Impact of Muscle Temperature on Muscle Growth and Breakdown at Rest: Influence of Local Muscle Cooling on Mitochondrial Related Gene Expression

Project Description

Temperature and exercise both impose stress on the body, especially the mitochondria. Mitochondrial stress induces adaptations called mitochondrial biogenesis (growth) and mitophagy (mitochondrial breakdown). An imbalance or dysfunction can lead to many diseases, such as vascular, metabolic, inflammatory, and neurodegenerative diseases, as well as aging (Meister et al., 2020).

Exercise promotes the balance of mitochondrial biogenesis and mitophagy to prevent these diseases. Endurance exercise, such as walking, running, and cycling stimulate the mitochondria and the genes associated with mitochondrial development, such as PGC1-α mRNA. PGC1-α mRNA is an indicator of mitochondrial growth and can be upregulated after an effective stimulus (Opichika et al., 2019). Exercise is an effective way to upregulate PGC1-α (Meister, not published); however, temperature variations, when combined with exercise, may alter the stimulus (Slivka et al., 2011).

Our lab has conducted studies on how both ambient (Slivka et al., 2011) and local (Shute et al., 2017) temperatures affect mitochondrial gene expression, during exercise. Cold ambient temperatures upregulate PGC1-α mRNA after exercise, while interestingly, hot ambient temperatures seem to be detrimental to PGC1-α (Slivka et al., 2011). Next we investigated the potential of local cooling applications before, during and after exercise for PGC1-α mRNA. Local muscle cooling is the process of lowering intramuscular temperature. The preliminary findings of local muscle cooling found that PGC1-α levels were unchanged during exercise, compared to its ambient counterpart (Shute et al., 2017). Environmental temperature affects core body temperature and causes a systemic reaction that stimulates an upregulation of PGC1-α mRNA (Slivka et al., 2011). Collectively, research indicates that there is more to mitochondrial related genes and their thermoregulatory mechanisms that we do not understand. Further research is needed to investigate the effects of local muscle cooling on gene expression at rest.

This study will be investigating the effect of local muscle cooling on mitochondrial related gene expression without an exercise stimulus. By adding localized cold to the muscle we can observe how decreasing muscle temperature impacts mitochondrial related gene expression without dramatically affecting core and whole body skin temperatures. *The purpose of this study is to apply localized cooling to the skeletal muscle during rest and examine the influence of local cooling on gene expression related to mitochondrial homeostasis.*

Methodology

Study Design and Participants: This proposed study will recruit 12 recreationally trained, apparently healthy individuals between the ages of 19 and 45 years old. IRB approval of this project has already been obtained. Participants will come to the exercise physiology lab at the University of Nebraska Omaha for two visits. This project will make use of the same subjects and data collection efforts that are part of a larger funded project (PI; Slivka) that is investigating the impact of temperature on muscle growth and breakdown.

Initial Visit: The initial visit will be to obtain informed consent and collect the descriptive data of the participants. Subjects will complete a risk stratification form based on ACSM guidelines and must be considered low risk in order to participate in this study. Height and weight will be measured using a standard stadiometer and medical scale. Body fat will be assessed with hydrostatic weighing using an electronic load cell based system correcting for estimated residual lung volume.

Experimental Visit: Participants will be instructed to arrive at the lab having consumed a standardized meal 90 minutes prior to arriving. They will be asked to avoid strenuous activity, alcohol consumption, tobacco use, and drug use for the 24 hour period leading up to experimental trial. Experimental procedures will include: two muscle biopsies, two intramuscular temperatures, two skin temperatures, and an ultrasound blood flow measurement. Subjects will be placed in a semi-reclined position and a wrap will be applied to the thigh areas of both legs. One wrap will be cooled (approximately 0°C) and the other not cooled (randomized assignment). After 4 hours the thermal wraps will be removed and blood flow will be analyzed using an ultrasound. A muscle biopsy will be obtained from each *vastus lateralis* (outer thigh) muscle. Dr. Slivka will take the biopsies after shaving the area surrounding the *vastus lateralis* and creating a sterile field. 1% lidocaine is then injected under the skin with a 25 ga hypodermic needle. Using sterile techniques, a small incision is made through the skin (~5mm) and the Bergstrom biopsy needle is inserted through the incision into the belly of the muscle, taking a small clip of muscle. After, excess blood and connective tissue is quickly removed, tissue samples will be immersed in liquid nitrogen and stored at − 80 °C for later analysis. Using the incision from the muscle biopsy, a hypodermic thermocouple will be inserted into the belly of the muscle to measure intramuscular temperature and skin temperature will be measured on the surface of each thigh using a skin thermistor. Afterwards, slight pressure is held over the incision for approximately 2 minutes and is then closed using steri-strips and a bandage with antibiotic ointment. The leg is further dressed with a compression bandage.

