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School of Health, Physical Education, and Recreation  
**Human Gene Response to Exercise in a Cold Environment**

**PROJECT DESCRIPTION**

**Overview and Objectives**

Cold stress in humans may enhance performance and aid in the treatment of diseases associated with mitochondrial dysfunction. There are three common methods to achieving cold stress in humans. One is applying cold locally to the area of interest, another is cold water immersion, and the third is the use of an environmental chamber (Herrera, Sandoval, Camargo, & Salvini, 2011) (Costello, Culligan, Selfe, & Donnelly, 2012). The proposed study will make use of a new environmental chamber installed in the exercise physiology lab and thus broaden the scope of research conducted in the exercise physiology lab. The information gained from the proposed experiment may have many applications. These applications may include but are not limited to exercise performance and prevention and treatment of disease. Thus a long term goal of this line of research is to develop novel temperature optimized training protocols in order to enhance performance, prevent disease, and/or treat disease.

The key to the effect of cold on health related parameters appears to reside in the mitochondria. Mitochondrial dysfunction has a role in peripheral arterial disease (Makris, et al., 2007), aging (Derbré, et al., 2011), obesity, diabetes and other diseases (Bullon, Newman, & Battino, 2014). Previous studies have shown that post-exercise recovery in a cold environment can lead to higher levels of oxygen uptake and higher levels of gene expression implicated in mitochondrial biogenesis such as PGC-1α. PGC-1α is often looked at because it is a key regulator of energy metabolism and likely involved in disorders such as obesity, diabetes, and cardimyopathy (Liang & Ward, 2006). Furthermore, cold exposure has shown to be effective in increasing PGC-1α in aged rats when the stimulus of exercise alone was unable to produce an increase (Derbré, et al., 2011). Cold exposure has also been shown to increase mitochondrial density in fish due to the increased demand of oxygen in a cold environment (O'Brien, 2011).

In a similar design to that proposed here, Slivka et al. found that PGC-1α was enhanced when human participants exercised for one hour and then recovered for three hours in a cold environment compared to a laboratory environment control condition (Slivka, Dumke, Tucker, Cuddy, & Ruby, 2012). This approach may not be a practical in actual application since most people would prefer to be at a more comfortable temperature when recovering and not have an additional three hours to dedicate to their rehabilitation or training. A more practical approach would be to eliminate the period of cold exposure during recovery. However, it is unknown if the cold exposure during recovery is necessary for enhanced PGC-1α expression and ultimately enhanced mitochondrial biogenesis. The three hour recovery time, regardless of environment, is needed to allow time for gene expression to peak after exercise.

The purpose of this study is to determine human mitochondrial related gene response to exercise in a cold environment. The hypothesis of the proposed study is that exercise in the cold environment will show higher expression of PGC-1α mRNA compared to exercise in a neutral control environment. When this data is taken with previous data from the Slivka lab, we can determine the need for recovery in a cold environment in order to enhance mitochondrial development. This study will represent a large step in the development of temperature optimized training protocols to treat the deleterious effects of mitochondrial dysfunction.
**Study Design**

This research project will recruit 12 recreationally trained apparently healthy males as participants between the age of 19 and 45. No recruitment of participants or procedures associated with this study will be undertaken until IRB approval has been obtained. Participants will come to the exercise physiology lab on three occasions, the initial visit will be to obtain informed consent and collect the descriptive data of the participants. The final two visits will consist of the experimental trials detailed below.

*Initial Visit* – Participant informed consent and descriptive data will be obtained during the first visit. The descriptive data will include height, weight, and percent body fat. Body fat will be assessed through hydrostatic weighing using an electronic load cell based system (Exertech, Dresbach, MN) correcting for residual lung volume. A maximal aerobic capacity test (VO2max) will be conducted on a Velotron cycle ergometer (Racermate, Seattle, WA) using a graded exercise protocol until volitional fatigue. The highest oxygen consumption recorded by a Parvo Medics TrueONE 2400 Metabolic Measurement System (Sandy, UT) will be defined as the VO2max. The workload for each ensuing trial will be set at 60% of the cycling workload associated with VO2max for one hour.

*Experimental Trials.* The experimental trials will involve one hour of exercise in either a 7°C or 20°C environment followed by three hours of recovery in standard laboratory conditions. These temperatures are selected because they have been used in previous research and are safe for the participants to withstand for an extended time without unsafe increases or decreases in core body temperature (Slivka, Dumke, Tucker, Cuddy, & Ruby, 2012). Three hours appears to be the minimal amount of time for peak expression of our target genes.

