AWARD NUMBER: W81XWH-15-2-0075

TITLE: Evaluation of the Physiological Challenges in Extreme Environments: Implications for Enhanced Training, Operational Performance and Sex-Specific Responses

PRINCIPAL INVESTIGATOR: Brent C. Ruby

CONTRACTING ORGANIZATION: The University of Montana

REPORT DATE: October 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE		*Form Approved OMB No. 0704-0188*
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**		
1. REPORT DATE OCTOBER 2019	**2. REPORT TYPE** Annual	**3. DATES COVERED** 30SEPT2018 – 29SEPT2019
4. TITLE AND SUBTITLE Evaluation of the Physiological Challenges in Extreme Environments: Implications for Enhanced Training, Operational Performance and Sex-Specific Responses		**5a. CONTRACT NUMBER** W81XWH-15-2-0075 **5b. GRANT NUMBER** **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Brent Ruby, Dustin Slivka E-Mail: brent.ruby@mso.umt.edu		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Montana, WPEM, Missoula, MT, 59812 University of Nebraska at Omaha, Omaha, NB, 68182		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		

14. ABSTRACT

The specific aim of this project series was to determine the impacts of environmental conditions on specific markers of exercise response under normobaria sea level, hypoxic, and high altitude derived hypobaria. This has culminated in the design of a final field trial that took place in Kailua-Kona, HI chosen because of its accessibility to both sea level and high altitude (~14,000 feet elevations. This trial effectively examined the translation of training/operational environments in reference to hypobaric and hypoxic environments. Recreationally active males (n=8) and females (n=8) served as study participants. Briefly, subjects were flown from the mainland United States to Kailua-Kona, HI. Subjects were studied on days 2, 3 and 4 after arrival. Subjects did not have their diets or physical activity controlled outside of time spent exercising restriction. In a repeated measures crossover design, three 90-minute altitude exposures were employed. It takes approximately 90 minutes to ascend Mauna Kea (~14,000 feet) by 4WD automobile. Therefore, the three interventions were a normobaric normoxia (sea-level control), graded hypoxic exposure (normobaric hypoxia) designed to mimic the gradual ascent to 14,000 feet, and a high altitude exposure (hypobaric hypoxia) inclusive of the time spent reaching 14,000 feet. Following baseline measurements, participants then completed 3 minutes of bench step exercise (39 cm step, 22 steps/min, estimated VO2=25 ml/kg/min). Key dependent variables included measures of muscle and brain oxygenation, HR, SV, CO, SpO2 and fluid shifts. Data were analyzed using 2-way (sex x trial) and 3-way (sex x trial x exercise time). A type I probability error of less than 5% was considered significant ($p < 0.05$). At the time of this report, data specific to main effects of trial and time have been evaluated. Specific sex comparisons have not yet been considered. Much of the initial data analyses was completed to provide information for the Annual meeting in October, 2019. Muscle oxygenations was altered by exercise but there were minimal differences across environments. In contrast, brain oxygenation was minimally influenced by exercise but demonstrated significant decreases during both normobaric and hypobaric hypoxia. Cardiac output was increased due to HR control during hypoxia and further by SV during hypobaric hypoxia. Plasma volume and body water shifts (decrease) were only evident during hypobaric hypoxia. These data demonstrate unique differences between normobaric and hypobaric hypoxia under semi-field conditions.

15. SUBJECT TERMS
environmental stress, hypoxia, hypobaria, oxygen saturation, sex differences

16. SECURITY CLASSIFICATION OF: Unclassified			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	26	

Table of Contents

Evaluation of the physiological challenges in extreme environments: Implications for enhanced training, operational performance and sex-specific responses

1. INTRODUCTION:

The implications of acute mountain sickness (AMS) and/or the reduced work capacity during high altitude operations diminish the effectiveness of the U.S. warfighter (in comparison to combatants native to these environments). The majority of acclimation strategies displace other key aspects of mission specific planning because of the time intensive requirements. Laboratory based research typically uses a hypoxic environment to simulate altitude. However, certain physiological responses may differ between hypoxic conditions and hypobaric conditions. Therefore, during task 1, our intention was to determine differences in our proposed markers of mitochondrial development and oxidative stress between normoxic normobaric conditions, hypoxic conditions, and hypobaric conditions. This preliminary project was established to ensure that the overall information gained from this project series has direct application to conditions in which high altitude military operations occur.

Task 4, using results from task 1, was the capstone project for this research series. This capstone project included actual field based trials in Kailua-Kona, HI due to the ease of access to both sea level and high altitude (~14,000 feet) elevations. This approach allows the information gained from this research series to have direct application to the proposed end-user, the warfighter. For this capstone project, w*e hypothesized that there will be no difference between hypoxic and hypobaric conditions and will further validate our methodological approach to the issues related to safety and performance during high altitude operations.*

The aim of this project is to translate our previous normoxic normobaric conditions, hypoxic conditions, and hypobaric conditions responses to short term (~90 minute) altitude exposures to determine if the observed laboratory responses translate to training/field based operations. This study involves both male (n=8) and female (n=8) participants. The inclusion of both males and females will enable considerable sex comparison measures that may exist when in the field. These results may also elucidate a low time cost and comparable acclimatization response when hypobaric exposure is considered against hypoxic exposure for training operations. This study effectively moves the focus of our research (and others) from a descriptive laboratory approach to an applied field approach for greater warfighter generalizability and usability.

2. KEY WORDS:

environmental stress, hypoxia, hypobaria, oxygen saturation, sex differences

3. ACCOMPLISHMENTS:

Despite delays in our sample analyses procedures due to the relocation of Dr. John Quindry from Auburn University to the University of Montana and dual testing location during years 2 and 3 (FY17 and FY18, respectively), we have completed study participant testing within previously projected time frames. We have achieved a cumulative sample size n=8 males and n=8 females for the capstone field project. Below outlined an up to date sequence of accomplishments progressing through the completion of data collection, initial analyses, and drafting of a manuscript.

Prior accomplishments are included below to show how the project sequence grew to specifically influence the final capstone project.

1. The 2017 USMRMC Extreme Environments program review was attended in October 2017 and an update on FY17 work and progress was delivered. At this time the SOW was discussed and revised to change the direction of the originally planned study 3. The revised SOW included a continuation of the environmental training study model but with the inclusion of an additional N=24 females (12 from each site location, UM and UNO) and became the revised **(Task 3)**.

2. Data collection for all three phases of study 2 was completed in November of 2017. This included the final group of males (n=12). Each phase of study 2 included testing and environmental specific training for a total sample size of N=36 males (n=18 from each site location, UM and UNO), **(Task 2)**.

3. UM and UNO IRB documents associated with the inclusion of female participants were submitted and approved in December of 2017. The inclusion of female participants serves as the basis for study 3 of the project series **(revised Task 3)**.

4. One abstract was prepared and submitted for presentation at the National ACSM meeting (Submitted November 1, 2017) - *Blood Oxidative Stress Following Exercise Recovery in Normobaric and Hypobaric Hypoxic Environments.*

5. The initial submission of a manuscript from study one was included in the Annual Report (2017). After the submission of the 2017 report, the final draft was completed and the manuscript was submitted for review (High Altitude Medicine and Biology) in December of 2017 **(Task 1)**. *Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Exercise.*

6. The initial reviewer comments of our first manuscript from study 1 *(Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After*

Exercise) were extremely delayed but finally received in the early spring of 2018. These were addressed and re-submitted to the journal for consideration.

7. Additional reviewer comments for the initial study 1 manuscript *(Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Exercise)* were received and the responses to the second round of reviewer comments were underway at the time of submitting the 2018 Annual Report.

8. The abovementioned manuscript *(Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Exercise)* was accepted for publication.

9. Results from **Task 1**, as outlined in the above-mentioned manuscript, were used to design the capstone **Task 4** project.

10. Capstone specific University approved IRB was received on August 16, 2018 with revised methods approved on December 6, 2018. Similarly, ARMY HRPO approval was received on December 19, 2018.

11. Planning, equipment inventory, and logistics for the capstone project was completed in August – December, 2019 and pilot testing occurred in a mock trial with associated travel in early December, 2019

12. Study subject recruitment began in late December (12/28/19).

13. Study travel and associated data collection occurred January 8, 2019 to February 8, 2019.

Accomplishments specific to FY 19 (year 4).

14. Participant recruitment began on 12/28/19

15. The research team traveled to Kaliua-Kona, HI from Missoula, MT and Omaha, NE on January 8, 2019

16. The first round of subjects arrived in Kailua-Kona, HI on January 11, 2019, effectively beginning data collection. Data collection was completed over 4 weeks, ending on February 6, 2019.

17. Data organization occurred concurrently while in Kailua-Kona, HI, and is ongoing at the time of this report.

18. Initial manuscript preparations are in place with anticipation of completion by mid-later, 2020.

Methodology:

The following represents the basic methodology for the data collection surrounding **Task 4**, the capstone project, of the study series.

Study 4: Effects of environmental temperature on exercise response and adaptation in males and females.

