Overview of the Project:

This project will use advanced imaging techniques to examine and compare brain function following both opioid use and exercise. This work will be completed at the Molecular Imaging Institute at Rutgers University. The funding requested for this proposal is for 40 hours of ultrasound and laboratory use, a stipend for my effort and for an undergraduate research assistant, the purchase of mice, drugs, and technical support. All animals for this study will be housed at Rutgers University. Drugs used during this study will be purchased through Rutgers University and kept at the Molecular Imagining Institute.

HYPOTHESIS:

Exercise and opioid abuse facilitate similar acute responses both involving the opioid system. My hypothesis is that acute exercise exhibits a similar effect on brain blood flow as acute opioid use.
Specific Aim 1: To assess differences in blood flow by use of molecular imaging techniques following bouts of acute exercise and opioid administration.
Specific Aim 2: To assess the role nitric oxide plays in brain blood flow by administration of a NO blocker.

Background:

Opioid addiction remains on the rise in the United States of America and is now solely responsible for decreasing the average life expectancy by 2 ½ months over the last year. In the past 15 years, fatal overdoses pertaining to opioid abuse have tripled, and opioid related mortalities are estimated to increase by next year. Overdoses from fentanyl, a powerful opioid pain medication, have risen from 9,945 in 2016 to 20,145 in 2017. The drug problem in America is a difficult one to solve, as it appears that opioid addiction is a multifactorial condition and the continued use of opioids have the potential to alter the function of the brain[1]. Although advances in the treatment of opioid addiction is needed the discovery of both novel and consistently effective treatments is difficult.

The role of the body's internal opioid system both during and following exercise is well established. Exercise also has been linked to at least three phenomena that are likely to involve opioid release; the 'athlete's high', increased pain tolerance, and addiction to exercise[2]. Exercise studies that have examined the effects of the opiate blocker naloxone, found that its administration prior to exercise alter these phenomena. An opioid blocker given before exercise abolishes the sense of well-being and reverses pain suppression that is associated with exercise[3]. These studies conclude that exercise naturally mimics many of the same effects as taking opioids.

Both exercise and opioid use can increase brain blood flow. An increase in brain blood flow leads to a subsequent rise in the potent vascular dilator nitric oxide (NO) which can facilitate a pleasure response and can further increase in opioid activation. Studies have found that NO activation can increase the effect of opioids[4]. To this point, nobody has directly compared brain blood flow nor the contribution of NO following exercise and opioid use. The further discovery of the overlapping effects between exercise and opioid use will provide insight into whether exercise can serve as a healthful intervention to include in the recovery of opioid addiction.

Background Work Accomplished:

I have worked on animal models of exercise at two separate universities. Both as a doctoral student (University of Delaware) and as a post-doctoral fellow (Rutgers Medical School). During this time, I was able to publish scholarly works at both institutions (please see attached CV). While a post-doctoral fellow I worked with the Molecular Imaging Center at Rutgers University to develop techniques to assess, cardiac and skeletal muscle blood flow using advanced ultrasound imaging. These will be the same techniques used to assess brain blood flow. Further demonstrating my advanced knowledge of this

equipment, I co-wrote a grant to the National Institute of Health grant to purchase an upgraded machine for the Cardiovascular Research Institute in the Department of Cell Biology and Molecular at Rutgers Medical School. Further, I helped developed new techniques for advanced data analysis.

Statement of the procedures/methodology:

IACUC Approval: Animals used in this study will be maintained and all experiments will be performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, Eighth Edition 2011). Institutional Animal Care and Use Committee (IACUC) approval is currently under review at Rutgers University.

Timeline: Brain blood flow measurements for the proposed project will be collected within a three-week period over the summer months. Data analysis for the proposed will be completed in a subsequent 3-4 weeks.

Exercise Bout: Mice will undergo a 50-minute bout of swimming which is an adequate exercise time to increase opioid release.

Drug Administration: Opioids will be injected in a subset of mice and 15 minutes will be allowed to pass in order to allow a peak effect to take place. In a separate subset of animals, an opioid blocker will be delivered 25 minutes prior to opioid administration or exercise.

Brain Blood Flow: We will employ blood flow imaging of brain circulation using an advanced ultrasound system. We will use advanced data analysis software to assess any changes in brain blood flow that occurs following opioid administration or exercise. Additional blood flow will be measured in the skeletal muscles, heart and stomach.

