EFFICACY OF EXERCISE MODALITIES IN OLDER AND ADULT MICE

by

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Introduction:

As you age it is inevitable that a decrease in muscle mass will occur. This loss of muscle results in loss of functionality and strength [8]. As people's bodies become weaker, independence is lost resulting in need for assistance to do activities of daily living. This also results in onset of disability, increased mortality, and decreased quality of life [7]. The age-related loss of muscle mass and strength is known as sarcopenia. Frailty, a common diagnosis alongside sarcopenia, is defined as the inability of the body to maintain homeostasis. Most individuals with frailty are also sarcopenic [7]. In 2000, it was estimated that $18.5 billion was attributed to sarcopenia in the United States [5]. The United States' demand for nursing homes is also increasing at a speed beyond its capacity[1].

Resistance training has been shown to help restore function in frail older adults in studies such as "Exercise training and nutritional supplementation for physical frailty in very elderly people" [2]. This study also supported exercise as therapy for sarcopenia. Evidence from research has also supported voluntary aerobic exercise in the form of wheel running to reverse frailty in older mice [4]. Cognitive function improvement has also been seen as a benefit of exercise through structural and functional enhancements in the brain [6]. Strength and endurance-based exercises are often used to combat the loss of muscle mass, yet it is not a cure for age-related loss of function [3]. There is much left to discover in regard to the relationship between exercise and the aging process. This research, performed in the lab of Dr. Ted Graber, was able to construct a mouse model analyzing the underlying molecular mechanisms involved in functional decline with age.

Animal models allowed the researchers involved to control the environment of the test subjects to accurately discover relationships between aging, frailty, sarcopenia, and exercise.

This allowed variability between subjects to be decreased. Rodents were able to be easily kept in the same conditions as each other especially as they are small and do not change much from animal to animal. The tissues of these subjects were also able to be ethically examined after the training program. This allowed changes in cell signaling pathways, gene expression, and other molecular mechanism to be analyzed after the training programs.

Hypotheses:

Working Hypothesis:

Exercise will reduce functional decline in older mice compared to a sedentary control.

Secondary Hypotheses:

Treadmill running will induce greater beneficial change to functional status than voluntary wheel running. Furthermore, we hypothesize that adult mice will receive greater benefit from an equal exercise dosage than older mice.

Methods:

This was a large team-based project with many individual components. I was involved working with both exercise modes in the older mice (each exercise mode having a team of researchers), as well as coordinating the data collection from both groups. My specific role in the first semester was to determine which exercise mode prevents functional decline better. I was in charge of the younger adult mice follow-up in the fall of 2020, as well as working on some biochemical experiments to determine mechanism of exercise response in the older mice.

We used 22-month-old male C57BL/6 mice at the start, who were 26 months old (approximately equivalent to a low to mid-70s human) and 10 months (approximately equivalent to an early 30's human), respectively, at the end of study 1.

Study 1 Efficacy of Exercise in Older Mice (semester 1)

Pre- and Post-Exercise Functional Testing:

CFAB (composite functional assessment battery):

Five tests were used to determine the functional fitness level of the mice before beginning exercise training. These tests included the rotarod, grip tests, treadmill test, inverted cling, and voluntary wheel running. Means and standard deviations from mice of 6-months of age were used to determine z-scores for each mouse. Each mouse's group of z-scores were then summed to create a CFAB score or a composite functional assessment battery (Graber, 2020). This represented each mouse's functional ability before beginning exercise training.

Rotarod

The rotarod test was used to determine overall motor function for each mouse. This included balance, coordination, stamina, and power testing. A Panlab LE820 rotarod was used. Each mouse was given 2 practice sessions before beginning testing. Testing day involved 3 trials which consisted of 5 minutes of increasing speed from 4 to 40 rpm. The best time of all three

trials was used to determine the mouse's overall motor function. There was also 15 minutes of rest between each trial and the rotarod was cleaned with 70% ethanol between each session.