Our previous DOD funding has allowed us to accumulate meaningful data from men in response to hypoxic exposure. We have demonstrated that muscle hypoxic signaling, oxidative stress, and markers of physical performance adaptations to be intact when exposed to a simulated altitude of 3000 m compared to ambient conditions. However, when exposed to an altitude of 5000 m these responses are severely blunted (Ruby and Slivka, in progress). Due to the known hormonal sex differences, women exhibit a smaller decrease in aerobic capacity when exposed to altitude (Bhaumik, Dass, Lama, & Chauhan, 2008), and a higher oxygen saturation of hemoglobin (Ricart, Pages, Viscor, Leal, & Ventura, 2008). The muscle mitochondrial and oxidative response in women may also be altered with altitude exposure. We propose to include an additional experimental group composed of women in this project in order to determine possible sex-differences. *We hypothesize that there will be differences between men and women in response to hypoxia and hypobaria.* Due to the different altitude induced responses in aerobic capacity and oxygen hemoglobin saturation women may have a more favorable response. However, the hormonal differences between men and women may dominate the muscle multi-systemic response and thus put women at a disadvantage. The response to our acute hypoxic and hypobaric protocols between men and women will allow us to determine the possible safety issues with regards to oxidative stress that may impact physical function during training for and completion of high altitude operations.

Participants. Participants include recreationally active males (n=8) and females (n=8). Testing occurred in Kailua-Kona, HI due to accessibility to both sea level and high altitude (~14,000 feet) elevations. All participants provided written informed consent approved by the University Institutional Review Board and USAMRMCC Office of Research Protections prior to commencement of testing.

Experimental Design. In a repeated measures cross over design, subjects were acutely exposed to 90 minutes of three different environmental conditions. Normobaric normoxia at sea level, graded normobaric hypoxia at sea level, and ascent via automobile to the top of Mauna Kea (~14,000 feet), graded hypobaric hypoxia. Study trials were completed on days 2, 3, and 4 upon arrival to Kona.

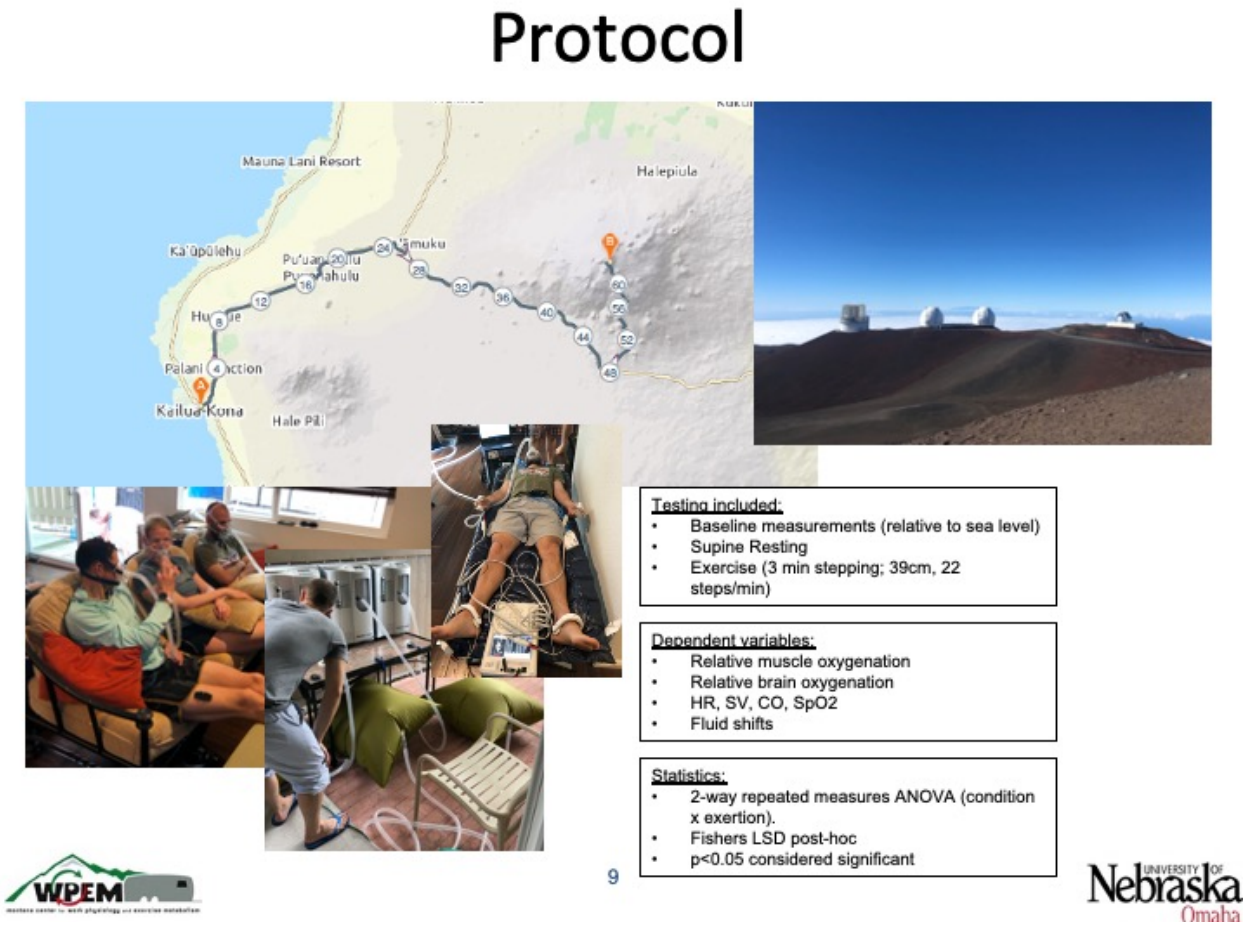
Participants

Eight males and eight females were recruited from the university and local communities to take part in the study. Participants were required to pass a pre-

screening Physical Activity Readiness-Questionnaire. Participants signed an informed consent form that was approved by the university Institutional Review Board and the Army HRPO office.

Experimental Protocol

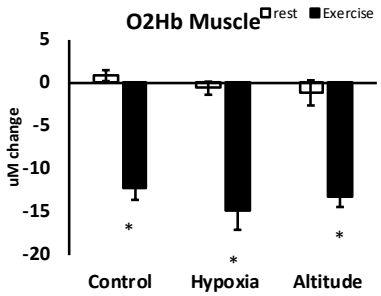
Participant diet was not controlled, however low-moderate intensity exercise was limited to 30 minutes per day. After arrival to Kailua-Kona, HI testing began on day 2 and was repeated on days 3 and 4.

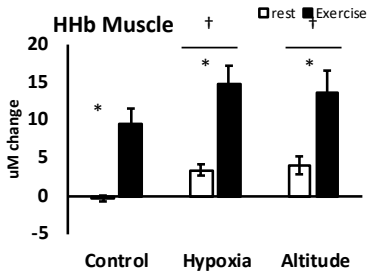


Results:

Results/Conclusions

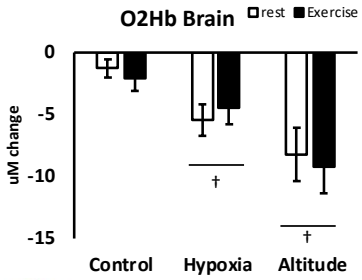
Muscle and Brain Oxygenation

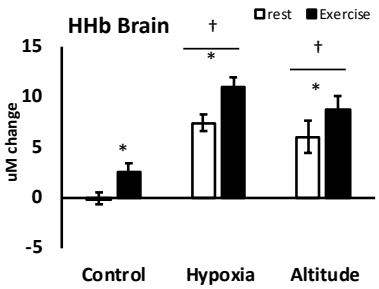




Muscle oxygenation is effected by exercise, but minimal effect of hypoxia or altitude.

Tissue Specific Response



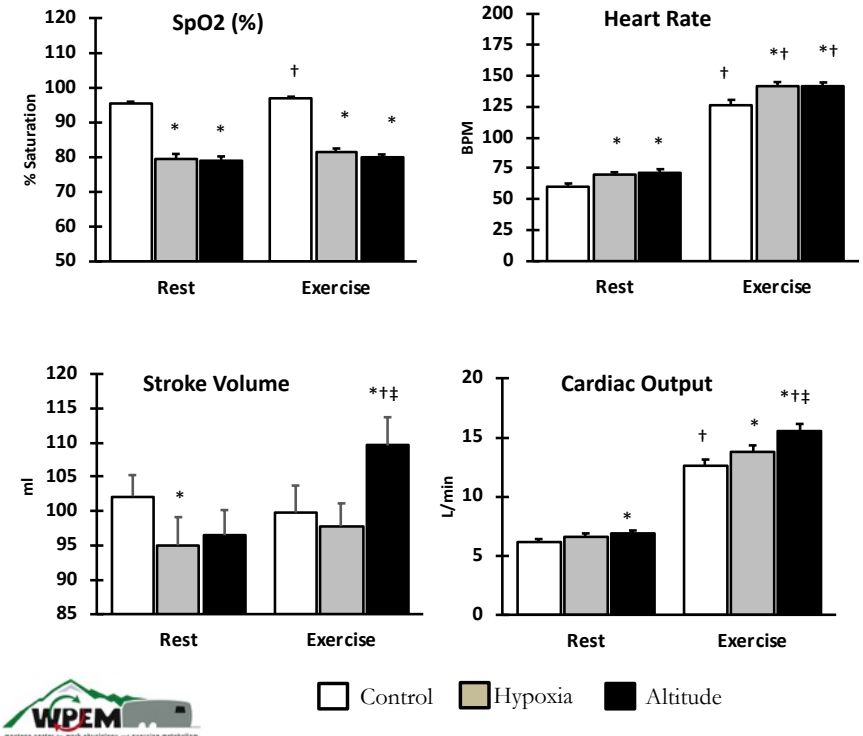


Brain oxygenation is effected by altitude, but minimal effect of exercise.

