similarly, female Sms-/- flies experienced reductions in SpdS and Odc1 expression, but they also demonstrated reduced SamDC expression and increased expression of Sat1/2 (Figure 5.4). Moreover, both male and female Sms-/- flies had significantly diminished spermine content and increased spermidine to spermine ratios, with females displaying an additional reduction in spermidine content

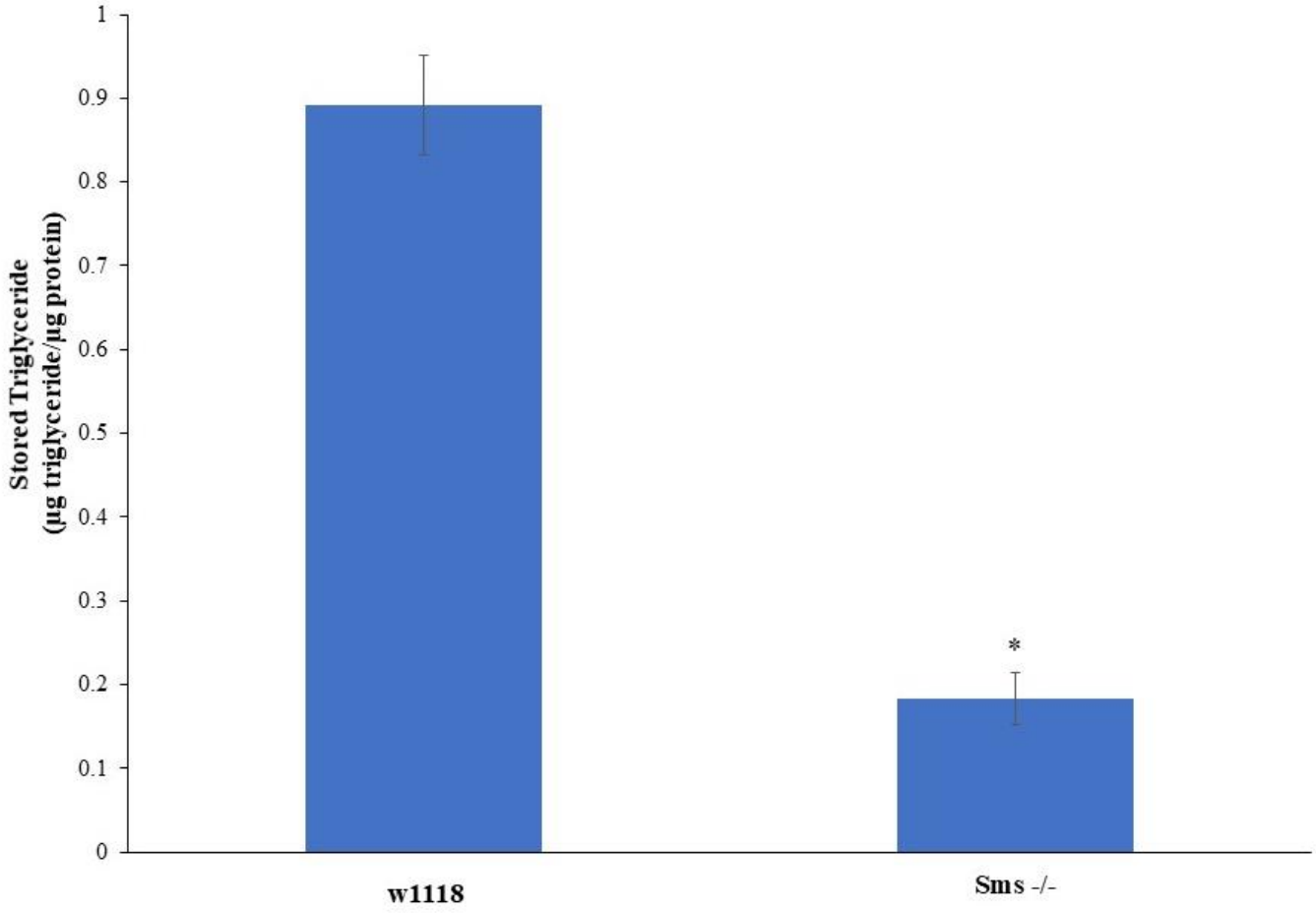

Figure 5. 5 Female Sms knockout flies store less triglycerides than control flies.

Triglycerides were measured in *Sms*^{c909} (*Sms -/-*) mutants by homogenizing pairs of one-week old adult female flies and *w1118* controls. Triglyceride measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Sms -/-* to *w1118* controls. (n=22-25).

Data collection by Erik Avis and Elise Paskowski.

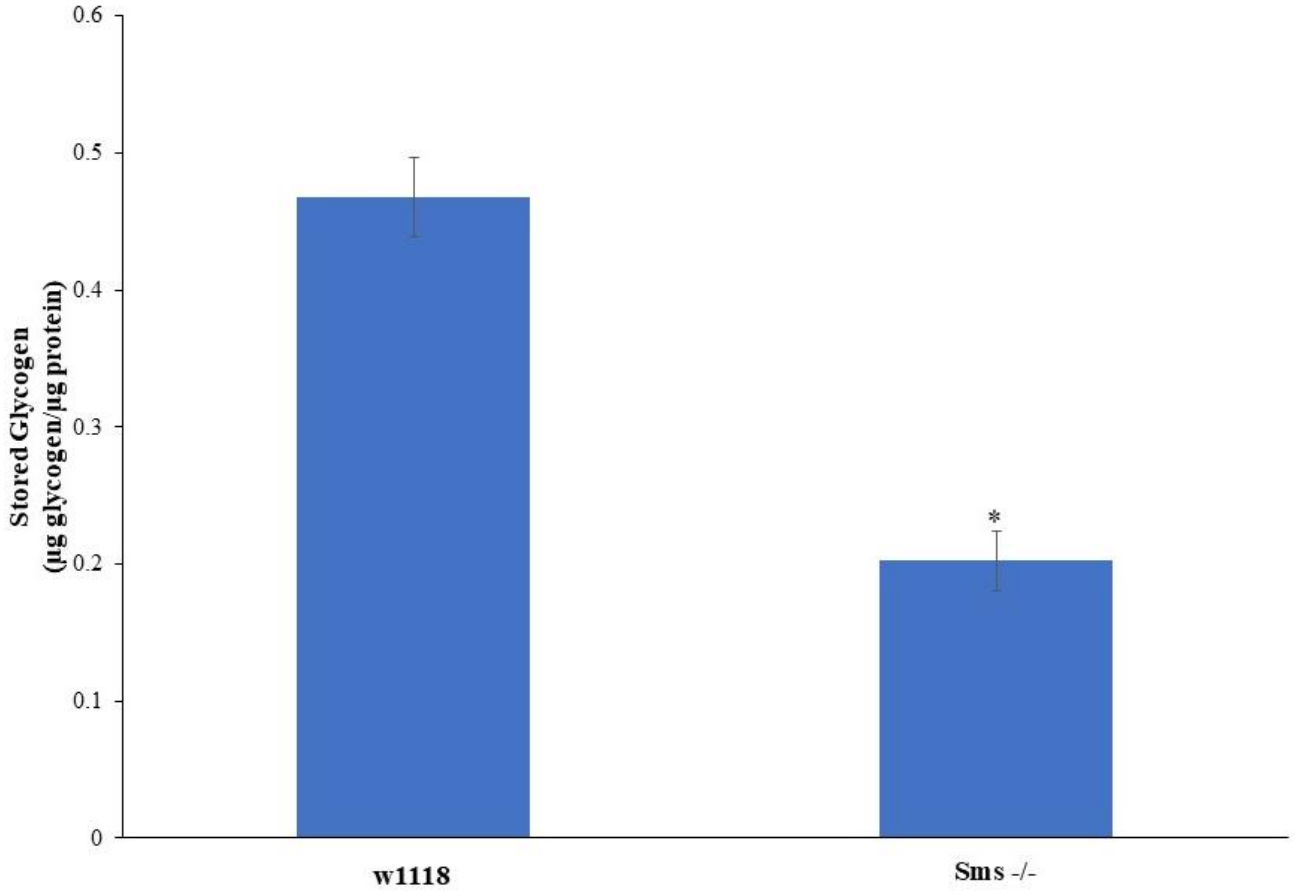

Figure 5. 6 Female Sms knockout flies store less glycogen than control flies.

Glycogen was measured in *Sms*^{c909} (*Sms -/-*) mutants by homogenizing pairs of one-week old adult female flies and *w1118* controls. Glycogen measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *Sms -/-* to *w1118* controls. (n=23-25).

Data collection by Erik Avis and Elise Paskowski.