Grip Test

To determine mouse forelimb strength, the grip test was used. 5 trials using a Bioseb GT3 model grip strength tester were used to determine (in Newtons) the strength of their grip. Each mouse was held gently by the tail and carefully placed so that its paws could grip the trapeze-style bar. They were then slowly pulled until they released the bar. Between each session the device and bar were cleaned with 70% ethanol.

Treadmill

The mice's endurance capacity was measured Using a Columbus Instruments Exer. 3/6 treadmill. Each mouse was given one to four days of practice sessions to acclimate to the treadmill. In these practice sessions they learned to walk and then run. A shock plate was used to motivate them to continue running. The shock was only used to startle and motivate the mice to keep running and was not painful. The maximum speed and time that the mice could withstand was recorded. They were each given a practice trial that was identical to the test session. This took place by increasing speed 1 cm/s from 6 cm/s. During test sessions this continued until the mice failed to avoid the shock grid three times, staying on it for more than two seconds each time. The trial was also stopped if the mouse sits on it for more than three seconds one time, or if the mouse cannot be manually prodded to get back on the treadmill.

Inverted Cling

For the inverted cling test, to determine mouse strength and endurance, mice will cling to a grid which will be placed over a padded floor and the amount of time before falling will be recorded.

If a mouse holds onto the grid for less than 10 seconds, the trial will not be recorded and will be considered a slip. Paper towels will also be placed on the padded floor in case of any animal waste. These will be removed and replaced between each cage of mice and the grid and floor will be cleaned with 70% ethanol between each session. The amount of time before falling will be measured in seconds and there will be three trials with 15-minute rests in between.

Voluntary Wheel Running

Mice were placed in cages by themselves over a one-week period to measure voluntary activity and exercise rate. This cage included a running wheel. The number of revolutions of the wheel were recorded in km/day. This was done by using a magnetic revolution counter and a computer.

Randomize Mice into Exercise Groups:

Mice were randomly selected to be in either the treadmill High Intensity Interval Training group (HIIT, 26m n=10, 10m n=8) or voluntary wheel running (VWR, n=8 in both ages) group. A sedentary 6-month-old control group (n=8) was also included. Mice that were unwilling to exercise or were unable to exercise were to be removed from the study, however, in this study no mice were removed.

Exercise for 12-14 weeks:

There were two kinds of modes for exercise training including voluntary and prescribed. The voluntary exercise group was allowed to train of their own volition and simulated an increased activity intervention group (walking or jogging program). The prescribed exercise HIIT group was given structured and planned exercise forcing the mice to train at a specified rate, level, and

length of time. These two different modes of exercise training were then compared to determine which was more positively impactful upon the functional ability and muscle retention of the older and adult mice.

A) Activity Wheel (voluntary wheel running)

The wheel running group represented a voluntary aerobic conditioning mode. The cages that included their own running wheel allowed the mice to decide when they wanted to train aerobically. The distance ran by each mouse each week was recorded in km/day by a magnetic revolution counter which was connected to a computer program. The mice were also given the weekends to go back to the cages with the other mice in their original housing. This allowed them to socialize as well as rest to reduce stress, resulting in a more comforting environment to return to exercise the next week. Cages with running wheels were cleaned with 70% ethanol between each mouse's 5-day session. The wheels within the cages were also taken apart and thoroughly cleaned before being put on a new cage.

B) HIIT (prescribed treadmill running)

The HIIT mode of training was represented by 3 planned treadmill runs each week for each mouse. These interval-training-based treadmill workouts were used to improve mouse power and endurance. A shock plate placed behind the treadmill was used as a motivational tool to coerce the mice to continue running. This shock plate delivered a mild shock to alert the mice rather than harm them. If they stayed on it for longer than 3 seconds, the treadmill and shock plate would turn off. This also occurred if the mouse touched the plate for equal to or longer than 2 seconds for 3 times in a row. If this happened, the mice were let rest, or their training session

would conclude for the day. Between bouts of treadmill training, the treadmill was cleaned thoroughly with 70% ethanol.