* p<0.05 vs. rest (main effect of exercise)
† p<0.05 vs. control (main effect of hypoxia)

Results/Conclusions

cardiovascular

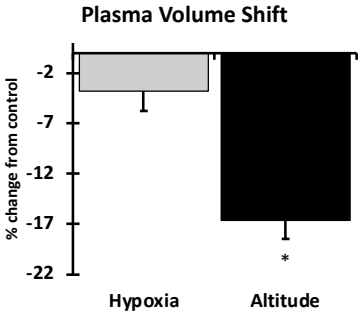


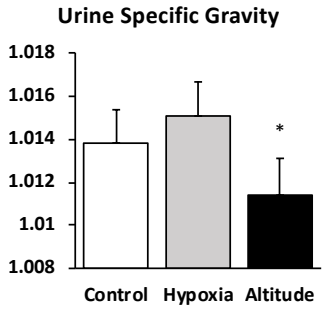
Cardiac Output is increased primarily by HR with hypoxia (typical observations) and then further by stroke volume with altitude.

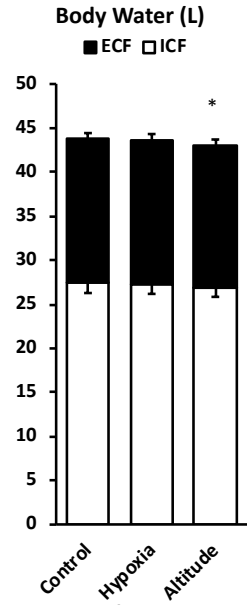
* $p<0.05$ from control
† $p<0.05$ from rest
‡ $p<0.05$ from hypoxia

Results/Conclusions

Fluid shifts







Greater fluid shifts (water loss) with altitude exposure than hypoxia and control

* $p<0.05$ from Control and Hypoxia

Opportunities for Training and Professional Development:

Nothing to report.

Dissemination of results:

Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Exercise) was accepted for publication during this reporting period.

Plans for the next reporting period:

With the completion of **Task 4** we plan to finalize remaining data analyses and drafting of additional manuscripts for journal submission. Additional abstracts and presentations are also planned for the next reporting period.

4. IMPACT:

The impact of this field project is yet to be determined based on the early, initial results. However, these data will provide foundational research describing the impacts specific exercise approaches may act to counter the deleterious impacts of high altitude or other environmental stressors. Moreover, at present, minimal sex specific responses have been noted. This may impact programmatic procedures related to training methodologies during high altitude or hot deployments and be uniformly applied to male and female warfighters.

Impact on the development of the principal discipline

Nothing to report at this time.

Impact on other disciplines

Nothing to report at this time.

Impact on technology transfer

Nothing to report at this time.

Impact on society beyond science and technology

Nothing to report at this time.

5. CHANGES/PROBLEMS:

There were limited changes or problems encountered during the accomplishment of task 4. Although travel logistics and flight delays can occur when the entire subject pool for the present study required relocation from the mainland to Kona, HI, we did not experience any delays or problems. Moreover, the weather at the summit of Mauna Kea is variable during January. Often the road to the summit is closed due to ice and snow if weather moves in. We experienced only 1 weather delay during our entire testing period. However, a weather system did move in during early February causing the summit to close for multiple weeks. All of our data collection was completed without issue.

Changes in approach

No changes are anticipated.

Delays and resolutions

No delays or resolutions are anticipated.

Changes on expenditures

The project was briefly expected to go over budget; however the necessary adjustments were made. The project, at the time of this reporting, has an available balance of . Cumulative spending has amounted to

Changes in human subjects

No changes in human subjects occurred for year 4 of the project.

6. PRODUCTS:

Other than publication of, *Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Exercise*, there are no products to report at this time.

See attached pdf.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Montana

Name: Brent Ruby
Project Role: PI
Researcher Identifier (ORCID ID): 0000-0002-3565-8094
Nearest person month worked: 5
Contribution to Project: Dr. Ruby coordinated study design, implementation, sample and data collection, and reporting.
Funding Support:

Name: Walter Hailes
Project Role: Research Associate
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 5
Contribution to Project: Mr. Hailes coordinated study participant recruitment and management and organized/conducted data collection.
Funding Support:

Name: John Quindry
Project Role: Co-investigator
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 2
Contribution to Project: Dr. Quindry is organizing data analyses for the oxidative stress markers.
Funding Support:

Nebraska

Name: Dustin Slivka
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 6
Contribution to Project: Dr. Slivka assisted in study design, implementation, sample analysis, statistical analysis, and reporting.
Funding Support:

Name: Roksana Zak
Project Role: Graduate Student (doctoral)
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 6
Contribution to Project: Ms. Zak performed skeletal muscle gene expression analysis
Funding Support:

Name: Caleb Ross
Project Role: Graduate Student (masters)
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 6
Contribution to Project: Mr. Ross assisted in the muscle processing and analysis.
Funding Support:

8. SPECIAL REPORTING REQUIREMENTS

Quad Chart: See attached.

Budget Update:

Total expenditures during FY 19 (October 1, 2018-October 1, 2019) were
.

Cumulative spending has amounted to

Evaluation of the Physiological Challenges in Extreme Environments: Implications for Enhanced Training, Operational Performance and Sex-Specific Responses

W81XWH-15-2-0075

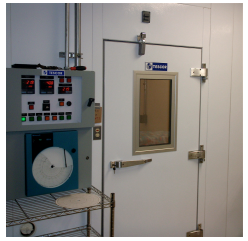
PI: Brent C. Ruby, Ph.D., FACSM **Org:** The University of Montana **Award Amount:** 2,652,591

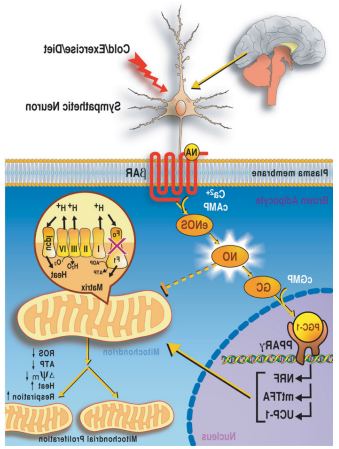
Study/Product Aim(s)

- To determine the physiological differences between hypobaria and hypoxia using markers of mitochondrial biogenesis and oxidative stress (including an evaluation of sex differences).
- To determine the effect of environmental temperature on exercise response and adaptation in untrained males (year 2) and females (year 3).
- To implement laboratory protocols in the field to ensure translation to the training/operational environment.

Approach

The overall aim of the proposed research is to determine the outcomes of specific training and environmental interactions that may serve as countermeasures to the known performance decrements in extreme environments. This project series extends our previous DOD funded research describing the negative physiological consequences of operations in extreme environments to include novel countermeasures to mitigate these effects. Additionally, this project adds novel insight into sex-differences during acute and extended exposure to varied environmental stress.





Accomplishments: Data collection has been completed for study II a and IIb. Initial manuscripts are being prepared for study I. Sample analyses are completed for study IIb. The field study was initiated and completed in January of 2019. Data analyses continues.

Timeline and Cost

Activities / CY	16	17	18	19
Study I (hypobaria vs. hypoxia)				
Study II (Environ. and adaptations - males				
Study III (Environ. and adaptations - females				
Study IV (Field study protocol)				
Estimated Budget ($K)	**$658**	**$637**	**$646**	**$712**

Updated: (October, 2019)

Goals/Milestones (Example)

CY16 Goal – Complete Study I – Hypobaria vs. Hypoxia (including a sex differences evaluation)

✔ Data collection
✔ Sample/data analyses and presentation/pub/annual report
✔ Initial manuscript prepared

CY17 Goals – Complete Study II – Environmental impact on muscle adaptations.

✔ HRPO approval, recruitment, data collection
✔ Sample/data analyses and presentation/pub/annual report
✔ Initiate study III

CY18 Goal – Complete Study III – Environmental training – female cohort

✔ HRPO approval, recruitment, data collection
✔ Sample/data analyses and presentation/pub/annual report

CY19 Goal – Complete Study IV – Field translation study

✔ HRPO approval, recruitment, data collection
✔ Sample/data analyses and presentation/pub/annual report

Comments/Challenges/Issues/Concerns

No timeline adjustments necessary.

