May 2018

Effect of Exercise and Hypoxia on Plasma Telomerase

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EFFECT OF EXERCISE AND HYPOXIA ON PLASMA TELOMERASE

By

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Bachelor of Science – Kinesiology
University of Nevada Las Vegas
2016

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science – Exercise Physiology

Department of Kinesiology and Nutrition Sciences
School of Allied Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
May 2018

UNLV | GRADUATE COLLEGE

Thesis Approval

The Graduate College
The University of Nevada, Las Vegas

April 13, 2018

This thesis prepared by

Charli D. Aguilar

entitled

Effect Of Exercise And Hypoxia On Plasma Telomerase

is approved in partial fulfillment of the requirements for the degree of

Master of Science – Exercise Physiology
Department of Kinesiology and Nutrition Sciences

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Abstract

Effect of Exercise and Hypoxia on Plasma Telomerase

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Introduction: Telomerase reverse transcriptase (TERT) is the enzyme that adds telomeric sequences to the end of linear chromosomes. Exercise has shown to upregulate acutely leukocyte TERT after just 30 minutes of running on a treadmill at 80% of VO_2max (Denham et al., 2016). Hypoxia inducible factor 1 (HIF-1) is also a mediator of TERT in *in vitro* (Nishi et al., 2004). Moderate acute exposure to hypoxia was associated with substantial increases in plasma TERT in a recent study on rats (Wang et al., 2014). The specific aim of the current study was to identify if acute hypoxia upregulates plasma TERT in healthy adult humans. We hypothesized that TERT would be increased after cycling exercise and that exercise in hypoxia would illicit a greater increase than exercise alone.

Methods: Ten healthy adults (5 male, 5 females 23.8 ± 4.5 yrs.) volunteered for the study. Each participant visited the lab on three separate occasions separated by 72 hours but no more than 2 weeks. The conditions were defined as normoxia (F_iO_2 = 20.5%) and normobaric hypoxia (F_iO_2 = 14.4%) created by Altitude simulation machine. On the first visit, graded exercise tests (GXT) were performed in each condition to determine the resistance at 75% of age predicted maximum heart rate (HRmax) by cycling on a cycle ergometer at 60 revolutions per minute. Exercise trials took place on subsequent visits, conditions were counterbalanced and randomized. Exercise trials were defined as 30 minutes cycling at 60 RPM at an intensity set to 75% of age predicted HRmax. 600 µL blood samples were taken from finger stick immediately before and 30 minutes after completion of exercise trials. Blood samples were then centrifuged and plasma aliquoted

and stored at -80°C until all samples were collected for later analysis. Statistical significance was accepted at $p < 0.05$.

Results: ELISA analysis did not detect any levels of TERT in the plasma samples for any of the unknowns. Work load was decreased in hypoxia compared to normoxia (110.7 ± 34.5 W, 125.8 ± 49.6 W, $p = 0.04$) but mean exercise heart rate was not different between conditions (144.4 ± 4.5 BPM, 146.9 ± 5.5 BPM, $p = 0.065$).

Discussion: Plasma TERT is not detectable by ELISA analysis in healthy adults. Intensity was matched between conditions and confirmed by mean heart rate. Further research is needed to determine if hypoxia has an effect on TERT in human tissue.

Acknowledgements

Special thank you to Dr. James Navalta for his mentorship and patience.

The author would also like to thank Gabriela Guzman for her time and assistance.

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CHAPTER 1: Introduction

Telomeres are tandem nucleotide repeats found at the end of linear chromosomes. They protect deoxyribose nucleic acid (DNA) against attrition from mitotic division and oxidative damage. If the length of the telomeres becomes too short, the replicative process of cells for growth and repair will cease. Extremely short telomere lengths are linked to many human age-related diseases, including cancer and cardiovascular disease (CVD) (Botha et al., 2012 & He et al., 2016). Telomerase reverse transcriptase (telomerase) is the only DNA polymerase that fully replicates the terminal ends of linear chromosomes and rebuilds telomeric sequences (Sanchis-Gomar & Lucia, 2015).

Biological age is measured by telomere length because of its ability to predict the onset of age related disease and mortality (Lin et al., 2015). Telomerase enzyme activity positively correlates with longer telomere length in healthy individuals (Kim et al., 2016). Telomeres are reportedly longer in individuals who are regularly physically active. A study from 2008 found that leukocyte telomere length (LTL) was positively correlated with increases in physical activity during leisure time between groups of twins. Additionally, the more active twins, possessed LTL 88 nucleotides longer than those of their less active sibling (Cherkas et al., 2008). This data emphasizes that exercise training protects against biological aging (Denham et al., 2016).

Increased physical activity and decreased sedentary time are two factors highly associated with longer telomeres (Ludlow et al., 2008). Many previous studies have found associations between endurance exercise and longer leukocyte telomere length in humans (Chilton et al., 2014, & Denham et al., 2016). Increases in leukocyte telomerase levels has been reported after just a single bout of treadmill running (Denham et al., 2016). Upregulation of telomerase activity was seen in leukocytes after only 30 minutes of moderate intensity cardiorespiratory exercise, and it remained highly active for 24 hours post exercise (Chilton et

al., 2014). Several studies support the idea that hypoxia-inducible factor-1 (HIF-1) is activated in response to endurance exercise and mediates concurrent changes in gene expression (Lindholm & Rundqvist, 2016). Results from *In vitro* studies indicate that HIF-1 has the action of regulating cell growth and survival by controlling telomerase activity (Minamino et al., 2001).

Altitudes above 3,000 meters cause hypoxia/hypoxic conditions due to reduced partial pressure of O_2 in the inspired air. Acute exposure to moderate altitude has shown to increase both HIF-1 levels and telomerase activity. Most notably, increases in telomere length and telomerase expression was detected in rats under different hypoxic conditions. Wang and others reported higher levels of plasma telomerase in rats after 24 hours and up to 30 days at a moderate altitude (2014). HIF-1 also increased in correlation with telomerase levels. The authors concluded that a mild hypoxic state may increase telomere length and telomerase activity (Wang et al., 2014). However, it is unclear if this effect exists in humans.

Results from a study by McGinnis and others published in 2014 support evidence that exercise and altitude elicit different oxidative stress responses in the body. Their participants performed three separate cycling tests at varying simulated altitudes. The authors report the possibility of an additive effect of exercise and hypoxia greater than exercise or hypoxia individually (McGinnis et al., 2014).