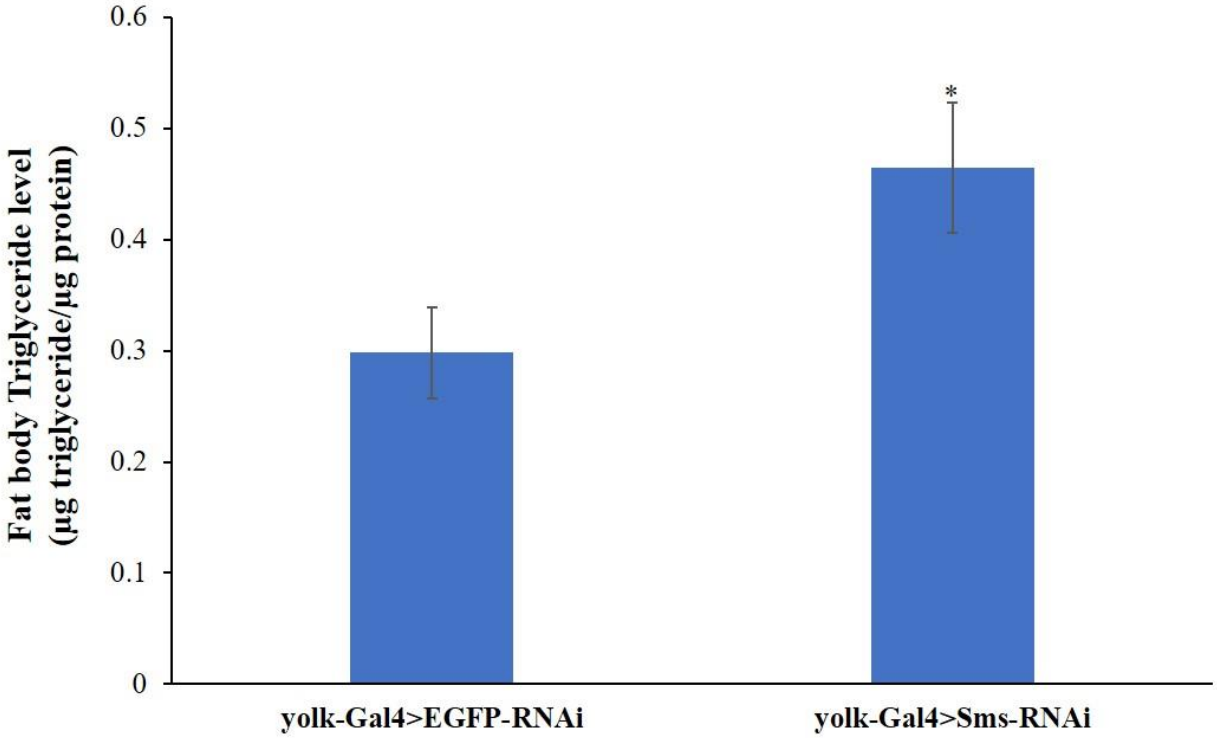

Figure 5. 7 Decreasing Sms in the fat body results in an increase in triglyceride storage in female flies.

Triglycerides were measured in one-week old female *yolk-Gal4>Sms-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Triglyceride measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>Sms-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=19-20).

Data collection by Erik Avis and Elise Paskowski.

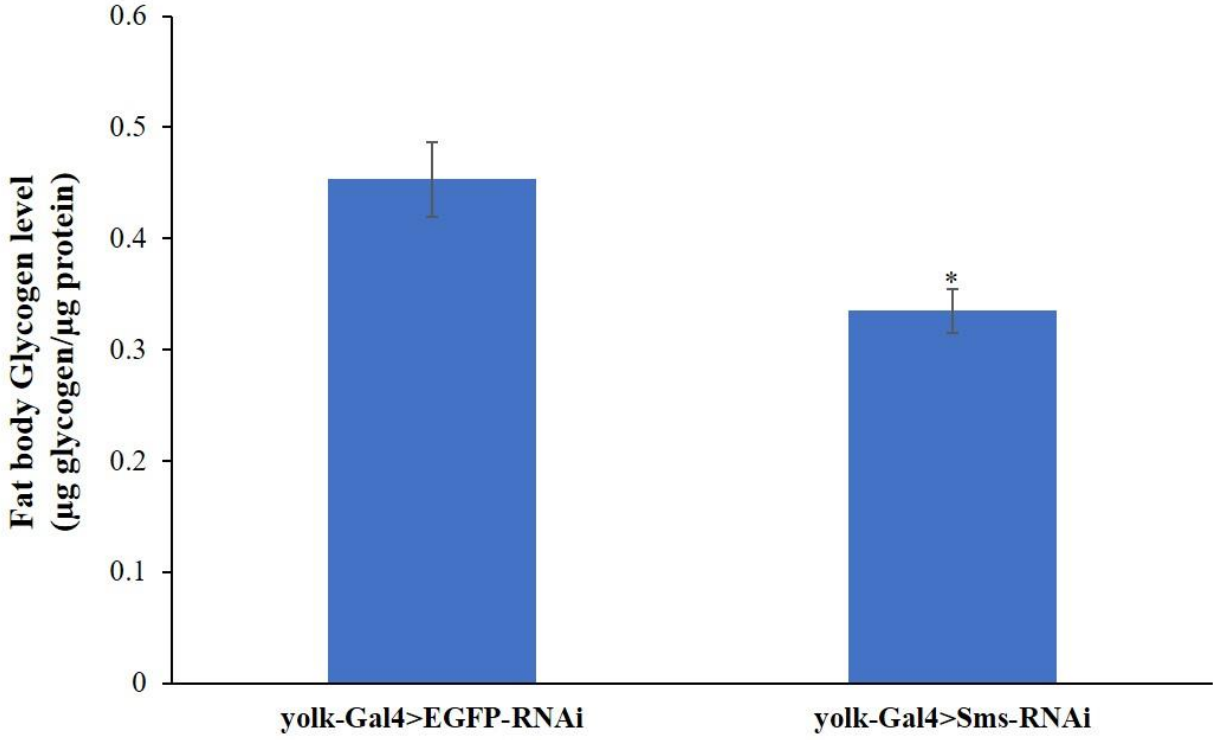

Figure 5. 8 Decreasing Sms in the fat body results in decreased glycogen storage in female flies.

Glycogen was measured in one-week old female *yolk-Gal4>Sms-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Glycogen measurements were normalized by dividing by total protein content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>Sms-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=20).

Data collection by Erik Avis and Elise Paskowski.

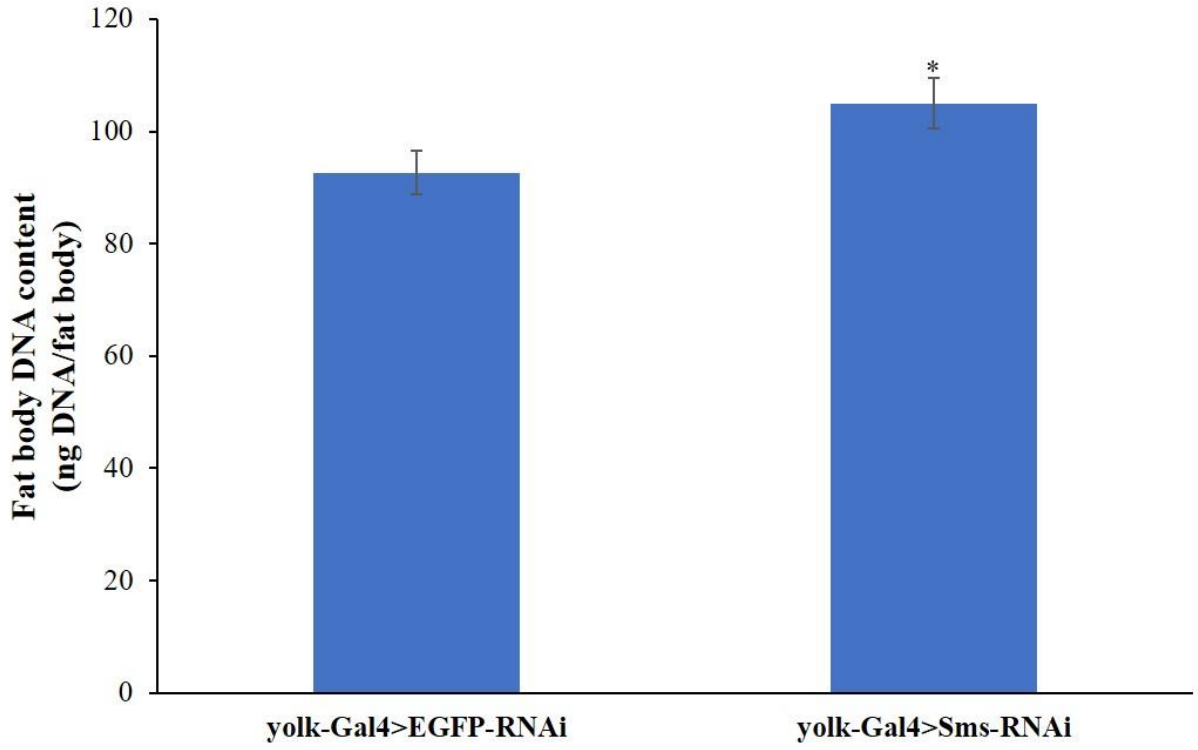

Figure 5. 9 Decreasing Sms in the fat body increased the number of fat body cells in female flies.

Total DNA content was measured in fat bodies dissected from one-week old female *yolk-Gal4>Sms-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Bars represent means ± S.E. *p<0.05 using student's t-test comparing *yolk-Gal4>Sms-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=54-55).

Data collection by Erik Avis and Elise Paskowski.