Budget Expenditure to Date –$ 2,596,022.44

HIGH ALTITUDE MEDICINE & BIOLOGY
Volume 20, Number 2, 2019

DOI: 10.1089/ham.2018.0147

Skeletal Muscle mRNA Response to Hypobaric and Normobaric Hypoxia After Normoxic Endurance Exercise

Caleb I. Ross,[1] Robert J. Shute,[1] Brent C. Ruby,[2] and Dustin R. Slivka[1]

Abstract

Ross, Caleb I., Robert J. Shute, Brent C. Ruby, and Dustin R. Slivka. Skeletal muscle mRNA response to hypobaric and normobaric hypoxia after normoxic endurance exercise. *High Alt Med Biol*. 20:141–149, 2019.
Background: The physiological effects of hypoxia may be influenced by how hypoxia is achieved. The purpose of this study was to determine the effects of recovery in hypobaric hypoxia (HH), normobaric hypoxia (NH), and normobaric normoxia (NN) after endurance exercise on gene expression related to mitochondrial biogenesis, myogenesis, and proteolysis.
Methods: Fifteen recreationally trained subjects each cycled for 1 hour before recovering for 4 hours in NN (laboratory atmospheric conditions, 975 m), HH (depressurized to simulate 4420 m), and NH (fraction of O_2 reduced to simulate 4420 m). Muscle biopsy samples were obtained before exercise and after 4 hours of recovery.
Results: Blood oxygenation (SpO_2) was lower in HH ($76.02 \pm 0.58\%$) than NH (79.45 ± 0.56, $p < 0.001$), which were both lower than in NN (96.3 ± 0.17, $p < 0.001$). Heart rate was higher in HH (82 ± 2 bpm) than NH (77 ± 1 bpm, $p < 0.001$), which were both higher than in NN (67 ± 1 bpm, $p < 0.001$). *Mitochondrial transcription factor A (TFAM)* mRNA was lower after NN than HH ($p = 0.034$) or NH ($p = 0.005$), but was not different between HH and NH ($p = 0.460$). *Myostatin (MSTN)* mRNA decreased from pre- to postexercise ($p < 0.001$) in all conditions and was lower in HH compared with NH ($p = 0.035$) and NN ($p = 0.017$). No other differences were noted in genes related to mitochondrial biogenesis, myogenesis, or proteolysis ($p > 0.05$).
Conclusion: *TFAM* mRNA is lower with hypoxia exposure, but effected by the type of hypoxia. *MSTN* gene expression is lower after exposure to HH than NH or NN. These data support previous work and caution the translation of NH data obtained in a NH environment to a HH environment.

Keywords: environment; mitochondrial biogenesis; myogenesis, proteolysis

Introduction

The environmental conditions, in which recovery takes place after exercise, may have implications on the cellular and physiological outcomes of that exercise bout. Previous researches from our laboratory have investigated a number of skeletal muscle responses following recovery from endurance exercise in environmental temperatures (Slivka et al., 2012; Zak et al., 2017), local temperature application (Tucker et al., 2012), and normobaric hypoxia (NH) environments (Slivka et al., 2014). However, achieving hypoxia through a lowered oxygen fraction such as in NH may be physiologically different from terrestrial high altitude or hypobaric hypoxia (HH) where oxygen fraction remains constant, but barometric pressure is lower.

Limited research has directly compared these different hypoxia modalities and a complete understanding is lacking. The research that does exist tends to indicate that HH is a more severe form of hypoxia than NH. Blood oxygen saturation (SaO_2) appears to be lower at rest in HH compared with NH (Evetts et al., 2005; Boos et al., 2016) and further be altered after exercise along with other measures of cardiac adaptations (Boos et al., 2016). The differences in the physiological responses to HH and NH hypoxia appear to translate

[1]Exercise Physiology Lab, University of Nebraska at Omaha, Omaha, Nebraska.
[2]Montana Center for Work Physiology and Exercise Metabolism, University of Montana, Missoula, Montana.

into applied effects. For example, cycling time trial performance is impacted more during exposure to HH than NH, despite similar blood oxygen saturation (SaO_2) and cardioventilatory parameters (Beidleman et al., 2014), and symptoms of acute mountain sickness have been reported to be higher with HH than with NH (DiPasquale, 2016). Furthermore, 1 week of NH sleep acclimation does not appear to translate into benefits in a HH environment (Fulco et al., 2011). While the effect of HH and NH on the cardiovascular system and performance is beginning to emerge, there is a lack of detail on the effects on the skeletal muscle.

One such effect on the skeletal muscle that is of interest in relationship to hypoxia is the production of mitochondria. Mitochondrial biogenesis occurs with cellular stress such as endurance exercise (Irrcher et al., 2003; Wright et al., 2007). However, hypoxic stress has demonstrated decrements in mitochondrial development (Ferretti, 1990; Hoppeler et al., 1990; Howald et al., 1990; Kayser et al., 1991). Previous evidence suggests that no differences occur in mitochondrial-related gene expression (*COX, PGC-1, FIS-1, MFN-1*, and *OPA-1*) between NH recovery compared with normobaric normoxia (NN) recovery from endurance exercise (Slivka et al., 2014). However, this study design did not compare these responses to a HH recovery environment. Therefore, it is currently unknown if HH recovery from exercise would regulate mitochondrial mRNA differently from NH or NN recovery.

Hypoxic conditions lead to specific signaling events with functional significance in skeletal muscle (Hoppeler et al., 2008). Hypoxia activates proteolytic regulator genes such as *FOXO3* to initiate cell death (Bakker et al., 2007; de Theije et al., 2013). Yet, there exists research that refutes the role of hypoxia in the activation of proteolysis (Favier et al., 2010; Manimmanakorn et al., 2013). Contradiction similarly exists on hypoxia's role in the regulation of myogenesis. Acute hypoxia exposure impairs myoblast differentiation and attenuates myofiber development (Di Carlo et al., 2004; Yun et al., 2005; Chaillou et al., 2014). However, additional literature suggests that exercise in hypoxic conditions improves myogenic activity by promoting muscle growth and repair in humans (Manimmanakorn et al., 2013). The differences in muscle mass regulating genes between HH and NH exposure have not been established and may aid in the interpretation of previous research as the method of achieving hypoxia is not consistent between studies.

The purpose of this study was to determine the response of key genes related to mitochondrial biogenesis and muscle mass regulation during HH, NH, and NN recovery after exercise. Determining the cellular responses of the skeletal muscle in HH and NH methods of achieving hypoxia will enhance the current literature and advance the understanding of hypoxia's effects on mitochondrial development and skeletal muscle mass regulation in addition to the interchangeability of NH and HH.

Materials and Methods

Subjects

Eight recreationally trained male and seven recreationally trained female subjects who engaged in endurance training for at least 30 minutes three times per week participated in this study. They were required to be between the ages of 18 and 40 years and have a VO_{2peak} of at least 45 mL/(kg · min). Those who previously experienced serious acute mountain sickness, or had a known risk factor for coronary artery disease assessed through a physical activity readiness questionnaire, were excluded from the study. Furthermore, female participants taking birth control influencing hormonal status or those who did not have a regular menstrual cycle in the past 8 months were excluded. Subjects signed the Institutional Review Board and USAMRMC Human Research Protections Office-approved informed consent form, which conformed to the Declaration of Helsinki, before testing.

Preliminary testing

Descriptive data included height (Seca 213 Statiometer; United Kingdom), weight (Befour PS-660 ST Digital Scale; Saukville, WI), body composition, and VO_{2peak}. Body composition for each subject was assessed through hydrostatic weighing using an electronic load cell-based system (Exertech, Dresbach, MN) correcting for estimated residual lung volume. Body density from this underwater weight was converted to percent body fat using the Siri equation (Siri, 1993). Peak oxygen uptake (VO_{2peak}) was obtained for each participant using a graded exercise protocol starting at 95 W and increasing by 35 W every 3 minutes on an electronically braked Velotron, cycle ergometer (RacerMate, Seattle, WA). Cycling continued until volitional fatigue, and the highest obtained oxygen uptake value was considered the VO_{2peak}. Maximum workload (Wmax) was calculated by taking the time completed in the last stage divided by the total stage duration (3 minutes) multiplied by 35 W and added to the watts of the last completed stage. Expired gases were analyzed every 15 seconds throughout the exercise test using a flow and gas calibrated metabolic cart (ParvoMedics TrueOne 2400; Sandy, UT).

Experimental protocol

All subjects completed three trials in a randomized, counter-balanced order. Each trial was separated by ~7 days to allow for biopsy recovery and to minimize carryover acclimation between trials. Subjects reported to the laboratory in the early morning following an overnight fast. Subjects maintained a 24 hours dietary and 48 hours activity log before the first trial and replicated these for the subsequent trials. The exercise trial consisted of a 60-minutes bicycle ride on a cycle ergometer at a constant intensity of 70% of their power associated with VO_{2peak} in ambient conditions (975 m laboratory environment). Each subject drank water *ad libitum* during the first ride, and the amount was replicated for all subsequent trials. Following cessation of exercise, 4 hours of passive recovery occurred inside a small ($32'' \times 7'$) tube shaped altitude chamber (Engineering Innovations, LLC, Littleton, CO) capable of lowering barometric pressure to simulate HH. This tube was located inside of an oxygen-controlled environmental chamber (Tescor, Warminster, PA) capable of lowering the fraction of oxygen in the air to simulate NH. They received one short break after 2 hours of recovery. The experimental recovery conditions were simulated to the following altitudes:

1. NN; 975 m (3200′) Atmospheric conditions by having both the hypobaric tube and oxygen controlled chamber off so that participants breathed ambient air.

2. HH; 4420 m (14,500′) HH by having the hypobaric tube depressurized altering the barometric pressure, while the oxygen-controlled chamber was set to off.
3. NH; 4420 m (14,500′) NH by having the hypobaric tube off and the oxygen controlled chamber on.

Oxygenation saturation and heart rate

Blood oxygen saturation (SpO_2) and heart rates were measured using a finger pulse oximeter (Nonin WristOx2 3150; Plymouth, MN) during exercise and again every 60 minutes during passive recovery. Oxygen saturation and heart rates were recorded on the hour by having the device placed on the finger and allowed to stabilize for ~30 seconds.

Biopsies

Muscle biopsies were taken from the vastus lateralis before exercise and after 4 hours of recovery in each trial. The second muscle biopsy was extracted from a separate incision ~2 cm proximal to the preexercise biopsy. Each sample was extracted using a 5 mm Bergstrom percutaneous biopsy needle with the aid of suction. The leg was chosen in a random, counter-balanced order. After cleaning the site, ~3–4 mL of 1% lidocaine was injected under the skin surface and around the muscle fascia before a small incision through the skin and muscle fascia was made. Once the muscle tissue had been obtained, the sample was quickly cleaned of excessive blood, connective tissue, and fat before being placed in All-protect (Qiagen, Hilden, North Rhine-Westphalia, Germany). Samples were placed overnight at 4°C and then transferred to −30°C for storage. All subsequent trials repeated this process by alternating legs.