Statement of Purpose

The purpose of this study is to determine whether exercise, combined with normobaric hypoxia, influences changes in circulating cell-free plasma telomerase levels in humans. Evidence points to hypoxia being a mechanism by which exercise stimulates telomerase to repair DNA. This could mean that hypoxia has an added effect on age attenuation. However, the acute physiological responses of telomerase to moderate-load resistance exercise in hypoxia have yet

to be explored. The present study, will measure alterations in plasma telomerase levels to assess for effects of hypoxia.

Hypothesis

Plasma telomerase will be greater following exercise in normobaric hypoxia (F_I0_2 = 14.4%) than exercise in normoxia (F_I0_2 = 20.9%)

Limitations

- Diet and stress level can acutely alter telomerase levels (Denham et al., 2016)
- Maximal heart rate (HR_{max}) will be predicted using 220 – age = HR_{max}
- Normobaric hypoxia does not accurately simulate altitude because there is no change in barometric pressure. Therefore, we cannot assume that decreasing the fraction of oxygen will have the same physiological response on telomerase levels as altitude.

Delimitations

- Participants will exercise on a cycle ergometer under two conditions.
    - Normobaric hypoxia (F_I0_2 = 14.4%)
    - Normoxia (F_I0_2 = 20.9%)
- Blood samples before (baseline) and after each test session
- Avoid physical activity 24 hours prior to testing
- Wash out period: > 48 hours < 1 week.
- Participants blinded to conditions
- Cadence: 60 revolutions per minute

- Work rate adjusted to maintain heart rate within ± 5 BPM of 75% age predicted HR_{max}

Further research on individuals living at various altitudes are needed before assumptions about the effects of altitude can be made. This study is the first to investigate the connection between hypoxia and telomerase levels in a living human model. Understanding the mechanism by which exercise, and physical activity influences aging could improve knowledge about how exercise can optimize age attenuation.

CHAPTER 2: Review of Related Literature

Aging is an inevitable part of human biology and is often defined as a progressive loss of function and by increasing morbidity. The aging process is very complex and is affected by both genetic and environmental factors. Furthermore, the rate and manner by which people age varies significantly (Shin, Lee, Song, & Jun, 2008).

Every time a cell divides the 3' end of each linear chromosome is not fully replicated by DNA polymerase. This leads to progressive shortening of the chromosomes each time the cell replicates. Eventually the continual shortening of chromosomes will disrupt genes and lead to senescence of division and/or apoptosis (Botha et al., 2012). As tissues lose the ability to renew and repair the organism ages and eventually dies from age related disease.

Telomeres are repetitive DNA sequences at the end of chromosomes that protect against DNA (gene) attrition and cellular death (Botha et al., 2012). When telomeres become too short, cells become susceptible to senescence and apoptosis. Telomere length often serves as an indicator of a cell or tissue's biological age and a predictor of lifespan potential (Shin, Lee, Song, & Jun, 2008). This has been seen in many twin studies where majority of the time a twin with the shorter telomeres will die first from age related complications (Chen et al., 2011). Telomere length is influenced by the balance between telomere attrition (mitotic divisions or exposure to oxidation) and telomere lengthening / repair (Wolkowitz et al., 2012).

Telomerase Reverse transcriptase (telomerase) the DNA polymerase that replicates the terminal ends of linear chromosomes and rebuild the telomeric sequences (Sanchis-Gomar & Lucia, 2015). Telomerase is a cellular ribonucleoprotein enzyme that promotes cellular viability and prevents telomere attrition (Wolkowitz et al., 2012). Telomerase is known to extends DNA telomere length in embryonic cells but was long believed to be inactive in adult cells. However,

recent data supports evidence that telomerase is active in many adult tissues and may serve a vital role in age attenuation (Kim et al., 2016).

Mild systemic hypoxia has shown to upregulate telomerase activity. Research by Nishi et. al., on human embryonic cells indicates that upregulation of telomerase by hypoxia is mediated through activity of Hypoxia-Inducible Factor 1 (HIF-1) (Nishi et al., 2004). Follow up research confirmed that hypoxia regulates growth and survival of human umbilical vein endothelial cells by modulating telomerase activity (Guan et al.,2012). Recently, in vivo studies found evidence that acute exposer to moderate altitude increases telomere length and telomerase activity in blood leukocytes and thymocytes (Wang et al., 2014).

Several studies support the idea that HIF-1 is activated in response to endurance exercise and mediates concurrent changes in gene expression (Lindholm & Rundqvist, 2016). The purpose of the following literature review is to investigate the relationship between physical activity, hypoxia, and rate of telomere attrition to understand how hypoxia affects the aging process.

Telomeres and Health

Telomere shortening, and loss of telomerase activity occurs throughout life. A survey of participants for the Helsinki Businessmen study have shown that obesity, high cholesterol levels, and smoking in midlife are related to shorter telomere length in old age (Savela et al., 2013). These telomeric changes in the elderly are associated with many age-related complications such as cardiovascular health, cancer, diabetes, and dementia (Botha et al., 2012 & Savela et al., 2013). Questionnaire data on 2,401 white twins reported that short leukocyte telomere length is highly associated with diseases such as increased oxidative stress, coronary artery disease,

diabetes mellitus, heart failure, and osteoporosis. They also found that short telomeres can even predict early myocardial infarctions (Cherkas et al. 2008).

Cardiovascular diseases (CVD) are the leading causes of death worldwide. Such diseases include; chronic heart failure, coronary artery disease and myocardial infarction. Aging is a major risk factor for the development of CVD. Telomerase has found to be active in healthy cells of the cardiovascular system (Werner et. al., 2008). Recent evidence suggests telomerase may have a protective effect against CVD and that interference with the action of this enzyme, contributes to the development of CVD (Zurek et. al., 2016). Short myocardial telomeres have been associated with an increased risk of developing heart disease in older adult humans as well (Sanchis-Gomar & Lucia, 2015).

Rapid telomere shortening has shown to have a strong association with the development of coronary artery disease. Telomere shortening can result in pathological cardiac remodeling and severe ventricular dysfunction. Conversely, coronary artery disease has affectively been treated with telomerase gene therapy in mice (Sanchis-Gomar & Lucia, 2015). When mice were treated with telomeric gene therapy they experienced improvements in myocardium remodeling.

Telomere length is emerging as a prognostic marker of disease risk, progression, and premature mortality in many types of cancer, including breast, prostate, colorectal, bladder, head and neck, lung, and renal cell (Ornish et. al., 2008). About 78% of all cancers are diagnosed in adults aged 60 years and older (Courneya, & Karvinen, 2007). Short telomeres and telomerase dysfunction can lead to the onset of certain cancers, furthermore, environmental factors known to promote cancer and cardiovascular disease also adversely affect telomere length and telomerase function (Allegra et al., 2017).