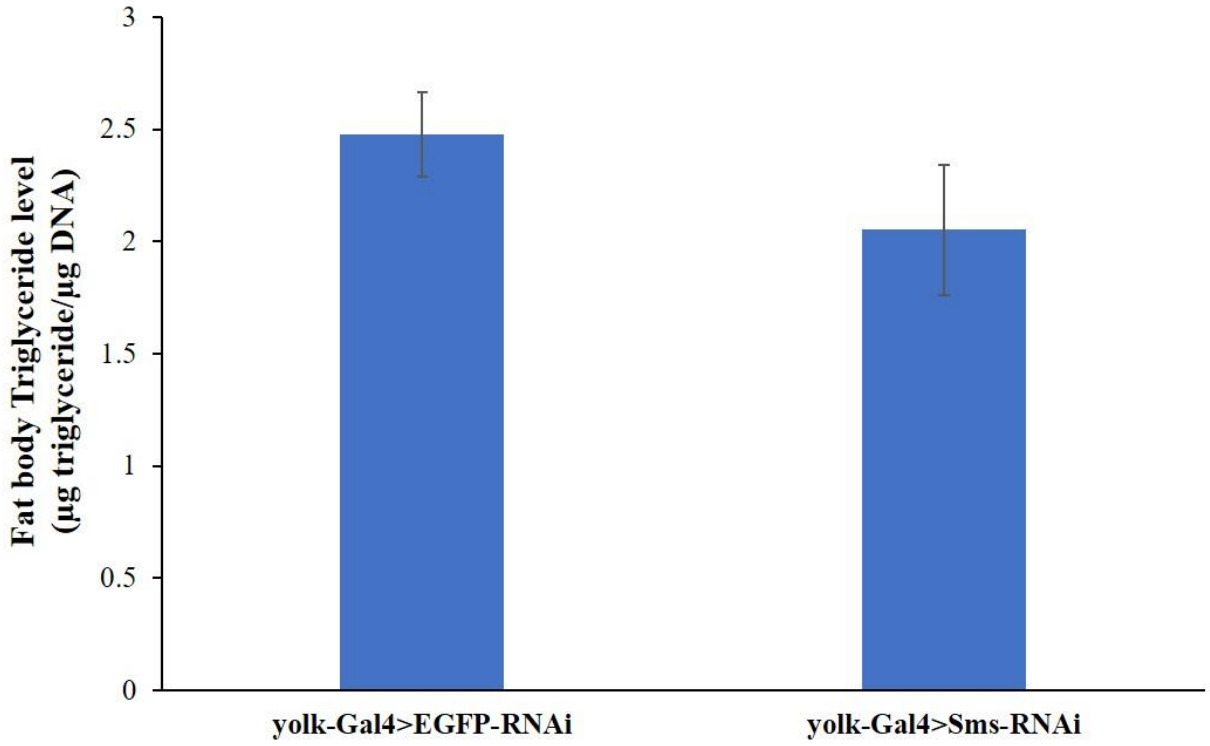

Figure 5. 10 Decreasing Sms in the fat body has no effect on the amount of triglyceride stored in each fat body cell in female flies.

Triglycerides were measured in fat bodies dissected from one-week old female *yolk-Gal4>Sms-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Triglyceride measurements were normalized by dividing by total DNA content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>Sms-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=54-55).

Data collection by Erik Avis and Elise Paskowski.

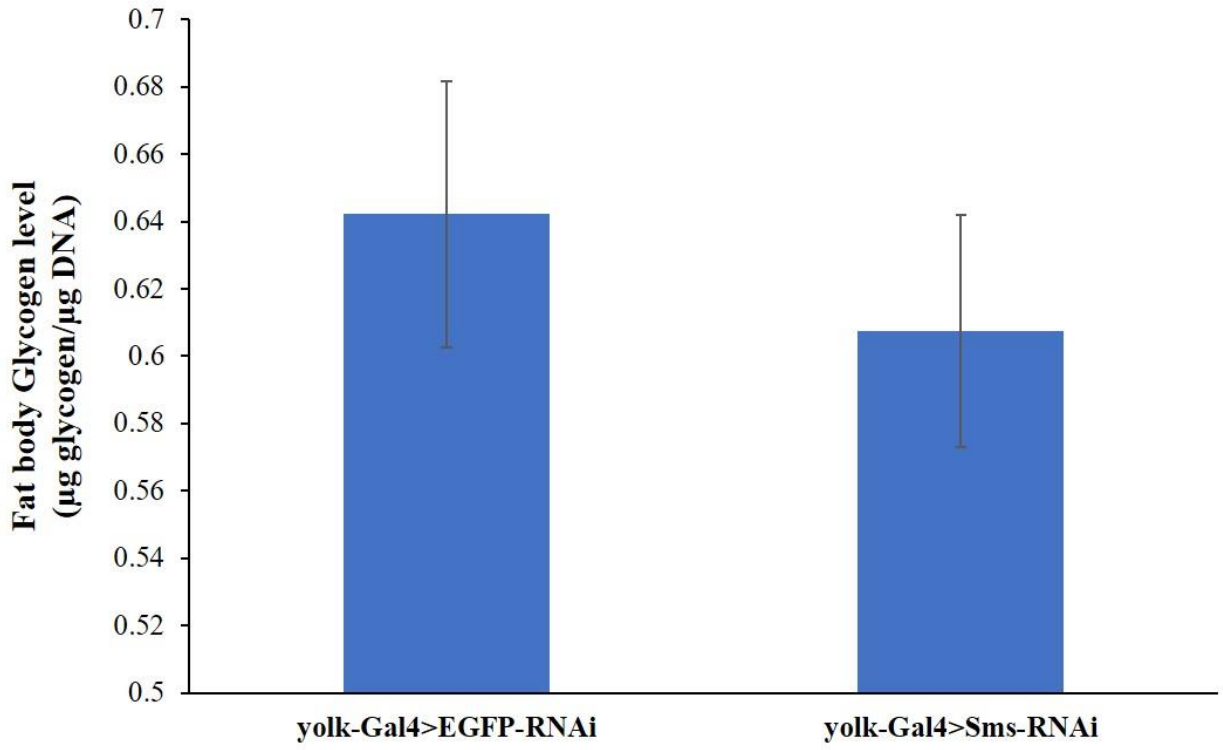

Figure 5. 11 Decreasing Sms in the fat body has no effect on the amount of glycogen stored in each fat body cell in female flies.

Glycogen was measured in fat bodies dissected from one-week old female *yolk-Gal4>Sms-RNAi* flies and *yolk-Gal4>EGFP-RNAi* controls. Glycogen measurements were normalized by dividing by total DNA content. Bars represent means ± S.E. *$p<0.05$ using student's t-test comparing *yolk-Gal4>Sms-RNAi* to *yolk-Gal4>EGFP-RNAi* controls. (n=54-55).

Data collection by Erik Avis and Elise Paskowski.

(Tables 5.1 and 5.2). Triglyceride and glycogen storage assays revealed significant reductions in triglyceride and glycogen content in Sms-/- female flies (Figures 5.5 and 5.6). However, fat body-specific knockdown of Sms resulted in increased triglyceride storage, decreased glycogen storage, and an increased number of fat body cells (Figures 5.7, 5.8, and 5.9). Despite the changes seen in universal triglyceride and glycogen storage, triglyceride and glycogen storage per fat body cell was unchanged in Sms knockout flies (Figures 5.10 and 5.11).

The data described here fit with previous studies of Sms-deficient model organisms. Interestingly, in both mammalian and yeast tissue culture systems the ablation of Sms activity did not hinder cell growth (Hamasaki-Katagiri et al., 1998; Pegg and Coward, 1985). However, in the Sms deficient Gy mice a reduced size was observed when the Gy mice were compared to littermates. The data here recapitulate these finding as the Sms-/- flies weighed less than wild-type flies (Wang et al., 2004; Figures 5.1 and 5.2). These findings support the hypothesis that Sms plays a pivotal role in growth.

Gene expression analyses revealed that both male and female Sms-/- flies showed decreased Odc1 and SpdS expression, yet females also showed decreased SamDC expression (Figures 5.3, and 5.4). Although SRS and Sms deficiencies have not been well characterized in females, the model demonstrates potentially novel impacts of Sms mutation in females as females, unlike males, exhibit decreased SamDC expression and increased Sat1/2 expression (Lyon et al., 1986; Figure 5.4). Interestingly, both males and females demonstrated significant increases in the expression of genes implicated in polyamine catabolism, specifically, PAO in males and Sat1/2 in females (Figures 5.3 and 5.4). The mechanism accounting for these differences is unclear, though it could potentially be due to differences in hormonal regulation.

Bastida et al. showed testosterone and other sex-specific hormones led to varying polyamine regulation between males and females (Bastida et al., 2007).

The roles of Sms and spermine in lipid formation was further investigated as both Sms-/- male and female flies experienced diminished spermine content and increased ratios of spermidine to spermine. This ratio was found to be elevated in 3T3-L1 adipocytes during differentiation, supporting its potential importance in lipid storage and regulation (Tables 5.1 and 5.2; Reviewed in [Pegg, 2014]; Ishii et al., 2012; Bethell and Pegg, 1981). Interestingly, the female Sms-/- flies demonstrated significantly decreased spermidine content (Table 5.2). This was surprising given the increased expression of genes involved in interconversion of polyamines, PAO and Sat1/2, which facilitate formation of putrescine and spermidine (Figures 5.3 and 5.4). The discrepancy seen between these data may be due to the polyamine transport system or due to the fact that previous studies involved male Gy mice and the current study focused on females.