Specific Aim 1: To assess differences in blood flow by use of ultrasound imaging following bouts of acute exercise and opioid administration.

Protocol: Mice will be divided into three subsets: (a) control mice receiving neither opioids nor exercise (b) opioid recipients (c) exercise recipients. Brain blood flow will be measured 24-48 hours before and after each experimental condition. Mice will be measured once more 24-48 hours later and an opioid blocker will be administered prior to opioid or exercise to test the efficacy of each treatment.

Specific Aim 2: To assess the role nitric oxide plays in cerebral blood flow by administration of a NO blocker.

Protocol: The Protocol described in Specific Aim 1 will be repeated following administration of an NO blocker.

Project Importance:

This project is important on several levels. As mentioned in the background section of this proposal, opioid addiction is a growing problem. Therefore, gaining a better understand of the actual biology involved with addiction is crucial. This project aims to investigate similarities between opioid use and exercise. These pilot data could help determine whether exercise can be successfully used as a behavioral intervention for recovering opioid addicts. I can also begin to explore if exercise can serve as an alternative adjunct therapy for pain management and in some cases replace opioids.

There are also a number of indirect benefits as well. This project allows for undergraduate students in the newly formed Exercise Science program to become exposed to research here at Stockton University and allows me to begin to develop an undergraduate research program. Three undergraduate students will assist me in the collecting and analyzing data over the summer months. The imaging proposed is innovative and, therefore, students who wish to participate can train on state of the art molecular imaging equipment The Exercise Science program has little to no resources currently available

for research. The funding of this project is important as it will also allow me to apply for external funds to support my program.

Students who wish to assist with the project will have the opportunity to submit research abstracts based on the findings of these pilot studies to the Mid Atlantic Regional Chapter of the American College of Sports Medicine (MARC-ACSM) conference. I am a member of the organization and nearly every exercise science program is represented from this region of the country. Last year alone over 1200 students attended this meeting. Students will have the opportunity to highlight the work completed at Stockton University during this event providing great exposure for the university. This is also an important opportunity to aid in the development of our undergraduate students. Lastly, the funding of this project will also allow me to continue to develop new imaging techniques I began as a post-doctoral fellow.

Outcome and Dissemination of Results:

The unique imaging techniques, proposed for this project, can capture many data points and yield a lot opportunity for presentations and publications. Therefore, my short-term goal would be to present these pilot data nationally at the Experimental Biology Conference in the spring of 2019. Further, I would encourage at least 2-3 undergraduate students who wish to assist me with this research to submit abstracts for presentations at the regional ACSM conference. This would be a rewarding experience for these students. My students and I will also apply to participate in the 2019 Day of Scholarship at Stockton University to share our findings. I also intend on submitting three manuscripts based off these works. One in collaboration with Dr. Sara Campbell of Rutgers University studying how exercise and opioid use influence stomach health. A second manuscript in the fall of 2019 to a suitable peer reviewed journal this will feature the brain blood flow data outline in this proposal. I will also aim to publish a third paper on novel data analysis that I will be able to explore during this project.

Future Research:

These data collected during this project will make be a competitive candidate for external funding. With successful funding of this proposal, I intend on applying for an American College of Sport Medicine (ACSM) New Investigator Award ($10,000), during the fall of 2018. This would provide additional funding for this work with the next aim being to study long-term effects of opioids and exercise. I am a member in good standing with the ACSM for a number of years. In addition, I had a Pre-Doctoral Investigator Award ($5,000) successfully funding through the same organization. In the spring of 2019, I intend on applying for an American Physiological Society Career Enhancement Award ($20,000). This provides another opportunity to receive external funding for my future project goals. My main goal over the next 2 years is to collect enough data to put forth a competitive proposal for National Institute of Health funding. I have also discussed the possibility of co-authoring another National Institute of Health grant with Dr. Sara C. Campbell of the Dept. of Kinesiology and Health at Rutgers University. This proposal would focus around data she collects in collaboration with me on gastrointestinal microbes and their reaction to opioids. Dr. Campbell is one of the leaders in exercise and gastrointestinal function in the country and we have already successfully co-authored a paper together (please see attached CV) with an additional manuscript in preparation.

References

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2. Hamer M., Karageorghis C., Psychobiological mechanisms of exercise dependence. *Sports Med.*,37(6) (2007), 477-485.
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4. Ide K, Horn A, Secher NH. Cerebral metabolic response to submaximal exercise. *J Appl Physiol* 87: 1604–1608, 1999.