Cantaurus, Vol. 28, 5-7, May 2020 © McPherson College Department of Natural Science

Effect of exercise and L-citrulline on heart rate in rats

Amari Heisser

ABSTRACT

Blood pressure is a good indicator of health in many organisms. Many diseases and disorders can be detected by the measurement of blood pressure and heart rate. This study focuses on the individual and combined effects of exercise and the amino acid L-citrulline on heart rate of mice. L-citrulline is found to produce nitric oxide in the body. Nitric oxide can be produced from different synthases in the body. The focus of this study is the effect of oral administration of L-citrulline with and without exercise on the heart rate of rats. Each rat was put into a swimming pool to evaluate their ability to swim continuously for fifteen minutes. Results shows that there no significant difference in the heart rates of rats who were treated with the above compound, compared to the untreated.

Keywords: *L-citrulline, heart rate, rats, exercise*

INTRODUCTION

Blood is an important component of many animals. The function of blood ranges from protection against diseases and elimination of wastes from the body to the transportation of nutrients throughout the body. Blood is a good indicator of health inside an individual. We can tell the condition of someone through blood pressure, heart rate and pulse.

There are many molecules that can affect the circulatory system, nitric oxide being one of these molecules. Nitric oxide (NO) is a free radical, its potential as an endothelium-derived relaxing factor was first proposed independently by Robert Furchgott and Louis Ignarro in 1986. (Zhao. 2015). Nitric Oxide functions to regulate blood flow and promote healthy blood pressure levels. Through years of research and experimentation, it is common knowledge that exercise helps to reduce resting heart rate.

Two very important parameters that doctor use when a patient goes in for a checkup are heart rate and blood pressure. When a person has high blood pressure it means that, there is a strain on the heart and blood vessels. Over time the built-up pressure could lead to a heart attack or other heart complications (Silverthorn. 2016). Heart rate is an equally important component of the circulatory system that doctors check. The rate at which the heart is pumping blood through the circulatory system can have an effect on the strain of the heart and blood vessels (Silvethorn. 2016).

A vasodilator is a molecule that relaxes the blood vessels, allowing more blood to flow through the vessels. To test the effects of a vasodilator there are few options, but nitric oxide become the final choice because of its ability to be produced in the body. Nitric oxide has vasodilator properties, which are beneficial to improving the condition of the heart (Shabeeh, 2016). It was found that nitric oxide is synthesized in the body through specific nitric oxide synthase pathways (Willoughby 2015).

Research has been done to manipulate the synthesis of nitric oxide and two amino acids have been found to cause the production of this molecule. L-arginine and L-citrulline were discovered to be the main cause of production of nitric oxide in humans (P Hwang, P, D Willoughby, 2015). A specific pathway called endothelial nitric oxide synthase (eNOS) is the site from production in the circulatory system. (Zhao. 2015). Nitric oxide can only be manipulated from inside the body since it can only be produced naturally inside the body.

Exercise has been a common option for reducing high blood pressure in people. It increases the strength of the cardiovascular system. It works by increasing blood circulation throughout the body, thus strengthening the heart muscles making them stronger and making it easier to pump blood. While many different types of exercise are recommended to lower blood pressure, studies show that low and high intensity are the most effective forms of exercise to accomplish the job. (Maior. 2015). Exercise does not only affect one part of the body. In a recent study, exercise affects the nervous system by reducing the sympathetic activities of the brain (Lemos, 2017).

If exercise and nitric oxide were combined, how would it effect heart rate? As stated before both exercise and nitric oxide have the ability to affect the cardiovascular system in some way. Therefore, the intent of this study is to test the combined effects of exercise and an amino acid that increases production of nitric oxide. The parameter being measured would be heart rate.

The applications of this study can be used in the medical field. This study could lead to further research in the understanding and function of the circulatory system and the molecule nitric oxide.

MATERIALS AND METHODS

Animals and initial procedures

Rats were obtained from a local pet store and transported immediately to a lab setting. Food and water were provided freely. Rats were handled every day for a week before the first experiment. They were also weighed using a special weight scale. The average weight amongst the rats was 144 grams.

