Effect of Local Cold Application during Exercise on Mitochondrial Gene Expression

Ben Meister, Robert Shute, Christopher Collins, Megan Johnson, Dustin Slivka

University of Nebraska at Omaha, School of Health and Kinesiology, Omaha, NE

Exercise Physiology Lab

ABSTRACT

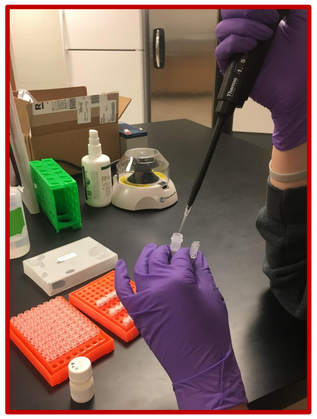
BACKGROUND: Different modes of exercise and temperatures can elicit different responses in mitochondrial gene expression. Stresses including exercise and cold exposure act to trigger the transcription of genes associated with mitochondrial growth (biogenesis). Traditional endurance exercise is known to have a potent effect on mitochondrial growth biogenesis. However, limited knowledge exists on the effect of local cold application during endurance exercise. Additionally, it is currently unknown how cold exposure impacts mitochondrial breakdown (mitophagy). **PURPOSE:** Therefore, the purpose of this study is to determine the impact of local muscle cooling during endurance exercise on human skeletal muscle gene expression related to mitochondrial homeostasis (mitophagy and biogenesis). **METHODS:** Twelve recreationally-trained males and females (age 19-45) will be recruited for the study. Subjects will complete a 60-min cycling protocol at 65% W_{peak} with thermal pads applied to their legs followed by 4 h of recovery. One leg will be cooled (ICE) and the other leg will be kept at thermoneutral conditions (CON). The leg that is cooled and the leg that serves as the control will be randomized. Intramuscular temperature will be recorded continuously. Intramuscular temperature will be recorded continuously. Muscle biopsies will be taken from each *vastus lateralis* before and after the cycling as well as at 4 hours post-exercise for the analysis of mitochondrial-related gene expression. **IMPLICATIONS:** This data may establish the effectiveness of using local cooling during endurance exercise. Furthermore, future research could determine the effectiveness of local cold application as a potential therapy for mitochondrial dysfunction.

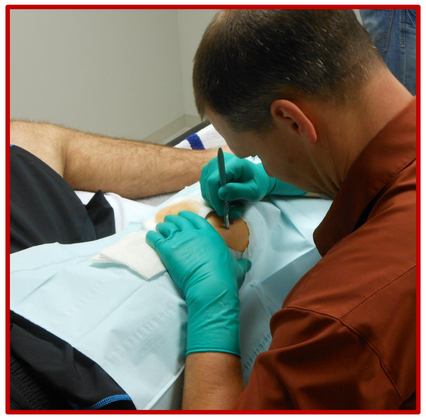
AIMS

- To compare transcription of genes related to mitochondrial function following exercise with local cold exposure and recovery at room temperature compared to exercise and recovery at room temperature
- To identify differences in mRNA expression for mitophagy and mitochondrial biogenesis after an acute exercise bout

INTRODUCTION

- A well-known adaptation to endurance exercise training is an increase in mitochondrial content in the active skeletal muscle fibers.
- Previous work from our lab suggest that mRNA expression of mitochondrial genes have a more favorable response to exercise in cold environments.
- However, it is unknown whether local cold temperature can lead to the same effects as environmental cold temperature.
- Additionally, there is a lack of research exploring the transcription of mitophagy with exercise and temperature combined.
- Core temperature remains constant in cold environments and local cold. Intramuscular temperature was significantly different in response to local cold application. Intramuscular temperature may have a role in the outcomes of gene expression in human skeletal muscle.





METHODS

- 12 recreationally active males and females between the ages of 19 and 45 will complete two visits to the Exercise Physiology Laboratory.
- During the initial visit, body composition will be determined through hydrostatic weighing. Subjects will also perform a graded exercise test until volitional exhaustion on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA), and expired gases will be measured using a metabolic cart (ParvoMedics, TrueOne Metabolic System, Sandy, Utah) to evaluate VO_{2peak}.

METHODS

- The initial visit and the experimental trial will be separated by at least 48 h to allow for recovery.
- During the exercise trial, thermal pads will be placed on both legs. One leg will randomly be assigned as the experimental leg.
- The pad on the experimental leg will be cooled (ICE).
- The other leg's pad will not be turned on and serve as a control (CON).
- Prior to exercise, a muscle biopsy will be taken from the *vastus lateralis* using the percutaneous needle biopsy technique with suction (Bergstrom, 1962).
- Intramuscular temperature will be monitored during the experimental trial.
- Following the pre-exercise biopsy, participants will cycle at 65% W_{peak} (determined from VO_2 max test) for 60 minutes on a Lode cycle ergometer.
- Post-exercise muscle biopsies will be taken immediately after cycling, and following 4-h of recovery in a supine position at 20 ºC.
- All mRNA analysis will be measured using qRT-PCR normalized using $2^{-\Delta\Delta CT}$ method.
- Differences will be analyzed via repeated measures two-way ANOVA (time x trial).
- If a significant F-ratio is detected, variance will be determined using Fishers protected least significant difference method.
- Data indicating a probability of less than 5% for type I error ($p<0.05$) will be deemed significant.

IMPLICATIONS

- Despite conflicting evidence of acute local cold application, ice is still commonly used because it is a cost-effective treatment that is easily obtainable and carried out at home. This data may establish the effectiveness of local cold application during endurance exercise.
- Furthermore, future research could determine the effectiveness of this protocol as a potential therapy for mitochondrial dysfunction.
- This work will be supported by the Nebraska IDeA Networks of Biomedical Research Excellence (INBRE), National Institute for General Medical Science (NIGMS P20GM103427) and a Graduate Research and Creative Activity Grant.