The triglyceride and glycogen data described are consistent with the 3T3-L1 adipocyte model (Figures 5.5 and 5.7). The observed decrease in overall triglyceride content was consistent with the decrease in lipid accumulation that was seen when 3T3-L1 cells were treated with a Sms inhibitor (Ishii et al., 2012). Interestingly, the fat body-specific knockdown data do not agree with the findings from the whole animal (Figures 5.5 and 5.7). One reason for this may be because the knockdown of Sms is not sufficient to create a similar phenotype seen in the knockout fly. In order to test this idea, Sms must be reduced to a threshold that will display the Sms-/- phenotype.

There were marked whole body and fat body-specific decreases in glycogen storage in the Sms deficient systems utilized in this study, and these findings represent a novel role of Sms

in regulating glucose metabolism (Figures 5.6 and 5.8). Glycogen storage disorders such as storage disorder type four (GSD IV) have been previously associated with hypotonia, and could suggest that glycogen storage malfunctions may contribute to the phenotypes associated with SRS and Sms deficiency (Wang et al., 2004; Peron et al., 2013; Burrow et al., 2006). Future studies could aid in better understanding the mechanism by which this glycogen storage phenotype may be associated with SRS and spermine deficiency.

In summary, the effect of knocking out Sms in *D. melanogaster* and its impact on polyamine content, storage of triglycerides and glycogen, and expression of genes implicated in polyamine biosynthesis was characterized and investigated. The analyses conducted suggest the role of Sms in regulating the expression of several pathway genes. However, the mechanisms surrounding the different ways in which males and females respond to Sms ablation through the polyamine pathway and the polyamines themselves remain elusive. This study also highlights the role Sms has in regulating lipid metabolism and glycogen storage. Future investigations are necessary to better understand the mechanism by which Sms impacts glycogen and lipid storage. Furthermore, a more robust study of these models is warranted to better characterize not only polyamine gene expression changes, but also changes to enzyme activity that result from the Sms downregulation.

Chapter 6

Discussion

6.1 General Summary of Findings and Conclusions

The primary goal of these studies was to better characterize the roles of the polyamine genes ornithine decarboxylase (Odc1), spermidine synthase (SpdS), and spermine synthase (Sms) in male and female *Drosophila melanogaster.* The effect that decreasing these genes had on triglyceride and glycogen metabolism, polyamine content, and the gene expression of other polyamine pathway enzymes was thoroughly investigated and described.

6.1.1 The influence of Odc1 knockdown and SpdS and Sms knockout on the polyamine pathway and the weight of flies.

In males, Odc1 reduction led to decreased spermidine content and increased spermine content without changes in the gene expression of other polyamine pathway enzymes (Table 3.1 and Figure 3.1). The ablation of SpdS in male flies resulted in the upregulation of polyamine oxidase (PAO) without affecting polyamine content (Figure 4.3 and Table 4.1). In the male SpdS -/- model, this shift in upregulating the expression of catabolic enzymes may be done, in part, to maintain polyamine homeostasis because PAO aids in the back conversion of polyamines (Reviewed in [Pegg, 2014]). As SpdS was no longer expressed in the model and would not be able to form the higher polyamines, PAO would aid in converting the existing polyamines back to less complex forms, such as putrescine. (Reviewed in [Pegg, 2014] ; Ikeguchi et al., 2006). This would aid in the maintenance of homeostatic polyamine levels.

Unlike their male Odc1+/- and SpdS -/- counterparts, a significant reduction in weight was observed in male flies deficient of Sms, a phenotype that was previously observed in Gy mice and is associated with SRS in humans (Figure 5.1; Reviewed in [Pegg, 2014]; Murray-Stewart et al., 2018; Schwartz et al., 2011). Additionally, polyamine pathway enzymes' gene expression varied more in Sms knockout flies with Sms -/- males showing reduced expression of Odc1 and SpdS and increased PAO expression (Figure 5.3). Whether the upregulation of PAO seen in this model is directly or indirectly due to the ablation of Sms is unclear, as a similar upregulation of PAO was observed in the SpdS knockout flies. The downregulation of biosynthetic genes (Odc1 and SpdS) could be circumvented by the upregulation of PAO and the back conversion of the polyamines (Figure 5.3; Ikeguchi et al., 2006; Tobias and Kahana, 1993; Reviewed in [Pegg, 2014]). This back conversion to spermidine and, potentially, putrescine could explain why these flies displayed decreased spermine content and a substantially increased spermidine to spermine ratio (Table 5.1).

The knockdown of Odc1 in females did not correlate with any appreciable differences in polyamine content, attesting to the importance of maintaining homeostatic polyamine levels in the model system (Table 3.2). However, it was observed that female flies showed a reduction in ornithine decarboxylase antizyme (Oda) expression (Figure 3.2). Given that Oda binds to Odc1 and aids in its degradation by the 26S proteasome, these results were not surprising (Reviewed in [Nowotarski et al., 2013]). For example, Zhu et al. showed similar results in a human mammary cell culture model (2012). Moreover, previous studies in L1210 leukemia cells have shown that Odc1 inhibition correlated with a downregulation of Oda expression, consistent with the findings described in this body of work (Nilsson et al., 1997). Thus, based on previous findings and the current study, it was hypothesized that the reduction in expression of Oda may allow the lower

level of Odc1 to function without inhibition and aid in maintaining homeostatic levels of the polyamines.

Female SpdS knockout flies showed decreased spermine/spermidine N_1-acetyltransferase (Sat1/2) expression along with undetectable spermine levels and a two-fold increase in putrescine content (Figure 4.4 and Table 4.2). These data make sense because SpdS converts spermidine to spermine. The downregulation of Sat1/2 may be an attempt by the fly to maintain homeostatic levels by blocking the catabolism of polyamines.

The knockout of Sms though led to a substantial increase in Sat1/2 expression, along with decreased SpdS, Odc1, and S-adenosylmethionine decarboxylase (SamDC) expression (Figure 5.4). The ablation of Sms in females also led to lower weights and substantial decreases in spermidine and spermine content while displaying an approximately two-fold increase in the spermidine to spermine ratio (Figure 5.2 and Table 5.2). As stated above, the weight phenotype in Sms knockout females is consistent with previous findings in Gy mice (Wang et al., 2004; Peron et al., 2013). These data highlight the complexity of how the polyamine pathway self-regulates in order to try to maintain threshold levels of polyamines.

It was interesting to discover that the polyamine pathway was regulated differently in males and females. For example, the male and female Sms knockout models both displayed the downregulation of Odc1 and SpdS and the upregulation of a gene involved in polyamine catabolism, albeit in the female model Sat1/2 was upregulated whereas the male model upregulated PAO (Figures 5.3 and 5.4). Although both processes facilitate back conversion, these data suggests that the regulation of these genes and mechanisms by which they are activated may vary between males and females. In addition, the downregulation of SamDC in Sms -/- females as well as the reduction of Oda in Odc1 +/- females further highlight that males

and females regulate the polyamine pathway differently (Figures 3.2 and 5.4). Previous studies have detailed potential contributors that influence the difference in polyamine regulation between the two sexes. For example, studies on mouse kidneys and adrenal tissue suggested that potential differences in polyamine regulation between males and females could be due to the presence of higher levels of testosterone in males (Bastida et al., 2007; Goldstone et al., 1982). The fly model used here further suggests that polyamine pathway regulation may be influenced by physiological and hormonal differences between males and females.

6.1.2 Spermidine synthase and spermine synthase influence triglyceride and glycogen storage in female Drosophila.

To determine the roles that SpdS and Sms had on the triglyceride and glycogen storage in the female animals, stored glycogen and triglycerides in the whole fly and fat body were measured. Females were used for these studies because they are larger and provide more material for the studies than male flies. Prior studies on Odc1+/- flies have shown that Odc1 impacts triglyceride storage (Leon et al., 2020). Here, novel data were shown to further characterize the role of polyamine enzymes in triglyceride and glycogen storage. SpdS-/- flies stored more triglycerides and fat-body specific SpdS knockdown flies stored more triglycerides but displayed decreased glycogen storage (Figures 4.5, 4.7 and 4.8). Interestingly, the number of fat body cells increased in female flies that had decreased SpdS expression (Figure 4.9).

In contrast, Sms-/- flies stored less triglycerides and glycogen when compared to control flies (Figures 5.5 and 5.6). These data are fitting as the flies weigh less (Figures 5.1 and 5.2). However, the knockdown of Sms in fat bodies revealed increased triglyceride storage, decreased glycogen storage and an increase in the number of fat body cells (Figures 5.7, 5.8, and 5.9).

These findings highlight the complexity of whole body versus tissue specific lipid storage in flies and suggest that Sms plays a role in lipid metabolism.

In the female flies, an increase in the spermidine to spermine ratio was observed (Table 5.2). These data are supported by previous studies conducted in 3T3-L1 adipocytes that showed an increased spermidine to spermine ratio when exposed to a Sms inhibitor (Ishii et al., 2012). In contrast, utilizing a SpdS inhibitor on 3T3-L1 adipocytes undergoing differentiation did not cause any changes to the ratio of spermidine to spermine and these data are consistent with the present findings. Moreover, the increased SSAT activity shown in 3T3-L1 cells treated with a SpdS inhibitor is in agreement with findings that Sat1/2 was upregulated in SpdS -/- female flies (Figure 5.2) (Ishii et al., 2012). Taken together, this model further underscores the potential role of the spermidine to spermine ratio in fat metabolism and potentially adipocyte development and the importance of the polyamine pathway in lipid metabolism.

