The Concurrent Effects of Dietary Restriction and Exercise on a *Drosophila melanogaster* Model of Spinal Muscular Atrophy

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ABSTRACT

Dietary restriction (DR) and exercise studies have shown that reducing caloric intake and increasing physical activity independently improve symptoms of neurodegenerative diseases. Spinal Muscular Atrophy (SMA) is a neuromuscular degenerative disease characterized by progressive muscle weakness, decreased metabolic rate, and worsened motor function. The effects of dietary restriction and exercise, concurrently, have not yet been analyzed in models of SMA. A 3-D *Drosophila* Rotation Device was designed and created to induce exercise at various intensities, and a respirometry device was created to determine relative metabolic rates in *Drosophila melanogaster* with SMA. This study found that the downregulation of the target of rapamycin (TOR) pathway through DR, compounded with moderate exercise, had a statistically significant effect on SMA symptoms presented by the flies. Data suggest that the optimal combination of DR and exercise can significantly improve the progressive symptoms associated with SMA.

INTRODUCTION

Aging is the natural degeneration of human tissue and progressive impairment of bodily functions, and one of the hallmarks of aging is neurodegeneration (Harman, 2006). Many interventions which prolong lifespan and delay aging can be used to successfully ameliorate a number of neurodegenerative disorders (Jahrling and Laberge, 2015). Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disorder characterized by progressive muscle weakness for which no cure exists (Finsterer, 2009). SMA results from mutations in the *survival motor neurons 1* (SMN1) gene (D'Amico et al., 2011) and in addition to progressive muscle weakness, SMA has been shown to decrease metabolic rate and worsen motor function capabilities (Arnold et al., 2015). Additionally, Zolgensma, the most effective treatment for SMA, is the costliest by-dose medical prescription in the world at $2.15 million (Nuijten, 2022). 1 in every 8,000 newborns are diagnosed with SMA and it is one of the most fatal diseases amongst infants (Verhaart et al., 2017). It is therefore encouraging that the effects of SMA can be mitigated by both environmental and genetic factors (Tosolini and Sleigh, 2017).

Drosophila melanogaster is a widely used model of human disease (Pandey and Nichols, 2011) as nearly 65% of their disease-causing genes are homologous to humans (Ugur, 2016). In addition to their genetic similarity, *Drosophila* require low maintenance (Hales et al., 2015), have a relatively short life span with fast reproduction rates (Linford et al., 2013), are highly accessible, and are easily manipulated (Venken et al., 2012). The flies are also composed of postmitotic cells, allowing for identifiable physical changes as the flies age (De Nobrega and Lyons, 2018). *Drosophila* has been used to model several neurodegenerative diseases (Muqit and Feany, 2002) such as Alzheimer's Disease, Parkinson's, and Huntington's Disease. Thus, making *Drosophila* an ideal model organism for studying complex human diseases such as SMA.

In *Drosophila*, CO_2 output has strong correlation with O_2 intake (Van Voorhies et al., 2004). Thus, measurement of CO_2 output can successfully be used to study metabolic rates (Hulbert et al., 2004). An inexpensive and simple system can be used to carry out these studies, in which expelled CO_2 reacts with an atmospheric absorbent, resulting in a slight drop in pressure in a sealed chamber. The volume of CO_2 expelled can be determined by measuring the distance a liquid migrates in a capillary allowing for both the measure of CO_2 produced by the flies and thus

of their metabolic rate (Hsia et al., 2013). Subsequently, allowing for the determination of relative metabolic rates under given conditions.

Motor function and metabolic rates are affected by a multitude of factors (Deguise et al., 2021), including exercise (Houdebine et al., 20019) and diet (Watson et al., 2008). Exercise improves cardiovascular health, neurological health, and ameliorates symptoms in neurodegenerative diseases such as Parkinson's and Alzheimer's Disease (Mahalakshmi et al., 2020). In *Drosophila*, exercise has been associated with numerous health benefits such as increased endurance, increased metabolic activity, and decreased gross fly weight. Dietary Restriction (DR) is one of the most effective methods utilized in counteracting the negative effects of the aging process. By limiting specific macronutrients, such as lowering the ratio of protein to carbohydrates, it has been shown that motor function is improved, lifespan is extended, and stress tolerance increases (Kapahi et al., 2017). A key modulator of this intervention is the target of rapamycin (TOR) pathway, which strongly responds to changes in amino acid availability. TOR signaling is reduced under conditions of lower dietary amino acid (Inoki et al., 2005). When TOR signaling is decreased, there is an increase in autophagy, a decrease in the rate of protein synthesis, and an increase production of cellular repair proteins. This has widespread effects, as low TOR signaling has beneficial effects in many diseases. In this study, the effects of both exercise and DR was measured using a *Drosophila* model of SMA.

While SMA is a neuromuscular disorder, mutations and symptoms are present in both the motor neurons and in the muscle tissue (Park et al., 2010). Furthermore, in this study SMA will be eliminated in either muscle or neural tissue to test whether the effect is restricted to loss of SMA in neural tissue or whether it also plays a role in muscle degeneration (Visser and Dieen, 2005). This will be done using a combined RNA interference (RNAi) (Haiyong, 2018) approach to down regulate the gene, and tissue specific delivery of the RNAi mediated by the GAL-4/upstream activating sequence (UAS) System (Southall et al., 2008). In determining the critical tissue for the progression of SMA symptoms, more specific treatments to target the critical tissue groups have potential to facilitate significant advancements in SMA treatment.

Studies concerning the effects of DR, exercise, and DR and exercise have not been conducted while using *Drosophila melanogaster* as a model organism for SMA. So, one objective of this study was to determine the relationship of DR and/or exercise with *Drosophila* models of SMA symptoms. It was hypothesized that when DR and exercise are introduced

individually, SMA symptoms would improve. However, when introduced simultaneously, it was hypothesized that strenuous exercise (SE) and DR would worsen symptoms. The other objective of this study was to determine mutations in which tissue are responsible for SMA symptoms. I hypothesized that using flies with SMA expressed in motor neuron tissue (mnSMA) will display more severe symptoms than flies with SMA expressed in muscle tissue (mSMA).