Muscle sample preparation and quantitative reverse transcription-polymerase chain reaction

A piece of skeletal muscle (11.5 ± 1.7 mg) was homogenized in 500 μL of TRIzol (Invitrogen, Carlsbad, CA) using an electric blender homogenizer (Bullet Blender; Next Advance, Inc., Averill Park, NY) utilizing 1.5 mL Red RINO tubes prefilled with RNase-free ceramic beads (Next Advance, Inc.). The samples were centrifuged at 12,000 *g* for 15 minutes, and the aqueous phase was then transferred to a fresh 1.5 mL tube and incubated overnight at −20°C. Samples were centrifuged the next morning, and the supernatant was removed. Ethanol was added followed by a 5 minutes centrifugation at 7500 *g*. The ethanol was then removed, the pellet dried, and then redissolved in 30 μL of RNase-free water. RNA concentration was quantified using a nanospectrophotometer (nanoDrop ND-2000; Thermo Scientific, Wilmington, DE). Average RNA yields were 165.9 ± 12.1 ng/μL. The average absorbance ratio at 260:280 was 1.90 ± 0.00 indicating high purity of the RNA. The RNA integrity of the samples were assessed using an Agilent RNA 6000 Kit and a 2100 Bioanalyzer (both from Agilent Technologies, Santa Clara, CA) according to manufacturer's instructions. The RNA integrity number was 8.0 ± 0.1, indicating that the RNA was intact.

First-strand cDNA synthesis was achieved using the Superscript IV first-strand synthesis system for RT-PCR Kit (Invitrogen) according to manufacturer's instruction. The resulting cDNA was diluted with the appropriate amount of RNase-free water to achieve a final cDNA concentration of 0.5 μg/μL in the PCR reaction. Each 10 μL quantitative reverse transcription-polymerase chain reaction (qRT-PCR) volume contained 0.5 μL of probe and primer mix (PrimeTime qPCR assay Integrated DNA Technologies, Coralville, IA), 5 μL qPCR Master Mix (Integrated DNA Technologies), and 4.5 μL of sample cDNA. PCR was run in triplicate on a Stratagene mx3005p PCR system (Agilent Technologies) using a two-step protocol (1 cycle at 95°C for 5 seconds followed by 60°C for 20 seconds for 50 cycles).

Mitochondrial biogenesis-related genes included *peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)*, *estrogen-related receptor alpha (ERRα)*, *GA-binding protein alpha (GABPA)*, *nuclear respiratory factor 1 (NRF-1)*, and *mitochondrial transcription factor A (TFAM)*. The myogenic genes of interest involved in muscle hypertrophy are *myogenic differentiation factor (MYOD)*, *myostatin (MSTN)*, *myogenin (MYOG)*, *myogenic factor 5 (MYF-5)*, and *myogenic factor 6 (MYF-6)*. Proteolytic genes of interest involved in muscle atrophy are *forkhead box O3 (FOXO3)*, *atrogin-1*, and *muscle ring finger 1 (MuRF-1)*.

Quantification of mRNA for genes of interest was completed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). For each participant, the geometric mean of five housekeeping genes *beta-actin (ACTB)*, *beta-2-microglobulin (B2M)*, *cyclophilin (CYC)*, *ribosomal protein S18 (RPS-18)*, and *glyceraldehyde-3 phosphate dehydrogenase (GAPDH)* was used as the stable reference point. This combination of genes was determined to be stable using NormFinder software (Andersen et al., 2004). Probe and primer sequences used for qRT-PCR are presented in Table 1.

Statistical analysis

Differences in gene expression among HH, NH, and NN condition trials were analyzed using two-way (time × trial) repeated-measures analysis of variances (ANOVAs). In the event of a significant F-ratio, Fisher's protected least significant difference method was applied to determine where differences occurred. All ANOVAs were performed using the Statistical Package for Social Sciences software (SPSS) for Windows Version 23.0 (Chicago, IL). A probability of <5% was considered significant ($p < 0.05$). For all significant comparisons, effect size (η^2 for ANOVA and Cohen's *d* for individual comparisons) was calculated. All data are reported as mean ± standard error.

Results

Participant descriptive data

Eight recreationally trained male and seven recreationally trained female participants ($n = 15$) completed this study. Male subjects were taller ($p < 0.001$, $d = 2.643$), weighed more ($p = 0.028$, $d = 1.378$), had a lower percent body fat ($p = 0.003$, $d = 2.063$), VO_{2peak} ($p = 0.001$, $d = 2.512$), and a higher cycling workload at VO_{2peak} ($p < 0.001$, $d = 3.440$) than female subjects. After initial analysis, there were no differences in any other of our dependent variables between males and females ($p < 0.05$) which is supported by previous research indicating that men and women have similar cardiopulmonary responses after acute hypoxia exposure (Boos et al., 2016). Therefore, male and female subjects were

Table 1. Probes and Primers Used for Real-Time Polymerase Chain Reaction

	Primer 1	*Primer 2*	*Probe*
Reference genes			
ACTB	AAGTCAGTGTACAGGTAAGCC	GTCCCCCAACTTGAGATGTATG	CTGCCTCCACCCACTCCCA
B2M	ACCTCCATGATGCTGCTTAC	GGACTGGTCTTTCTATCTCTTGT	CCTGCCGTGTGAACCATGTGACT
CYC	TCTTTCACTTTGCCAAACACC	CATCCTAAAGCATACGGGTCC	TGCTTGCCATCCAACCACTCAGTC
RPS18	GTCAATGTCTGCTTTCCTCAAC	GTTCCAGCATATTTTGCGAGT	TCTTCGGCCCACACCCTTAATGG
GAPDH	TGTAGTTGAGGTCAATGAAGGG	ACATCGCTCAGACACCATG	AAGGTCGGAGTCAACGGATTTGGTC
Mitochondrial genes			
PGC-1α	TGTCTGTATCCAAGTCGTTCAC	GAGTCTGTATGGAGTGACATCG	ACCAGCCTCTTTGCCCAGATCTTC
ERRα	TCTCCGCTTGGTGATCTCA	CTATGGTGTGGCATCCTGTG	TGGTCCTCTTGAAGAAGGCTTTGCA
GABPA	TGTAGTCTTGGTTCTAGCAGTTTC	TGGAACAGAGAAAGCAGAGTG	TGGTTCATTGATGTCTATGGCCTGGC
NRF-1	GTCATCTCACCTCCCTGTAAC	GATGCTTCAGAATTGCCAACC	ATGGAGAGGAACAAAATTGGGC
TFAM	GCCAAGACAGATGAAAACCAC	TGGGAAGGTCTGGAGCA	CGCTCCCCCTTCAGTTTTGTGTATTT
Myogenic genes			
MYOD	GAGATGCGCTCCACGATG	CGGAACTGCTACGAAGGC	ACAGGCAGTCTAGGCTCGACAC
MYOG	AGAAGTAGTGGCATCTGTGG	GACAGCATCACAGTGGAAGA	ATGCCCGGCTTGGAAGACAATCT
MSTN	TCGTGATTCTGTTGAGTGCT	TGTAACCTTCCCAGGACCA	TCTTTTTGGTGTGTCTGTTACCTTGACCT
MYF-5	GGCATATACATTTGATACATCAGGAC	CACCTCCAACTGCTCTGATG	TGCTGTCAAAAGTACTGCTCTTTCTGGA
MYG-6	CTACTCGAGGCTGACGAATC	CAGCTACAGACCCAAACAAGA	TGATAACGGCTAAGGAAGGAGGAGCA
Proteolytic genes			
FOXO3	CGTGCCCTACTTCAAGGATAAG	ATTCTGGACCCGCATGAATC	AGGTTGTGCCGGATGGAGTTCTTC
Atrigin-1	TCAGCCTCTGCATGATGTTC	CAACAGACTGGACTTCTCAACT	CACTGACCTGCCTTTGTGCCTACA
MURF-1	GCAACTCACTTTTCTTCTCATCC	TGCAGACCATCATCACTCAG	ACCTGGTGACTGTTCTCCTTGGTC

PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *ERRα, estrogen-related receptor alpha*; *GABPA, GA-binding protein alpha*; *NRF-1, nuclear respiratory factor 1*; *TFAM, mitochondrial transcription factor A*; *MYOD, myogenic differentiation factor*; *MSTN, myostatin*; *MYOG, myogenin*; *MYF-5, myogenic factor 5*; *MYF-6, myogenic factor 6*; *FOXO3, forkhead box O3*; *MuRF-1, muscle ring finger 1*; *ACTB, beta-actin*; *B2M, beta-2-microglobulin*; *CYC, cyclophilin*; *RPS-18, ribosomal protein S18*; *GAPDH, glyceraldehyde-3 phosphate dehydrogenase*.

pooled together for further analysis. Descriptive data are presented in Table 2.