Telomere length is governed mostly by genetic factors, with majority of the initial length being determined at birth (Botha et al., 2012). Telomeres are also particularly susceptible to aging. Yearly telomere reduction is between 30 to 60 base pairs. When telomeres become too short, the cell will no longer be able to transcribe functional telomerase (Sanchis-Gomar & Lucia, 2015). However, it is apparent that telomere length is dynamic in nature. The rate at which they shorten is dependent on many lifestyle factors. For instance, a sedentary life style in addition to smoking, high body mass index, and low socioeconomic status, have all show to be strongly correlated with faster telomere attrition (Cherkas et al., 2008).

Lifestyle habits such as smoking, sleep patterns, body composition, and physical activity levels alter telomerase activity (Botha et al., 2012). Exposure to oxidative stress increases the rate of telomere attrition whereas reduction in stress can promote telomere lengthening. Oxidative stress can be brought on by environmental factors such as chemicals in pollution or in the diet, and cigarette smoking that may accelerate the aging process (Kim et al., 2016). Conversely, healthy habits such as proper nutrition, physical activity, and healthy sleep patterns can reduce age related telomere attrition and promote repair of telomeres, thereby, slowing down aging and decreasing risk of developing age-related diseases.

Physical Activity and Aging

Regular physical activity is considered a foundational health behavior. The US guide lines recommend a minimum of 30 minutes of moderate-intensity physical activity at least 5 days per week. They state that this level of physical activity can have significant health benefits (Cherkas et al., 2008 and Du et al., 2012). Physical exercise, adequate to the metabolic capability of an individual at a given stage of life, has been proposed as a remedy to counteract aging because exercise has shown to facilitate the reduction of many age-related diseases (Ludlow et

al., 2008). Likewise, inactivity is a major risk factor for the development of many aging-related diseases (Cherkas et al., 2008 and Loprinzi, & Sng, 2016 and Denham, 2017).

Physical activity and physical fitness decrease the risk of morbidity and mortality from a variety of age related causes, with associated increases in longevity (Ludlow et al., 2008). Upregulation of leukocyte telomerase expression correlates with both a reduction in age and increases in exercise volume (Denham et al., 2016). Telomere length in leukocytes has even shown to increase after 5 years of moderate exercise intervention, implying reversal of aging (Ornish et al., 2008). Researchers propose that repeat bouts of endurance exercise training may reprogram telomerase gene expression and increase its activity, leading to lengthening of telomeres and essentially attenuating ageing (Denham et al., 2016).

Many researchers are looking to identify the proper volume and mode of exercise needed to optimize telomere protection. In a 2008 study by Ludlow and others, the correlation of physical activity levels with telomere length and telomerase enzyme activity was investigated. Sixty-nine participants between 50 and 70 years of age were assessed for weekly exercise energy expenditure using the Yale Physical Activity Survey. Participants who reported an exercise energy expenditure of 991 to 2340 kcal per week for at least five years or more, exhibited the longest telomere lengths. These results indicate that moderate physical activity levels may provide a protective effect to leukocyte telomere length over both low and high exercise energy expenditure levels (Ludlow et al., 2008).

Fitness level positively correlates with telomere length. The authors of another study examined cross-sectional associations among activity level, sedentary behavior, and leukocyte telomere length from 7,813 women aged 43–70 years in the Nurses' Health Study. Participants self-reported activity by questionnaire in 1988 and 1992 and sedentary behavior in 1992. They

measured telomere length in peripheral blood leukocytes. Analysis revealed that women who reported a greater volume of moderate- or vigorous-intensity activity (calisthenics or aerobics) had increased leukocyte telomere length (Du et al., 2012). A plateau in leukocyte telomere length has been identified. Participants with the highest volume of aerobic activity per week and highest leukocyte telomerase levels did not have telomeres longer than individuals who performed a moderate amount of aerobic activity each week. Because of this, the researchers in this study suggest that moderate amounts of exercise (running: 10–25 km/wk; cycling: 30–200 km/wk) may be as sufficient as large amounts of exercise to prevent age-associated telomere erosion (Denham et al., 2016).

These collections of data indicate a linear relationship between increase in physical activity and increased telomere length. Majority of current studies have found associations between endurance type exercise and longer telomere length in various cell types, including but not limited to, buccal cells, skeletal muscle cells, and leukocytes. However, some studies have failed to find any association between telomere length and physical exercise (Denham, 2017). This may be evidence that modes other than aerobic activity have little to no effect on telomere length. Also, could demonstrate that telomerase activity is limited to specific mitotic cell types.

The purpose of a 2016 study by Loprinzi & Sng was to investigate whether there is a mode specific association of physical activity on telomere length in cells of the immune system. Data from the National Health and Nutrition Examination Survey (1999-2002) were analyzed. Nine separate modes of physical activity were classified as aerobics, basketball, bicycling, dance, running, stair climbing, swimming, walking, and weight lifting. Results found that the only mode of physical activity that was significantly correlated with leukocyte telomere length was running at a minimum of 2000 metabolic equivalent min per month. The researchers concluded that

running based physical activity may help to prevent premature mortality (Loprinzi, & Sng, 2016).

Leukocyte telomere length is preserved in individuals who perform regular aerobic exercise and telomere length is positively correlated with maximal aerobic exercise capacity (VO_2max). A study published in Mechanisms of Ageing and Development, found that when they analyzed leukocyte telomere length in groups of young and older endurance athletes, telomere length was positively related to VO_2max. Stepwise multiple regression analysis revealed that VO_2max was the only independent predictor of leukocyte telomere length. In fact, maximal aerobic exercise capacity predicted 20% of telomere length while age predicted only 13% (LaRocca, Seals, & Pierce, 2010).

Ultra-endurance athletes stress their bodies beyond may physical limits. In cross-sectional research by Denham and others, they hypothesized that exercise training may prevent disease through telomere length maintenance and sought to find if long term endurance performance is beneficial to telomere length. The research quantified the leukocyte telomere length and analyzed the expression of telomere-regulating genes in endurance athletes and healthy controls. After analysis, they found that a group of ultra-marathon runners had telomeres 11% longer than healthy age matched controls. The 11% length corresponds to 16 years of age prevention. They also noted significantly higher levels of telomerase activity (Denham et al., 2016).

Acute bouts of exercise at a moderate intensity can also increase the activity level of telomerase temporarily (Denham et al., 2016). One study found that a single bout of exercise at 80% of an individuals' VO_2Max can increase leukocyte telomerase activity 20-fold in healthy individuals immediately and 60 minutes following the exercise session (Chilton et al., 2014).