MATERIALS AND METHODS

Drosophila Stocks and Diets

All fly strains were obtained from the Bloomington Stock Center at Indiana University. In order to modulate the expression of SMA a conditional expression system, Gal-4 Upstream Activation Sequence (UAS) (Brand and Perrimon) was used. In this system the desired phenotype is achieved by crossing two strains of flies each contributing one component of the expression system. The driver strains contain a tissue specific regulatory sequence controlling the expression of the yeast transcriptional activator Gal-4. Since Gal-4 is not found in *Drosophila,* expression of Gal-4 has no effect on the flies in which it is expressed. The receiver strain contains the Gal-4 responsive UAS sequences controlling the expression of the gene whose activity is to be modulated, however, since there is no Gal-4 present in the fly this construct has no effect on its host. Upon crossing the two strains (driver + responder) the F1 progeny will now express the gene whose activity is controlled by Gal-4. In these experiments, the sequences under control of UAS encoded a hairpin RNA which would lead to post-transcriptional gene silencing of the expressed sequence. Two driver strains were obtained from the Bloomington Stock center: Bloomington stock number 1767, also known as *24B*, which expresses Gal-4 in muscle tissue (mSMA); and 8816 or *D42* which expresses Gal-4 in motor neurons (mnSMA). A receiver strain 36621 or SMA RNA-I, which expresses a short hairpin RNA corresponding to the SMA gene in *Drosophila.* A lab control strain (3605, *w1118*) was used as the control for all experiments.

To culture flies and obtain sufficient individuals to do the experiments, flies were maintained in bottles for 3-5 days and then removed to allow the progeny to emerge as described in (Partridge and Fowler, 1992). To perform the crosses, virgins were collected from either of the two drivers and these were then crossed to either the SMA RNA-I strain or to the control. Three groups were then used in experiments. Control flies consisting of either driver strain crossed to *w1118*, flies with reduced SMA expression in motor neurons obtained by crossing the D42 driver to SMA-RNA (from now referred to as motoneuron SMA), and flies with reduced SMA expression in muscles obtained by crossing mSMA flies to SMA-RNA-I (from now referred to as muscle SMA) bottle groups: SMA strain for population growth, virgin collection, muscle expressors, motor neuron expressors, and control flies. All fly strains and crosses were cultured on Caltech fly media, consisting of 87 g cornmeal (Augason Farms Bakery Cornmeal), 75 g

sucrose (Fisher Science Education Crystalized Purified Sucrose), 15 g live yeast (Red Star Active dry yeast), 4.5 g agar (Sigma-Aldrich), 10 mL fly acid (mix of propionic and phosphoric acid), per 1 L of distilled water (Lewis 1960). A Mettler Toledo electronic scale was used to measure each macronutrient. The solution was then placed in a microwave for 1 minute then stirred, until it reached a boil. Food was then poured into empty bottles and vials, allowed to solidify, and cool to room temperature prior to introducing the flies.

Genetic Manipulation

SMA responder flies ordered have a non-expressed dormant mutation on the SMN1 gene. These responder flies have cis-regulatory (non-coding DNA sequence in a gene required for expression of that gene, UAS) binding sites, while the driver flies obtain a Gal-4 transcription factor which binds to the responder sites, expressing the targeted gene in progeny. In this study, female SMA flies were used for their UAS, and male *D42* and *24B* flies were used for their Gal-4 transcriptional factors.

Fly Crossing and Genetic Manipulation

Prior to crossing SMA females and male drivers, female SMA virgins need to be collected, since females can store sperm once they have mated. Therefore, virgin collection is an essential step in fly crossing. Half of the SMA population was allocated to virgin collection, while the other half was allocated to growing populations. Fly bottles were left untouched for 3-5 days, allowing for fertilization and for larvae to emerge. Once a considerable number of larvae and pupae were observed in a bottle, all adult flies were removed from the bottle, ensuring that only larvae or pupae are present. Since flies begin mating 8 hours after emerging, virgin collection is extremely time sensitive. At the start of each day, bottles were emptied, and virgins were collected in 4–8-hour intervals, ensuring that only virgins are collected. Female virgin SMA flies were stored on Caltech fly food until enough were collected to be mated. To cross the flies, virgin female receivers were placed in vials with either of the two driver males. Under a Genesee Nikon SMZ645 microscope and CO_2 anesthetic on a FlyStuff Flypad, the sex of the flies was identified, and crosses were set up in bottles with new food. The female to male ratio was 2:1. 10-20 grains of live yeast were then sprinkled into each reproduction bottle to encourage

female flies to lay eggs. The procedure for collecting experimental progeny was the same for collecting virgins.

	Dietary Restriction	**Normal Diet**
No Exercise	mSMA, mnSMA, Control	mSMA, mnSMA, Control
Moderate Exercise	mSMA, mnSMA, Control	mSMA, mnSMA, Control
Strenuous Exercise	mSMA, mnSMA, Control	mSMA, mnSMA, Control

**mSMA = muscle SMA; mnSMA = motor neuron SMA*

Table 1: Nutritional and Activity Profile of Fly Strains. Each nutritional profile consists of one of two diets (normal diet (ND) or protein restricted, dietary restriction (DR)). Each activity regimen consists of three different intensities (no exercise (NE), moderate exercise (ME), or strenuous exercise (SE)). These 6 profiles were applied to three different fly strains, yielding 18 experimental groups for the analysis of progressive SMA symptoms. 30 flies were tested in each of the 18 experimental groups, in each of the two testing rounds, totaling for 1,080 flies used in this study.

