Post-Exercise Metabolic Response to Acute Hypoxic Interval Bouts

by

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Abstract

An overwhelming number of investigations have examined the effect of low arterial partial pressure of oxygen (P_aO_2) on cardiorespiratory response and athletic performance. Less attention has, however, been given to the effect of hypoxic interval exercise on post-exposure substrate oxidation. The study, therefore, examines the effects of hypoxic interval exercise on post-exercise substrate partitioning and energy expenditure. Endurance trained athletes (age: 28±5 yrs; height: 178±7 cm; weight: 75±6 kg; BMI: 24±1 kg•m^{-2}) underwent a ramp cycling test in normoxia to determine maximal oxygen uptake ($\dot{V}O_{2max}$: 4.3±0.4 L•min^{-1}) and peak power output (PPO: 331±30 W). Participants were then assigned to a randomized, controlled crossover design experiment consisting of a 45-min basal metabolic rate (BMR), followed by a 60-min cycling interval exercise protocol (3-min @70%PPO, 4.5-min @35%PPO), and a 60-min post-exercise metabolic rate (PEMR). The treatment (hypoxic interval exercise) and the control (normoxic interval exercise) were performed under moderate hypoxic (F_iO_2= 0.15) and normoxic (F_iO_2= 0.2094) conditions, respectively. To control for the thermic effect of food, the participants consumed a standardized meal (780 Kcal; 26g fat, 98g carbohydrate, and 28g protein) between 18:30 and 19:00 the night before and fasted for 12-hrs prior to exercising. Post-hypoxic interval exercise glucose oxidation significantly decreased by 140±44 mg•min^{-1} from BMR to PERM while no change was observed post-normoxic interval exercise (Δ2±16 mg•min^{-1}). A corollary of these outcomes resulted in a significant increase in fat oxidation (Δ72±38 mg•min^{-1}) from BMR to PEMR post-hypoxic interval exercise with a non-significant increase post-normoxic interval exercise (Δ14±20 mg•min^{-1}). Energy expenditure was not significantly different from BMR to PEMR (Δ0.14±0.22 Kcal•min^{-1} and Δ0.03±0.22 Kcal•min^{-1} in hypoxic and normoxic interval exercises, respectively). In conclusion, hypoxic interval exercise affected substrate partitioning up to one hour after exercising. This result could be explained by higher reliance on endogenous glucose during exercise under hypoxia compared to normoxic condition at the same absolute workload. These results might lead to development of a non-pharmacological approach to weight loss management.

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List of Symbols, Nomenclature or Abbreviations

ATP: Adenosine-triphosphate
BMR: Basal metabolic rate
CHO: Carbohydrate
EE: Energy Expenditure
EPOC: Excess post-oxygen consumption
HIIT: High-Intensity Interval-training
HYP: Hypoxia
NOR: Normoxia
PEMR: Post-exercise metabolic rate
PPO: Peak power output
PRO: Protein
RPE: Rate of perceived exertion
RPM: Rotations per minute
SP: substrate partitioning
$\dot{V}CO_2$: Carbon dioxide production
$\dot{V}O_2$: Oxygen uptake
$\dot{V}O_{2max:}$ Maximal oxygen uptake

Chapter 1: Thesis Overview

1.1: Overview of the Thesis

This thesis report, "Post-exercise metabolic response to high-intensity interval training under normoxic and hypoxic conditions" is presented in manuscript form.

Chapter 2 focuses on a review of literature regarding the main components of this study. The main areas that are reviewed within this section are as follows: hypoxia (HYP), exercise modalities, indirect calorimetry, substrate partitioning (SP) (during and post-exercise), and energy expenditure (EE) (pre and post-exercise).

Chapter 3 is the manuscript of the study. This chapter includes: the introduction, experimental design and approach, the techniques used, results, and a discussion of the results. Overall, it examines the effect of hypoxic cycling bouts on post-exercise substrate contribution to energy production in seven male endurance athletes.

Chapter 4 is the overall conclusion from the research conducted. It includes the response to the hypothesis, a summary of the overall thesis, as well as, the limitations of the study.

1.2: Background of Study

Researchers with an interest in exercise physiology are continually searching for the best method of training to enhance exercise performance. Within this discipline, environmental conditions and the effects on the human body are observed closely.

The study of HYP began decades ago. It was after the 1968 Summer Olympics, in Mexico City, that a piqued interest in the effects of HYP on exercise performance was heightened. During these Olympics Games no records were broken. Athletes had a difficult time competing in the

environmental conditions, the high altitude (2200m), respectively (Parker, 2004). It was after these Olympic Games that the importance of the body's acclimation to different environmental conditions in the elite athlete were recognized. A series of questions surrounding exercise and HYP arose. The effects of HYP and high-intensity interval training (HIIT) on SP and EE post-conditions, are the main focus for this research project.