Oxygen saturation and heart rate

No differences in SpO_2 occurred between trials at baseline, during exercise, or immediately postexercise ($p > 0.05$, average $d = 0.250$) as no experimental interventions had been introduced at these time-points. During each hour of recovery, arterial oxygen saturation in NN was higher than both HH ($p < 0.001$, average $d = 6.546$) and NH ($p < 0.001$, average $d = 5.662$). Furthermore, oxygen saturation was lower in HH compared to NH at hours 1 ($p = 0.004$, $d = 1.059$), 3 ($p = 0.032$, $d = 0.772$), and 4 ($p = 0.008$, $d = 0.886$). No differences were observed at hour 2 ($p = 0.293$, $d = 0.343$) between HH and NH. Oxygen saturation data are presented in Figure 1.

No differences occurred in heart rate between trials ($p > 0.05$, average $d = 0.200$) at baseline, exercise, or immediately postexercise, as no experimental intervention had been introduced at these time points. Heart rate in the NN condition was lower than HH ($p < 0.001$, average $d = 1.427$) and NH ($p < 0.05$, average $d = 1.005$). Furthermore, heart rate was lower in NH compared with HH at recovery hours 2 ($p = 0.020$, $d = 0.586$), 3 ($p = 0.029$, $d = 0.517$), and 4 ($p = 0.041$, $d = 0.525$). Heart rate data are presented in Figure 2.

Gene expression

There were no differences in mitochondria-related gene expression of *PGC-1α* ($p = 0.816$, $\eta^2 = 0.014$), *ERRα* ($p = 0.978$, $\eta^2 = 0.002$), or *GABPA* ($p = 0.925$, $\eta^2 = 0.006$). *NRF-1* decreased due to exercise ($p = 0.003$, $\eta^2 = 0.473$), but was not different between trials ($p = 0.748$, $\eta^2 = 0.020$). *TFAM* decreased after NN ($p = 0.048$, $d = 0.821$), increased after NH ($p = 0.024$, $d = 0.974$), but did not change with HH ($p = 0.093$, $d = 0.680$). Furthermore, *TFAM* gene expression was lower in NN compared with HH ($p = 0.034$, $d = 1.047$) and NH ($p = 0.005$, $d = 1.301$), but not different between HH and NH ($p = 0.460$, $d = 0.308$).

There were no differences in the myogenesis-related gene expression of *MYOD* ($p = 0.787$, $\eta^2 = 0.017$), MYF-5 ($p = 0.073$, $\eta^2 = 0.170$), or *MYOG* ($p = 0.837$, $\eta^2 = 0.013$) between trials or after exercise ($p = 0.327$, $\eta^2 = 0.069$; $p = 0.262$, $\eta^2 = 0.089$; and $p = 0.624$, $\eta^2 = 0.018$; respectively). *MYF-6* was higher after exercise ($p = 0.004$, $\eta^2 = 0.457$), but not different between trials ($p = 0.971$, $\eta^2 = 0.002$). *MSTN* decreased from pre- to postexercise ($p < 0.001$, $\eta^2 = 0.831$) in all conditions and was lower in HH than both NH ($p = 0.035$, $d = 0.0626$) and NN ($p = 0.017$, $d = 0.907$). NH and NN conditions were not different from each other ($p = 0.718$, $d = 0.140$). There were no differences in the proteolysis-related gene expression of *atrogin-1* with exercise ($p = 0.821$, $\eta^2 = 0.004$) or between trials ($p = 0.318$, $\eta^2 = 0.079$). FOXO3 ($p = 0.015$, $\eta^2 = 0.356$) and MuRF-1 ($p < 0.001$, $\eta^2 = 0.836$) gene expression increased with exercise but were not different between the three conditions ($p = 0.361$, $\eta^2 = 0.070$; and $p = 0.196$, $\eta^2 = 0.110$ for *FOXO3* and *MuRF-1*, respectively). Gene expression data are presented in Table 3.

Discussion

The purpose of the present study was to determine the response of key genes related to mitochondrial development and muscle mass regulation during recovery in HH, NH, and NN after exercise. The data from this investigation indicate that SpO_2 is lower and heart rate is higher in HH recovery compared with NH. In addition, both hypoxic recovery conditions had lower arterial oxygen saturations and higher heart rates than NN recovery. We also observed increased *TFAM* gene expression in hypoxic conditions compared to control conditions. Furthermore, we found *myostatin*, a negative regulator of myogenesis, to be suppressed in HH to a greater extent than NH and NN. While these differences among HH, NH, and NN were observed, several other genes associated with exercise adaptation were not affected by hypoxia, regardless of the factor used to create the hypoxic environment.

As expected, no differences in peripheral oxygen saturation or heart rate occurred at baseline, during exercise, or immediately postexercise because cycling took place in ambient conditions during each trial. However, hypoxic recovery in HH produced the lowest oxygen saturation and highest heart rate compared to NH and NN conditions. Our study is in agreement with previous literature, but not all, suggesting that cardioventilatory differences exist between hypoxic types (Tucker et al., 1983; Loeppky et al., 1997; Savourey et al., 2003). Like the current study, previous data suggest a specific response of HH compared with NH. A lowered barometric pressure modifies fluid circulation and the trans-alveolar-capillary membrane flux (Levine et al., 1988) causing constriction of the pulmonary blood vessels and decreased oxygen diffusion (Millet et al., 2012) leading to reduced arterial oxygen saturation. Therefore, further increases in heart rate during HH compensate for the reduced oxygen saturation to meet metabolic demand.

Recovery in hypoxia increased *TFAM* gene expression in the current study independent of *PGC-1α* or the method in which hypoxia was achieved. *TFAM* mRNA has previously

Table 2. Participant Descriptive Data

	Males (n = *8*)	*Females* (n = *7*)	*Combined* (n = *15*)
Age (years)	24 ± 1	24 ± 2	24 ± 1
Height (cm)	184 ± 2*	166 ± 3	178 ± 3
Weight (kg)	79.0 ± 2.7*	65.2 ± 4.1	72.5 ± 3.6
Body fat (%)	11.6 ± 1.7*	23.9 ± 2.2	17.4 ± 2.2
VO_2 peak (L/min)	4.24 ± 0.15*	3.04 ± 0.15	3.60 ± 0.20
Watt max (W)	335 ± 9*	219 ± 15	281 ± 18

Data are means ± SE.
*$p < 0.05$ males different from females.
SE, standard error.

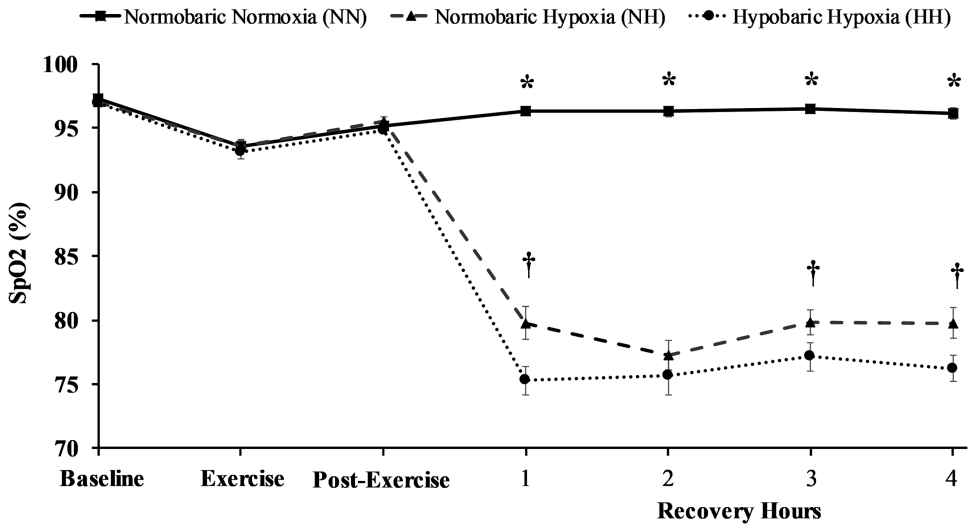


FIG. 1. Oxygen saturation between trials at baseline, during exercise, immediately post-exercise and each hour of recovery. *$p < 0.05$ NN from NH and HH, †$p < 0.05$ NH from HH. NN, normobaric normoxic; NH, normobaric hypoxic; HH, hypobaric hypoxia.

been shown to increase even in the absence of increased *PGC-1α* (Arany et al., 2005; Yin et al., 2008), postexercise (Pilegaard et al., 2003), or under hypoxic conditions (Gutsaeva et al., 2008; Yin et al., 2008; Zhu et al., 2010). Hypoxia increases *TFAM* gene expression, but does not alter *PGC-1α* in mice with ischemic brain injury (Yin et al., 2008). These data support the role of *TFAM* mRNA regulation during hypoxia exposure regardless of whether hypoxia was achieved with an alteration in barometric pressure or inspired oxygen fraction.