Study 2 Efficacy of Exercise in Adult Mice (semester 2)

This study followed a single cohort of 10-month-old mice as they exercise for 3 months in the mode of exercise demonstrated to be more beneficial in the older mice, and sedentary controls. Procedure was the same as for Study 1.

Results

Exercise Program:	VWR		HIIT		Sedentary/Control
Test	26m	10m	26m	10m	10m
Rotarod(Seconds)	382% ±324.2	-33% ± 4.9	165% ± 130.2	41% ± 25.4	-37% ± 5.2
Grip Test(Newtons)	18% ± 8.2	-9%± 6.2	-25% ± 7.14	-32%± 3.18	-24% ± 4.3
Treadmill(Seconds)	45% ±20.4	12% ± 11.9	70% ± 18.9	31% ± 12.2	-18% ± 11.9
Voluntary Wheel Running(km/Day)	54% ±101.1	15% ± 41.9	7% ± 41.3	5675%±4809.8	1097% ± 801.4
Inverted Cling (Seconds)	81% ±44.8	-22% ± 14.6	263% ± 270	-15% ± 11.1	-43% ± 24.5
Body Mass(grams)	-15% ± 5.2	22% ± 4.8	-12%± 1.9	22% ± 2.1	34% ± 3.5
Fat %	-42% ± 4.9	52%± 13.2	-26%±	101% ± 26.3	101% ± 40.5
CFAB Scores(SD)	3.4 ± 0.96	-3.3 ± 1.4	0.8 ± 1.8	-1.7 ± 0.6	-7.3 ± 1.4

Table 1. Exercise vs. Function Status Pre- and Post-Training: The graphs are representative of the information in this table. Key: SE = standard error of the mean, different letters indicate significance, * = $p<0.05$, # = $0.05<p<0.10$, p-values are from ANCOVA adjusted for body mass,

SD = Standard Deviation, 26m = 26-month-old mice (older adults equivalent to humans in the mid-70's), 10m = 10-month-old mice (adults, equivalent to humans in the mid-30's).

Figure 1. Exercise vs. Mean Fat Mass (25-month-old).