To date, research is limited on how HYP and interval training affects SP and EE, post-exercise. It has been shown previously that interval training has an effect on SP and EE post-exercise; in that carbohydrates (CHO) are depleted and the body relies heavily on fat oxidation for energy, until the body replenishes the glycogen that was depleted (Kuo *et al.*, 2005). It has also been shown that EE is higher post-exercise, and can become even more significant in post-interval training style exercise than in steady state (Gore and Whiters, 1990). Research has shown that hypoxic conditions can alter SP during and post-exposure, as well as, effect EE (Tschop *et al.*, 2001). An increase in EE has been observed both during and post- hypoxic exposure; although the amount of time EE remains raised is still uncertain due to variability of research protocols (exposure level and duration) (Bernardi *et al.*, 2001; Clanton *et al.*, 2001; Levine, 2000).

Substrate partitioning has been examined thoroughly during exposure; however, the research is still very limited in post-exposure effects. Research has been conducted to show that post-exposure to a hypoxic condition can cause glycogen depletion, therefore raising fat oxidation to provide the body with the energy necessary to recover and support the function of vital organs (Workman and Basset, 2013). Examining how the body reacts post-exercise, specifically how the body reacts post-hypoxic intervals, will lead us to evaluate further the effects of SP and EE. Through evaluation of

basal metabolic rates (BMR) and post-exercise metabolic rates (PEMR) ofnormoxic exercise and hypoxic exercise, we would expect to see a significant positive effect on the human body.

1.3: Purpose of Study

Different styles of cardiovascular training have different effects on the human body.For example, training in high altitude will have a different effect on the body compared to sea level training. It has been shown that interval training has a positive effect on the body (Kuo *et al.*, 2005). Training under hypoxic exposure has been shown to enhance athletic performance, as well as aid as a weight loss strategy (Bernardi *et al.*, 2001; Millet *et al.*, 2010; Tschop *et al.*, 2001). It is important to note that both conditions (optimal altitude and intensity) need to be manipulated so the maximal benefits will be reaped. Training at optimal altitude (~2000m-moderate altitude) and at optimal intensity to increase EE (>70%), will yield such results (Gore *et al.*, 1990; Warren *et al.*, 2009). It can be predicted that if HIIT and hypoxic exposure are combined at optimal levels, greater enhancement in performance will be achieved, as well as greater EE and in return, greater weight loss.

The purpose of this study was to compare substrate partitioning and energy expenditure pre- and post- exercise under normoxic (NOR) and HYP conditions in an endurance-trained population. Throughout the study, SP, EE, heart rate (HR), oxygen saturation (SpO_2), and rate of perceived exertion (RPE) will be monitored and observed under both conditions (NOR and HYP). It was hypothesized that in this current study: 1) there will be a difference in SP between NOR and HYP conditions; 2) there will be an increase in EE in HYP compared to NOR; and 3) there will be a fat oxidation shift during post-exercise recovery in both conditions (greater in HYP compared to NOR).

1.4: Significance of Study

Results shown in this current study have great application towards enhancing endurance performance. It has been shown that in post-HYP exercise, there is a significant decrease in glucose oxidation, therefore a decreased amount of CHO available to fuel the body during the recovery period. The popular approach of "carb loading" could be a useful tactic in such circumstances, although not specific to this current research. Following such protocol would ensure that a participant would have enough CHO for fuel, to complete the task at hand. From the given results, this current study has applicable use, not only for an enhancement in endurance training, but also for potential weight-loss protocol. It can be difficult to implement strenuous exercise regimes on obese individuals, due to greater health risks, as well as the untrained capacity to perform certain exercises (Urdampilleta *et al.*, 2012). If an obese or overweight individual is well monitored and/or aware of their own health, training under a HYP condition could be valuable to them (Netzer, Chytra, & Kupper, 2008; Urdampilleta *et al.*, 2012). Weight-loss can be achieved through manipulation of EE and SP. Creating a greater EE and a shift towards lipid oxidation have been shown to positively correlate to weight-loss. Therefore, using a combination of exercise and HYP will allow individuals to create this negative energy balance by increasing their post-exercise EE and shifting their SP towards lipid oxidation. It is worth mentioning that dietary intake needs to be balanced (energy intake needs to be equal or less than energy output) with this protocol in order for it to be successful (Urdampilleta *et al.*, 2012).