Gene Expression Analysis: The two muscle biopsies (per subject) will be used to analyze transcriptional changes of genes associated with mitophagy (PINK 1, PARK2, NIX and BNIP3) and mitochondrial biogenesis (PGC-1α, TFAM, ERRα, VEGF, NRF1 and NRF2). Muscle mRNA will be determined using real-time, quantitative reverse transcriptase polymerase chain reaction (Real-Time RT-PCR). Samples will be homogenized and mRNA will be extracted using standard procedures. This will be followed by purification and then quantification of RNA with a nano-spectrophotometer (nano-drop ND-1000, Wilmington, DE). A Superscript-first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA) will synthesize RNA to cDNA. Each sample within a subject will be adjusted to contain a standard RNA concentration by dilution using RNase free water. Each RT-PCR 10μL reaction volume will contain 500 nM of primers, a 250 nM probe (PrimeTime qPCR assay, Integrated DNA technologies), Brilliant III Ultra-Fast QPCR master mix (Agilent Technologies Inc., Santa Clara, CA), and 2.5 mL of sample cDNA. Samples will be analyzed real time PCR detection system (Agilent Technologies Inc., Santa Clara, CA) running 1 cycle at 95 °C for 3 minutes, then 40 cycles of 95 °C for 5 seconds and 60 °C for 20 seconds. Quantification of mRNA for genes of interest will be computed on pre and four hour post muscle samples using the $2^{-\Delta\Delta CT}$ method and compared to stable reference housekeeping genes. NormFinder software (Version 0.953) (Aarhus University Hospital,

Denmark) will be used to examine housekeeping gene stability. Probes and Primers that will target the specific gene sequences will be attained from Integrated DNA technologies (Coralville, Iowa).

Statistical Analysis: A paired t-test will be used to compare the control and experimental limbs. A probability of type 1 error less than 5% will be considered significant ($p < 0.05$).

Contributions

This research project will contribute to the developing topic of mitochondrial growth, biogenesis, and breakdown, mitophagy. Mitochondrial biogenesis has been a huge topic of interest during the past 20 years. The scope of the mitochondrial research has grown to include not only biogenesis, the building of new mitochondria, but also mitophagy, the degradation of dysfunctional mitochondria, in order to understand mitochondrial homeostasis. This study will use a human model with local cooling application to provide further insight in the field of mitochondrial health. When this data is paired with previous research, a more complete understanding of the balance of the mitochondrial network will be established. This study will further examine how local cooling of the muscle in the absence of exercise changes mitochondrial gene expression. Based on this, future research could determine how local cooling may be incorporated into prevention or rehabilitation of mitochondrial related disorders.
If funded, this research project will be used to fulfill thesis requirements and will be submitted for publication in a peer reviewed journal in the spring of 2022. This project will also aid in my understanding in the field of exercise physiology and muscle biology.

Project Timeline

	2020		2021						2022
	Fall		Spring and Summer			Fall			Spring
Subject Recruitment									
Data Collection									
Data Analysis									
Manuscript Preparation									
Present at 2022 Student Research and Creative Activity Fair									

Roles of Student and Faculty Mentor

Student: I will be responsible for coordinating and leading data collections while adhering to Institutional Review Board (IRB) standards and protocols listed in the application, ordering the products needed to perform this research, homogenizing all muscle samples and extracting mRNA, performing PCR analysis on the muscle samples, running statistical analysis, and preparing the manuscript for publication.

Faculty: Dr. Slivka will serve as the faculty mentor for this project. Dr. Slivka will be responsible for collecting muscle samples, supervising data analysis, serving as a senior author on the manuscript, supervising the descriptive and experimental trials, reviewing all manuscript preparations, and providing any additional supplies needed to complete this study.