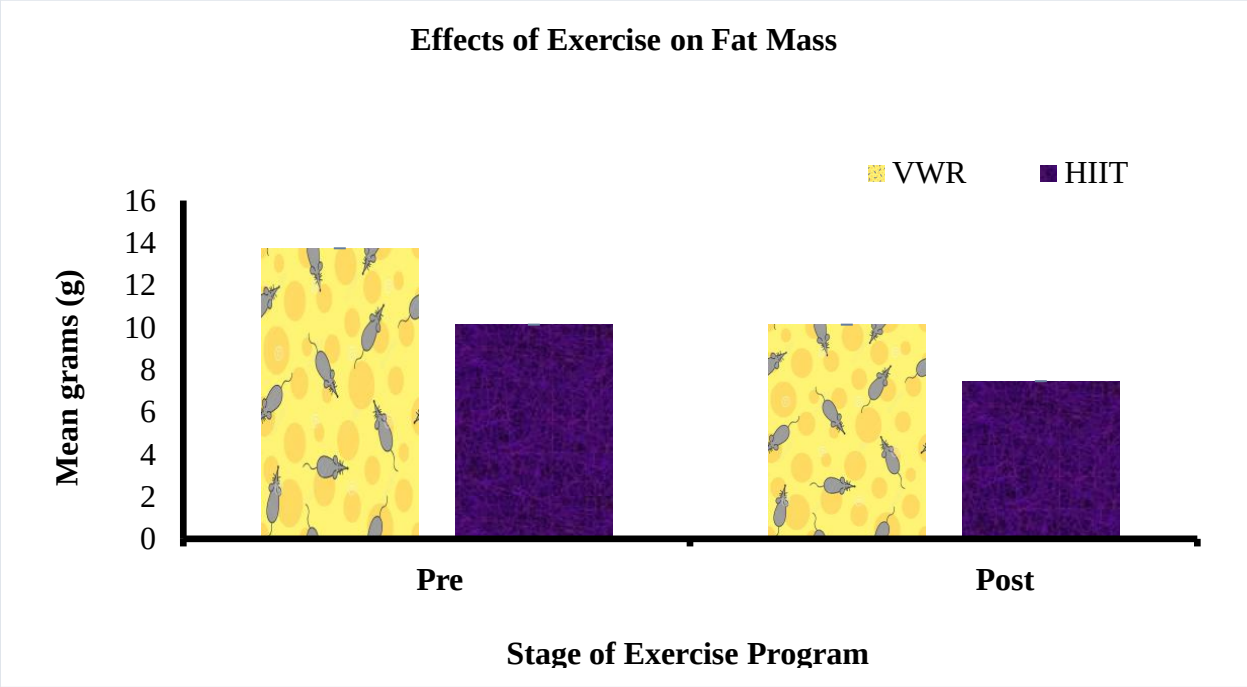


Figure 2. Exercise vs. Rotarod Time (25-month-old).

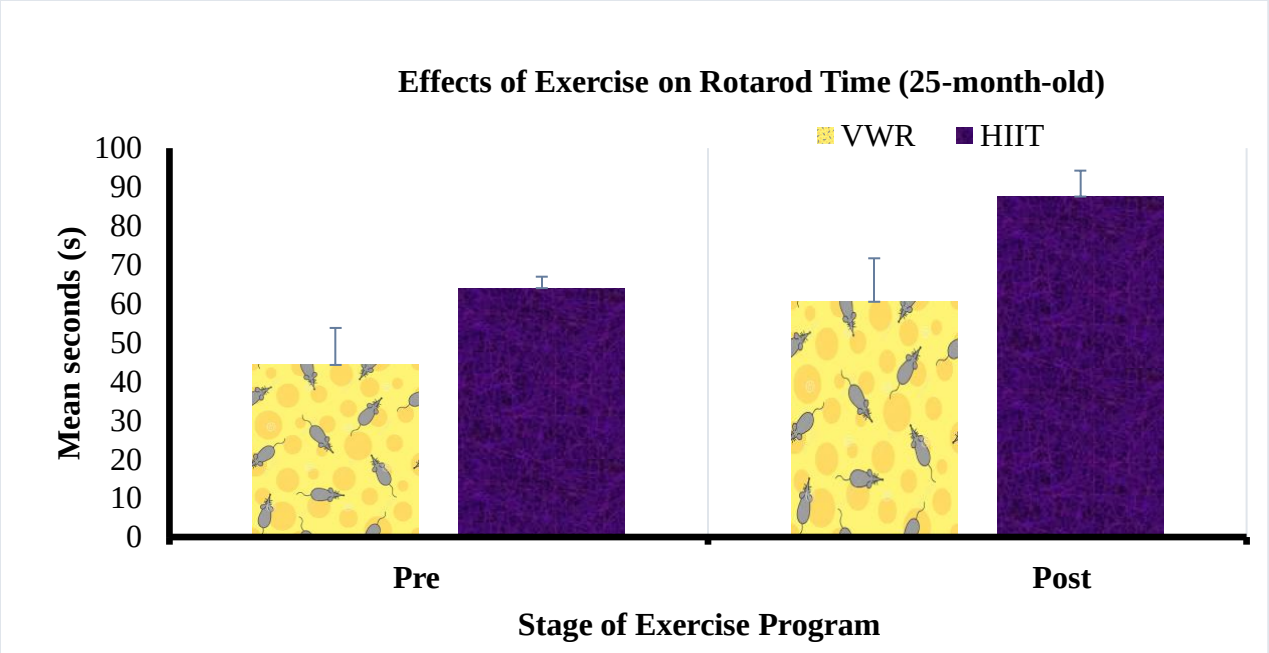


Figure 3. CFAB score change for 10-month and 25-month-old mice after training.