Experimental Setup and Pre-Testing

Cross 1 was defined as 24B x SMA (mSMA), Cross 2 was defined as D42 x SMA (mnSMA). After crosses produced enough larvae, bottles were emptied. Only male F1 (first generation, ensured half of genes were from each of the desired strains) progeny were collected. ND contained 5 g sucrose, 5 g yeast, 1.2 g agar, and 1 mL fly acid per 100 mL. DR contained 5 g sucrose, 1 g yeast, 1.2 g agar, and 1 mL fly acid per 100 mL. The lack of yeast in the DR group decreases the amount of protein flies consume, thus decreasing the TOR pathway activity (Katewa and Kapahi, 2011). As SMA is a neurodegenerative disorder, and symptoms are age-progressive, age matching was a crucial step prior to testing. All fly strains for testing were collected within 2 days of each other, ensuring a similar age range. Flies were then put on their respective diets for the remainder of their life in vials. Flies were then tested for motor function,

fatigue, and metabolic rate at day 5, 10, 15, and 20 after being placed on their respective diets. Old food was replaced with new respective food the day after each testing day as well.

DRD/Geotaxis Assay

In order to test motor function and endurance in flies with SMA, the *Drosophila* Rotation Device (DRD) was 3-D printed to induce exercise in all flies (Lin, 2021, unpublished thesis). 10 flies were placed in each vial with their respective diets (ND or DR). For all flies, ND or DR, on the NE regimen, they were tested for motor function with no prior activity. For each experimental group, 3 geotaxis times were recorded, allowing 1.5 minutes in between each test to allow fly recovery. It was reported that short intervals of exercise split by five-minute breaks induced significant exercise for *Drosophila* (Mendez et al., 2016). So, all flies on the ME regimen were spun in the DRD at 5 RPM for 15 minutes, then allowed to rest for 5 minutes before completing rounds of the geotaxis assay. Lastly, flies on the SE regimen were spun for 15 minutes, rested for 5 minutes, spun for 15 more minutes, and rested 5 additional minutes prior to completing the geotaxis assay. Fly geotaxis times were recorded in seconds in an excel spreadsheet. The time recorded indicates the time it took the median fly to cross the half-way mark on the vial. Fly vials were tapped 3 times to bring all flies to the bottom of the vial. Time started directly after the third tap and ended after the median fly crossed the 4 cm mark. An iPhone 13 Pro was used to record videos, and the SlowMo Video application was used. After testing, all flies were returned to their respective diets, which were changed after every testing day to ensure the food would not spoil. This process was repeated at fly age 5, 10, 15, 20, and 25.

A) Geotaxis Assay **B) Drosophila Rotation Device**

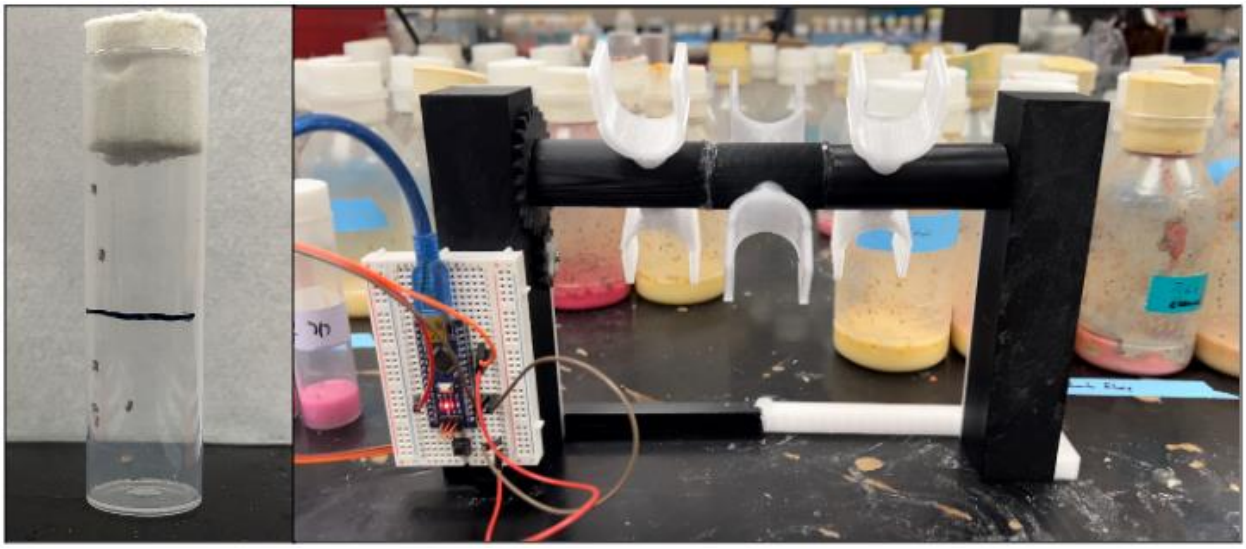


Figure 1: Diagram of the DRD/Geotaxis Assays. The geotaxis assay (A) and DRD apparatus (B) can both be seen in this image. 6 vials were placed in the 3-D printed clamps of the DRD and spun for respective times. The DRD was powered by a Microsoft Pro 7, an Arduino Nano, breadboard, tactile push button, wires, and servo motor.

DRD/Respirometry Assay

In order to test the effect of diet and exercise on metabolic rate, a respirometer was made to derive relative metabolic rates (Yatsenko et al., 2014). Four flies were placed in each respirometer and left untouched for 1 hour and 30 minutes. A control respirometer was also placed in the chamber to account for the presence of atmospheric pressure. The change in height of the red solution was recorded and used in the following equation to determine individual fly metabolic rates:

$$\frac{\left((\pi \times R^2) \times (\Delta d) - (\Delta c)\right) \times 1000}{n \times h}$$

**Where R = radius of micropipette tube in centimeters, Δd = distance liquid traveled in experimental respirometers in centimeters, Δc = distance liquid traveled in control respirometer in centimeters, n = number of flies per respirometer, and h = time in hours* (Yatsenko et al., 2014).

