4-24-2020

Marathon Mice

Joshua Juaire
College of Saint Benedict/Saint John's University, JJUAIRE001@CSBSJU.EDU

Sydney Sherek
College of Saint Benedict/Saint John's University, SSHEREK001@CSBSJU.EDU

Marathon Mice

Josh Juaire, Sydney Sherek, and Professor Clark Cotton
Biology Department
College of Saint Benedict | Saint John's University

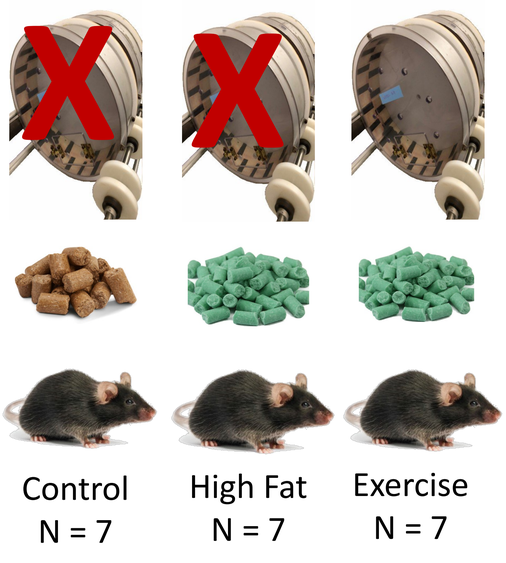
Abstract

Skeletal muscle phenotypes can change based on individual training and diet. Expression of fast twitch, glycolytic muscle fibers (type IIB) fibers, can be upregulated through fast paced, explosive movements. Expression of slow twitch, oxidative muscle fibers (type I) fibers, can be upregulated by low intensity endurance training. In addition to low intensity, endurance training, studies have suggested that a high fat diet can also prompt upregulation of oxidative muscle type I fibers (Hoppeler et al. 2011). In this study we aim to see whether or not the combination of a high fat diet and endurance exercise would increase expression of more slow twitch, oxidative fiber types with longer contraction and relaxation times combined with increased fatigue resistance. We started with 21 mice broken into three groups: A control group (normal diet / no exercise), high fat group (high fat diet / no exercise), and an exercise group (high fat diet / exercise). After thirty days on the specified diet and exercise regimens, we measured twitch contraction strength, contraction time, relaxation time, and fatigue time using isolated tibialis anterior muscles. . We found that mean mean contraction time was significantly higher for mice in the exercise group when compared to the control group. Although not significant, we also found changes in mean relaxation times consistent with an increase in more slow, oxidative fiber types. We did not observe any changes in contraction strength or fatigue resistance. Future studies using more sophisticated in vitro techniques combined with muscle fiber typing may more accurately reveal the potential effects of a high fat, endurance training regimen on expressed muscle fiber-type.

Introduction

- Skeletal muscle phenotypes can be changed based on the type of training an individual does and the nutrients consumed.
- We wanted to test if more type I muscle fibers (slow twitch) would be produced in mice with an endurance workout regiment and a high fat diet (HFD).
- In mice, HFD increases peroxisome proliferator-activated receptor (PPAR) which increases the amount of type I fibers and mitochondrial development. Low-intensity endurance type exercise leads to qualitative changes of muscle tissue characterized mainly by an increase in structures supporting oxygen delivery and consumption (type I fibers). ([CC2] Hoppeler et al. 2011)
- The increase of slow twitch fibers and mitochondrial biogenesis are produced by prolonged high-Ca levels and altered AMP:ATP ratios, altering PGC-1alpha which produces the slow fiber and high mitochondria paradigm.

Methods



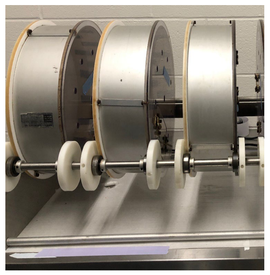


Figure 1. The mouse treadmill apparatus had 5 wheels driven by an electric motor. We were able to control: max speed in meters per minute (mpm), time to max speed X10 seconds, and exercise duration in minutes. We gradually increased max speed and exercise duration over the course of 4 weeks for the exercise group as indicated in Table 1. Control and High Fat groups did not run on the treadmill. All animals received *ad libitum* water and food for the duration of the experiments.

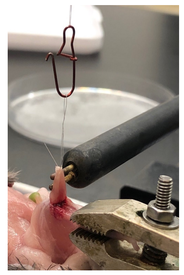


Figure 2. *In vitro* contraction setup. We euthanized mice with CO_2, and then isolated the tibialis anterior muscle. We connected the muscle to a force transducer and stimulated with external electrodes.

Table 1. Weekly mouse treadmill protocol for the exercised mice.

	Max Speed (mpm)	Time to max speed (x10 Seconds)	Exercise Duration (minutes)
Week 1	10	30	30
Week 2	8	50	40
Week 3	12.5	50	45
Week 4	14.5	50	60

Results

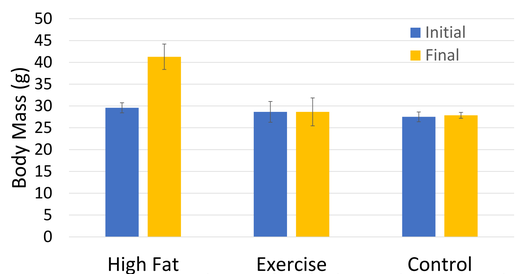


Figure 3. Average initial versus final body mass between each experimental group. N = 7 for each group. The p-value between the initial and final body mass's for the high fat diet group is 0.0022, the exercise group is 1, and the control group is 0.798.