Initial Heart Rate Measurements

Rats were allowed time to explore the open environment to familiarize themselves with the space. Since the experiment consisted of making the rats swim in ## degree water, they were acclimated to the same temperature water for 20 minutes as well.

After 3 days of acclimation, initial heart rates were taken. Heart Rate measurements were taken with the PT-104 plethysmograph that is plugged into the IX-TA-220 Recorder and connected to a desktop via USB port. The plethysmograph will be attached to the base of the rat's tail via a Velcro strap. The rat will be restrained using a rat holder. (PT-104, IX-TA-220. IWorx. Dover, New Hampshire).

Experimental Design

A swimming exercise regimen was imposed on the rats. This regimen consisted of a 15-minute session per day over a 5 days period. Heart rate was taken immediately after the 5 day period. This is done as heart rate can change very fast depending on the recovery rate of the rat.

Exercise Protocol

A swimming apparatus was set up using a 36(L) x 21(D) x 19(W) plastic container. This depth was used to ensure that rats are convinced there is no bottom, so they continuously swim instead of float. Each rat was tested one at a time. Water was maintained at room temperature which was at 20º – 22º degrees Celsius.

Making L-citrulline solution.

A solution of L-citrulline solution was made to administer to rats. A simple mixture of water and L-citrulline powder from Beyond Raw Labs. Water was the solvent and L-citrulline powder was the solute. To make this solution the serving size was kept the same which was 3 grams per 60 kilograms of human body weight, converted to be 0.05mg of powder per gram of human.

The optimal amount of water to dissolve the powder is was 3 grams per 8 ounces of water. 8 ounces is equivalent to 29.58ml. Converting that to grams per milliliter it comes out to 0.1268 grams per milliliter or 12.68 milligrams per milliliter. A 100-milliliter stock solution was made for easy administration which required 1.268 grams per 100 milliliters. This is the concentration for the solution

Each rat's weight was taken into consideration when administering the L-Citrulline solution. A stock solution of citrulline was diluted to match with each rat's weight.

Administering the solution

During the administration of the citrulline solution, the Rats were put into to a holder which restricted their movements. A 5 mL syringe was used to administer the appropriate amount of the citrulline solution orally.

RESULTS

Heart Rate

Of the 10 rats the participated only 6 complete all treatments.

Table 1. Group treatment per week

Week	Exercise	L-Citrulline	Avg HR
1			102bpm
2	X		105bpm
3		X	105bpm
4	X	X	114bpm

There were no significant differences in the heart rates between the treatment groups or between the control and the treatment groups. There were also no trends aside from the slight increase of the average HR of the L-Citrulline (only) treatment group to exercise and L-Citrulline treatment group.

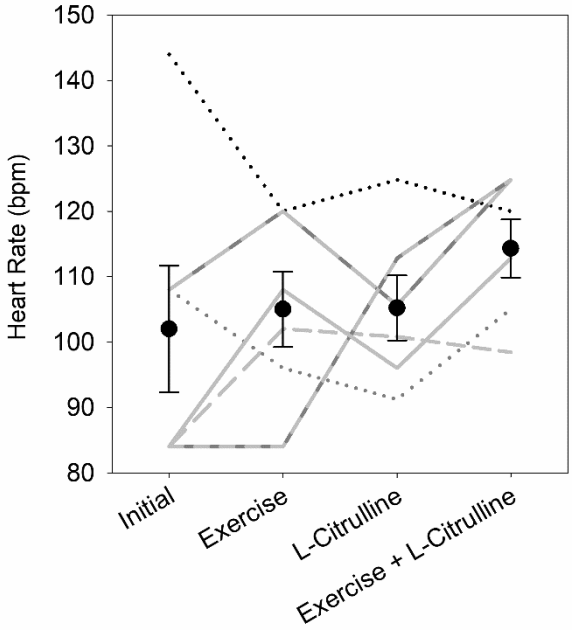

Figure 1. Heart rate measurements of six rats from three different treatments.