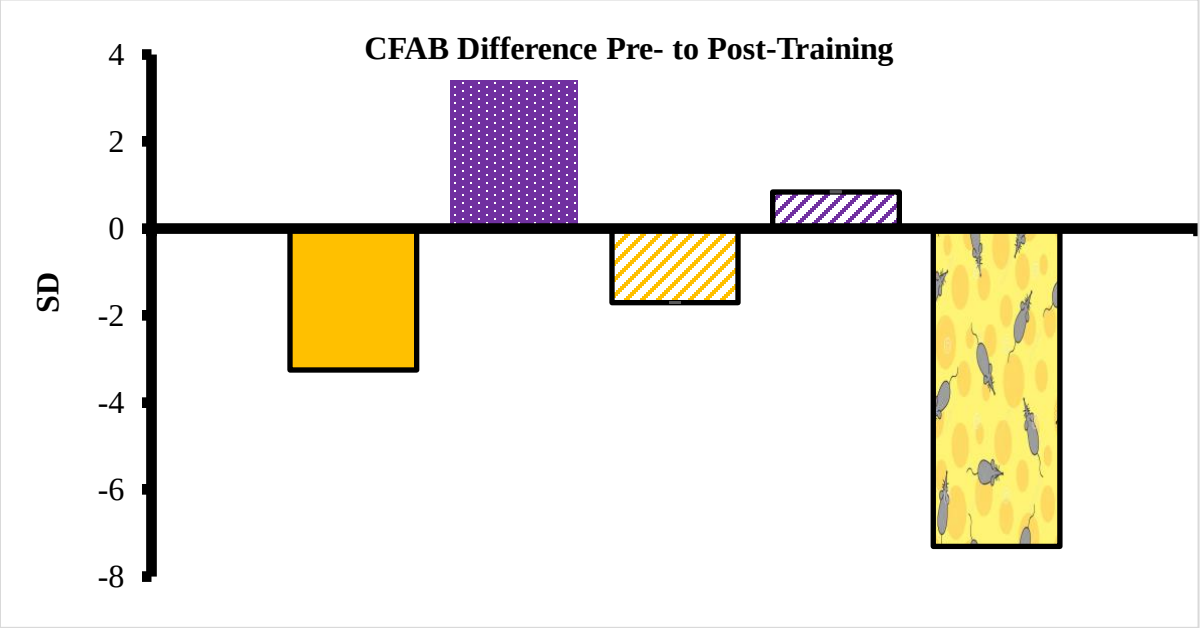


Figure 4. Body mass % change for 10-month and 25-month-old mice after training.