Upregulation of telomerase activity has been found in leukocytes after only 30 minutes of moderate intensity cardiorespiratory exercise and it remained active for 24 hours post exercise (Chilton et al., 2014). This evidence supports the hypothesis that telomerase is activated to repair oxidative damage post exercise.

Telomeres have high amounts of guanine and cytosine content, which is a major target of reactive oxygen species, therefore, telomeric DNA may be more susceptible to oxidative damage. During exercise, there is an increase in metabolism and the production of reactive oxygen species, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Collins et al., 2003). Science has shown a lot of interest in biomarkers of oxidative stress as an effect of exercise ever since the first study discovered them (Lindholm & Rundqvist, 2016).

Exercise and Hypoxia

The components of acute exercise that act as triggers for adaptation are still largely unknown. Early research believed that local hypoxia could act as a possible stimulus for exercise adaptation (Desplanches et al., 1993; Sundberg,1994). Cellular responses to hypoxia are induced partially by activation of the hypoxia-sensitive transcription factor HIF-1, which is highly conserved between species and expressed in most tissues. Several studies support the idea that HIF-1 is activated in response to endurance exercise and mediates concurrent changes in gene expression (Lindholm & Rundqvist, 2016). One study found that HIF-1 is activated in human skeletal muscle immediately post and up to 6 h after a single bout of exercise. The investigators believe that HIF-1α protein in human skeletal muscle might be induced by exercise-dependent reduction in oxidative stress (Ameln et al., 2005).

It has been previously reported that the exposure of human cells to hypoxic conditions *in vitro* results in the induction of telomerase activity. HIF-1α triggering has been identified as one

mechanism by which hypoxia affects telomerase. In many cells, HIF-1α has shown to mediate upregulation of telomerase and induces cell proliferation (Nishi et al., 2004 & Minamino, Mitsialis, & Kourembanas, 2001). Other experiments found that different levels of hypoxia regulate telomere length and telomerase activity (Guan et al., 2012).

Although several reports demonstrated HIF-1 mediated upregulation of the human telomerase gene under hypoxia *in vitro*, whole animal systems *in vivo* models are limited. The first study to explore *in vivo* results was published in 2006. The researchers found that hypoxia induced telomerase gene expression in non-tumor fish tissues (Yu et al., 2006).

A follow up experiment in 2014 was published in the Journal of Physiological Anthropology by Wang and others, aimed to evaluate the effects of altitude on telomere length in living mice. They reported changes of telomere length and telomerase levels in mice tissue when kept in various simulated altitudes. Results showed that mice in the moderate altitude group exhibited greater leukocyte telomere length than those in the sea level or super high-altitude groups. Plasma telomerase levels and HIF-1α levels were significantly higher in moderate altitude mice but not in the sea level and super high-altitude specimens (Wang et al., 2014). These results indicate that there may be an optimum altitude/hypoxic dosage for telomere lengthening. Research has yet to explore whether this holds true for humans.

Conclusion

The purpose of this literature review was to investigate the connection between exercise, hypoxia, and telomerase levels. Telomeres shorten with age, eventually leading to cellular death. Unhealthy lifestyle habits increased the rate of natural telomere attrition. Telomerase reverse transcriptase, is an enzyme known for having the function of preventing DNA telomere shortening and some evidence supports its ability to lengthen telomeres over time. Levels of

telomerase have shown to increase with bouts of exercise, primarily endurance type exercise, in various cell types in human studies. Physical activity is also linked to hypoxia related factors that stimulate telomerase activity. Telomerase may also be a mechanism that protects against genetic stress induced by hypoxia

When biological age is measured by telomere length, adults who partake in regular physical activity are on average, biologically younger than sedentary adults the same age (Cherkas et al., 2008). Currently, the optimal amounts of physical activity and exercise needed for antiaging benefits needs further investigation. Some studies found that both low physical activity and high physical activity levels were associated with shorter telomeres (Savela et al., 2013). This is an indicator that an optimum amount and type of physical activity needed for telomere attrition may exist.

A long-term prospective study monitoring intensity, duration, and frequency of exercise along with many environmental factors, in participants over different periods of life, would help in the understanding of the complex relationship and the key periods of life for maximal benefit of exercise. It is unknown whether exercise under hypoxic conditions provides added benefit to telomere length? Additionally, the role of physical and psychological stress on telomere attrition still needs investigation. Also, the question remains about how long after a bout of endurance exercise does telomerase activity remain elevated? (SaBenroth et al., 2015 and Denham et al., 2016). Answering these questions and more will help make it possible to prescribe physical activity as an antiaging strategy one day.

CHAPTER 3: Methodology

Participants

Previous data (Wang et al., 2015) indicated a *priori* power analysis (effect size = 0.938, beta = 0.80, alpha = 0.05) indicated a required n=5 to detect significant differences in telomerase levels (G*Power, version 3.1.5). 10 healthy adults (5 male, 5 female) volunteers were recruited from the UNLV student population by word of mouth. All participants were 18-44 years of age non-smokers free of asthma and classified as low risk by the ACSM Health Risk Questionnaire. Volunteers were excluded if they had any underlying risk factor as indicated on the medical health history questionnaire. Exclusion criteria also included diagnosed heart disease, uncontrolled hypertension, and women who were pregnant or may have been pregnant. This study was approved by the University of Nevada Las Vegas Institutional Review Board approval, (IRB # 1131335-3).

Baseline Testing

Potential participants were screened using the ACSM Health Risk Questionnaire, and those who are low risk (no signs/symptoms of or diagnosed cardiovascular disease, pulmonary, and/or metabolic disease) were provided informed consent forms.

Participants reported to the Exercise Physiology Laboratory for testing on three separate occasions at least 72 hours apart but no more than 2 weeks. Participants were asked to report to the lab well rested and hydrated. They were instructed to refrain from physical activity, caffeine, and alcohol for 24 hours prior to testing. On the first visit, resting heart rate and blood pressure was measured (Omron, Lake Forest, IL, USA). Two graded exercise tests (GXT) were

performed to determine the initial work rate for testing subsequent days for each condition. The two conditions are defined as, normoxia (F_iO_2 = 20.5%) and normobaric hypoxia (F_iO_2 = 14.4%). An altitude simulating machine (Hypoxico 5570 Everest Summit II Altitude training system: New York, NY, USA) connected through a mask was worn during the activity in both conditions and programed to the desired fraction of oxygen. Participants pedaled on a cycle ergometer (Watt bike Pro, Waukesha, WI, USA) at a rate of 60 RPM, set by a metronome. Every two-minutes, resistance was increased by 15 watts. Testing stopped when the participant reached a goal HR of 75% of their age predicted HR_{max}.