DISCUSSION

The differences in the mean values of the average heart rates among the treatment groups are not great

enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.379).

I expected the heart rate to increase for the rats treated with L-Citrulline alone and also the rats treated with the combination of both L-Citruline and exercise regimen. This result could have been due to a multitude of reasons. One could be administering the L-Citrulline orally. Rats could pick and choose if they wanted to swallow the solution or not. Another reason could be due to determination of if rats were calm or not during the measurement of heart rate. To add on to this point the modified heart rate measurement device could have caused some error. When looking at other articles they were able to take and relate both heart rate and blood pressure. This is something I lacked in my experiment. Lastly regarding the exercise protocol, weights were supposed to be implemented to force exhaustion in rats. The problem with this was attaching weight to the tails of rats proved to be more of a problem than anticipated

The purpose of this research was to see if there were any effects on heart rate from exercise or L-Citrulline. The graph shows a slight trend of heart rate increasing from treatment of only L- Citrulline to treatment of L-Citrulline and exercise. Due to the p-value this trend is not enough of a difference to be significant.

ACKNOWLEDGEMENTS

I would like to acknowledge firstly my senior classmates Miaya Sample, Garrett Owen and Sebia Kalambayi for helping me with my test. I would also like to thank my advisor Manjula Koralegedara for correcting all of my mistakes. I would like to thank my co-advisor Jonathan Frye for helping me solve problems and analyze data. I am thankful for the science faculty for all of the amazing feedback. Finally, I would like to thank McPherson College Natural Science Department for funding this project.

LITERATURE CITED

Lemos, M, G da Mota, M Marocolo, C Sordia, R Chirguer, O Neto. 2018. Exercise training attenuates sympathetic activity and improves morphometry of splenic arterioles in spontaneously hypertensive Rats. Arquivos Brasileiros de Cardiologia. 110:3 pp 263 - 269

Liptak, B, B Kaprinay, Z Gaparova. 2017. A rat friendly modification of the non-invasive tail-cuff to record blood pressure. LabAnimal. 46:6 pp 251

Maior, A, R Simao , M.S.R Martins, B De Sallies J Willardson . 2015. Influence of blood flow restriction during low-intensity resistance exercise on postexercise hypotensive response. The Journal of Strength and Conditioning Research. 29:10 pp 2894 - 2899.

Olah, A, B Nemeth, C Matyas, E Horvath, L Hidi, E Birtalan, D Kellermayer, M Ruppert, G Merkely, G Szado, B Merkely, T Radovits. 2015. Cardiac effects of acute exhaustive exercise in a rat model. International Journal of Cardiology. 182:258 - 266.

Shabeeh, H, S Khan, B Jiang, S Brett, N Melikian, B Casadei, P J Chowienczyk, A Shah, A. 2017. Blood pressure in healthy human is regulated by neuronal NO synthase. American Heart Association Journal. pp 970 - 976.

Silverthorn, D. 2016. Human Physiology an Integrated Approach. Pearson Education, London, 503 - 525 pp.

Veskoukis, A, A Kyparos, V Paschalis, M Nikolaidis, M. 2018, A Novel Swimming Performance Test in Rats. Chinese Journal of Physiology 61(3): 144-15.

Willoughby, D, P Hwang. 2015. Intracellular mechanistic role of nitric oxide: a comparative analysis of effectiveness of L-Arginine and L-Citrulline supplementation of nitric oxide synthesis and subsequent exercise performance in humans. International Journal of Food and Nutritional Science. 2:1 40 - 47.

Zhao, Y, P Vanhoutte, S Leung, S. 2015, Vascular nitric oxide: beyond eNOS. Journal of Pharmacological Sciences. 129: 83 - 94.

April. 2019. Basic rat handling and technique workshop. UNC Chapel Hill. https://research.unc.edu/files/2017/03/Rat-Handling-and-Techniques.pdf (21 April, 2019).