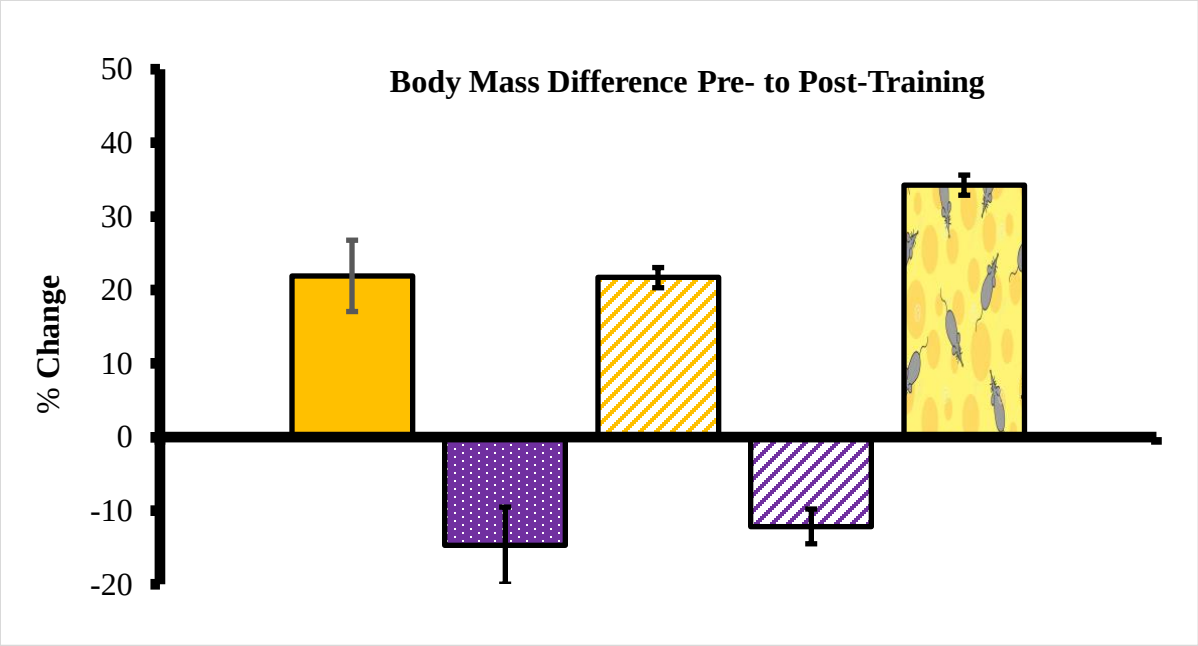


Figure 5. Treadmill time % change for 10-month and 25-month-old mice after training.

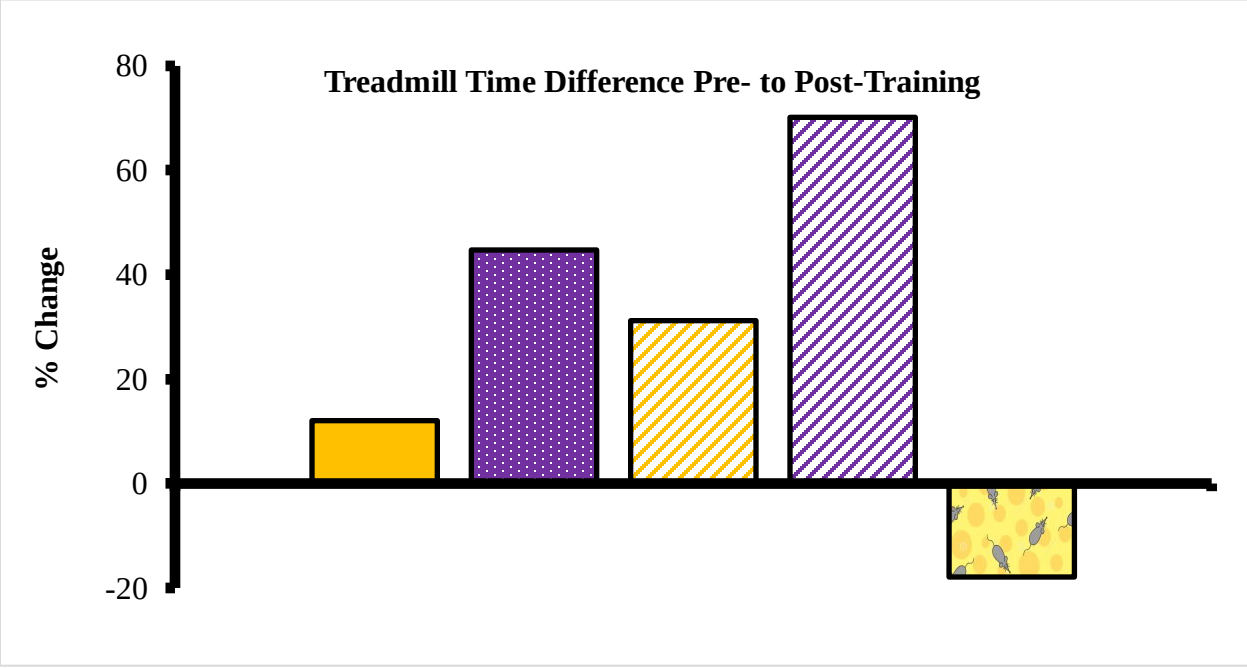
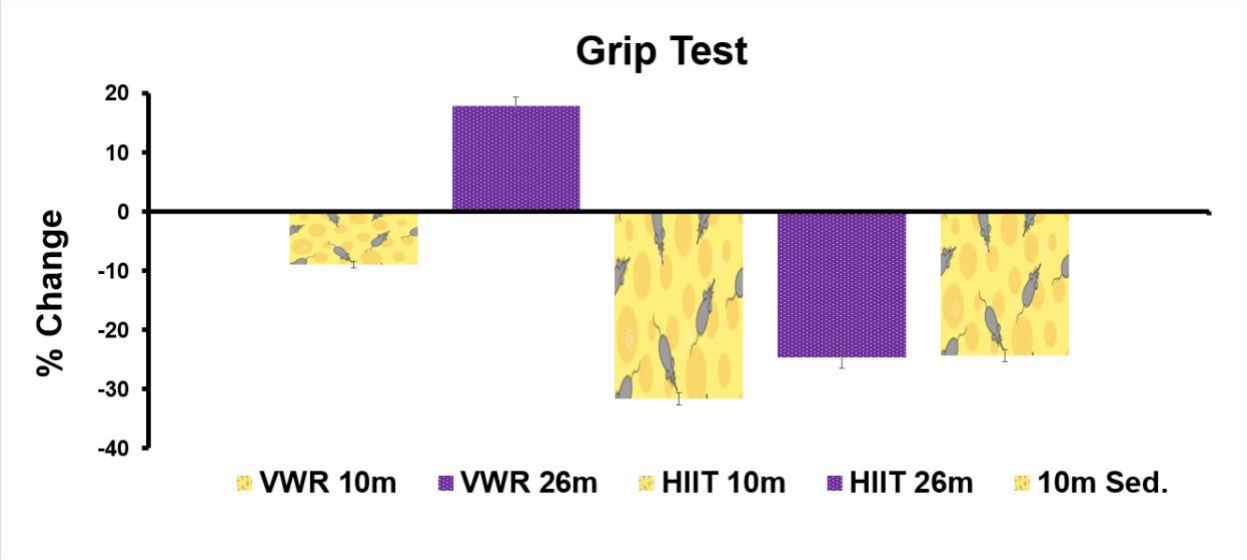