Chapter 2: Review of Literature

2.1: Introduction

Research involving the effects of high altitude training on athletic performance increased significantly after the 1968 Olympics in Mexico City (Parker, 2004). The results of endurance trials in these Olympic Games were not as outstanding, as they had been for previous Olympics. This could have been due to the athletes not being accustomed to the lowered partial pressure of oxygen, at an altitude of 2250m (Parker, 2004). Partial pressure of inspired oxygen at sea level is 149 mmHG (Hale, T, *62-63*, 2003), whereas at ~2250 m partial pressure of inspired oxygen is 110-120 mmHG (Rusko, Tikkanen, & Peltonen, 2004). Bailey *et al.* (1998) stated that acclimation to a reduced inspiratory partial pressure of oxygen initiates metabolic and cardiorespiratory adaptations that influence O_2 transport and utilization. Altering an athletic environment prematurely would significantly impact these adaptations.. These adaptations and athletic environments are major areas in research to date, including HYP and athletic performance, HYP and weight-loss, and HYP and human metabolism.

Hypoxic exercise and how it affects key components of human metabolism, EE and SP, still requires further investigation. Being able to understand how human metabolism reacts to HYP exercise can aid in preparing athletes who need to compete at higher altitudes. The knowledge gained regarding human metabolism (SP and EE), can also benefit individuals who are overweight or obese. As HYP and intense levels of exercise have been observed to aid in weight-loss (Netzer *et al.*, 2008). Overall, HYP has yielded positive results in multiple studies; however, there still remain inconsistencies. There are many components, which need to be considered when studying the topic and that should be introduced to fully grasp the concept of this research project. Main considerations are HYP (exposure level and duration), interval-training type exercise, SP, EE, and metabolism

(BMR vs. PEMR). Understanding how these components are manipulated, to reap health and/or athletic benefits, will allow researchers to construct proper regimes in current and future studies.

2.2: Hypoxia

Hypoxia has been studied throughout the decades, however the true pique in athletic performance and how HYP affects the human body began shortly after the 1968 Olympic Games. Parker (2004) stated that it was still unclear at the time of the games, how the athletes would react to the environmental condition. Since then, HYP and its effect on the human body have been highly studied in multiple physiological parameters, including athletic performance, SP, and weight management.

Hypoxia can be induced by two main approaches: (1) affecting the partial pressure of gases, that is, lowering the effect of gravity using altitude or hypobaric chamber or (2) affecting gas concentrations using the nitrogen dilution technique (Kelly *et al.*, 2013). Hypoxia is classified into grades of elevation and duration of exposure. Levine (2002) discusses the HYP elevation levels and which are the most beneficial; altitudes less than 1600m are too low to stimulate an effective response, and altitudes greater than 3000m can result in negative effects of altitude acclimatization. Therefore, altitudes between 1600m and 3000m are the most beneficial and are considered as moderate altitude in most studies (Levine, 2002; Millet *et al.*, 2010; Wilber, 2001). Clanton *et al.* (2001) evaluated the difference between intermittent HYP and long-term exposure to severe HYP. Clanton *et al.* (2001) found that prolonged exposure to severe HYP (>3000m) can progress to cell injury and deterioration, whereas short-term exposure does not have this effect. Therefore, it is important to overview the duration of exposure to a HYP environment.

Hoppeler *et al.* (2003) best categorize duration of exposure into five groups: 1) Individuals who are 'native' to the HYP climate, is any individual who has been exposed to HYP for generations and could possibly be genetically equipped to be at altitude (e.g. Sherpa's); 2) 'Permanent' HYP conditions for individuals who live in a HYP environment from birth to death, and may only be exposed to normoxia for brief periods during their lifespan; 3) 'Long-term' HYP is when an individual is brought into HYP and remains under the condition for a duration of weeks to months (Mountaineering expedition); 4) 'Short-term' HYP which is referred to as continuous bouts of HYP exposure, lasting only minutes to hours, the remainder of the individual's day is spent under NOR conditions. 'Short-term' HYP has been most commonly used for athletics; and 5) 'Intermittent' HYP, which is during experimental protocols where short periods of HYP are implied as well as short periods of NOR, lasting only up to minutes for each condition. Clanton *et al.* (2001) described intermittent HYP as a condition of repeated, transient reductions in O_2 that might play a role in triggering the responses to certain environmental or pathological conditions.