6.2 Future Directions

The observations made in this study support the need for further research to more precisely determine the roles of Odc1, SpdS, and Sms in lipid metabolism to enhance the understanding of how the polyamine pathway self-regulates. The experiments described here provide some ideas for future research.

6.2.1 Determining the role of the polyamine transport system in the current model systems.

Future studies could be directed towards characterizing the polyamine transport system in the various model systems. In all of the models, altered gene expression occurred in one or

more polyamine enzymes; however, not all of the utilized model systems displayed changes to the levels of the polyamines themselves. This could be, in part, due to changes to the polyamine transport system in the various models. As the polyamine transport system has not been fully characterized in higher organisms, future studies could investigate the use of the transporter(s) in Odc1, SpdS, and Sms deficient models to observe how exogenous polyamines may be mobilized to compensate for losses within a cell (Belting et al., 2003; Poulin et al., 2012).

6.2.2 Understanding the influence of sex in the self-regulation of the polyamine pathway.

An additional area of future investigation should be determining the mechanisms involved in creating different responses in males and females when the polyamine pathway is altered. The data here provide rationale for investigating the potential roles hormones play in the self-regulation of the polyamine pathway, showing that polyamine enzyme expression and polyamine concentrations between male and females can differ. Such differences in polyamine regulation have previously been described in mice (Bastida et al., 2007; Goldstone, 1982). One future project would be expanding these findings to include *Drosophila melanogaster*.

6.2.3 Determining the interplay of the polyamine pathway and lipid metabolism.

A more thorough understanding of the roles of SpdS and Sms on fatty acid metabolism is another area of potential research for the Nowotarski research laboratory group. Odc1 knockdown has been previously shown to increase the expression of genes involved in fatty acid synthesis and overall triglyceride storage (Leon et al., 2020). Moreover, previous studies have shown that mice on a high fat diet that were given spermine displayed reduced adiposity and had

improved glucose tolerance (Sadasivan et al., 2014). Exogenous spermidine administration also improved glucose tolerance and reduced lipid accumulation in another study of mice fed on a high fat diet (Ma et al., 2021). In another study, spermidine was shown to attenuate weight gain and comorbidities of diabetes (Fernandez et al., 2017). These previous findings, as well as the current data presented here, support the need to further investigate the link between polyamines and lipid metabolism. Additionally, it is necessary to further characterize the complex mechanism of whole-body lipid metabolism and how this is similar or different to the tissue-specific lipid metabolism in flies.

6.3 Overall Conclusions

In summary, the results of this study have further characterized the roles of Odc1, SpdS, and Sms in the self-regulation of the polyamine pathway. Moreover, the influence of SpdS and Sms on triglyceride and glycogen storage was shown. Overall, the data suggest that the regulation of the enzymes involved in the polyamine pathway as well as the concentration of the polyamines themselves is highly complex and differs between male and female flies, attesting to the importance of maintaining tightly regulated levels of the polyamines. Additionally, the metabolic phenotypes that were observed support potential roles of SpdS and Sms in regulating triglyceride and glycogen storage. The exact mechanisms by which SpdS and Sms influence triglyceride and glycogen metabolism are unknown and remain an area of interest. These findings further support the necessity of polyamine pathway research by highlighting the role that the polyamine pathway plays in lipid metabolism.

BIBLIOGRAPHY

American Diabetes, A. (2013). Economic costs of diabetes in the U.S. in 2012. *Diabetes care, 36*(4), 1033-1046. doi:10.2337/dc12-2625

Appling, D. R., Anthony-Cahill, S. J., & Mathews, C. K. (2017). *Biochemistry: concepts and connections* (Second edition. ed.).

Arena, J. F., Schwartz, C., Ouzts, L., Stevenson, R., Miller, M., Garza, J., . . . Lubs, H. (1996). X-linked mental retardation with thin habitus, osteoporosis, and kyphoscoliosis: linkage to Xp21.3-p22.12. *American journal of medical genetics, 64*(1), 50-58. doi:10.1002/(SICI)1096-8628(19960712)64:1<50::AID-AJMG7>3.0.CO;2-V

Aryal, B., & Lee, Y. (2019). Disease model organism for Parkinson disease: Drosophila melanogaster. *BMB reports, 52*(4), 250-258.

Bachrach, U. (2010). The early history of polyamine research. *Plant physiology and biochemistry, 48*(7), 490-495. doi:10.1016/j.plaphy.2010.02.003

Basler, K., & Struhl, G. (1994). Compartment boundaries and the control of Drosophila limb pattern by hedgehog protein. *Nature, 368*(6468), 208-214. doi:10.1038/368208a0

Bastida, C. M., Cremades, A., Castells, M. T., Lopez-Contreras, A. J., Lopez-Garcia, C., Sanchez-Mas, J., & Penafiel, R. (2007). Sexual dimorphism of ornithine decarboxylase in the mouse adrenal: influence of polyamine deprivation on catecholamine and corticoid levels. *American journal of physiology- endocrinology and metabolism, 292*(4), E1010-1017. doi:10.1152/ajpendo.00316.2006

Belting, M., Mani, K., Jonsson, M., Cheng, F., Sandgren, S., Jonsson, S., . . . Fransson, L. A. (2003). Glypican-1 is a vehicle for polyamine uptake in mammalian cells: a pivotal role for nitrosothiol-derived nitric oxide. *Journal of biological chemistry, 278*(47), 47181-47189. doi:10.1074/jbc.M308325200

Bethell, D. R., & Pegg, A. E. (1981). Polyamines are needed for the differentiation of 3T3-L1 fibroblasts into adipose cells. *Biochemical and biophysical research communications, 102*(1), 272-278. doi:10.1016/0006-291x(81)91517-5

Bjelakovic, G., Beninati, S., Bjelakovic, B., Sokolovic, D., Jevtovic, T., Stojanovic, I., . . . Zivic, S. (2010). Does polyamine oxidase activity influence the oxidative metabolism of children who suffer of diabetes mellitus? *Molecular and cellular biochemistry, 341*(1-2), 79-85. doi:10.1007/s11010-010-0439-0

Burnette, M., Brito-Robinson, T., Li, J., & Zartman, J. (2014). An inverse small molecule screen to design a chemically defined medium supporting long-term growth of Drosophila cell lines. *Molecular biosystems, 10*(10), 2713-2723. doi:10.1039/c4mb00155a

Burrow, T. A., Hopkin, R. J., Bove, K. E., Miles, L., Wong, B. L., Choudhary, A., . . . Chen, Y. T. (2006). Non-lethal congenital hypotonia due to glycogen storage disease type IV. *American journal of medical genetics, A, 140*(8), 878-882. doi:10.1002/ajmg.a.31166

Casero, R. A., Jr. (2018). Targeting the aryl hydrocarbon receptor/polyamine biosynthesis axis of evil for cancer therapy. *Journal of clinical investigation, 128*(10), 4254-4256. doi:10.1172/JCI123266

Casero, R. A., Jr., Murray Stewart, T., & Pegg, A. E. (2018). Polyamine metabolism and cancer: treatments, challenges and opportunities. *Nature reviews. Cancer, 18*(11), 681-695. doi:10.1038/s41568-018-0050-3

Cason, A. L., Ikeguchi, Y., Skinner, C., Wood, T. C., Holden, K. R., Lubs, H. A., . . . Schwartz, C. E. (2003). X-linked spermine synthase gene (SMS) defect: the first polyamine deficiency syndrome. *European journal of human genetics, 11*(12), 937-944. doi:10.1038/sj.ejhg.5201072

Chen, Z. P., & Chen, K. Y. (1997). Marked elevation of hypusine formation activity on eukaryotic initiation factor 5A in v-HA-RAS transformed mouse NIH3T3 cells. *Cancer letters, 115*(2), 235-241. doi:10.1016/s0304-3835(97)04741-1

DiAngelo, J. R., & Birnbaum, M. J. (2009). Regulation of fat cell mass by insulin in Drosophila melanogaster. *Molecular and cellular biology, 29*(24), 6341-6352. doi:10.1128/MCB.00675-09

Dion, A. S., & Herbst, E. J. (1967). The localization of spermidine in salivary gland cells of Drosophila melanogaster and its effect on H3-uridine incorporation. *Proceedings of the national academy of sciences, 58*(6), 2367-2371. doi:10.1073/pnas.58.6.2367

Dudley, H. W., Rosenheim, M. C., & Rosenheim, O. (1924). The chemical constitution of spermine. I. The isolation of spermine from animal tissues, and the preparation of its salts. *Biochemical journal, 18*(6), 1263-1272. doi:10.1042/bj0181263

Feith, D. J., Shantz, L. M., & Pegg, A. E. (2001). Targeted antizyme expression in the skin of transgenic mice reduces tumor promoter induction of ornithine decarboxylase and decreases sensitivity to chemical carcinogenesis. *Cancer research, 61*(16), 6073-6081.