Changes in *myostatin* are another key outcome of the current study. *Myostatin* is a negative regulator of myogenic signaling—meaning deficiency or inhibition of this gene leads to a muscle growth stimulus and increases in this gene leads to muscle atrophy (Sandri, 2008; Gumucio and Mendias, 2013). Previous research observes decreases in skeletal muscle mass after exposure to HH during mountaineering expeditions (Hoppeler et al., 1990; Howald and Hoppeler, 2003), where other factors such as exercise and nutrition cannot be controlled. It is unknown if the reductions in skeletal muscle mass during these expeditions are associated with increases in *MSTN* expression. Interestingly, *MSTN* mRNA decreased in all conditions of the current study and was lower in HH than NH and NN. This suggests recovering from exercise in a lower barometric pressure may suppress *MSTN* to a greater extent than NH or NN recovery. Myostatin attenuation in these environments may provide a myogenic stimulus, particularly after exercise. Therefore, if HH recovery from exercise further attenuates *MSTN*, protocols may be developed to suppress *MSTN* expression further and potentially lead to a greater muscle-building stimulus. Applied research investigating the changes in muscle mass and strength incorporating HH into a training regimen is needed to further develop this hypothesis. The results from such re-

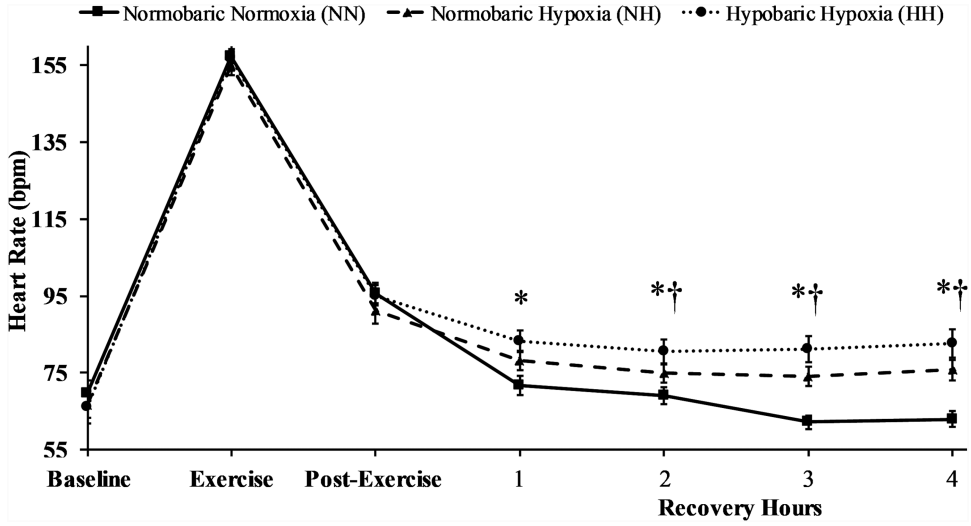


FIG. 2. Heart rate between trials at baseline, during exercise, immediately post-exercise and each hour of recovery. *$p < 0.05$ NN from NH and HH, †$p < 0.05$ NH from HH.

Table 3. Fold Change in Genes Related to Mitochondrial Development, Myogenesis, and Proteolysis in Normobaric Normoxic, Normobaric Hypoxic, and Hypobaric Hypoxia Before and Four Hours After Endurance Exercise

	NN		*NH*		*HH*	
Genes	*Pre*	*4 Hours post*	*Pre*	*4 Hours post*	*Pre*	*4 Hours post*
Mitochondrial						
PGC-1α	1.006 ± 0.001	1.098 ± 0.279	1.011 ± 0.005	1.283 ± 0.296	1.019 ± 0.012	0.888 ± 1.006
ERRα	1.047 ± 0.016	1.133 ± 0.297	1.014 ± 0.005	1.146 ± 0.209	1.049 ± 0.017	1.090 ± 0.209
GABPA	1.017 ± 0.007	0.877 ± 0.100	1.014 ± 0.005	1.129 ± 0.228	1.015 ± 0.008	1.124 ± 0.232
NRF-1	1.011 ± 0.005	0.819 ± 0.116*	1.010 ± 0.003	0.942 ± 0.102*	1.023 ± 0.012	1.008 ± 0.810*
TFAM	1.008 ± 0.002	0.895 ± 0.079*	1.021 ± 0.008	1.508 ± 0.177*,†	1.012 ± 0.003	1.311 ± 0.155†
Myogenesis						
MYOD	1.047 ± 0.014	2.430 ± 0.825	1.058 ± 0.018	1.631 ± 0.416	1.065 ± 0.014	1.593 ± 0.334
MSTN	1.029 ± 0.011	0.495 ± 0.093*	1.017 ± 0.006	0.489 ± 0.088*	1.008 ± 0.002	0.284 ± 0.051*,†,‡
MYOG	1.005 ± 0.002	1.139 ± 0.206	1.015 ± 0.008	1.026 ± 0.104	1.017 ± 0.011	1.148 ± 0.140
MYF-5	1.023 ± 0.011	1.025 ± 0.275	1.021 ± 0.008	1.862 ± 0.618	1.016 ± 0.007	1.174 ± 0.258
MYF-6	1.013 ± 0.006	1.671 ± 0.209*	1.021 ± 0.010	1.734 ± 0.381*	1.012 ± 0.005	1.901 ± 0.446*
Proteolysis						
FOXO3	1.016 ± 0.006	1.892 ± 0.372*	1.022 ± 0.009	1.338 ± 0.159*	1.019 ± 0.009	2.567 ± 0.802*
Atrogin-1	1.105 ± 0.078	2.047 ± 0.522	1.025 ± 0.009	0.920 ± 0.141	1.079 ± 0.053	2.441 ± 1.012
MURF-1	1.022 ± 0.007	4.245 ± 0.828*	1.013 ± 0.005	2.627 ± 0.529*	1.006 ± 0.001	3.775 ± 0.669*

Data are means ± SE.
*$p < 0.05$ from pre-, †$p < 0.05$ from 4 hours post-NN, ‡$p < 0.05$ from 4 hours post-NH.
NN, normobaric normoxic; NH, normobaric hypoxic; HH, hypobaric hypoxia.

search may have practical implications for improving muscle mass in various populations with limited exercise intensity capabilities.

Despite the differences that occurred among HH, NH, and NN, we observed no other effects of hypoxic recovery after exercise on select skeletal muscle gene expression. We observed a lower than expected exercise-stimulated response in the genes we measured. However, the exercise response in human gene expression appears to be affected by several variables and is generally in agreement with previous literature on mitochondrial biogenesis (Tunstall et al., 2002; Pilegaard et al., 2003; McGee and Hargreaves, 2004; Cartoni et al., 2005; Hock and Kralli, 2009), myogenesis (Yang et al., 2005; Coffey et al., 2006), and proteolysis (Sandri et al., 1995; Raue et al., 2007; Harber et al., 2009). Indeed, a subject population or exercise protocol that yielded a more robust response in gene expression may lead to a differential effect of hypoxia. Furthermore, the current subject population consisted of both males and females, and while no differences were noted, these results should be interpreted with caution as this study may be underpowered to detect these differences.

This study was completely done in the laboratory and thus factors other than barometric pressure may exist between actual terrestrial altitude and the simulations that were incorporated in this study. This study incorporated a relatively small chamber for inducing HH and thus factors such as CO_2 accumulation may have played a role. The effects of this small chamber were controlled for by having subjects placed in this tube during the NH and NN trials as well. Furthermore, the laboratory, in which this research was conducted, is located at 975 m and thus may not be a true NN control. However, all subjects resided at this elevation and were therefore acclimated to this altitude. While there are limitations to this laboratory-based design, it allowed for safe and well-controlled muscle sampling and data collection conditions.

Conclusion

When recovery from endurance exercise takes place in HH, lower oxygen saturations and higher heart rates occur compared to normobaric hypoxic recovery. In addition, Hypobaric hypoxic recovery attenuates *myostatin* to a greater extent than NH and NN after exercise. *TFAM* expression is enhanced with hypoxia with no differences between the type of hypoxia. These data suggest and support the notion that a lowered barometric pressure initiates a greater hypoxic response compared to a lowered fractional oxygen concentration. However, hypoxia did not affect several other genes associated with exercise adaptation, regardless of the factor used to create the hypoxic environment.

Acknowledgments

We thank John Cuddy and Walter Hailes for their assistance in data collection and Roksana Zak for her assistance in gene expression analysis. The research was funded by the Department of Defense United States Army Medical Research and Materiel Command (DOD USAMRMC: W81XWH-15-2-0075) and by a Graduate Research and Creative Activity grant from the University of Nebraska at Omaha.

Authors Contributions

All authors have substantially contributed to this article and have reviewed and approved this submission. C.I.R. was the primary author and wrote the majority of the article, aided in data collection, and sample analysis. R.J.S. was the primary person responsible for gene expression sample and data analysis and statistical analysis and subsequently much of the methods section. B.C.R. and D.R.S. were responsible for the conceptualization of the project, obtaining muscle samples, aiding in the data collection, analysis, and writing.

Author Disclosure Statement

No competing financial interests exist.

References

Andersen CL, Jensen JL, and Ørntoft TF. (2004). Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 64:5245–5250.

Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, Ahmad F, Matsui T, Chin S, Wu P, Rybkin II, Shelton JM, Manieri M, Cinti S, Schoen FJ, Bassel-Duby R, Rosenzweig A, Ingwall JS, and Spiegelman BM. (2005). Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. Cell Metab 1:259–271.

Bakker WJ, Harris IS, and Mak TW. (2007). FOXO3a is activated in response to hypoxic stress and inhibits HIF1-induced apoptosis via regulation of CITED2. Mol Cell 28:941–953.

Beidleman BA, Fulco CS, Staab JE, Andrew SP, and Muza SR. (2014). Cycling performance decrement is greater in hypobaric versus normobaric hypoxia. Extrem Physiol Med 3:8.

Boos CJ, Mellor A, O'Hara JP, Tsakirides C, and Woods DR. (2016). The effects of sex on cardiopulmonary responses to acute normobaric hypoxia. High Alt Med Biol 17:108–115.

Boos CJ, O'Hara JP, Mellor A, Hodkinson PD, Tsakirides C, Reeve N, Gallagher L, Green NDC, and Woods DR. (2016). A four-way comparison of cardiac function with normobaric normoxia, normobaric hypoxia, hypobaric hypoxia and genuine high altitude. PLoS One 11:e0152868.