Figure 6. Grip test % change for 10-month and 25-month-old mice after training.



VWR 10m VWR 26m HIIT 10m HIIT 26m 10m Sed.

Key:

VWR = Voluntary Wheel Running group

HIIT = High Intensity Interval Training group

Sed. = Sedentary Control

10m = 10 months old at post-training

26m = 26 months old at post-training

% Change = change from pre- to post-training

Statistics: ANCOVA adjusted for body mass at pre- and post-training or ANOVA.

Different letters = significance from 10m sedentary control; * = statistically significant ($p<0.05$), # = a trend ($0.05<p<0.10$).

Conclusion

This investigation shows promising evidence for endurance exercise as effective anti-aging therapy for both groups of mice. This form of therapy improves body composition and physical function, potentially leading to increased independence ultimately resulting in improved quality of life. Both voluntary wheel running and high intensity interval training on treadmills demonstrated improvements which are similar to those seen in humans. With this information we hope to also investigate the gene expressions related to changes with age and how they are affected by consistent exercise. Between adult and older mice, both experienced either improvement or preservation of function. Both groups saw great improvements in body composition with training, with older mice seeing a significant decrease in their body fat percentage while adult mice were able to maintain their current body-fat percentages while increasing muscle mass. There were some outlying differences between VWR and HIIT group improvements, including more improvement on the grip test for wheel-running mice. This could be due to the ridges on their wheels that they had to grab with their paws. This allowed them to strengthen their grip throughout the training program. The treadmill mice did not have these ridges to hold on to, in fact, their paws were adapting to an especially flat surface. This resulted in a lack of improvement in grip strength compared to wheel-running mice. It was also discovered that older mice were found to have more pronounced CFAB score improvements after training. This could be due to their lower level of activity, function, and muscle mass at the beginning of the training program, giving them more room for improvement compared to the 10-month-old mice.

Although this investigation found great results for exercise as therapy, there were some caveats involved. A 26-month-old sedentary group was not included, although a 10-month-old sedentary group of mice was. High intensity interval training mice were also treated very

carefully when being trained as to not overtrain them. If not trained hard enough, maximal potential of improvements could not have been discovered. In the future, we hope to make the interval training more challenging for treadmill mice.

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