Fernandez, A. F., Barcena, C., Martinez-Garcia, G. G., Tamargo-Gomez, I., Suarez, M. F., Pietrocola, F., . . . Marino, G. (2017). Autophagy couteracts weight gain, lipotoxicity and pancreatic beta-cell death upon hypercaloric pro-diabetic regimens. *Cell death & disease, 8*(8), e2970. doi:10.1038/cddis.2017.373

Fortini, M. E., Skupski, M. P., Boguski, M. S., & Hariharan, I. K. (2000). A survey of human disease gene counterparts in the Drosophila genome. *Journal of cell biology, 150*(2), F23-30. doi:10.1083/jcb.150.2.f23

Fozard, J. R., Part, M. L., Prakash, N. J., Grove, J., Schechter, P. J., Sjoerdsma, A., & Koch-Weser, J. (1980). L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. *Science, 208*(4443), 505-508. doi:10.1126/science.6768132

Gardiner, T. A., Canning, P., Tipping, N., Archer, D. B., & Stitt, A. W. (2015). Abnormal Glycogen Storage by Retinal Neurons in Diabetes. *Investigative ophthalmology & visual science, 56*(13), 8008-8018. doi:10.1167/iovs.15-18441

Goldstone, A., Koenig, H., & Lu, C. (1982). Testosterone-dependent sexual dimorphism of the mouse kidney is mediated by polyamines. *Biochemical and biophysical research communications, 104*(1), 165-172. doi:10.1016/0006-291x(82)91954-4

Goodwin, A. C., Destefano Shields, C. E., Wu, S., Huso, D. L., Wu, X., Murray-Stewart, T. R., . . . Casero, R. A., Jr. (2011). Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. *Proceedings of the national academy of sciences, 108*(37), 15354-15359. doi:10.1073/pnas.1010203108

Griffiths, A. J. F., Doebley, J. F., Peichel, C. L., & Wassarman, D. A. (2020). *Introduction to genetic analysis* (Twelfth ed.). New York, NY: W.H. Freeman & Company/Macmillan Learning.

Hader, T., Muller, S., Aguilera, M., Eulenberg, K. G., Steuernagel, A., Ciossek, T., . . . Bronner, G. (2003). Control of triglyceride storage by a WD40/TPR-domain protein. *EMBO reports, 4*(5), 511-516. doi:10.1038/sj.embor.embor837

Haghighi Poodeh, S., Alhonen, L., Salonurmi, T., & Savolainen, M. J. (2014). Ethanol-induced impairment of polyamine homeostasis--a potential cause of neural tube defect and intrauterine growth restriction in fetal alcohol syndrome. *Biochemical and biophysical research communications, 446*(1), 173-178. doi:10.1016/j.bbrc.2014.02.079

Hamasaki-Katagiri, N., Katagiri, Y., Tabor, C. W., & Tabor, H. (1998). Spermine is not essential for growth of Saccharomyces cerevisiae: identification of the SPE4 gene (spermine synthase) and characterization of a spe4 deletion mutant. *Gene, 210*(2), 195-201. doi:10.1016/s0378-1119(98)00027-4

Hegde, S. S., Chandler, J., Vetting, M. W., Yu, M., & Blanchard, J. S. (2007). Mechanistic and structural analysis of human spermidine/spermine N1-acetyltransferase. *Biochemistry, 46*(24), 7187-7195. doi:10.1021/bi700256z

Herbst, E. J., & Dion, A. S. (1970). Polyamine changes during development of Drosophila melanogaster. *Annals of the New York academy of sciences, 29*(4), 1563-1567.

Ikeguchi, Y., Bewley, M. C., & Pegg, A. E. (2006). Aminopropyltransferases: function, structure and genetics. *Journal of biochemistry, 139*(1), 1-9. doi:10.1093/jb/mvj019

Ishii, I., Ikeguchi, Y., Mano, H., Wada, M., Pegg, A. E., & Shirahata, A. (2012). Polyamine metabolism is involved in adipogenesis of 3T3-L1 cells. *Amino acids, 42*(2-3), 619-626. doi:10.1007/s00726-011-1037-5

Kabra, P. M., Lee, H. K., Lubich, W. P., & Marton, L. J. (1986). Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phase liquid chromatography: improved separation systems for polyamines in cerebrospinal fluid, urine and tissue. *Journal of chromatography, 380*(1), 19-32. doi:10.1016/s0378-4347(00)83621-x

Korhonen, V. P., Halmekyto, M., Kauppinen, L., Myohanen, S., Wahlfors, J., Keinanen, T., . . . Janne, J. (1995). Molecular cloning of a cDNA encoding human spermine synthase. *DNA and cell biology, 14*(10), 841-847.

Lam, G., & Thummel, C. S. (2000). Inducible expression of double-stranded RNA directs specific genetic interference in Drosophila. *Current biology, 10*(16), 957-963. doi:10.1016/s0960-9822(00)00631-x

Lambertos, A., Ramos-Molina, B., Lopez-Contreras, A. J., Cremades, A., & Penafiel, R. (2018). New insights of polyamine metabolism in testicular physiology: A role of ornithine decarboxylase antizyme inhibitor 2 (AZIN2) in the modulation of testosterone levels and sperm motility. *PLoS one, 13*(12), e0209202. doi:10.1371/journal.pone.0209202

Leon, K. E., Fruin, A. M., Nowotarski, S. L., & DiAngelo, J. R. (2020). The regulation of triglyceride storage by ornithine decarboxylase (Odc1) in Drosophila. *Biochemical and biophysical research communications, 523*(2), 429-433. doi:10.1016/j.bbrc.2019.12.078

Lewandowski, N. M., Ju, S., Verbitsky, M., Ross, B., Geddie, M. L., Rockenstein, E., . . . Small, S. A. (2010). Polyamine pathway contributes to the pathogenesis of Parkinson disease. *Proceedings of the national academy of sciences, 107*(39), 16970-16975. doi:10.1073/pnas.1011751107

Li, C., Brazill, J. M., Liu, S., Bello, C., Zhu, Y., Morimoto, M., . . . Zhai, R. G. (2017). Spermine synthase deficiency causes lysosomal dysfunction and oxidative stress in models of Snyder-Robinson syndrome. *Nature communications, 8*(1), 1257. doi:10.1038/s41467-017-01289-7

Lima, G., & Shiu, R. P. (1985). Role of polyamines in estradiol-induced growth of human breast cancer cells. *Cancer research, 45*(6), 2466-2470.

Lorenz, B., Francis, F., Gempel, K., Boddrich, A., Josten, M., Schmahl, W., . . . Strom, T. M. (1998). Spermine deficiency in Gy mice caused by deletion of the spermine synthase gene. *Human molecular genetics, 7*(3), 541-547. doi:10.1093/hmg/7.3.541

Lyon, M. F., Scriver, C. R., Baker, L. R., Tenenhouse, H. S., Kronick, J., & Mandla, S. (1986). The Gy mutation: another cause of X-linked hypophosphatemia in mouse. *Proceedings of the national academy of sciences, 83*(13), 4899-4903. doi:10.1073/pnas.83.13.4899

Ma, L., Ni, Y., Hu, L., Zhao, Y., Zheng, L., Yang, S., . . . Fu, Z. (2021). Spermidine ameliorates high-fat diet-induced hepatic steatosis and adipose tissue inflammation in preexisting obese mice. *Life sciences, 265*, 118739. doi:10.1016/j.lfs.2020.118739

Mackintosh, C. A., & Pegg, A. E. (2000). Effect of spermine synthase deficiency on polyamine biosynthesis and content in mice and embryonic fibroblasts, and the sensitivity of fibroblasts to 1,3-bis-(2-chloroethyl)-N-nitrosourea. *Biochemical journal, 351 Pt 2*, 439-447.

Madeo, F., Eisenberg, T., Pietrocola, F., & Kroemer, G. (2018). Spermidine in health and disease. *Science, 359*(6374). doi:10.1126/science.aan2788

Majumdar, R., Lebar, M., Mack, B., Minocha, R., Minocha, S., Carter-Wientjes, C., . . . Cary, J. W. (2018). The Aspergillus flavus spermidine synthase (spds) gene, is required for normal development, aflatoxin production, and pathogenesis during infection of maize kernels. *Frontiers in plant science, 9*, 317. doi:10.3389/fpls.2018.00317

Mandal, S., Mandal, A., & Park, M. H. (2015). Depletion of the polyamines spermidine and spermine by overexpression of spermidine/spermine N(1)-acetyltransferase 1 (SAT1) leads to mitochondria-mediated apoptosis in mammalian cells. *Biochemical journal, 468*(3), 435-447. doi:10.1042/BJ20150168

Miao, H., Ou, J., Peng, Y., Zhang, X., Chen, Y., Hao, L., . . . Liang, H. (2016). Macrophage ABHD5 promotes colorectal cancer growth by suppressing spermidine production by SRM. *Nature communications, 7*, 11716. doi:10.1038/ncomms11716

Miller-Fleming, L., Olin-Sandoval, V., Campbell, K., & Ralser, M. (2015). Remaining mysteries of molecular biology: the role of polyamines in the cell. *Journal of molecular biology, 427*(21), 3389-3406. doi:10.1016/j.jmb.2015.06.020

Minois, N., Rockenfeller, P., Smith, T. K., & Carmona-Gutierrez, D. (2014). Spermidine feeding decreases age-related locomotor activity loss and induces changes in lipid composition. *PLoS One, 9*(7), e102435. doi:10.1371/journal.pone.0102435

Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (2019). Drosophila melanogaster: a model organism to study cancer. *Frontiers in genetics, 10*, 51. doi:10.3389/fgene.2019.00051

Monson, E. T., de Klerk, K., Gaynor, S. C., Wagner, A. H., Breen, M. E., Parsons, M., . . . Willour, V. L. (2016). Whole-gene sequencing investigation of SAT1 in attempted suicide. *American journal of medical genetics, B, Neuropsychiatric genetics, 171*(6), 888-895. doi:10.1002/ajmg.b.32462

Morgan, T. H. (1915). *The Mechanism of Mendelian heredity*. New York: H. Holt and company.