All trials will occur in a temperature and humidity controlled environmental chamber (Darwin, St. Louis, MO). The order of the trials will be randomized and three muscle biopsies will occur during each of the two experimental trials. The muscle biopsies will be taken from the vastus lateralis before the trial, after the exercise protocol, and after the three hour recovery period. Other measures taken during exercise and recovery will be core body temperature using ingestible thermister capsules, oxygen uptake measurement for substrate use and metabolic rate, heart rate using polar heart rate monitors, and rating of perceived exertion using the Borg 6-20 scale.

*Sample Analysis.* The oxygen consumption during the trial will determine substrate use, and muscle biopsy samples for will be used to analyze mRNA and muscle glycogen. The primary genes of interest will be PGC-1α, COX, MFN2, UCP3, ERRα, NRF1, and NRF2 due to their relation to mitochondrial development. We will be using standard real-time PCR protocols to analyze the genes of interest relative to stable reference genes (GAPDH, B-actin).

*Statistical Analysis.* A repeated measures two-way ANOVA (time x trial) will be used for measurements. If the F-ratio values are found to be significant a Fisher’s protected LSD post hoc will be performed to evaluate where significance occurs. A probability of type I error of less than 5% will be considered significant (p<0.05). All statistical data will be analyzed using the Statistical Package for Social Sciences software (SPSS 18.0).

**Contributions**

This research project will contribute to the field by determining the optimal and realistic stimuli needed for enhancing mitochondrial biogenesis. When this data is taken with previous research using similar temperatures for exercise but including exposure to cold during recovery we can identify the dynamics
associated with enhanced mitochondrial function and develop therapies to combat mitochondrial related disorders. We will distinguish whether temperature during exercise is the main contribution to the changes observed in previous research such as the expression of PGC-1α or if the recovery in the cold temperature after exercise is necessary.

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 2015. This project will also aid in my understanding in the field of exercise physiology and serve as a foundation for my potential future career in the field.

**Project Timeline**

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<th>2014</th>
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<th>2015</th>
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<td></td>
<td>May</td>
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<td>Write IRB</td>
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<td>Recruitment of Subjects</td>
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<td>Presentation of Research at 2015 Student Research and Creative Activity Fair</td>
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**Figure 1:** Project Timeline

**Roles of Student and Faculty Mentor**

I will be responsible for the bulk of the work associated with this project. My faculty mentor, Dr. Slivka, will assist me in this project and work with me to learn and perform the analytic techniques described here.

**Student** – I will be responsible for:

- Writing the IRB
- Writing a thesis
- Recruiting and scheduling participants
- Conducting the descriptive and experimental trials
- Assisting in muscle biopsies
- Performing the muscle sample analysis
- Ordering the products needed to perform this research

**Faculty** – Dr. Slivka will be the faculty member involved with the project. Dr. Slivka will be responsible for:

- Reviewing the IRB
- Reviewing the thesis and manuscript
- Serve as the senior author on the manuscript
- Supervising the descriptive and experimental trials
- Performing all muscle biopsies
- Supervising sample and data analysis
REFERENCES


**BUDGET JUSTIFICATION**

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<th>Item</th>
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**Figure 2: Budget Breakdown**

We request a total of $5000.00 to complete the proposed research. No student stipend or travel funds are requested for this grant. The experiments will take place in the Exercise Physiology Lab and the sample analysis will take place in the Exercise Biology lab in the Health, Physical Education, and Recreation building. The existing ParvoMedics metabolic cart, and newly built environmental chamber will be used in the experiments. The funding for the proposed experiment will go towards essential supplies needed to complete the experiment for data collection, sample analysis and for participant stipend. Gene expression analysis supplies needed include probes and primers, mastermix, Rnasy, mini kit, Dnase set, first strand cDNA kit, pipette tips and expendables. If funded, this will be one of the first projects on campus to take advantage of the new environmental chamber and the technologies associated with it. Due to the magnitude of this project, we realize that additional expenses beyond the requested $5000 are associated with this project (medical supplies for biopsies). Dr. Dustin Slivka, my faculty mentor, has committed laboratory funds to cover the added expense if this GRACA proposal is funded.