A) Respirometer **B) Respirometry Chamber**

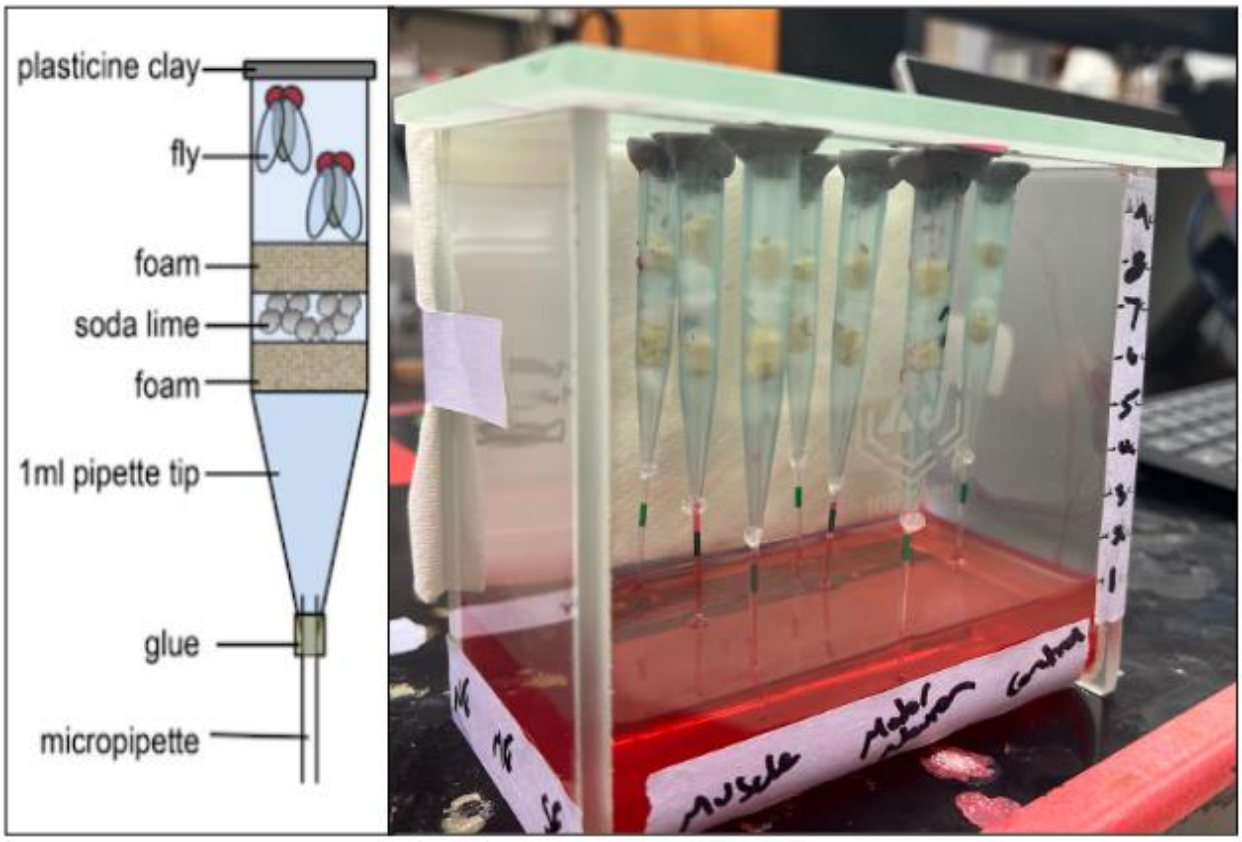


Figure 2: Diagram of the Respirometry Assays. Individual respirometers (A) were created to house the flies in a vacuum sealed thin-layer chromatography chamber (B). The red dye-water solution was used to observe changes in gas pressure within each respirometer. 0.3 g of NaOH was used in each respirometer.

Critical Tissue Determination and Statistical Analysis

Python, Excel, and GraphPad Prism 9 were all used for statistical analyses. Multivariable t-tests were run to compare the mean performance index across testing days between the various exercise regimens and diets in each trial. Additionally, a multivariable linear regression was conducted to observe the rate of change over testing days for each experimental group. A Pairwise Tukey test was then completed to determine where significant differences occurred in the data. An α of 0.05 was used for all p-values.

RESULTS & INTERPRETATIONS

This study aimed to investigate the combined effects of DR and various intensities of exercise on aging *Drosophila* with SMA. This study also sought to determine the critical tissue for SMA by expressing the disease in motor neuron tissue and muscle tissue, and by examining local SMA symptoms. It was hypothesized that individually, DR and ME would improve symptoms of SMA, however when introduced concurrently with DR and SE, symptoms would worsen. It was also hypothesized that mSMA flies would have similar results as control flies, while mnSMA flies would have worsened symptoms.

After each testing round, data was plotted over time beginning on day five which acted as the baseline measurement for each group. Two different approaches were taken to analyze the data. Each genotype group (Control, mSMA, and mnSMA) was isolated, and the ND/NE group was deemed the control regimen. For every increment of exercise and diet, a new data set was created, thus allowing for the analysis of each intervention independently as well.

This study aimed to analyze consistency in SMA groups across different symptoms: motor function, metabolic rate, and muscle weakness. An increased time in geotaxis results suggest weaker flies, as more time was required to cover a given distance (Figures 3-5). The combined effects of DR and SE bear significantly worse motor function abilities than did DR and ME. Furthermore, a decreased output in CO_2 suggests a weaker fly, as lower metabolic rates are associated with decreased fly health (Stahl et al., 2017).

It was predicted that DR and ME would mitigate the effects of SMA in *Drosophila* models while DR and SE would worsen SMA symptoms. Due to past success of low TOR signaling and exercise individually (Spring et al., 2019), along with the long-standing history of these interventions in neurodegenerative disease treatments, analyzing the combined effects would be novel. It was hypothesized that the combination of the two would be overbearing at a high intensity, thus exacerbating SMA symptoms. The data presented suggest multiple novel findings. Primarily, there was correlation between mSMA and Control groups (Figures 7-10). This suggests that when eliminating the SMN protein, mutating the SMN1 gene in muscle tissue, SMA will not persist. In mnSMA flies, however, when eliminating the SMN protein in motor neuron tissue, the symptoms of SMA were significantly exacerbated (Figure 6). These results confirm previous studies that motor neurons are the critical tissue in the onset of SMA (Shababi

et al., 2013), and so future treatments should target motor neurons rather than the rehabilitation of muscle tissue.

This study highlighted a consistency in the effects of my two apparatuses, as the geotaxis results seemed to predict the respirometry results. Over time, symptoms in mnSMA flies were more severe than mSMA and Control flies. To observe the effect of ME, all ND/NE times were subtracted from ND/ME times, the mean difference was taken, and incremental effects were recorded and plotted (Figures 6 & 13).