Protocol

At the second visit, body composition estimated through bioelectrical impedance analysis (SECA mBCA 515: SECA Hamburg, Deutschland). The order of the interventions was counterbalanced, and participants were not told which condition they were given. Participants initially rested for 15 minutes before they cycled for 30 minutes following 5-minute cycling warm up. Cadence was set at 60 revolutions per minute (RPM) and work rate was initially set to match predetermined resistance from GXT and was then periodically adjusted to maintain heart rate within 5 beats of goal HR (75% of age predicted HR_{max}). Participants then rested in normoxia for 30 minutes. The same protocol was repeated on the third visit for the opposite condition.

Heart rate was obtained through telemetry (Polar Electro Bethpage, NY, USA) and, blood oxygen saturation analysis was gathered with a fingertip pulse oximeter (Hypoxico, New York, NY, USA) every minute throughout the duration of the exercise. Mean power output, cadence, and distance was provided by the cycle ergometer monitor.

Blood Sampling and Telomerase Assay

Peripheral whole blood was drawn from a finger stick prior to and 30 minutes after cycling on both visits to the laboratory. The site of the finger stick was cleansed with an alcohol swab, lanced, and whole blood collected into a 600 µL capillary tube (Sarstedt Inc, Nümbrecht, Germany) lined with lithum heparin to prevent clotting. Whole blood was centrifuged (Eppendorf centrifuge 55415 D: Brinkmann Instruments Inc, Westbury, NY, USA) for 4 minutes, and hematocrit was estimated from zipocrit scale. Plasma was aliquoted and stored at -80 C° until subsequent analysis. Plasma samples were analyzed in duplicate for telomerase levels using an 96 well ELISA kit (Cloud-Clone Corp., Katy, TX, USA). Reagents, samples and standards were prepared per instructions. 100µL of each sample was added to each well and incubated for 1 hour at 37°C then aspirated. 100µL detection reagent A was added and incubated again for 1 hour at 37°C. We aspirated the plate again and washed 3 times with wash buffer. Then 100µL of detection reagent B was added and incubated for 30 minutes at 37°C. It was aspirated and washed 5 times, then 90µL of substrate solution was added in the dark and incubated 20 minutes at 37°C until a visible color gradient appeared in the standard wells. Finally, 50µL of stop solution was added and the plate was immediately read at 450nm with plate reader (Epoch: BioTec Instruments Inc, Winooski, VT, USA). Plate reader optical density (OD) values were calculated for substrate levels and mean concentrations.

Statistical Analysis

All data are presented as mean ± SD and were analyzed using the SPSS version 25 statistical analysis package (SPSS Inc., Chicago, IL, USA). Repeated measures ANOVA was used to compare means of telomerase levels. Duplicates that recorded a coefficient or variance greater

than 10 percent were omitted. Dependent t-tests were used to evaluate differences in mean power, blood oxygen levels, and HR during activity. Pearson's correlations were used to examine associations between telomerase levels and performance measures. Statistical significance was accepted $p \leq 0.05$.

CHAPTER 4: Results

Ten participants, half male and half female completed the protocol. Table one lists descriptive differences between male and female participants note that body fat % (BF) was not different between the two groups.

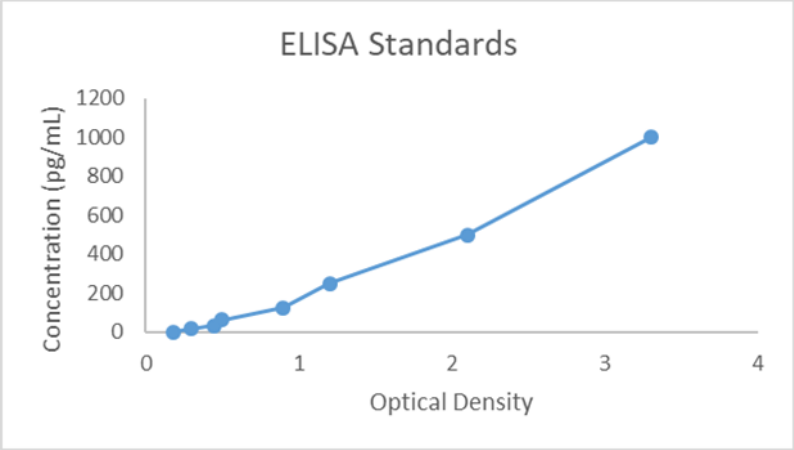
Table 1. Participants description

Sex	Age (yrs)	Height (cm)	Weight (kg)	BF (%)	HEMO .
Male n = 5	25 ± 6.3	177.9 ± 3.8	85.8 ± 4.5	24.7 ± 6.1	42.2 ± 2.2
Female n = 5	22.6 ± 1.7	159.8 ± 2.8	61.7 ± 13.6	27.2 ± 10.5	36.6 ± 4.9
Combined n = 10	23.8 ± 4.5	168.9 ± 10.1	73.7 ± 15.9	25.9 ± 8.2	39.2 ± 5.1
Significance	$p = 0.453$	$p < 0.001$*	$p = 0.014$*	$p = 0.655$	$p = 0.011$*

Data are expressed in means and standard deviations. Hematocrit (HEMO) reported was measured prior to exercise on 2nd visit to lab. Differences between sexes and significance* accepted at $p < 0.05$

ELISA analysis resulted in no detection of cell-free circulating plasma telomerase in any of the unknown samples (see appendix). All unknowns were determined to be below zero. The ELISA standards produced an expected curvilinear graph. ANOVA analysis of OD values found no interactions between conditions or for any time interval ($p = 0.807$).

Figure 1. ELISA Standard Curve



Standard curve used to calculate unknown concentrations from optical densities

Blood oxygen saturations (SPO_2) were significantly lower during hypoxic exercise than during normoxic exercise (average SPO_2 = 80.58 ± 4.3 in hypoxia and 95.23 ± 0.97 in normoxia) $p < 0.001$ (Cohen's d = 4.8). Average cycle wattage was also significantly decreased during hypoxic exercise (110.7 ± 34.5, compared to 125.9 ± 49.6) $p = 0.044$ (Cohen's d = 0.31). Heart rate (HR) was not significantly different between the two conditions. Caloric (CAL) expenditure was greater in normoxia than hypoxia. Table 2 lists the means and mean differences of the values collected during the 30-minute cycle trials.

