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Comparison of Cardiac Muscle between Male and Female Rats with Exercise and Aging: Analyzing Vitals, GDNF Expression, NGF Expression, RNA Expression, Sympathetic Innervation, Parasympathetic Innervation, and Sensory Innervation

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Abstract

The direction of my research-based thesis answers the question of how exercise affects the cardiovascular system with aging in male and female rats. My study would highlight the important differences of the cardiovascular system of both sexes which could lead to sex-specific insight on the cardiovascular disease epidemic which prevails as the most common cause of death in the United States. The studies completed will test the hypothesis: neurotrophic factor will be higher in females than males due to the higher presence of estrogen in females. The trophic factor is predicted to increase in both males and females with regular cardiovascular exercise. I will observe changes of expression of GDNF, NGF, and innervation patterns over the course of a 3-month aging study. Use of vertebrae animals in research required all student researchers at Western Michigan University to complete Collaborative Institutional Training Initiative (CITI) for Research Involving Animals as part of Institutional Animal Care and Usage Committee (IUCAC). The subjects tested will be rats ranging from 6-week, 8-week, and 12-week-old of both male and female sexes. One group will be the experimental group of (n=6) exercised rats that are placed in isolated cages with access to a running wheel. The control group of (n=6) sedentary rats are placed in cages without running wheel access. These rats are euthanized once aged properly via asphyxiation using a carbon dioxide chamber. Vitals (heart rate, blood pressure, and weight) were taken of each rat at the 4-week, 8-week, and 12-week periods of life. Ideally, I would've analyzed neurotrophic factor for my results but due to Covid-19 limitations to lab time and graduation time restrictions, the only data I was able to collect and analyze was their monthly vitals. Despite this loss of further analysis, this study's results still allude to sex differences of

cardiovascular health and levels of neurotrophic factor. This data shows that my initial study is still relevant for studying in the future.

Introduction

Cardiovascular disease runs ramped amongst the many generations of United States populations and it affects both sexes. About 610,000 people die of heart disease in the United States every year which equates to 1 in every 4 deaths; it is the leading cause of death for both American men and women (CDC, NCHS, 2015). The main concern is that about half or 47% of Americans have at least one of the 3 key risk factors such as high blood pressure, high cholesterol, and smoking with underlying medical conditions that make you more prone (Fryar CD, Chen T, Li X, 2012). The American Heart Association (AHA) has been attempting to reduce risk through the critical components of diet modifications and increased physical activity (Krauss RM et al, 2000). The most recent AHA Diet and Lifestyle Recommendations have been created as a result of new scientific evidence relative to the previous publication in 2000 that now intentionally meet the needs for growth, development, and aging. As the cardiac muscle ages, just as any muscle ages, there is a decrease in muscle quality (muscle strength per unit of muscle mass) that leads to functional decline and mortality (Goodpaster et al. 2006). Physical activity specifically cardiovascular exercise and resistance training have been found to be reliable treatment to reverse the decline of muscle quality during the aging process (Peterson et al. 2010). Exercise has been found to significantly increase muscle mass, strength, and functional mobility of skeletal muscle equally in both men and women (Leenders et al, 2013). It has been evident that both can reduce these effects up until the ninth decade of life (Fiatarone et al. 1990). One underlying mechanism that this is responsible for the positive adaptation in muscle quality is the activity-induced protection of large motor units (MU). As we age, the mode of denervation preferentially selects larger and faster conducting MUs that lead to larger scale atrophy of myofibers. Neurotrophic factor promotes survival of these MUs following exercise. Two important survival factors for the autonomic nervous system include glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF). NGF plays a crucial role in supporting the development and survival of sympathetic innervation. NGF protein content has been shown to increase during pulmonary hypertension (Freund-Michel et al, 2015). Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for the autonomic nervous system, the motor nervous system, and the sensory nervous system (Rebimbas- Cohen, 2005). GDNF has been shown to be regulated by physical activity in skeletal muscle (Wehrwein et al, 2002).