Nonparametric linear regressions were performed on all study groups for geotaxis data. There was no significant slope between ME mSMA groups and ME Control groups; $p = 0.2207$ and $p = 0.3160$ respectively, however significance was established for mnSMA groups; $p = 0.0354$. However, the immediate effect of DR and SE was proven significant; $p = 0.0402$ and $p = 0.0137$ respectively. For groups mentioned having a significant regression slope, further analysis was not warranted. However, when comparing the slopes of the DR/ME group (Figure 11) to the slopes of the DR/SE regimen (Figures 8, 9, & 10), the DR/ME group had a significantly lower slope than the DR/SE group. This suggests that, over time, the DR/ME regimen was effective in slowing the progression of SMA symptoms, as symptoms were not as severe at any timepoint as DR/SE flies experienced.

Multivariable t-Tests were performed on groups lacking significance in regression slopes (Figure 12). For all Control, mSMA, and mnSMA groups, a significant difference was observed between mean geotaxis times/CO_2 output at day 5 and day 25. This confirms previous studies that DR and exercise, independently, improve neurodegenerative symptoms, while concurrently, they exacerbate symptoms. Lastly, the consistency in results amongst mSMA groups and Control groups helps support the notion that motor neurons are the critical tissue for SMA.

***Strenuous Exercise and Dietary Restriction Exacerbate SMA Symptoms Across Genotypes* (Figures 3-6)**

In a comparison of all six regimens, only dietary restriction in combination with strenuous exercise (DR/SE, orange) showed a significant difference from the normal diet, no exercise regimen (ND/NE, blue) for all genotypes (mnSMA, mSMA, and Control).

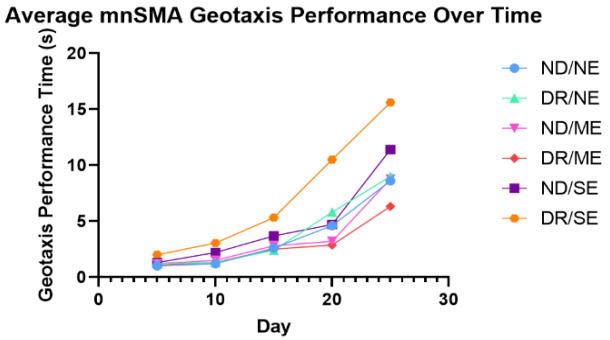


Figure 3: Concurrent Effect of Regimens on Average Geotaxis Performance Times in mnSMA Flies.

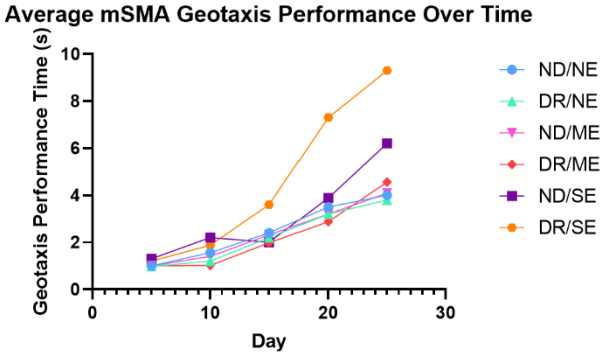


Figure 4: Concurrent Effect of Regimens on Average Geotaxis Performance Times in mSMA Flies.

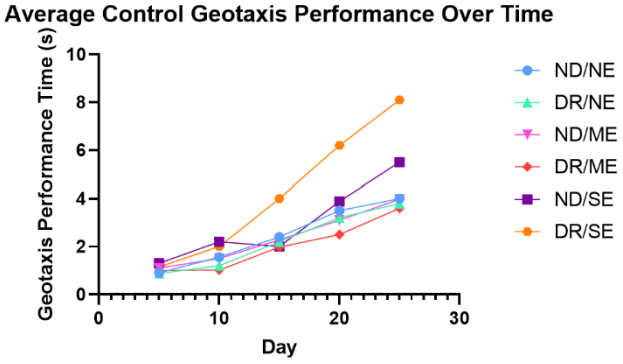


Figure 5: Concurrent Effect of Regimens on Average Geotaxis Performance Times in Control Flies.

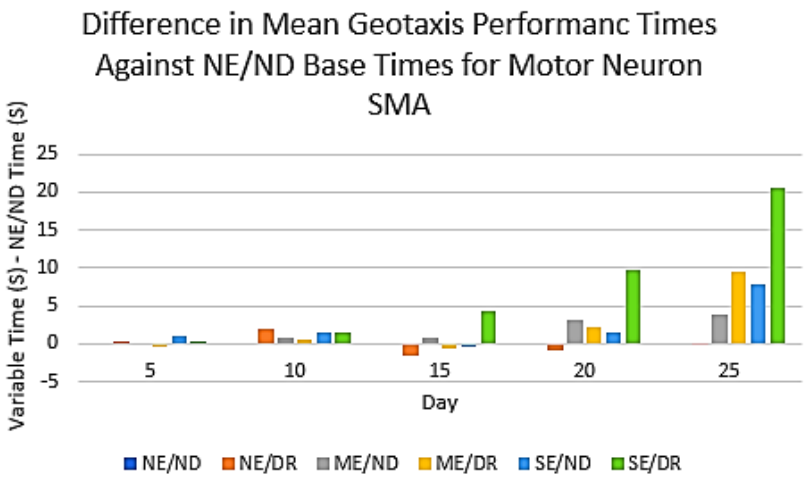


Figure 6: Incremental Value Chart of mnSMA Interventions. SE/DR has a discernible difference from baseline NE/ND.