There are four different training protocols that are taken into consideration when trying to enhance athletic performance. Hochachka *et al.* (1998) described these different levels of training effects as; (a) acute and acclimation responses to HYP, (b) highlander acute and acclimation responses, (c) phenotype for HYP tolerance, and (d) similar phenotypes for HYP tolerance and for endurance performance. Hochachka *et al.* (1998) divided the groups based on current living altitude, as well as genetically equipped adaptations to high altitude; similar to that explained by Wilber (2001). Wilber (2001) described the levels of training as (a) live low-train low (LLTL) (sea-level standard), (b) live low-train high (LLTH) (HYP is incorporated into training regimes by intermittent HYP training), (c) live high-train low (LHTL) (gain an increase in endogenous erythropoietin and resultant increase in erythrocyte volume from living at altitude, while simultaneously inducing beneficial metabolic and

neuromuscular adaptations from training at sea level), and (d) live high-train high (LHTH) (live and train in a natural/terrestrial hypobaric HYP environment at moderate altitude).

Bailey *et al.* (1998) compared endurance athletes training in a sustained moderate altitude to endurance athletes training at sea-level, to assess any enhancement in athletic performance. Chronic hypoxia did not influence performance during or following recovery from the maximal exercise regime after return to sea-level (Bailey *et al.*, 1998). A decrease in lactic acidosis during submaximal exercise at sea-level was found in the group that trained at altitude. Bailey *et al.* (1998) reported that the decrease in lactic acidosis was due to a decrease in lactate flux from skeletal muscle to blood. They concluded overall that a 4-week exposure to moderate altitude training did not improve performance following recovery from either submaximal or maximal exercise. Furthermore, supramaximal exercise performance was impaired following return to seal-level (Bailey *et al.*, 1998). Although Bailey *et al.* (1998) did not support the enhancement of athletic performance through LHTH mechanism, Bernardi *et al.* (2001) and Millet *et al.* (2010) observed a more beneficial method of training using IHT.

Bernardi *et al.* (2001) was one of the pioneers to research this training regime, and its benefits. Bernadi *et al.* (2001) defines IHT as repeated short periods (5-7min) of steady or progressive HYP, interrupted by similar periods of rest/recovery. It was shown, that in order to trigger positive responses from HYP, a sequence of HYP/NOR is necessary (Bernardi *et al.*, 2001). Intermittent HYP exposure displays similar results as those obtained by training at low altitude, while living at high altitude or in hypobaric HYP for 30-min/day (Bernardi *et al.*, 2001).

More recently, Millet *et al.* (2010) stated that altitude exposure in conjunction with the exercise training could work synergistically to induce the adaptations necessary for increase in performance,

further supporting Wilber (2001) and Hochachka *et al.* (1998). Millet *et al.* (2010) discussed in detail LLTH training strategies that are similar to the protocol used for this current study. Intermittent hypoxic training (IHT) is a method of LLTH. Intermittent hypoxic training is effective, even though the time spent in HYP is not as sufficient as the alternative training regimes (ie. LHTL, LHTH) (Millet *et al.*, 2010). There are many ways HYP affects the human body. Bernardi *et al.* (2001) stated that IHT can enhance muscle tissue by muscle fibre size, capillarity, myoglobin concentration, and muscle oxidative capacity. Millet *et al.* (2010) concluded that athletes who engaged in endurance sports could take advantage of IHT, at an altitude of 2500-3000m. Overall, there still remain several unanswered questions regarding the optimal combination of training, level of exposure, level of duration, and intensity to gain all benefits from IHT.

Hypoxia has shown multiple effects on the human body, which can be altered, depending on duration and degree of exposure. When studying HYP and exercise, there are multiple areas of concern. The most common research in this field looks at increased performance post-treatment under HYP conditions, oxygen consumption ($\dot{V}O_{2MAX}$) improvements, muscle performance, and substrate alteration during and/or post-exposure and/or alterations in EE (Millet *et al.*, 2010; Tschop *et al.*, 2001; Basset *et al*., 2006).

There are some major concerns when training under a HYP environment. Acute mountain sickness can affect training intensity and duration. Basset *et al.* (2006) states that headaches, loss of appetite, sleeplessness, and queasiness have been diagnosed after long-term exposure of HYP in humans. Symptoms of acute mountain sickness include dizziness, light headedness, nausea, weight loss, decrease in appetite, headaches, sleeplessness, and typically happen in individuals who are at an altitude above 1600m (Tschop *et al.*, 2001). There remains some uncertainty regarding the perfect

combination of exercise (intensity and duration) and level of exposure. Determining the effects of both conditions on human metabolism are most efficiently done through indirect calorimetry. Determining PEMR through this technique during recovery post-exposure allows researchers to assess SP and EE.