The neuromuscular function of the heart is regulated by both the parasympathetic and sympathetic nerve fibers. During exercise, cardiovascular regulation is important to maintaining adequate blood flow to all body tissues. In addition to the heart's role, the circulatory system transports nutrients in blood and aids in temperature regulation. Both of these conditions are relevant while working out. During exercise, the demand for oxygen to the muscles is 15 to 25 times greater than at rest. The heart rate is sympathetically risen to account for the elevated breathing rate delivering the demand of oxygen. Exercising for any duration will increase your heart rate and will remain elevated for as long as the exercise is continued. To meet the demand for oxygen, two major adjustments of blood flow are made, an increase in the amount of blood being pumped per minute by the heart or cardiac output and a redistribution of blood flow from inactive organs to the active skeletal muscle. During exercise, the release of epinephrine and norepinephrine stimulate receptors in the heart which causes heart rate to increase while your body removes the parasympathetic stimulation, which also enables the heart rate to gradually increase. As you exercise more strenuously, the sympathetic system “kicks in” to accelerate your heart rate even more to sustain cardio level activity. Regular participation in cardiovascular exercise over an extended period of time can decrease your resting heart rate by increasing the heart size, the contractile strength and the length of time the heart fills with blood. The reduced heart rate results from an increase in activity of the parasympathetic nervous system, and perhaps from a decrease in the activity of the sympathetic nervous system. It is now important to understand that these conditions occur regardless of sex differences.

Studies from the American Heart Association revealed that there are sex differences with risk. However, both sexes can prevent heart disease with exercise. The muscle quality of the cardiac muscle tissue needs to be further evaluated with age and exercise. The quality of the cardiac muscle tissue can also decrease in quality over time making hearts more vulnerable. The risks of heart disease are dramatically exaggerated with age. Heart health can be prolonged with regular cardiovascular exercise. Exercised male and female hearts have responded slightly different to healthy stress exercise causes. The Mayo Clinic research team conducted between 1993 and 2006 took a sample of 25,000 patients, both men and women between the ages 40-89. It revealed that although everybody’s peak heart rate declines with age, the decline is more gradual in women. The study also showed that younger men have a lower resting heart rate and higher peak heart rate than women and that men’s heart rates rise more dramatically during exercise and return to normal more quickly after stopping. The study did not investigate the physiological reasons behind the differences, although the researchers suggest hormones, especially testosterone, may play a role. In another study, ejection fraction and fractional shortening were significantly greater in male hearts due to larger masses of heart tissue.

Now this study aims to reveal patterns of GDNF Expression, NGF Expression, RNA Expression, Sympathetic Innervation, Parasympathetic Innervation, and Sensory Innervation changes within cardiovascular muscle with age and exercise in both biological sexes of male and female. The studies completed will test the following hypothesis: neurotrophic factor will be higher in females than males due the hormonal differences. The trophic factor will increase regardless of sex with continued exercise as any heart would respond. I will observe expression of GDNF, NGF, and innervation patterns over the course of a 3-month aging study.

Methods

I. Use of Vertebrate Animals in Research

All animals used in this study were maintained according to protocol approved by Western Michigan University's Institutional Animal Care and Usage Committee (IUCAC). Before completing studies using animals, all researchers completed the Collaborative Institutional Training Initiative (CITI) for Research Involving Animals.

II. Animal Subjects

This study consisted of three animal groups of three different ages: one month old, two-month-old, and three-month-old male and female Sprague Dawley rats. First, twelve male four-week- old Sprague Dawley rats were obtained and euthanized. A female one-month group. Then, twelve six-month-old Sprague Dawley rats were randomly placed in exercised (n=6) and sedentary groups (n=6). Exercised rats were placed into a cage alone with voluntary access to a running wheel. Distances ran by each rat were monitored throughout using software. Sedentary rats were placed in cages without access to a running wheel. Both groups were maintained for up to three months with access to food and water *ad libitum (*Rebimbas-Cohen, 2005). The body weights, blood pressure, and heart rate were collected as monthly vitals. The only group that could not be measured due to lack of proper fitting blood pressure cuffs, was the 4-week female group. Both groups were euthanized at different age groups ranging from 1 to 3 months of age. Tissues were collected immediately after animals were sacrificed to affirm good integrity of tissue.