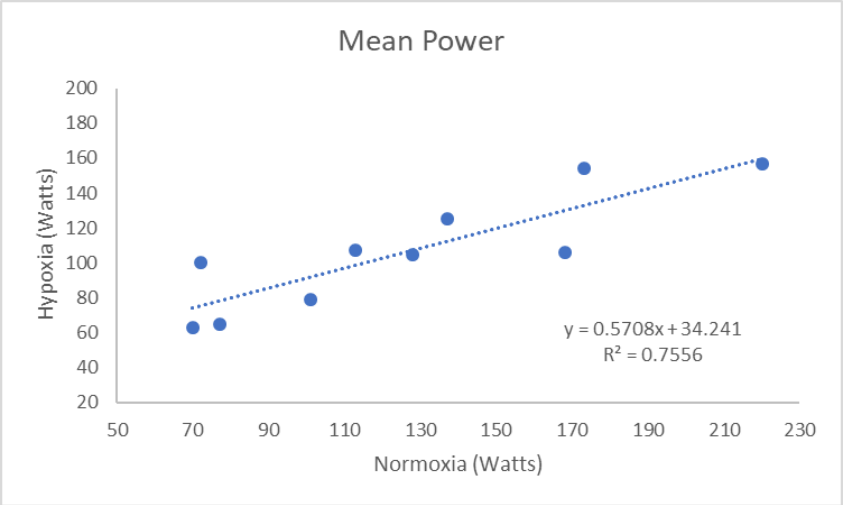
Table 2. Exercise Performance Measures

Condition	Mean Watts	SPO_2	Mean HR	CAL
Normoxia	125.9 ± 49.6	95.2 ± 0.9	144.4 ± 4.5	326.6 ± 85.2
Hypoxia	110.7 ± 34.5	80.6 ± 4.3	146.9 ± 5.6	293.2 ± 57.6
Significance	p = 0.044*	p < 0.001*	p = 0.65	p = 0.047*

Data are expressed in mean and standard deviation, significance* accepted at $p < 0.05$. SPO2 blood oxygen saturation (SPO_2) expressed in percentage. Heart rate (HR) in beats per minute. CAL estimated for the 30 min exercise bout by Watt bike.

Correlations were ran to identify relationships between performance variable. One significant correlation was identified. A positive linear correlation was found between average power output in normoxia and average power in hypoxia for each cycling trial. This relationship is significant with Pearson's $r = 0.869$ and $R^2 = 0.7556$ ($p < 0.001$). Figure 2. Is a graph that represents the linear relationship between these variables.

Figure 2. Prediction of Power in Hypoxia from Power in Normoxia



Mean power output in normoxia to wattage in hypoxia, $r^2 = 0.7556$. Pearson's correlation = 0.869 $p < 0.001$

CHAPTER 5: Discussion

The purpose of this study was to investigate the effect of exercise under moderate hypoxia on plasma telomerase levels. One of our primary aims was to detect changes in circulating cell-free human telomerase between conditions. We hypothesized that plasma telomerase levels would be elevated after exercise. Also, we hypothesized that telomerase levels would be greater after exercise in hypoxia than normoxia. However, plasma telomerase was not detected in any sample by ELISA analysis and therefore neither of our hypotheses were accepted. The results of the ELISA were unexpected, as a previous investigation utilizing rat plasma (Wang et al., 2014) led us to believe that similar results were feasible

All participants were healthy and had no detectable levels of plasma telomerase at any point in the data collection. The present investigation concurs with previous findings by Miura and others who detected elevated levels of human telomerase by reverse transcription polymerase chain reaction (RT-PCR) in patients with carcinoma and none in the serum of 34 healthy controls (2003). These authors indicated that lymphocytes and circulating normal cells express very low levels of telomerase that are difficult to detect (Miura et al., 2003). The same team hypothesized that telomerase is not detectable in serum, possibly due to enzymatic instability by RNase in the serum (Miura et al., 2003). The research team later developed a more sensitive test for detecting telomerase using real-time quantitative reverse transcription-PCR amplified with SYBR Green. The new assay detected small levels (2 parts /0.02 ml) of serum telomerase in samples from fifty healthy individuals, including 12 females (22-83 years old; mean age, 58 years) (Miura et al., 2005). Another study that sought to identify differences in telomerase between cancer patients and healthy controls, found that telomerase was not detected in the plasma of samples taken from 21 healthy participants age 30-73 years (7 males, 14

females) (Chen et al., 2000). Furthermore, none of the 20 plasma samples from healthy volunteers (equal number of males and females mean age 64 years) presented any detectable telomerase in another study using RT-PCR (Tani et al., 2007). In our design, we sought to increase levels of plasma telomerase in healthy individuals by exercise and hypoxic exposure. We report for the first time, that telomerase is not detectable in human plasma in healthy individuals that completed a protocol theoretically designed to increase levels.

Our protocol may not have resulted in increases in plasma telomerase due confounding variables that were not accounted for. Previous literature shows increase in leukocyte telomerase after exercise (Zeitzer et al., 2016 & Denham et al., 2016). However, the optimum time for collecting samples after exercise, varies within the literature. In this study, we collected blood sampled 30-minutes after the exercise trial. This may not have been enough time for the enzyme to leave the cells and enter the plasma. We also did not control for time of day. Participants visited the lab at various times ranging from early morning to early evening. Therefore, we cannot estimate the affect that circadian rhythms may have had on our results. Genotype for our participants was not assed but there is evidence that genotype plays a role in telomerase response to the exercise as well (Ludlow et al., 2008). Moreover, Telomere length was not measured in the present study and results from a recent study support the idea that longer telomeres can limit telomerase expression (Kim et al., 2016). Our participants were reasonably young and reportedly healthy. It is safe to assume that their telomeres were could have been long and telomere homeostasis may have suppressed their telomerase levels. Given the number of confounding variables, we believe that controlling for more of them, may have resulted in detection of plasma telomerase.

Most studies to date, that investigated human telomerase levels used cells of the immune system (Chen ea al., 2011, Cherkas et al., 2008, Denham et al., 2016, La Rocca et al., 2010, Loprinzi et al., 2016, SaBenroth et al., 2015, Wolkowitz et al., 2012). This is because leukocytes are highly mitotic, cells of the immune system are important to aging, and the tissue is less invasive to analyze than many others. Many assays have been shown effective in detecting changes in leukocyte telomerase expression and activity (Chilton et al., 2014). The TRAP assay being one of the most used to detect and measure telomerase activity (Fajkus, J. 2006). Telomerase expression is regularly measured by detecting the RNA component of telomerase with TaqMan PCR (Denham et al., 2016 & Kim et al, 2016). Future exploration into the effect of hypoxia on telomerase would be more pragmatic to investigate leukocyte telomerase changes with a validated human assay.