2.3 : Indirect Calorimetry

Simonson *et al.* (1990) stated that BMR is the greatest determinant of overall daily EE, it is mainly dependent on the lean body mass of the subject; however, age, sex, and familial factors also play a role. Measuring BMR can be done by direct calorimetry and indirect calorimetry. Direct calorimetry involves the heat generated by the body within an insulated environment (Simonson *et al.*, 1990). This method of measurement is identical to that of the simple bomb calorimeter, in which the caloric content of a substance is determined during the process of combustion (Simonson *et al.*, 1990). Simonson *et al.* (1990) states that the heat generated when an essential nutrient (carbohydrates (CHO), lipids, protein, lactate, or ketone body) is oxidized to CO_2 and H_2O within the body, is the same process, which happens through the bomb calorimeter. Protein is best measured through urinary collection because the greater end products of protein catabolism are urea, uric acid, and creatinine (Simonson *et al.*, 1990). Although direct calorimetry has great potential in measuring caloric amounts, there are some disadvantages that accompany the process. One major disadvantage of direct calorimetry is that the apparatus used is not easily set up and requires the subject to remain in a physically confined space for an extended period of time (Simonson *et al.*, 1990). Also, direct calorimetry, being a measure of heat, only measures conductive, convective, and radiant heat loss from the body; there is no measurement of the heat released when the water of perspiration is converted to its gaseous phase (Simonson *et al.*, 1990). It should be noted that it could be corrected for by measuring the water content of the chamber and by knowing the molar heat of vaporization of

water; requiring more work by the researcher (Simonson *et al.*, 1990). Another important factor to take into consideration is that the researcher must account for the heat stored in the body, as the measurement should reflect the change in body temperature. This can be corrected by recording core body temperature and skin temperature (Simonson *et al.*, 1990). Simonson *et al.* (1990) also stated that direct calorimetry does not measure energy expended during the performance of external work. Finally, Simonson *et al.* (1990) concludes that this process does not provide any information about the nature of the substrates being oxidized to generate energy within the body.

Indirect calorimetry is the method of determining energy (heat) production by measuring O_2 uptake and CO_2 production rather than directly measuring heat transfer (Simonson *et al.*, 1990). In earlier stages of using indirect calorimetry, a closed circuit design determined the rates of O_2 uptake and CO_2 production through changes in volume or pressure in the closed system (Simonson *et al.*, 1990). Today, this method is rarely used when testing humans; therefore, an open circuit was created. The open circuit method has both ends of the system open to the atmospheric pressure, and separates the subject's inspired and expired air by a three-way respiratory valve (Simonson *et al.*, 1990). Simonson *et al.* (1990) stated that the difficulty with this method is that untrained subjects involuntarily hyperventilate when requested to breathe through a mouthpiece. This yields inappropriately high rates of O_2 uptake and CO_2 production. To avoid such circumstances, a ventilated hood system has been introduced. The ventilated hood is placed over the subjects' head and is made airtight. The air is then drawn through the system by an adjustable speed fan (Simonson *et al*., 1990). Simonson *et al*. (1990) concluded that indirect calorimetry has a major advantage for the study of nutrition and clinical physiology, since it can provide quantitative information about the type of substrates that are oxidized.

Ferrannini (1988) stated that indirect calorimetry was on the rise in clinical and physiological aspects. Indirect calorimetry has been one of the main systems used to evaluate metabolic rates in humans. Ferrannini (1988) defines indirect calorimetry as the measurement of metabolic free energy conversion. It is the method of determining the energy production through measuring O_2 uptake and CO_2 production (Ferrannini, 1988). The measurements of substrates are important in understanding how the body retains and produces energy and how the substrates are utilized for certain activities. To further support Simonson *et al.* (1990), Ferrannini (1988) stated that indirect calorimetry supports further research in the nutrition field; thermogenesis, the energetic of physical exercise, as well as the pathogenesis of obesity and diabetes. Ferrannini (1988) discusses early theories on energy production and systems related to CHO depletion. Ferrannini (1988) defined energy production as the conversion of the chemical free-energy of nutrients into the chemical energy of ATP plus loss of some energy during the oxidation process. Eventually, all energy will be converted into heat (Ferrannini, 1988). When looking at the oxidation rates of the three main fuel sources (glucose, fat and protein), there are several aspects that need to be taken into consideration. The respiratory quotient (ratio of CO_2 to O_2) is 1.00 for glucose, 0.70 for fat, and 0.80 for protein (Ferrannini, 1988). Caloric or ATP equivalents of O_2 can then be calculated to determine how much energy or ATP we can generate with 1L (or 1 mol) of O_2 (Ferrannini, 1988). Through these calculations, it was concluded that glucose has the highest equivalent, followed by palmitate (a form of fatty acid) and protein (Ferrannini, 1988). Ferrannini (1988) reinforces the importance of this calculation, stating that the most efficient way of utilizing O_2 to produce reusable energy is to oxidize glucose. Fat and protein oxidation are more costly, in terms of O_2 currency. Exchange of energy is a reoccurring event that happens constantly between the body and the environment. It is important that this is done as efficiently as possible. Therefore, understanding what substrates will generate the most energy with