Budget Justification

Item:	Price/Unit:	Unit:	Price:
Probes and Primers	$200	10	$2,000
Student Stipend	$12.50/hr	240	$3,000
TOTAL:			**$5,000**

We request a total of $5000 to complete the proposed research. The experiments will take place in the Exercise Physiology Lab and the Sample analysis will take place in the Exercise Biochemistry Lab in the Health and Kinesiology building at UNO. Dr. Slivka has committed to provide space in the lab, general laboratory chemicals, and other expendables. The specific probes and primers will be paid for by this grant and are necessary for us to compare our 10 genes of interest (PINK 1, PARK2, NIX, BNIP3, PGC-1alpha, TFAM, ERR alpha, VEGF, NRF1 and NRF2). The student stipend will be used to compensate for my work over the summer. At 20 hours per week for 12 weeks it comes to 240 hours and $12.50 an hour. Due to the magnitude of this project, we realize that there are additional expenses beyond the requested $5000 associated with this project and these supplies will be covered by Dr. Slivka. This additional funding that will pay for the supplies needed for subject stipends, biopsies, thermoregulatory equipment and other molecular analyses.

References

Meister, B., Collins, C., Mcglynn, M. L., & Slivka, D. R. (2020). Effect of Local Cold Application During Exercise on Gene Expression Related to Mitochondrial Homeostasis. Applied Physiology, Nutrition, and Metabolism. doi:10.1139/apnm-2020-0387

Opichka, M., Shute, R., Marshall, K., & Slivka, D. (2019). Effects of exercise in a cold environment on gene expression for mitochondrial biogenesis and mitophagy. Cryobiology, 90, 47-53. doi:10.1016/j.cryobiol.2019.08.007

Shute, R., Heesch, M., Laursen, T., & Slivka, D. (2017). Local muscle cooling does not impact expression of mitochondrial-related genes. *Journal of Thermal Biology, 67*, 35-39. doi:10.1016/j.jtherbio.2017.04.008

Shute, R., Marshall, K., Opichka, M., Schnitzler, H., Ruby, B., & Slivka, D. (2020). Effects of 7°C environmental temperature acclimation during a 3-week training period. *Journal of Applied Physiology, 128*(4), 768-777. doi:10.1152/japplphysiol.00500.2019

Slivka, D., Dumke, C., Tucker, T., Cuddy, J., & Ruby, B. (2011). Human mRNA Response to Exercise and Temperature. *International Journal of Sports Medicine, 33*(02), 94-100. doi:10.1055/s-0031-1287799

University of Nebraska Omaha

SCHOOL OF HEALTH AND KINESIOLOGY

November 5, 2020

Graduate Research and Creative Activity (GRACA)
Letter of Mentor support for Larry Robins

GRACA Review Committee:

I write this letter in support of the GRACA proposal titled, "Impact of Muscle Temperature on Muscle Growth and Breakdown at Rest: Impact of local muscle cooling on Mitochondrial Related Gene Expression" submitted by Larry Robins. Lary is in his first semester and is volunteering with me in the exercise physiology laboratory. Larry came to me to get involved in research. He has been a very dedicated volunteer and has essentially been working unpaid taking on the same type of tasks that a graduate assistant would. He has been spending his first semester learning the background of our lab and the type of molecular techniques we use. He is now ready to incorporate these techniques in conducting his own research project.

Larry proposes to conduct his research on some muscle samples that will be collected as part of a larger project. The general data collection is funded. However, the other aspects related to the development of mitochondria does not have funding. In order for Larry to investigate this area of interest he seeks GRACA funding. Although this proposal seeks funding for only some of the basic science aspects, Larry will be involved with the complete project and is expected to publish the findings in a peer reviewed journal. This approach allows students to leverage the funding and work currently being done in the lab and add their research on to these projects. Ultimately, leading to a higher impact than could be accomplished with GRACA alone.

Larry will need continued mentorship as he completes this project. I will mentor him to the best of my ability. My hope is that this mentorship and guidance now will allow him to reach his next level goals. I have committed to Larry to provide this mentorship and the facilities of the exercise physiology laboratory. I am fully committed to fill the mentorship role to help him further develop academically and professionally. This project represents a critical step in developing his career in research. I am confident that Larry has prepared a realistic proposal/budget and that he will complete this project with the ultimate outcome of a published peer-reviewed journal article.

Thank you for your consideration,

Dustin R. Slivka, Ph.D., FACSM
Associate Professor, Exercise Physiology Laboratory Director
School of Health and Kinesiology| H&K Building #207R
College of Education
University of Nebraska at Omaha | www.unomaha.edu

402.554.3224 (office)
513.260.8271 (cell)
402.554.3693 (fax)
dslivka@unomaha.edu