We predicted from pilot data that work rate would decline during hypoxic exercise. Our results confirmed this with mean work rate decrease being an average of 21% (W) from normoxia to hypoxia. Hypoxic exercise is often characterized by workload decrements. As individuals ascend to higher altitudes, oxygen becomes less biologically available. As a result, absolute exercise intensity decreases (Wehrlin & Hallen, 2006).

Previous researchers that sought to identify the individual effects of exercise and hypoxia on oxidative stress markers, noted an average of 10% decrease in cycling workload in 11 active male adults (McGinnis et al., 2014). These researchers also had their participants perform graded cycle test but in contrast measured VO_2peak tests in simulated environments (normoxic = 975 m and hypoxic = 3,000 m) to prescribe subsequent matched intensities at 60%

of VO_2peak for steady-state trials. They report a decrease in absolute workload during hypoxic VO2peak that therefore reduced the steady-state workload (McGinnis et al., 2014).

For McGinnis and others, pulse oximetry data indicated significant oxygen desaturation during exercise in hypoxia (87 ± 0.6%) compared with normoxic trials (96 ± 0.4%) (2014). Our pulse oximetry data indicated 15.37% ± 4.8% reduction in SPO_2 from normoxic exercise to hypoxic exercise. Our greater reduction in oxygen saturation may be due to a higher exercise intensity (75% vs 60%). Exercise alone did not cause much oxygen desaturation. Therefore, hypoxia only occurred in hypoxic exercise trials and is similar to findings by Martin and others in 2015.

We anticipated that participants would have difficulty completing a steady state cycling protocol in hypoxia. There was a noted reduction in work rate and SPO_2 as predicted. Therefore, our intentions for adjusting cycle ergometer resistance during exercise was to ensure the exercise intensities were matched between hypoxia and normoxia trials. Heart rates between the two trails were not statistically different, supporting the view that matched exercise intensities were achieved.

A positive correlation between mean cycling power in normoxic conditions and hypoxic conditions was discovered. This relationship suggests that average power performed in one condition predicts 75.56% of power in the second condition. However, the sample size for the present study was not large enough to generalize this data. With follow up, this data could be used to develop an equation for prescribing normobaric hypoxic workload from normoxic workload. Researches should keep in mind that nomobaric hypoxia is not the same thing as altitude and the effects of one should not be generalized to the other.

Other limitations of this study were the fact that we used age predicted heart rate max (220 – age = HR_{max}) to prescribe relative intensity. A more accurate method for prescribing exercise intensity would be to first estimate VO_2peak for each condition using a metabolic cart. Evidence that this method accurately matches exercise intensities between exercise trials in normoxia and hypoxia is seen in previous research. McGinnis and others report mean heart rates at the end of exercise sessions at 60% of VO_2peak in normoxia were 146.0 ± 4.8 and 148.2 ± 4.0 for hypoxia ($p = 0.770$). Another limitation for this study is that rate of perceived exertion was not evaluated. This information could be helpful in determining if intensity was truly matched between trials.

We hypothesized that exercise would upregulate plasma telomerase post exercise and that exercise in hypoxia would upregulate plasma telomerase post exercise to a greater extent than normoxic exercise. The protocol for the current study produced the expected performance results but the assay did not provide any data. This is most likely due to the type of tissue used. Although detectable is rats, human telomerase is not detectable by ELISA analysis, therefore, future studies should focus more on changes to cellular based telomerase. HIF-1 responses would also add understanding to the effect of hypoxia on the body during exercise. Future research could compare telomerase and HIF-1 levels during exercise in and out of hypoxia to identify a relationship. Further research is still needed to determine if acute hypoxia affects telomerase levels in other human tissue. For now, the question whether altitude has an effect on aging, still remains.

Appendix

UNLV

UNLV Biomedical IRB - Expedited Review
Approval Notice

DATE:	February 12, 2018
TO:	James Navalta, Ph.D
FROM:	UNLV Biomedical IRB
PROTOCOL TITLE:	[1131335-3] The Effect of Hypoxia on Telomerase
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	February 12, 2018
EXPIRATION DATE:	February 11, 2019
REVIEW TYPE:	Expedited Review

Thank you for submission of New Project materials for this protocol. The UNLV Biomedical IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a protocol design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

PLEASE NOTE:
Upon approval, the research team is responsible for conducting the research as stated in the protocol most recently reviewed and approved by the IRB, which shall include using the most recently submitted Informed Consent/Assent forms and recruitment materials. The official versions of these forms are indicated by footer which contains approval and expiration dates. If your project involves paying research participants, it is recommended to contact Carisa Shaffer, ORI Program Coordinator at (702) 895-2794 to ensure compliance with subject payment policy.

Should there be *any* change to the protocol, it will be necessary to submit a **Modification Form** through ORI - Human Subjects. No changes may be made to the existing protocol until modifications have been approved.

ALL UNANTICIPATED PROBLEMS involving risk to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed.

All NONCOMPLIANCE issues or COMPLAINTS regarding this protocol must be reported promptly to this office.

This protocol has been determined to be a Minimal Risk protocol. Based on the risks, this protocol requires continuing review by this committee on an annual basis. Submission of the **Continuing Review Request Form** must be received with sufficient time for review and continued approval before the expiration date of February 11, 2019.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway . Box 451047 . Las Vegas, Nevada 89154-1047
(702) 895-2794 . FAX: (702) 895-0805 . IRB@unlv.edu