Correlation Between mSMA and Control Fly Motor Function **(Figures 7-10)**

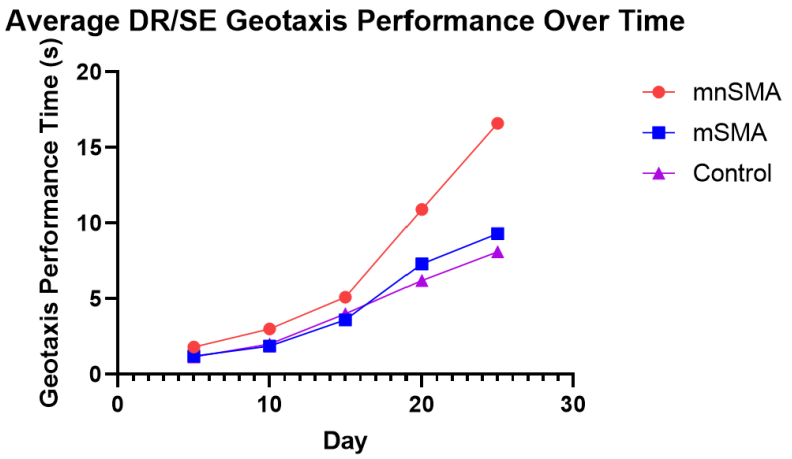


Figure 7: Concurrent Effect of Regimens on Average Geotaxis Performance Times Across Fly Genotypes.

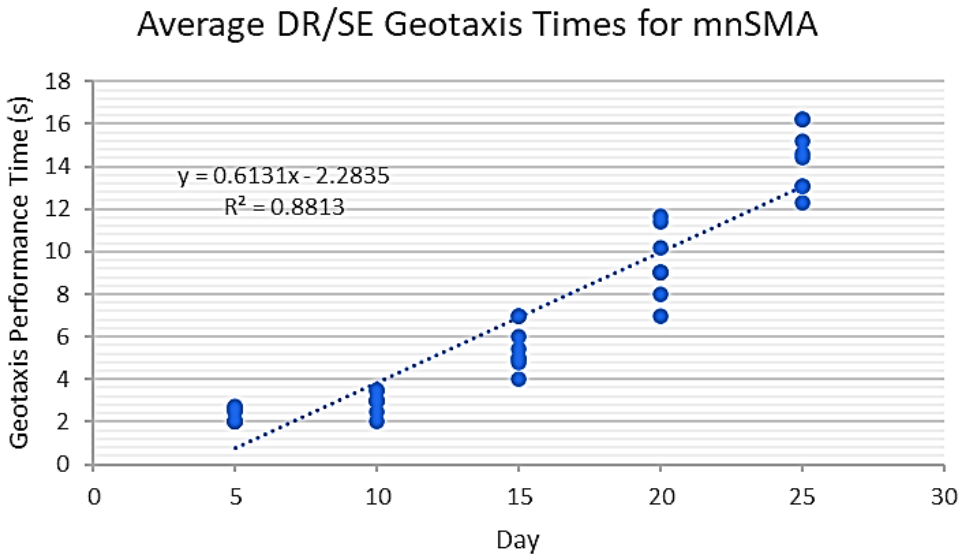


Figure 8: Geotaxis Performance Time and Days Elapsed are Positively Correlated for mnSMA Flies Under DR/SE Regiment. Significant change in times from day 5 to 25 ($p < 0.05$) and association ($R^2 = 0.8813$) is strong.

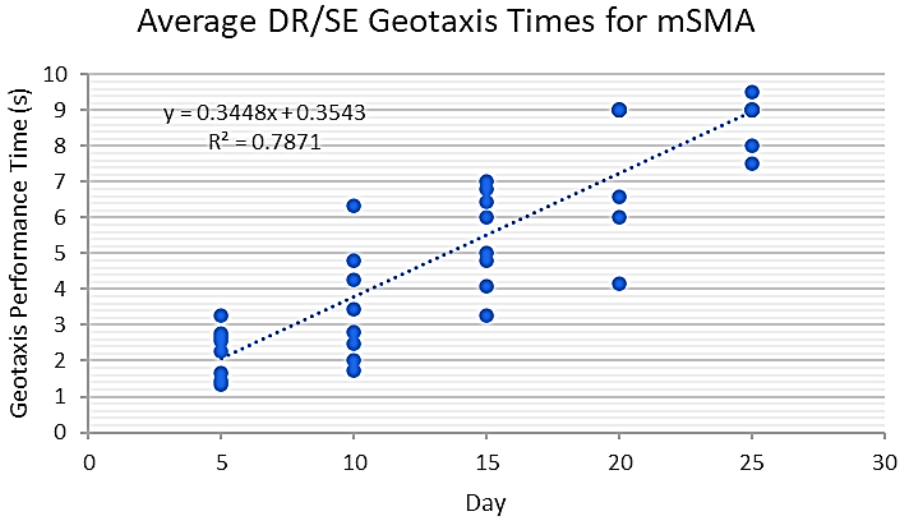


Figure 9: Geotaxis Performance Time and Days Elapsed are Positively Correlated for mSMA Flies Under DR/SE Regiment. Significant change in times from day 5 to 25 ($p < 0.05$) and association ($R^2 = 0.7871$) is strong.

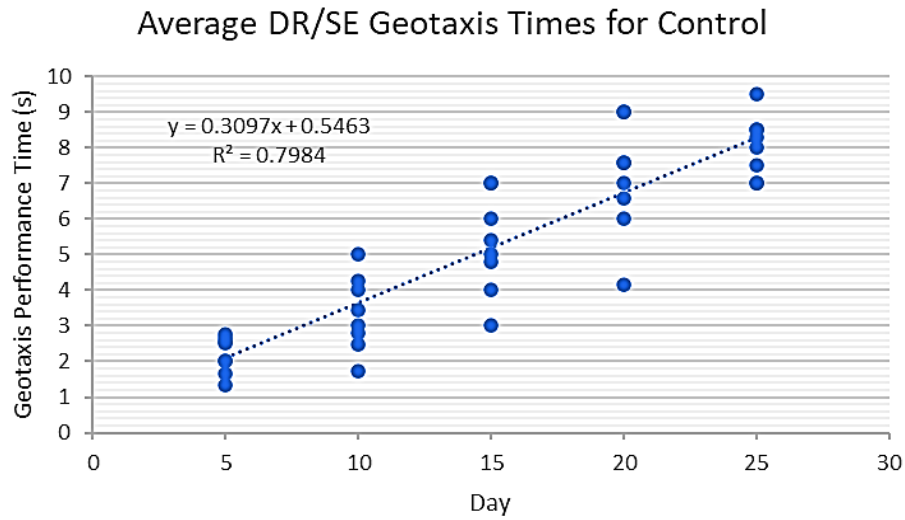


Figure 10: Geotaxis Performance Time and Days Elapsed are Positively Correlated for Control Flies Under DR/SE Regiment. Significant change in times from day 5 to 25 ($p < 0.05$) and association ($R^2 = 0.7981$) is strong. Data strongly correlated with mSMA results.