III. Tissue Collection

Animals were asphyxiated using a carbon dioxide chamber. The rat was pinned out on a Styrofoam board on all 4 paws. Fur was rinsed with DI water to dampen fur prior to precisely map incisions. An incision is made with scissors under the rib cage by using tweezers to pull skin and cut a small incision around the diaphragm area. Avoid cutting this area too deep as it will puncture the abdominal wall. Using a scalpel, shred connective tissue off and the superficial skin off of the abdominal wall. Using the tweezers again to pull up the skin/ connective layer and a scalpel to poke underneath, the rat was confirmed dead with the puncturing of the diaphragm. Using the scissors, the skin was cut along the midline of the rat's ribcage along the sternum up until the neck just superior to the pectoralis major. The same gentle shredding motion with the scalpel helps separate the fascia from the skin. The thoracic cage should be isolated from excess fur and skin. The rib cage was cut with surgical scissors to expose the heart. To surgically remove the heart, the apex was pinched and pulled until taught with tweezers. The other hand, holding the scalpel cut the superior vena cava, inferior vena cava, aorta, pulmonary arteries, pulmonary veins, and coronary arteries. Blood was immediately collected from the cut vessels. The heart was successfully removed and placed in a Petri dish of PBS. The heart was rinsed in phosphate buffered saline (PBS) pH 7.2. The heart was pinned out with one pin on the apex of the heart, one pin on the ventricles on the septum, and one pin in the upper ventricular region.

Due to the variability in size of heart between male and female hearts these number of pins were variable as well as the size of the tissue sample acquired. Six test microtubes were collected per heart separating it into upper ventricle, lower ventricle, medial left/right atria, and lateral left/right atria

Results

Due to Covid-19 restricting lab access, the monthly vital measurements are the only data points I have to use in this analysis. Weights were collected by placing the rats into the weighing cage and onto the scale. Heart rate and blood pressure were collected using blood pressure tail cuffs. Due to improper fitting of the blood pressure cuff, the data from youngest animals was unattainable and omitted from this study. We expected to see sex differences with body weight, heart rate, and trophic factor due to the anatomic differences.