the least amount of work is extremely important. Using the previous values, the rates of substrate oxidation are calculated through stoichiometry equations derived from a series of algebraic equations. The equations are as follows (Simonson *et al.*, 1990):

$$G\ (g\ min^{-1}) = (4.55 \bullet \dot{V}CO_2) - (3.21 \bullet \dot{V}O_2) - (2.87 \bullet N)$$

$$L\ (g\ min^{-1}) = 1.67(\dot{V}O_2 - \dot{V}CO_2) - (1.92 \bullet N)$$

$$P\ (g\ min^{-1}) = (6.25 \bullet N)$$

Oxidation rates ($g \bullet min^{-1}$) of CHO and lipid were calculated through the above equations' where; $\dot{V}O_2$ ($l \bullet min^{-1}$) and $\dot{V}CO_2$ ($l \bullet min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (Workman *et al.*, 2012). Simonson *et al.* (1990) stated that N content of mixed proteins is ~16% it is generally assumed that each gram of urinary nitrogen is stoichiometrically derived from the oxidation of 6.25g of mixed proteins, the same value given by Ferrannini (1988). All equations are congruent with the equations stated by Simonson *et al*. (1990) when calculating the substrate partitioning of these three major fuels. It is important to note that there are times that protein is per-assumed as a standard constant, since the indirect calorimetry of gas exchange does not measure the protein oxidation. The standard value for protein is 0.066g (Haman *et al.*, 2004). Since it is well known that protein content rarely varies from occasion to occasion, the N component can be removed from the stochiometry equations when calculating the SP of the glucose and lipids components (Simonson *et al*., 1990). Ferrannini (1988) stated that it is important to measure the urinary non-protein nitrogen excretion in order to eliminate any variation that could occur, and ensuring all values are as accurate as possible. Ferrannini (1988) further discussed restrictions, requirements, and advantages of the method of indirect calorimetry.

Ferrannini (1988) stated five different technical requirements that are essential to accurately measure indirect calorimetry: (a) an air-tight canopy with a constant air flow to be adjusted; (b) sensitive stable O_2 and CO_2 analyzers for continuous sampling of expired air; (c) a calibration routine using standard gas mixtures; (d) a system that traps or condenses out the moisture of the expired air line feeding into the sensors; and (e) a software to store and manipulate the data in any small desktop computer. It is reiterated that humidity can alter the fractional gas concentrations; therefore, recent metabolic carts monitor humidity within the system as well as within the atmosphere. Finally, it is critical to calibrate technical equipment to assure the system is correctly set-up, as well as, to aid the researcher and apparatus to analyze any O_2 or CO_2 drift that occurs throughout the study (Ferrannini, 1988).

A simple method of measuring O_2/CO_2 drift is to take baseline measurements 15-min prior to testing. During the testing, take 1 to 2-min baselines every 15-min, then post-testing take a final baseline for another 15-min. Having these baseline measurements allows the researchers to compare values from the beginning to the end of testing to see if there is a significant delta occurring. If drift is determined, it can then be corrected. Ferrannini (1988) briefly discussed one major concern with the urinary protein analysis; the timing of the urine collection is crucial. Urine output is better determined over longer periods of time. Ferrannini (1988) notes that, over an extended period of time, the metabolic state of the studied subject invariably changes, and the assumption that protein oxidation remains constant can become more questionable. Ferrannini (1988) stated seven major findings that summarize indirect calorimetry:

1. We have exact equations to calculate lipid, glucose, and protein oxidation.

2. The equation giving the energy production rate is one and the same when the metabolic processes involving gaseous exchange are those in previous calculations.

3. Approximate corrections can be applied to the measured gas flows to account for ketone body and lactate metabolism.

4. The presence of gluconeogenesis from amino acids and lactate whether occurring along or in the combination with net lipid synthesis from any source.

5. There are equations that give the coefficients for the known variables ($\dot{V}O_2$, $\dot{V}CO_2$, Glucose, etc) when the unknown (ie: gluconeogensis) are present.