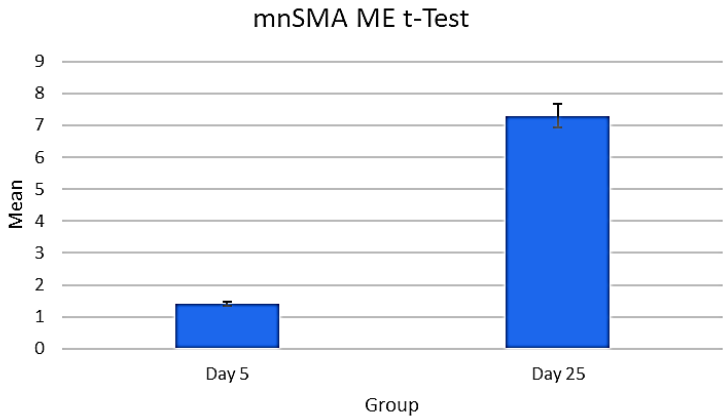
***Dietary Restriction and Moderate Exercise Slow the Progression of SMA Symptoms with Age* (Figures 11 &12)**

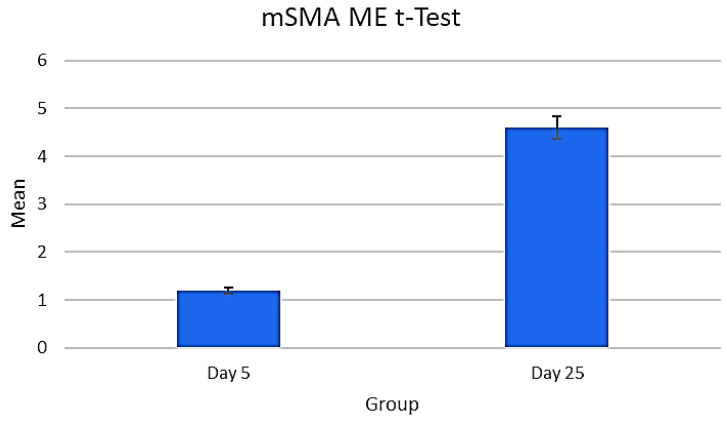
DR/ME - mnSMA	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	7.835434295	2.141436683	3.65896146	0.127246772	-1.378424097	17.04929269	-1.378424097	17.04929269
Slope	-12.67484027	2.452567194	-5.167988995	0.254621439	-23.2273852	-2.122295336	-23.2273852	-2.122295336

DR/ME - mSMA	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	13.02921496	3.539619995	3.680964334	0.16652344	-2.200540675	28.25897059	-2.200540675	28.25897059
Slope	0.516157596	0.292490942	1.764696001	0.219661406	-0.742329354	1.774644546	-0.742329354	1.774644546

DR/ME - Control	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	9.194841611	6.895529137	1.333449751	0.313973952	-20.47422565	38.86390888	-20.47422565	38.86390888
Slope	3.381808641	2.55050777	1.32593544	0.31602861	-7.592140578	14.35575786	-7.592140578	14.35575786

Figure 11: Linear Regression Outputs on ME/DR Incremental Values on Geotaxis Data. Using the Excel Analysis ToolPak, no significant slopes were determined for ME/DR data across all genotypes.





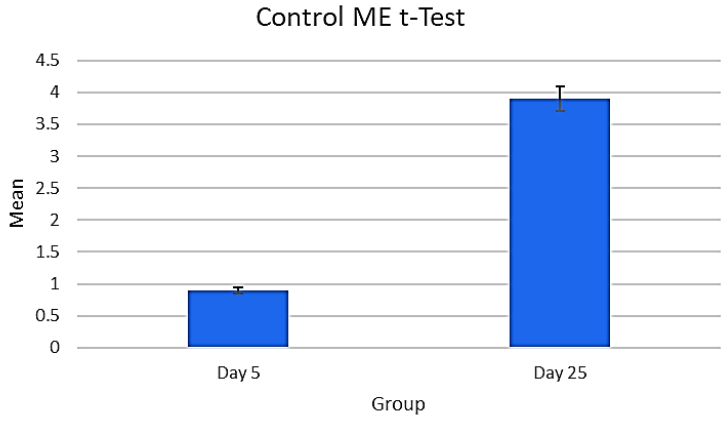
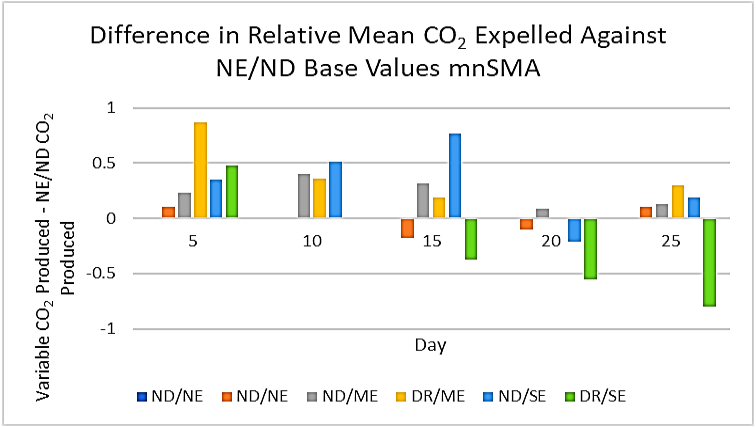