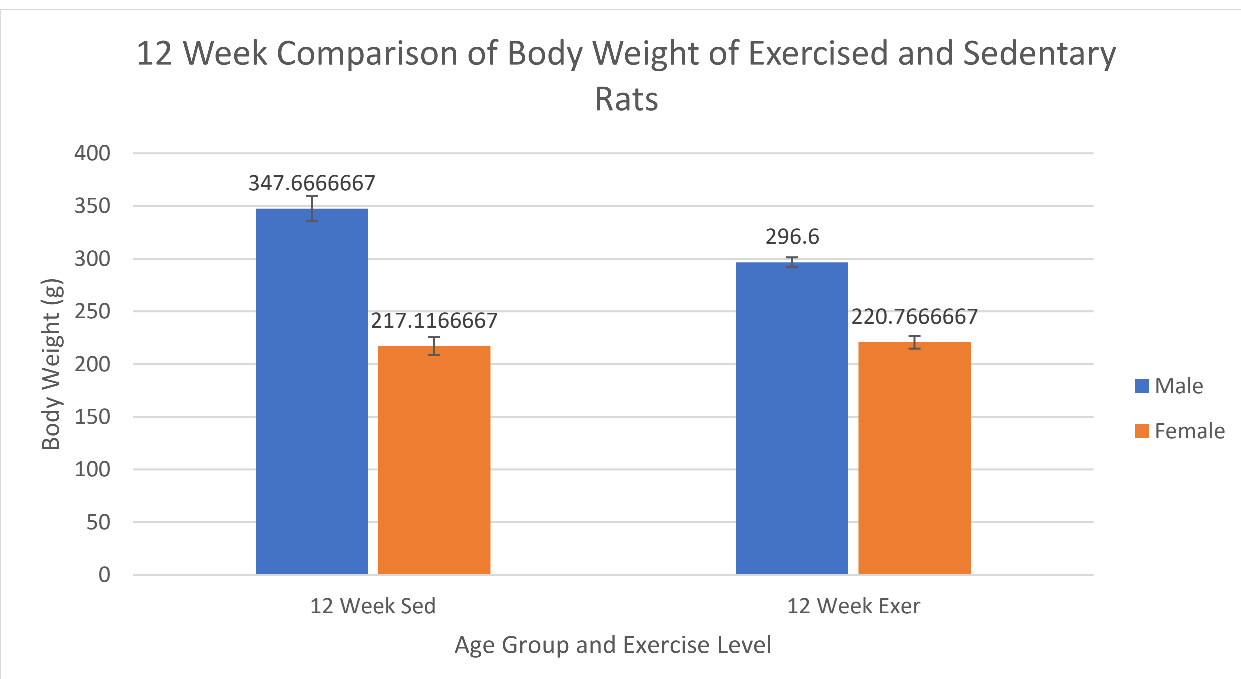

Figure 1. Comparison of body weight of exercised and sedentary rats at 12 weeks of age.

Discussion

The goal of this study was to understand the underlying factors of trophic factor expression in both male and female hearts. This aimed to compare the anatomic differences between male and females but observing the changes in the first developmental stages of life where physical and chemical changes occur the most. I hypothesized that female hearts would have higher trophic factors than male due to higher amounts of slow twitch muscle fibers as well as protective qualities of estradiol. However, due to graduation timeline restrictions as well as inaccessibility to the lab during Covid-19, I was unable to conduct further imaging studies analyzing the GDNF Expression, NGF Expression, RNA Expression, Sympathetic Innervation, Parasympathetic Innervation, and Sensory Innervation in both male and female rats.

Body Weights

Despite these setbacks, after analyzing the body weights collected each month, there is evidence that there are sex differences of the cardiovascular system with exercise given the differences in weight. Both sexes positively responded to exercise as the musculature that developed during exercise formed which increased lean muscle mass versus large cumulations of fat. Both groups of rats regardless of sex were the same weight at around one month of age. With aging and exercise, a gap between weights began to show. The male group had the most dynamic change over time in comparison to the female group given the weight differences between exercised and sedentary. The oldest group, 12-week-old rat body weights, in Figure 1, were higher overall in male rats as expected due to their larger anatomic features. Females had less of a weight difference between the sedentary and exercised groups, but this can allude to the need of longitudinal future studies analyzing changes throughout the rat’s life instead of just into young adulthood.

Heart Rate and Blood Pressure

The heart rate and blood pressure data, that I was no longer given access to, indicated that exercised rats regardless of sex overall had lower blood pressures and lower heart rates. This indicates that as the musculature of the heart developed stretch and strength from the exercise overtime the efficiency of the heart increased which we expected. These vital measurements were significantly greater in male hearts at all activity levels given their larger anatomic size differences. The results indicate that intrinsic cardiac function is moderately greater in male rats compared to female rats. Blood pressure increased with age, and sedentary rats had higher blood pressure than rats who exercised.

Trophic Factor

In previous studies, GDNF levels in fast twitch myofiber decrease following short-term exercise at lower velocities (McCullough et al. 2011). However, with increasing the intensity and resistance, GDNF expression increases in fast twitch myofibers with increasing velocity and resistance of exercise training (Gyorkos, 2014). Since this study did not change the resistance or intensity, we would expect to see more GDNF expression in younger individuals since GDNF plays a crucial role in inducing sympathetic innervation at earlier stages of life (Miwa et al. 2010). Aerobic exercised rats regardless of sex would show higher levels of trophic factor expression due to the increase of recruitment of muscle groups (Rodnick et al. 1989, Bagby et al. 1986). However, comparing sedentary individuals, we would expect premenopausal females to have higher trophic factor content because of the protective factors of estradiol (Duabl, et al. 1999). In the future, a longitudinal age study could investigate these sex differences later in life to investigate the effects of exercise on trophic factor expression without the abundance of estradiol. With this further analysis, this question of sex differences of the aging cardiovascular can be accurately answered. Even without this further analysis, it is evident that regardless of sex, exercise can offer the protective benefits of trophic factors that can combat cardiovascular disease epidemic.