6. Gluconeogenesis from protein sources causes an underestimation of lipid oxidation. Protein oxidation is definitely overestimated if gluconeogenesis is active

7. Estimation of energy production rate is relatively more resistant to the impact of gluconeogenesis.

Although the methods established from Simonson *et al.* (1990) and Ferrannini (1988) were almost three decades ago, their work is still the major basis of multiple studies. Horton *et al.* (1998) utilized these methods of indirect calorimetry established to calculate CHO and fat oxidation from the volume of O_2 consumed and the volume of CO_2 expired with correction for protein oxidation. Both introduced and well established by Simonson et al. (1990) and Ferrannini (1988). It was also noted through the research of Horton *et al.* (1998) that ketones may lead to errors in the estimation of fuel oxidation after exercise, but reassured that any errors reported would likely be minimal, which is also further supported by their previous research (Simonson *et al.*, 1990; Ferrannini, 1988).

Butler *et al.* (2004) further established that these methods are the most valid measurements of metabolic rate within the physiology field. Finally, Workman and Basset (2012) also used the methods established by these researchers to evaluate metabolic rate in overweight individuals after sedentary hypoxic exposure. It can be concluded that the methods proposed by Simsonson *et al.* (1990) and Ferrannini (1988) are valid, reliable, and useful for all studies within the physiology field that require basal and resting metabolic rate measurements.

Overall, indirect calorimetry does have limitations and potential for error. However; the limitations involved with indirect calorimetry can be kept to a minimum in order to yield the most accurate results possible. Both, Ferrannini (1988) and Simonson *et al.* (1990) agree that indirect calorimetry is the most effective method in measuring SP and EE estimation in nutrition and physiology, due to the flexibility it allows to conduct research on a human body in motion (ie. during exercise).

2.4: Exercise Modalities

Physical activity is defined as any bodily movement produced by contraction of skeletal muscle that substantially increases EE (Howley, 2000). Howley (2000) subcategorized physical activity to "exercise" or exercise training as planned, structured, and repetitive bodily movements that are performed to improve or maintain one or more components of physical fitness. Exercise has several components to consider, two very important components being intensity and duration.

The determination of exercise intensity (a) refers to whole-body metabolic rate; (b) should be categorized as: low-intensity (<50%), moderate (>50% and <75%), and high (>75%) (Basset & Boulay, 2000). Submaximal endurance training is exercise performed at 65-75% of the individuals VO_{2MAX} (Laursen & Jenkins, 2002). Laursen *et al*. (2002) state that through submaximal endurance

training an individual can experience increased delivery of O_2 to exercising muscles, even though changes in $\dot{V}O_{2MAX}$ occur after several weeks of training.

Steady-state training is an aerobic exercise in which the intensity of the exercise remains the same for the full duration of the exercise (Kelly *et al*., 2014). Interval training differs by having periods of intense exercise, coupled with periods of rest or less intense exercise. High-intensity interval training is described as brief, intermittent bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise (Gibala *et al.*, 2012). Perry *et al.* (2008) stated HIIT is repeated exercise intervals at high, submaximal intensities of 80%-90% $\dot{V}O_{2peak}$. Gibala *et al.* (2012) stated that factors such as intensity, duration, and number of intervals that are performed during the session could become major factors when assessing EE, SP, and enhancements of athletic endurance.

2.5: Substrate Partitioning during Exercise

Living organisms, including humans, are constantly in energy exchange with the environment (Simonson *et al.*, 1990). Metabolism is the sum of all of chemical processes within the human body, which controls how the energy taken in is liberated to generate energy for daily functions and activities. The major source of this energy is stored within C-H bonds of carbohydrates, lipids and protein (Simonson *et al.*, 1990). This energy is generated through oxidative pathways and is expended in four major responses (Simonson *et al.*, 1990); (a) Basal metabolism: the work performed for ion transport to maintain electrochemical gradients across the cell membrane, energy required for growth and repair, energy expended through interconversion of metabolic substrates, and the mechanical work performed by the cardiovascular and respiratory systems. (b) External work done by muscular contractions (i.e. exercise). (c) Thermoregulatory mechanisms, to maintain core body temperature. (d) The thermic effect of food.

Three macronutrients (lipids, protein and CHO) are the major fuel sources that allow the human body to function as a whole. Carbohydrates are either an endogenous energy source from the muscle and liver glycogen, or exogenous glucose from blood glucose (Brooks & Mercier, 1985). Lipids are adipose and intra-muscular triglycerides (Brooks *et al.*, 1994). Amino acids are in the blood, muscle, and liver pools; they also compose human protein (Brooks *et al.*, 1985). The human body uses these three macronutrients to produce adenosine triphosphate (ATP) in order to maintain homoeostasis. The timing of utilization of the three macronutrients varies depending on energy requirement.