Murakami, Y., & Hayashi, S. (1985). Role of antizyme in degradation of ornithine decarboxylase in HTC cells. *Biochemical journal, 226*(3), 893-896. doi:10.1042/bj2260893

Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., . . . Ichihara, A. (1992). Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. *Nature, 360*(6404), 597-599.

Murillo-Maldonado, J. M., & Riesgo-Escovar, J. R. (2017). Development and diabetes on the fly. *Mechanisms of development, 144*(Pt B), 150-155. doi:10.1016/j.mod.2016.09.004

Murray-Stewart, T., Dunworth, M., Foley, J. R., Schwartz, C. E., & Casero, R. A. (2018). Polyamine homeostasis in Snyder-Robinson syndrome. *Medical sciences (Basel), 6*(4). doi:10.3390/medsci6040112

Musselman, L. P., & Kuhnlein, R. P. (2018). Drosophila as a model to study obesity and metabolic disease. *Journal of experimental biology, 221*(Pt Suppl 1). doi:10.1242/jeb.163881

Nilsson, J., Koskiniemi, S., Persson, K., Grahn, B., & Holm, I. (1997). Polyamines regulate both transcription and translation of the gene encoding ornithine decarboxylase antizyme in mouse. *European journal of biochemistry, 250*(2), 223-231.

Nishimura, K., Lee, S. B., Park, J. H., & Park, M. H. (2012). Essential role of eIF5A-1 and deoxyhypusine synthase in mouse embryonic development. *Amino acids, 42*(2-3), 703-710. doi:10.1007/s00726-011-0986-z

Nishimura, K., Nakatsu, F., Kashiwagi, K., Ohno, H., Saito, T., & Igarashi, K. (2002). Essential role of S-adenosylmethionine decarboxylase in mouse embryonic development. *Genes to cells, 7(1),* 41-47. doi:10.1046/j.1356-9597.2001.00494.x

Nitta, T., Igarashi, K., & Yamamoto, N. (2002). Polyamine depletion induces apoptosis through mitochondria-mediated pathway. *Experimental cell research, 276*(1), 120-128. doi:10.1006/excr.2002.5517

Nowotarski, S. L., & Shantz, L. M. (2010). Cytoplasmic accumulation of the RNA-binding protein HuR stabilizes the ornithine decarboxylase transcript in a murine nonmelanoma skin cancer model. *Journal of biological chemistry, 285*(41), 31885-31894. doi:10.1074/jbc.M110.148767

Nowotarski, S. L., Woster, P. M., & Casero, R. A., Jr. (2013). Polyamines and cancer: implications for chemotherapy and chemoprevention. *Expert reviews in molecular medicine, 15*, e3. doi:10.1017/erm.2013.3

Park, M. H., Cooper, H. L., & Folk, J. E. (1981). Identification of hypusine, an unusual amino acid, in a protein from human lymphocytes and of spermidine as its biosynthetic precursor. *Proceedings of the national academy of sciences, 78*(5), 2869-2873. doi:10.1073/pnas.78.5.2869

Pegg, A. E. (2006). Regulation of ornithine decarboxylase. *Journal of biological chemistry, 281*(21), 14529-14532. doi:10.1074/jbc.R500031200

Pegg, A. E. (2014). The function of spermine. *IUBMB life, 66*(1), 8-18. doi:10.1002/iub.1237

Pegg, A. E. (2016). Functions of polyamines in mammals. *Journal of biological chemistry, 291*(29), 14904-14912. doi:10.1074/jbc.R116.731661

Pegg, A. E., & Coward, J. K. (1985). Growth of mammalian cells in the absence of the accumulation of spermine. *Biochemical and biophysical research communications, 133*(1), 82-89. doi:10.1016/0006-291x(85)91844-3

Pegg, A. E., & Williams-Ashman, H. G. (1969). On the role of S-adenosyl-L-methionine in the biosynthesis of spermidine by rat prostate. *Journal of biological chemistry, 244*(4), 682-693.

Pendeville, H., Carpino, N., Marine, J. C., Takahashi, Y., Muller, M., Martial, J. A., & Cleveland, J. L. (2001). The ornithine decarboxylase gene is essential for cell survival during early murine development. *Molecular and cellular biology, 21*(19), 6549-6558.

Peron, A., Spaccini, L., Norris, J., Bova, S. M., Selicorni, A., Weber, G., . . . Mastrangelo, M. (2013). Snyder-Robinson syndrome: a novel nonsense mutation in spermine synthase and expansion of the phenotype. *American journal of medical genetics, A, 161A*(9), 2316-2320. doi:10.1002/ajmg.a.36116

Poulin, R., Casero, R. A., & Soulet, D. (2012). Recent advances in the molecular biology of metazoan polyamine transport. *Amino acids, 42*(2-3), 711-723. doi:10.1007/s00726-011-0987-y

Recasens-Alvarez, C., Ferreira, A., & Milan, M. (2017). JAK/STAT controls organ size and fate specification by regulating morphogen production and signalling. *Nature communications, 8*, 13815. doi:10.1038/ncomms13815

Reiter, L. T., Potocki, L., Chien, S., Gribskov, M., & Bier, E. (2001). A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome research, 11*(6), 1114-1125. doi:10.1101/gr.169101

Russell, D., & Snyder, S. H. (1968). Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. *Proceedings of the national academy of sciences, 60*(4), 1420-1427. doi:10.1073/pnas.60.4.1420

Sadasivan, S. K., Vasamsetti, B., Singh, J., Marikunte, V. V., Oommen, A. M., Jagannath, M. R., & Pralhada Rao, R. (2014). Exogenous administration of spermine improves glucose utilization and decreases bodyweight in mice. *European journal pharmacology, 729*, 94-99. doi:10.1016/j.ejphar.2014.01.073

Schuber, F. (1989). Influence of polyamines on membrane functions. *Biochemical journal, 260*(1), 1-10. doi:10.1042/bj2600001

Schwartz, C. E., Wang, X., Stevenson, R. E., & Pegg, A. E. (2011). Spermine synthase deficiency resulting in X-linked intellectual disability (Snyder-Robinson syndrome). *Methods in molecular biology, 720*, 437-445. doi:10.1007/978-1-61779-034-8_28

Sever, S., Weinstein, D. A., Wolfsdorf, J. I., Gedik, R., & Schaefer, E. J. (2012). Glycogen storage disease type Ia: linkage of glucose, glycogen, lactic acid, triglyceride, and uric acid metabolism. *Journal of clinical lipidology, 6*(6), 596-600. doi:10.1016/j.jacl.2012.08.005

Shantz, L. M. (2004). Transcriptional and translational control of ornithine decarboxylase during Ras transformation. *Biochemical journal, 377*(Pt 1), 257-264.

Shantz, L. M., Feith, D. J., & Pegg, A. E. (2001). Targeted overexpression of ornithine decarboxylase enhances beta-adrenergic agonist-induced cardiac hypertrophy. *Biochemical journal, 358*(Pt 1), 25-32.

Snyder, R. D., & Robinson, A. (1969). Recessive sex-linked mental retardation in the absence of other recognizable abnormalities. Report of a family. *Clinical pediatrics, 8*(11), 669-674. doi:10.1177/000992286900801114

Soulet, D., Gagnon, B., Rivest, S., Audette, M., & Poulin, R. (2004). A fluorescent probe of polyamine transport accumulates into intracellular acidic vesicles via a two-step mechanism. *Journal of biological chemistry, 279*(47), 49355-49366. doi:10.1074/jbc.M401287200

Tang, H., Ariki, K., Ohkido, M., Murakami, Y., Matsufuji, S., Li, Z., & Yamamura, K. (2009). Role of ornithine decarboxylase antizyme inhibitor in vivo. *Genes to cells : devoted to molecular & cellular mechanisms, 14*(1), 79-87. doi:10.1111/j.1365-2443.2008.01249.x

Tholl, D., Harms, R., Ludwig, A., & Kaiser, A. (1998). Retarded growth of an Escherichia coli mutant deficient in spermidine synthase can be unspecifically repaired by addition of various polyamines. *World journal of microbiology & biotechnology, 14*(6), 857-863.