Conclusion

If my hypothesis was correct, greater trophic factor content in females may explain why females are protected in earlier in life. This can be groundbreaking in the world of age reversing and injury healing at all stages of life in both sexes. This can help us identify how to reverse these changes in our bodies changed as we develop. Exercise should be implemented in eldery lifestyles to promote healthy regeneration of these nerve fibers to have pro Neurotrophic factor is extremely crucial in reversing the effects of denervation with aging. With exercise, these motor units can be preserved with the promotion of GDNF and NGF. More studies must be conducted to reveal the interplay between neurotrophic factors and innervation patterns of both male and female heart activity with exercise.

Works Cited

Miwa, K. (2010). Glial cell line-derived neurotrophic factor (GDNF) enhances sympathetic neurite growth in rat hearts at early developmental stages. *Biomedical Research, 31*(6), 353-361. doi:https://doi.org/10.2220/biomedres.31.353

Bagby, G. J., J. L. Johnson, B. W. Bennett, and R. E. Shepherd. 1986. Muscle lipoprotein lipase activity in voluntarily exercising rats. J. Appl. Physiol. 60:1623–1627.

Dubal, Dena B., Wilson, Melinda E., and Wise, Phyllis M. 'Estradiol: A Protective and Trophic Factor in the Brain*'. 1 Jan. 1999 : 265 – 274.

Rodnick, K. J., G. M. Reaven, W. L. Haskell, C. R. Sims, and C. E. Mondon. 1989. Variations in running activity and enzymatic adaptations in voluntary running rats. J. Appl. Physiol. 66:1250–1257.

Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen KA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tibble DL, Bazzarre TL. AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. **Circulation**. 2000; 102: 2284–2299

Fryar CD, Chen T, Li X. Prevalence of Uncontrolled Risk Factors for Cardiovascular Disease: United States, 1999–2010 Cdc-pdf[PDF-323K]. NCHS data brief, no 103. Hyattsville, MD: National Center for Health Statistics. 2012.

CDC, NCHS. Underlying Cause of Death 1999-2013 on CDC WONDER Online Database, released 2015. Data are from the Multiple Cause of Death Files, 1999-2013, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program.

Kingma, J. G., Simard, D., & Rouleau, J. R. (2017). Influence of cardiac nerve status on cardiovascular regulation and cardioprotection. World journal of cardiology, 9(6), 508–520. doi:10.4330/wjc.v9.i6.508

Klabunde, R. (2007, April 06). Normal Heart Rhythm. Retrieved October 19, 2020, from https://www.cvphysiology.com/Arrhythmias/A002

Klabunde, R. (2007, April 06). Ganglionic Blockers. Retrieved October 19, 2020, from https://cvpharmacology.com/vasodilator/Ganglion

Sheng, Y., & Zhu, L. (2018, March 10). The crosstalk between autonomic nervous system and blood vessels. Retrieved October 19, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5871626/

Armour, C. (2020). Chapter 01: Heart-Brain Communication. Retrieved October 19, 2020, from https://www.heartmath.org/research/science-of-the-heart/heart-brain-communication/

Fleg, J., Science, L., O'Connor, F., Gerstenblith, G., Becker, L., Clulow, J., . . . Pickering, G. (1995, March 01). Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. Retrieved October 19, 2020, from https://www.physiology.org/doi/abs/10.1152/jappl.1995.78.3.890

Casteel, B. (2014, March 27). The Heart Responds Differently to Exercise in Men vs. Women. Retrieved October 19, 2020, from https://www.acc.org/about-acc/press-releases/2014/03/27/12/29/allison-peak-hr-pr

Gyorkos, A. M. 2014. Glial cell line-derived neurotrophic factor (GDNF) expression and NMJ plasticity in skeletal muscle following endurance exercise. Neuroscience 257:111.