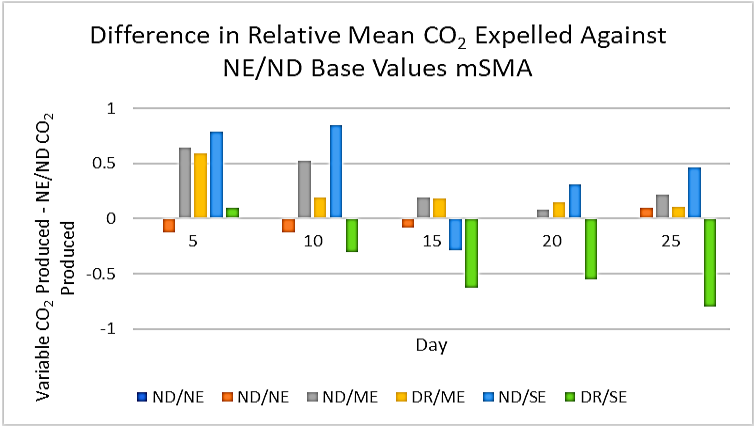
Figure 12: Two-Sample t-Test Assuming Equal Variances for ME Groups, Day 5 and Day 25 Geotaxis Times. Significant difference ($p < 0.05$) for days 5 and 25. However, change is not as significant as SE/DR groups.

Respirometry Results are Consistent with Exercise Results **(Figures 13a-c)**

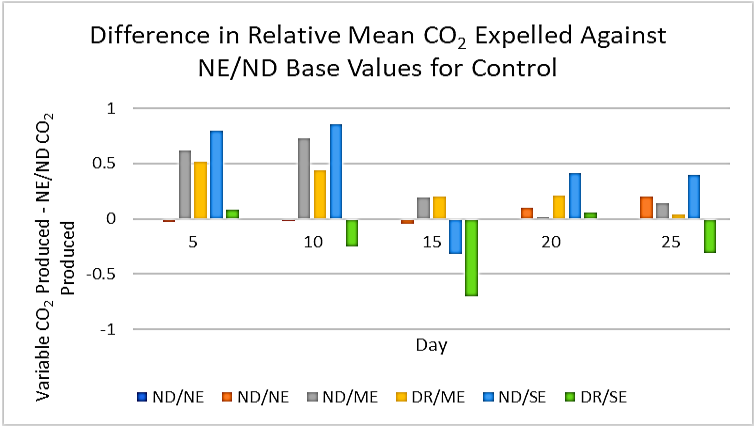
Figure 13: Incremental Differences of CO_2 Output Amongst Regiment Groups Across Genotypes. DR/SE presented a decrease in CO_2 output as compared to other regimens in all genotypes.



A) Motor Neuron SMA CO_2 Output Incremental Values.



B) Muscle SMA CO_2 Output Incremental Values.



C) Control CO_2 Output Incremental Values.

DISCUSSION

This study sought to examine multiple interactions between diet, exercise, and genotype in *Drosophila* models of SMA. This is the first study to utilize both a 3-D printed device, the DRD, and a constructed respirometer in studying the symptoms of SMA. This is also the first study to test the combined effects of dietary restriction and multiple magnitudes of exercise intensity on any neuromuscular degenerative disease. The DRD was shown to successfully induce exercise in fruit flies, and the respirometer was a successful method of displaying relative metabolic rates. The effect of exercise on *Drosophila* is consistent with findings in previous studies that used alternate exercise devices such as the TreadWheel (Lowman et al., 2018) and the Power Tower (Tinkerhess et al., 2012).

The use of *Drosophila* as a model organism for SMA also proved to be extremely advantageous. Due to a high reproduction of fruit flies (Voigt et al., 2013), it was relatively easy to obtain the 1,080 flies used in this study. Additionally, the *D42* and *24B* GAL-4 fly strains were a reliable model in the targeted expression of SMA in motor neurons and muscle tissue. Additionally, because this study involved intensive exercise with simultaneous DR, the use of an invertebrate organism ensured an absence of cruelty in the experimental design. The fruit fly was a reliable model of human disease, and the analysis of their behavior was effective in observing the progressive symptoms of SMA.

The Gal-4/UAS mechanism allowed for the targeted expression of SMA in F1 flies. Because of mutations across generations, only F1 flies were used in this study, and only males were analyzed. Strains of flies were used to reduce SMA expression, however the magnitude of that expression on a cellular level was not measured. In addition, a limited amount of DR food was present in each fly-contained vial, and it was inevitable that some flies would consume more food than others. So, it is likely that DR had a greater effect on some flies than it did on others. In this study, it was the goal to generate a mild phenotype of SMA, which allowed the flies to survive for a long period of time. However, because the phenotype was subtle, the SMA deficiency was most observable under the most extreme conditions (DR/SE). Furthermore, the phenotype could be enhanced using different driver flies or developing the flies under higher temperatures, as the Gal-4 phenotype is temperature dependent (Schinko et al., 2010). In this study, to maintain the mild phenotype, flies were kept at room temperature as opposed to being developed in an incubator. Future experiments could be conducted under conditions where the

phenotype was more severe to further dissect the disease, however in a shorter time. Lastly, due to time constraints and a large number of experimental groups, this experiment featured two testing rounds. More testing rounds would have increased experimental accuracy, and more time would have allowed for the analysis of other SMA symptoms, such as longevity and its fatality amongst infants.

CONCLUSION

This is the first study to 3-D print an exercise apparatus and create a respirometry device for *Drosophila*. It was found that, individually, exercise and DR improved the symptoms of SMA with age. It was also found that SE and DR accelerated SMA symptoms, while ME and DR slowed the progression of SMA symptoms. It was confirmed that motor neurons are the critical tissue for SMA, suggesting that long-term, effective treatments for SMA should only target motor neurons, and not muscle tissue. Additionally, the respirometry chamber and the DRD are viable apparatuses for studying *Drosophila* behavior as their results were predictable across the two. Ultimately, the downregulation of the TOR Pathway coupled with exercise affects a similar physiological pathway in *Drosophila*, and the fruit fly was a reliable model for studying the effects of these interventions in SMA. These findings do hold implications that exercise can be counterproductive if a proper diet is not sustained, and vice-versa.

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