Tobias, K. E., & Kahana, C. (1993). Intersubunit location of the active site of mammalian ornithine decarboxylase as determined by hybridization of site-directed mutants. *Biochemistry, 32*(22), 5842-5847. doi:10.1021/bi00073a017

Tsuji, T., Usui, S., Aida, T., Tachikawa, T., Hu, G. F., Sasaki, A., . . . Wong, D. T. (2001). Induction of epithelial differentiation and DNA demethylation in hamster malignant oral keratinocyte by ornithine decarboxylase antizyme. *Oncogene, 20*(1), 24-33. doi:10.1038/sj.onc.1204051

Uemura, T., Stringer, D. E., Blohm-Mangone, K. A., & Gerner, E. W. (2010). Polyamine transport is mediated by both endocytic and solute carrier transport mechanisms in the gastrointestinal tract. *American journal of physiology. Gastrointestinal and liver physiology, 299*(2), G517-522. doi:10.1152/ajpgi.00169.2010

Uemura, T., Yerushalmi, H. F., Tsaprailis, G., Stringer, D. E., Pastorian, K. E., Hawel, L., 3rd, . . . Gerner, E. W. (2008). Identification and characterization of a diamine exporter in colon epithelial cells. *Journal biological chemistry, 283*(39), 26428-26435. doi:M804714200 [pii]
10.1074/jbc.M804714200

Vigne, P., & Frelin, C. (2008). The role of polyamines in protein-dependent hypoxic tolerance of Drosophila. *BMC physiology, 8*, 22. doi:10.1186/1472-6793-8-22

Wahlfors, J., Alhonen, L., Kauppinen, L., Hyvonen, T., Janne, J., & Eloranta, T. O. (1990). Human spermidine synthase: cloning and primary structure. *DNA and cell biology, 9*(2), 103-110.

Wang, X., Ikeguchi, Y., McCloskey, D. E., Nelson, P., & Pegg, A. E. (2004). Spermine synthesis is required for normal viability, growth, and fertility in the mouse. *Journal of biological chemistry, 279*(49), 51370-51375. doi:10.1074/jbc.M410471200

Wang, X., Levic, S., Gratton, M. A., Doyle, K. J., Yamoah, E. N., & Pegg, A. E. (2009). Spermine synthase deficiency leads to deafness and a profound sensitivity to alpha-difluoromethylornithine. *Journal of biological chemistry, 284*(2), 930-937. doi:10.1074/jbc.M807758200

Wu, H., Min, J., Ikeguchi, Y., Zeng, H., Dong, A., Loppnau, P., . . . Plotnikov, A. N. (2007). Structure and mechanism of spermidine synthases. *Biochemistry, 46*(28), 8331-8339. doi:10.1021/bi602498k

Wu, H., Min, J., Zeng, H., McCloskey, D. E., Ikeguchi, Y., Loppnau, P., . . . Plotnikov, A. N. (2008). Crystal structure of human spermine synthase: implications of substrate binding and catalytic mechanism. *Journal biological chemistry, 283*(23), 16135-16146. doi:10.1074/jbc.M710323200

Wu, H. Y., Chen, S. F., Hsieh, J. Y., Chou, F., Wang, Y. H., Lin, W. T., . . . Chan, N. L. (2015). Structural basis of antizyme-mediated regulation of polyamine homeostasis. *Proceedings of the national academy of sciences, 112*(36), 11229-11234. doi:10.1073/pnas.1508187112

Xu, H., Chaturvedi, R., Cheng, Y., Bussiere, F. I., Asim, M., Yao, M. D., . . . Wilson, K. T. (2004). Spermine oxidation induced by Helicobacter pylori results in apoptosis and DNA damage: implications for gastric carcinogenesis. *Cancer research, 64*(23), 8521-8525. doi:10.1158/0008-5472.CAN-04-3511

Yamaguchi, M., & Yoshida, H. (2018). Drosophila as a model organism. *Advances in experimental medicine and biology, 1076*, 1-10. doi:10.1007/978-981-13-0529-0_1

Zabala-Letona, A., Arruabarrena-Aristorena, A., Martin-Martin, N., Fernandez-Ruiz, S., Sutherland, J. D., Clasquin, M., . . . Carracedo, A. (2017). mTORC1-dependent AMD1 regulation sustains polyamine metabolism in prostate cancer. *Nature, 547*(7661), 109-113. doi:10.1038/nature22964

Zahedi, K., Bissler, J. J., Wang, Z., Josyula, A., Lu, L., Diegelman, P., . . . Soleimani, M. (2007). Spermidine/spermine N1-acetyltransferase overexpression in kidney epithelial cells disrupts polyamine homeostasis, leads to DNA damage, and causes G2 arrest. *American journal of physiology, cell physiology, 292*(3), C1204-1215. doi:10.1152/ajpcell.00451.2006

Zhu, Q., Jin, L., Casero, R. A., Davidson, N. E., & Huang, Y. (2012). Role of ornithine decarboxylase in regulation of estrogen receptor alpha expression and growth in human breast cancer cells. *Breast cancer research and treatment, 136*(1), 57-66. doi:10.1007/s10549-012-2235-x

ACADEMIC VITA

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EDUCATION

The Pennsylvania State University — May 2021
B. S. Biochemistry and Molecular Biology (Molecular Biology Option)
Honors in Biochemistry and Molecular Biology
Minor: Business

HONORS, AWARDS, AND ACCOMPLISHMENTS

Eric A. and Josephine S. Walker Award Finalist	Spring 2021
Craig and Maxine Miller Award Recipient	Spring 2021
Martin Luther King Jr. Service Award Recipient	Spring 2021
Outstanding Internship in Biochemistry Award Recipient	Spring 2020
Campus Life Spirit Award	Spring 2020
Outstanding Organic Chemistry Student Award Recipient	Spring 2019
First-Year Advocate Award	Spring 2019
Outstanding General Chemistry Student Award Recipient	Spring 2018
President's Freshman Award	Spring 2018
Campus Life Rising Star Award	Spring 2018
Dean's List	Fall 2017- Present
Penn State Berks Honors Program	Fall 2017- Present

RESEARCH PROJECTS, PUBLICATIONS, AND RELEVANT EXPERIENCE

Undergraduate Honors Thesis — Spring 2020 - Present

Characterizing the impacts of ornithine decarboxylase, spermidine synthase, and spermine synthase on polyamine pathway regulation and cell metabolism changes in Drosophila melanogaster

- Performed qRT-PCR measuring the expression of several polyamine pathway genes in null and knockdown *Drosophila* models
- Conducted data analysis and assembled findings on gene expression, macromolecule, and HPLC measurements

Submitted Publications

The role of spermidine synthase (SpdS) and spermine synthase (Sms) in regulating triglyceride storage in Drosophila

- Tahj S Morales, Erik C Avis, Elise K Paskowski, Hamza Shabar, Shannon L Nowotarski *, Justin R DiAngelo *
- * Denotes Corresponding Author

Undergraduate Research Assistant- Nowotarski Lab — Fall 2018 - Present

- Conducted tissue culture, western blotting, agarose and polyacrylamide gel electrophoresis experiments in *Drosophila* models
- Presented data at ASCB/EMBO Cell Bio Virtual 2020 Meeting — Dec. 2020

Project Coordinator- Penn State College of Medicine — Sept. 2020 – Present

- Aided in development of a flu vaccination education and promotional campaign tailored for the local Hispanic and Latino community
- Assisted in coordination of mobile vaccination clinics
- Conducted research on perceptions of flu vaccination

Community Health Intern- Penn State Health St. Joseph Mar. 2020 - Sept. 2020

- Assisted in implementation and evaluation of intervention programs to address needs for improved access to healthy foods and exercise in the local Latino Community
- Conducted community-based research on health resource accessibility

Summer Research Intern- Penn State College of Medicine May 2019- Aug. 2019

- Conducted DNA and protein analysis on Acute Myeloid Leukemia and Amyotrophic Lateral Sclerosis samples to confirm presence of detected mutations and track neuronal development.
- Presented research at an undergraduate poster session

19th Annual Undergraduate HECBC Research and Creativity Conference Spring 2018

- Presented a literary analysis of chemistry principles in *Alice in Wonderland*

LEADERSHIP AND DEVELOPMENT EXPERIENCE

Penn State Berks Lion Ambassador Spring 2018 – Present

- Welcomed and gave tours to visitors, including prospective students, families, and employees
- Served as the Social Director for 1.5 years organizing group events, fundraisers, and community service events for the organization
- Served as the Executive Director for 1 year, leading the organization and its members, setting large scale goals, and serving as the organizational liaison to administration

Penn State Berks Chancellor's Circle Dinner Speaker Fall 2017 – Fall 2020

- Spoke about my experience and involvement at Berks to potential and existing campus donors through the Office of Development

Undergraduate Student Mentor Fall 2018 – Fall 2020

- Guided first-year and second-year students and provided in-class and out-of-class workshops and discussions on adjusting to college
- Mentored for Honors, non-Honors, and accelerated first-year seminar and Honors upper-level technical writing courses.

Penn State Berks Welcome Weekend Orientation Leader Fall 2018 – Fall 2020

- Assisted incoming students during their first week(s) on campus, including move-in, finding classes, and forming connections with peers of their class.
- Served as a member of the Lead Team training other orientation leaders on how to facilitate breakout sessions with incoming students
- Served as a Color Captain, leading my own group of orientation leaders and training them within a smaller group setting as opposed to within the large group

Penn State Berks Benefitting THON Fall 2019 - Spring 2020

- Served as an Outreach Captain and led a group to help raise funds for the larger THON organization

Chemistry Teaching Assistant Spring 2018 - Fall 2019

- Worked to tutor and guide students in and out of class on general chemistry lecture and organic chemistry laboratory information

Penn State Berks Student Government Association Fall 2017 - Spring 2019

- Worked to draft policies and initiatives on community service, diversity and inclusion, and campus renovations
- Served as First-Year Representative for 1 year and Chief of Staff for 1 year

TEDxPSUBerks, Assistant Marketing Coordinator Fall 2018

- Assisted Team Management and Marketing Director in organizing social media and other outreach initiatives for the TEDxPSUBerks event