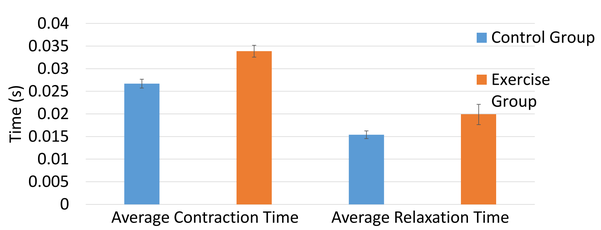


Figure 4. Average twitch contraction and relaxation time (1/2 relaxation from max twitch strength) in seconds of the anterior tiialis between a control and exercise group of mice. The muscle was connected to a force transducer and stimulated with 10 volts in a 1 msec pulse. The twitch was recorded at high speeds. N = 7 for each group. The p-value between the exercise and control group is 0.001 for the average contraction time and 0.087 for the average relaxation time.

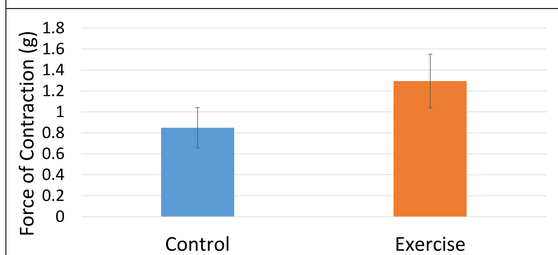


Figure 5. Average twitch contraction strength in grams of the anterior tibialis between a control and exercise group of mice. The muscle was connected to a force transducer and stimulated with 10 volts in a 1 msec pulse. The twitch was recorded at high speeds. N = 7 for each group. The p-value is 0.189.

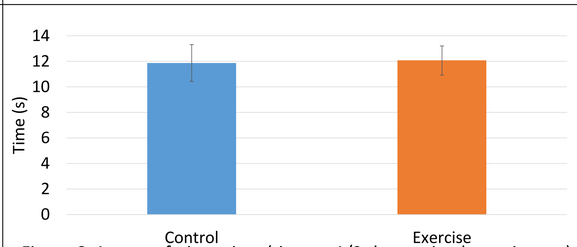


Figure 6. Average fatigue time (time to 1/2 the maximal recruitment) in seconds of the anterior tibialis between a control and exercise group of mice. The muscle was connected to a force transducer and stimulated with 10 volts, 1msec pulses at 40 Hz frequency. The stimulation continued until complete fatigue. N = 7 in the exercise group and 6 in the control group. The p-value is 0.922.

Discussion

- We found a significant increase in twitch contraction time along with an increased relaxation time, showing a shift in the fiber type in the mouse anterior tibialis towards slow twitch type I fibers. We believe the high fat diet along with exercise increase the PPAR and PGC-1α (Hoppeler et al. 2011) which led to the increased slow twitch fibers.
- While the high fat group experienced a 33% increase in body mass (p-value = 0.002), neither the exercise or control group experienced a similar change in body mass. The additional caloric expenditure due to exercise was sufficient to balance the higher caloric intake therefore not increasing the body mass of the exercised mice.
- The in vitro testing revealed a significant difference in the average contraction time of a twitch between the control and exercise group. The p-value is 0.001 favoring a longer contraction in the muscles of the exercised mice.
- There was no significant difference in fatigue time between the control and exercise groups.
- The mean contraction strength (p = 0.189), mean relaxation time(p = 0.087), and mean contraction strength during tetanus (p = 0.149) were all larger for the exercise group as compared to the control group, and each had relatively small p-values. Further testing would need to be done to determine if there is a significant difference.
- One improvement that could have been made is running the mice on the treadmill prior to their 4 weeks in order for the mice to learn to run on the treadmills properly.
- We could have improved the in vitro measurements by including optimal length tension and max recruitment. Additionally, our in vitro methods were suboptimal and led to rapid muscle deterioration. Ideally we would have found a better method.
- A source of error that we had during our in vitro testing was we did not find each muscle's optimal length tension. This means some of the muscles could have contracted better than we measured just because they were not at their optimal length for maximal contraction.
- In the future we would like to replicate the study with the addition of muscle cross sectional staining in order to get a better idea of how the muscle composition evolved.

Acknowledgements

This research was made possible by the Biology Department. Special thanks to Dr. Clark Cotton and the rest of the staff that helped us obtain mice and other materials.

Sources

Baar K. 2006. Training for endurance and strength: Lessons from cell signaling. Med Sci Sports Exerc. Vol 38. 1939-1944
Hoppeler et al. 2011. Molecular Mechanisms of Muscle Plasticity with Exercise. Comprehensive Physiology. Vol 1. 1383-1412
Thomas et al. 2014. Early oxidative shifts in mouse skeletal muscle morphology with high-fat diet consumption do not lead to functional improvements. Physiological Reports. Vol 2. 1-11