Well Category	Well	Well Position	Well Optical Density	Mean Concentration	Confidence Interval	ID #	Condition
Unknown	U1	A3,A4	0.188,0.283	0	No data	1	HA
Unknown	U2	B3,B4	0.216,0.247	0	No data	1	HB
Unknown	U3	C3,C4	0.27,0.212	0	No data	1	NA
Unknown	U4	D3,D4	0.271,0.195	0	No data	1	NB
Unknown	U5	E3,E4	0.251,0.291	0	No data	2	HA
Unknown	U6	F3,F4	0.259,0.305	0	No data	2	HB
Unknown	U7	G3,G4	0.289,0.27	0	No data	2	NA
Unknown	U8	H3,H4	0.257,0.245	0	No data	2	NB
Unknown	U9	A5,A6	0.308,0.399	0	No data	3	HA
Unknown	U10	B5,B6	0.309,0.286	0	No data	3	HB
Unknown	U11	C5,C6	0.287,0.273	0	No data	3	NA
Unknown	U12	D5,D6	0.275,0.268	0	No data	3	NB
Unknown	U13	E5,E6	0.222,0.203	0	No data	4	HA
Unknown	U14	F5,F6	0.259,0.24	0	No data	4	HB
Unknown	U15	G5,G6	0.294,0.332	0	No data	4	NA
Unknown	U16	H5,H6	0.249,0.25	0	No data	4	NB
Unknown	U17	A7,A8	0.305,0.548	0	No data	6	HA
Unknown	U18	B7,B8	0.233,0.368	0	No data	6	HB
Unknown	U19	C7,C8	0.241,0.296	0	No data	6	NA
Unknown	U20	D7,D8	0.252,0.233	0	No data	6	NB
Unknown	U21	E7,E8	0.251,0.266	0	No data	7	HA
Unknown	U22	F7,F8	0.252,0.276	0	No data	7	HV
Unknown	U23	G7,G8	0.37,0.303	0	No data	7	NA
Unknown	U24	H7,H8	0.298,0.295	0	No data	7	NB
Unknown	U25	A9,A10	0.297,0.205	0	No data	8	HA
Unknown	U26	B9,B10	0.292,0.266	0	No data	8	HB
Unknown	U27	C9,C10	0.248,0.29	0	No data	8	NA
Unknown	U28	D9,D10	0.261,0.297	0	No data	8	NB
Unknown	U29	E9,E10	0.344,0.303	0	No data	9	HA
Unknown	U30	F9,F10	0.347,0.363	0	No data	9	HB
Unknown	U31	G9,G10	0.371,0.378	0	No data	9	NA
Unknown	U32	H9,H10	0.352,0.371	0	No data	9	NB
Unknown	U33	A11,A12	0.287,0.272	0	No data	10	HA
Unknown	U34	B11,B12	0.291,0.302	0	No data	10	HB
Unknown	U35	C11,C12	0.27,0.324	0	No data	10	NA
Unknown	U36	D11,D12	0.291,0.319	0	No data	10	NB
Unknown	U37	E11,E12	0.362,0.406	0	No data	12	HA
Unknown	U38	F11,F12	0.379,0.489	0	No data	12	HB
Unknown	U39	G11,G12	0.388,0.405	0	No data	12	NA
Unknown	U40	H11,H12	0.358,0.378	0	No data	12	NB
Standard	S1	A1,A2	3.916,3.992	1000	N/A		
Standard	S2	B1,B2	3.078,3.18	500	N/A		
Standard	S3	C1,C2	1.441,0.171	250	N/A		
Standard	S4	D1,D2	0.971,0.987	125	N/A		
Standard	S5	E1,E2	0.633,0.567	62.5	N/A		
Standard	S6	F1,F2	0.502,0.386	31.25	N/A		
Standard	S7	G1,G2	0.292,0.296	15.625	N/A		

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Curriculum Vitae

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EDUCATION

Master of Science, Exercise Physiology Anticipated: May 2018
University of Nevada, Las Vegas (UNLV) GPA: 4.0

Bachelor of Science, Kinesiology June 2016
University of Nevada, Las Vegas (UNLV) GPA: 4.0

Associate of Science, Biological Sciences Dec 2014
College of Southern Nevada, (CSN) GPA: 4.0

PROFESSIONAL EXPERIENCE

University of Nevada Las Vegas: Las Vegas, NV Aug 2017 - Current
Graduate Assistant

- Instructing Exercise Physiology Laboratory
- Assisting collogues with data collection
- Recruitment for research studies

University of Nevada Las Vegas: Las Vegas, NV Aug 2016 - Current
Teaching Assistant

- Instruction of 2 credit hours of Anatomy & Physiology Laboratory
- Exam and quiz proctoring
- Grading exams and quizzes

Premier Exhibitions: Las Vegas, NV Nov 2015 - Dec 2016
Docent in Bodies Exhibit

- Greet guest with a friendly demeanor
- Guide guests through the exhibition
- Answer questions regarding the bodies

University of Nevada Las Vegas: Las Vegas, NV Jan 2014 - Jan 2016
Student Athlete Tutor

- Tutor: Biology, Anatomy & Physiology, and Microbiology
- Helped students with comprehension of biological concepts
- Gave guidance on studying and critical thinking

College of Southern Nevada: Las Vegas, NV Jan 2014 - Jan 2016
Biology Tutor

- Tutor: Biology, Anatomy & Physiology, and Microbiology
- Helped students with comprehension of biological concepts
- Gave guidance on studying and critical thinking

Terry Reilly Health Services: Boise, ID
Medical Receptionist Oct 2011 - Jul 2012

- Provided patient services, greeted, and checked in patients
- Answered telephones and scheduled medical appointments
- Worked with team to ensure timely and professional service

PROFESSIONAL PRESENTATIONS PUBLISHED ABSTRACTS

Aguilar, C.D., Woita, A.C., Montes, J., Bodell, N.G., Tanner, E.A., MacDonald, G.A., Thomas, C., Manning, J.W., Taylor, J., Navalta, J.W. Prediction of Mechanical Efficiency from Body Fat Percentage and Years of Experience in Male and Female Rock Climbers. Annual Meeting of the Southwest American College of Sports Medicine, Costa Mesa, CA, 2016.

Tallent, R.C., Woita, A.C., Aguilar, C.D., Young, J., Navalta, J.W., Bodell, N.G., Montes, J., Tanner, E.A., MacDonald, G.A., Thomas, C., Manning, J.W., Taylor, J. Comparison of Mechanical Efficiencies from Steady State and Rapid Speed Rock Climbs. Annual Meeting of the Southwest American College of Sports Medicine, Costa Mesa, CA, 2016.

RELEVANT EXPERIENCE

Teaching Assistant Internship, UNLV Exercise Physiology Lab Jan 2016 - Jun 2016

- Helped instruct and facilitate four of the kinesiology 491 lab sections
- Practiced writing lesson plans, instructing, and grading papers
- Gained familiarity with lab equipment and procedures

Undergraduate Research, **UNLV Exercise Physiology Lab** Oct 2015 - Dec 2015

- Tested hypothesis and ran statistical analysis
- Developed peer review research writing and reading skills
- Presented abstract at Spring 2016 Student Research Forum

ACHIEVEMENTS

National Education for Women's Leadership Alumni Class of 2015

Women's Research Institute of Nevada	2015 - 2017
Phi Theta Kappa Honors Society	2012 - Current
President's List	2012 - Current
CSN Biology Club President	2013 - 2014

SERVICE

International Journal of Exercise Science	2017 - Current
Desert Dash Trail Running	2016 - Current
Wetlands Park Clean-up	2012 - Current
Clark County Wash Green Up	2012 - Current
Three Square Food Bank	2012 - 2013