ACUTE HIGH INTENSITY TREADMILL EXERCISE INCREASES APPETITE AND NPY/AgRP AND TH NEURON ACTIVITY IN UNTRAINED FEMALE MICE

by

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Introduction

Exercise is commonly prescribed as a means for weight loss, however, exercise programs frequently have mixed success rates. Exercise alone rarely results in significant weight loss and likely needs to be paired with healthy diet changes to show a reduction in weight. Recent studies have shown exercise intensities differentially modulate appetite and appetite-regulating neurons in the hypothalamus. Furthermore, these studies have primarily focused on the effects in male mice, and the effects in female mice are unknown. As a result, the research conducted will examine the effects of different acute treadmill exercise intensities; sedentary, low, moderate, and high, on appetite regulation in female mice.

Background

Anatomical similarities between mice and humans

Biologically, mice and humans share almost all the same genes. Mice and humans have the same organs and organ systems, making it ideal for research regarding human anatomy and physiology, such as human development, disease progression, or biological responses to stimuli to be studied using mice models. Because of the genetic similarity, mice inherit traits the same way as humans. This can provide quicker insight into the chronic disease progression over a lifetime, since the average lifespan of a mouse is about 2 years.

Hypothalamic neurons

There are several neurons in the hypothalamus involved in regulating feeding behavior because of their stimulatory or inhibitory effects on the paraventricular nucleus. The paraventricular nucleus is often referred to as the satiety center, as it is involved in the regulation hunger. Neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons are both located in the arcuate nucleus of the hypothalamus. These neurons make peptides that may stimulate food intake by both increasing NPY signaling and increasing AgRP signaling to reduce melanocortin signaling. NPY/AgRP neurons have inhibitory effects on the paraventricular nucleus. Proopiomelanocortin (POMC) neurons in the arcuate nucleus are activated by leptin. Leptin is a protein involved in regulating energy balance via the inhibition of hunger, therefore, POMC neurons are involved in satiety and have an excitatory effect on the paraventricular nucleus. Tyrosine hydroxylase (TH) neurons in the arcuate nucleus have an orexigenic effect on hunger regulation. TH neurons have been shown to directly modulate the activity of other hypothalamic neurons also involved in regulating feeding behavior by releasing dopamine or Gamma aminobutyric acid (GABA). As a result, TH neurons increase food intake and have an inhibitory effect on the paraventricular nucleus. Single-minded 1 (SIM1) neurons in the paraventricular nucleus of the hypothalamus have excitatory effects and are associated with satiety.

Immunohistochemistry

NPY/AgRP, POMC, TH, and SIM1 neurons can be detected through immunohistochemical processes. cFOS is a protein that is expressed in these neurons in response to a stimulus. In order to detect for c-FOS, the secondary antibody is conjugated with an enzyme that acts on a substrate. The product of the enzymatic reaction is developed by a chromogen, which stains the brain slices and can be observed under light microscopy.

Purpose of the Study

The purpose of the study conducted is to determine if varying exercise intensities influence food intake following exercise. Furthermore, if differences are found, the study will aim to investigate the effects that low intensity exercise, moderate intensity exercise, and high intensity exercise have on appetite and appetite regulating neurons. The study will provide insight as to if exercise can be used as an effective means for weight loss for females, and if so, what exercise intensities provide the best conditions for weight loss.

Research questions or hypotheses

The hypothesis for the study is that higher exercise intensities will likely yield increased food intake in female mice, due to increases in NPY/AgRP neuron activity in the arcuate nucleus. This is theorized from evidence in previous studies observing male mice, because such studies observed increased food intake due to the elevated NPY/AgRP neuron activity in exercised mice as compared to mice that were sedentary.

Methodology

In order to determine how exercise intensity affects appetite, 11 8-week-old female mice are used for the study. The untrained female mice participated in a randomized-crossover trial of various intensities. Each mouse underwent sedentary activity, low intensity exercise, moderate intensity exercise, and high intensity exercise for 60-minute sessions in a randomized order. The sedentary activity session involved the mice sitting in their cages, on top of the treadmill for 60 minutes while it is running to expose the mice to the same auditory stressors involved with running on the treadmill. For the low intensity exercise, the mice ran on the treadmill at a speed of 10 meters per minute for 60 minutes. The moderate intensity exercise session consisted of the mice running at a speed of 14 meters per minute for 60 minutes. The high intensity exercise required the mice to run at a speed of 18 meters per minute for 60 minutes. Each intensity was performed only once by each mouse, for a total of 4 different exercise sessions per mouse. There was 7 days between each trial to allow for the mice to recover. Prior to each trial, the mice were fasted for 10 hours. The food intakes of the mice were measured following each exercise session to determine if each exercise intensity either increases, decreases, or has no effect on appetite. The weight of the food to be consumed by each mouse post-exercise was measured on a scale in grams prior to consumption. The food was measured at 1, 2, 3, 6, 12, 24, and 48 hours after exercise to determine the amount of food consumed by each mouse. An additional cohort of mice were perfused with PBS and formalin 3 hours post-exercise and their brains were removed to stain for appetite regulating neurons in the hypothalamus. Immunohistochemical detection for cFOS was performed in these mice to determine changes in NPY/AgRP, POMC, TH, and SIM1 neuron activity in response to exercise.

Results

Compared to sedentary trials, cumulative 24-hour food intake was greater following both moderate intensity exercise and high intensity exercise. This is predominantly due to the increased food intake 6-12 hours post-exercise in the female mice. The greatest increases in food intake were observed in mice exercising at a high intensity, with increases in food intake observed up to 48 hours following the 60-minute exercise bout. These increases in food intake were associated with increases in activity of the orexigenic NPY/AgRP and TH neuron populations in the arcuate nucleus of the hypothalamus. The cFOS staining for both the

NPY/AgRP and TH neurons indicated higher levels of activity in high intensity exercise as compared to sedentary activity. When looking at the anorexigenic POMC neuron population, staining for cFOS indicated no significant difference in neuron activity between sedentary and high intensity exercised mice, ultimately resulting in no significant changes in the satiety level of the mice. Interestingly, no effects on appetite were observed in response to low intensity exercise during the study.

Conclusion

These results indicate that higher exercise intensities increase 24-hour food intake post-exercise in female mice, possibly explaining the low success rates of exercise-focused weight loss programs. Low intensity exercise may be a useful exercise regimen due to the absence of compensatory increases in appetite.

Closure

In conclusion, there have been similar studies to show the effects of exercise intensity on appetite regulation in male mice, but the effects in female mice were unknown. This study aimed to further investigate into the impacts that various exercise intensities have on appetite and appetite regulating neurons in the hypothalamus in female mice. Because of the anatomical similarities between mice and humans, a female mouse model can provide insight into how female humans regulate appetite as a result of varying exercise intensities. This study's focus was on 8-week-old, fasted, untrained female mice. Future directions could include studying trained versus untrained female mice, fed versus fasted female mice, male versus female mice, or varying age groups of mice. Specific workouts, such as high intensity interval training versus constant low intensity exercise could provide specific insight into the benefits of interval training, and its effect on appetite regulation. The research conducted in this study regarding exercise intensities differentially modulating appetite and appetite-regulating neurons in the hypothalamus was presented at the 2021 East Carolina University Research Creativity and Achievement week as a poster presentation, the 2021 East Carolina University Pre-Professional Student Virtual Research Symposium, and at the 2021 Kinesiology Research Day.

Citations

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