Cartoni R, Léger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener J, Luthi F, Dériaz O, Zorzano A, Gobelet C, Kralli A, and Russell AP. (2005). Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. J Physiol (Lond) 567(Pt 1):349–358.

Chaillou T, Koulmann N, Meunier A, Pugnière P, McCarthy JJ, Beaudry M, and Bigard X. (2014). Ambient hypoxia enhances the loss of muscle mass after extensive injury. Pflugers Arch 466:587–598.

Coffey VG, Shield A, Canny BJ, Carey KA, Cameron-Smith D, and Hawley JA. (2006). Interaction of contractile activity and training history on mRNA abundance in skeletal muscle from trained athletes. Am J Physiol Endocrinol Metab 290:849.

de Theije CC, Langen RCJ, Lamers WH, Schols, Annemie MWJ, and Köhler SE. (2013). Distinct responses of protein turnover regulatory pathways in hypoxia- and semistarvation-induced muscle atrophy. Am J Physiol Lung Cell Mol Physiol 305:82.

Di Carlo A, De Mori R, Martelli F, Pompilio G, Capogrossi MC, and Germani A. (2004). Hypoxia inhibits myogenic differentiation through accelerated MyoD degradation. J Biol Chem 279:16332–16338.

Di Pasquale D, Strangman G, Harris N, and Muza S. (2016). Acute mountain sickness symptoms depend on normobaric versus hypobaric, hypoxia. Biomed Res Int. 2016:6245609.

Evetts G, Hartley A, Keane S, Keegan J, Simpson A, Taylor A, Hurley A, and Ernsting J. (2005). A comparison of acute hypoxia induced by lowconcentrations of oxygen at ground level, 10,000 feet and by air at 25,000 feet. implications for military aircrew training. SAFE (Europe).

Favier FB, Costes F, Defour A, Bonnefoy R, Lefai E, Baugé S, Peinnequin A, Benoit H, and Freyssenet D. (2010). Down-regulation of akt/mammalian target of rapamycin pathway in skeletal muscle is associated with increased REDD1 expression in response to chronic hypoxia. Am J Physiol Regul Integr Comp Physiol 298:1659.

Ferretti G. (1990). On maximal oxygen consumption in hypoxic humans. Experientia 46:1188–1194.

Fulco CS, Muza SR, Beidleman BA, Demes R, Staab JE, Jones JE, and Cymerman A. (2011). Effect of repeated normobaric hypoxia exposures during sleep on acute mountain sickness, exercise performance, and sleep during exposure to terrestrial altitude. Am J Physiol Regul Integr Comp Physiol 300: 428.

Gumucio JP, and Mendias CL. (2013). Atrogin-1, MuRF-1, and sarcopenia. Endocrine 43:12–21.

Gutsaeva DR, Carraway MS, Suliman HB, Demchenko IT, Shitara H, Yonekawa H, and Piantadosi CA. (2008). Transient hypoxia stimulates mitochondrial biogenesis in brain subcortex by a neuronal nitric oxide synthase-dependent mechanism. J Neurosci 28:2015–2024.

Harber MP, Crane JD, Dickinson JM, Jemiolo B, Raue U, Trappe TA, and Trappe SW. (2009). Protein synthesis and the expression of growth-related genes are altered by running in human vastus lateralis and soleus muscles. Am J Physiol Regul Integr Comp Physiol 296:708.

Hock MB, and Kralli A. (2009). Transcriptional control of mitochondrial biogenesis and function. Annu Rev Physiol 71: 177–203.

Hoppeler H, Kleinert E, Schlegel C, Claassen H, Howald H, Kayar SR, and Cerretelli P. (1990). Morphological adaptations of human skeletal muscle to chronic hypoxia. Int J Sports Med 11 Suppl 1:3.

Hoppeler H, Klossner S, and Vogt M. (2008). Training in hypoxia and its effects on skeletal muscle tissue. Scand J Med Sci Sports 18(Suppl 1):38–49.

Howald H, and Hoppeler H. (2003). Performing at extreme altitude: Muscle cellular and subcellular adaptations. Eur J Appl Physiol 90:360–364.

Howald H, Pette D, Simoneau JA, Uber A, Hoppeler H, and Cerretelli P. (1990). Effect of chronic hypoxia on muscle enzyme activities. Int J Sports Med 11(Suppl 1):10.

Irrcher I, Adhihetty PJ, Joseph A, Ljubicic V, and Hood DA. (2003). Regulation of mitochondrial biogenesis in muscle by endurance exercise. Sports Med 33:783–793.

Kayser B, Hoppeler H, Claassen H, and Cerretelli P. (1991). Muscle structure and performance capacity of himalayan sherpas. J Appl Physiol 70:1938–1942.

Levine BD, Kubo K, Kobayashi T, Fukushima M, Shibamoto T, and Ueda G. (1988). Role of barometric pressure in pulmonary fluid balance and oxygen transport. J Appl Physiol 64: 419–428.

Livak KJ, and Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25:402–408.

Loeppky JA, Icenogle M, Scotto P, Robergs R, Hinghofer-Szalkay H, and Roach RC. (1997). Ventilation during simulated altitude, normobaric hypoxia and normoxic hypobaria. Respir Physiol 107:231–239.

Manimmanakorn A, Manimmanakorn N, Taylor R, Draper N, Billaut F, Shearman JP, and Hamlin MJ. (2013). Effects of resistance training combined with vascular occlusion or hypoxia on neuromuscular function in athletes. Eur J Appl Physiol 113:1767–1774.

McGee SL, and Hargreaves M. (2004). Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. Diabetes 53:1208–1214.

Millet GP, Faiss R, and Pialoux V. (2012). Point: Hypobaric hypoxia induces different physiological responses from normobaric hypoxia. J Appl Physiol 112:1783–1784.

Pilegaard H, Saltin B, and Neufer PD. (2003). Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol (Lond) 546(Pt 3):851–858.

Raue U, Slivka D, Jemiolo B, Hollon C, and Trappe S. (2007). Proteolytic gene expression differs at rest and after resistance exercise between young and old women. J Gerontol A Biol Sci Med Sci 62:1407–1412.

Sandri M. (2008). Signaling in muscle atrophy and hypertrophy. Physiology (Bethesda) 23:160–170.

Sandri M, Carraro U, Podhorska-Okolov M, Rizzi C, Arslan P, Monti D, and Franceschi C. (1995). Apoptosis, DNA damage and ubiquitin expression in normal and mdx muscle fibers after exercise. FEBS Lett 373:291–295.

Savourey G, Launay J, Besnard Y, Guinet A, and Travers S. (2003). Normo- and hypobaric hypoxia: Are there any physiological differences? Eur J Appl Physiol 89:122–126.

Siri WE. (1993). Body composition from fluid spaces and density: Analysis of methods. 1961. Nutrition 9:491; discussion 480, 492.

Slivka DR, Dumke CL, Tucker TJ, Cuddy JS, and Ruby B. (2012). Human mRNA response to exercise and temperature. Int J Sports Med 33:94–100.

Slivka DR, Heesch MWS, Dumke CL, Cuddy JS, Hailes WS, and Ruby BC. (2014). Human skeletal muscle mRNAResponse to a single hypoxic exercise bout. Wilderness Environ Med 25:462–465.

Tucker A, Reeves JT, Robertshaw D, and Grover RF. (1983). Cardiopulmonary response to acute altitude exposure: Water loading and denitrogenation. Respir Physiol 54:363–380.

Tucker TJ, Slivka DR, Cuddy JS, Hailes WS, and Ruby BC. (2012). Effect of local cold application on glycogen recovery. J Sports Med Phys Fitness 52:158–164.

Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, and Cameron-Smith D. (2002). Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol Endocrinol Metab 283:66.

Wright DC, Han D, Garcia-Roves PM, Geiger PC, Jones TE, and Holloszy JO. (2007). Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1alpha expression. J Biol Chem 282:194–199.

Yang Y, Creer A, Jemiolo B, and Trappe S. (2005). Time course of myogenic and metabolic gene expression in response to acute exercise in human skeletal muscle. J Appl Physiol 98:1745–1752.

Yin W, Signore AP, Iwai M, Cao G, Gao Y, and Chen J. (2008). Rapidly increased neuronal mitochondrial biogenesis after hypoxic-ischemic brain injury. Stroke 39:3057–3063.

Yun Z, Lin Q, and Giaccia AJ. (2005). Adaptive myogenesis under hypoxia. Mol Cell Biol 25:3040–3055.

Zak RB, Shute RJ, Heesch MW, La Salle DT, Bubak MP, Dinan NE, Laursen TL, and Slivka DR. (2017). Impact of hot and cold exposure on human skeletal muscle gene expression. Appl Physiol Nutr Metab 42:319–325.

Zhu L, Wang Q, Zhang L, Fang Z, Zhao F, Lv Z, Gu Z, Zhang J, Wang J, Zen K, Xiang Y, Wang D, and Zhang C. (2010). Hypoxia induces PGC-1α expression and mitochondrial biogenesis in the myocardium of TOF patients. Cell Res 20:676–687.

Address correspondence to:
Dustin R. Slivka, PhD
Exercise Physiology Lab
University of Nebraska at Omaha
6001 Dodge Street
Omaha, NE 68182

E-mail: dslivka@unomaha.edu

Received December 20, 2018;
accepted in final form February 1, 2019.