Substrate utilization and exercise intensity are directly correlated. The crossover concept is the premise that there is an interaction between exercise intensity and endurance training status, with the net effect of two opposing influences determining the relative contributions of CHO and lipids to energy metabolism during exercise (Coggan, 1997). Brooks (1997) further stated that the concept represents an attempt to integrate the seemingly divergent effects of exercise intensity, nutritional status, gender, age, and prior endurance training on the balance of CHO and lipid metabolism during sustained exercise. The theory behind the crossover concept is that there is an essential changing point when the human body begins to use CHO as an energy supply instead of lipids. At the commencement of exercise, both fat and CHO are required. As intensity of the exercise increases, SP alters (Brooks, 1997). Generally, this crossover point happens at 50% $\dot{V}O_{2MAX}$, but it can be altered due to the above mentioned variables, that is, intensity, modality of exercise, and/or training profile, etc. (Brooks, 1997). Training status can have an effect on when the crossover of substrate utilization happens. Prior endurance training allows an individual to perform an exercise at a relatively lesser intensity, and therefore, their shift from lipids to CHO occurs later in the exercise regime (Brooks, 1997). Substrate utilization has been evaluated extensively during exercise, exhibiting important findings that allow researchers and athletes understand the timings of energy fuel utilization.

As stated previously, training has been shown to have an effect on the crossover concept. It has also been demonstrated that HYP exposure can alter substrate utilization during exercise. Louis & Punjabi (2009) conducted a single-blind study involving healthy volunteers, who were exposed to intermittent HYP for eight hours one testing day and to ambient air on another testing day (one week separation). It was found that intermittent HYP can negatively impact glucose disposal by affecting insulin-dependent and insulin-independent mechanisms of glucose disposal; and sustained HYP has a negative impact on glucose metabolism (Louis *et al*., 2009).

Benso *et al.* (2007) indicated that during exposure to high altitude and physical exercise there appeared to be a dependence on blood glucose as a fuel. This dependence had an associated increase in insulin sensitivity and lipolysis, which was coupled with a decreased reliance on lipid substrate. It is worth noting that the altitude in which this study was performed was higher than moderate altitude (>3000m). It has yet to be shown that altitudes greater than 3000m yield positive effects on human metabolic responses (Levine, 2000).

Investigators have proposed that a change in the regulation of metabolic pathways to favour greater dependence on CHO utilization at altitude would aid in maintaining homeostasis by optimizing the energy yield per unit O_2 (Katayama *et al.,* 2010). It was shown that, during exercise at moderate altitude, respiratory exchange ratio (RER) values were slightly but significantly higher compared with those at sea level (Katayama *et al.*, 2010). This further suggests a greater increase in CHO utilization. Therefore, it appears that the body adjusts accordingly to become as efficient as possible when exercise and HYP are introduced.

Overall, it appears that substrate utilization is altered during when a HYP environment is present. Substrate utilization post-exercise needs to be further researched in order to understand how the body recovers from a given activity.

2.6: Post-Exercise Metabolic Rate

Excess post-oxygen consumption (EPOC) was first introduced by Gaesser *et al.* (1984). It included the more prolonged increase in oxygen uptake that may be observed for hours after exercise (Borsheim & Bahr, 2003). Excess post-oxygen consumption is defined as the elevation in O_2 consumption above the resting level, after exercise. This was initially thought to contribute significantly to the energy cost of exercise (Laforgia *et al.*, 1997).

Previously, Chad and Wenger (1988) investigated different durations of exercise (30-, 45-, and 60-min) at the same intensity (70% $\dot{V}O_{2MAX}$) using the same modality (ergometer cycle). It was concluded that the EPOC increased by 2.3- and 5.3-fold, respectively, when the exercise duration was increased from 30-minutes to 45-minutes and 60-minutes (Chad *et al.*, 1988). Several studies have shown (Borsheim *et al*., 2003; Gore and Whiters, 1990; Warren *et al*.,, 2009) that the longer the duration of exercise, the greater the EE, which results in a greater EPOC. Although it has been shown that the duration of the exercise is highly important in gaining a greater EE and EPOC, it appears that intensity of the exercise can be just as important to focus on.

Gore and Whiters (1990) defined EPOC as a substantial percentage of the net EE of exercise. They compared the durations and intensities of a given exercise to retrieve which factor had the greatest effect on EE and EPOC. The intensities used ranged from 30% to 70% $\dot{V}O_{2MAX}$, while the durations ranged from 20- to 80-minutes. Three different intensities were chosen to exemplify three different types of common modalities: (a) 30% $\dot{V}O_{2MAX}$-short walk, (b) 50% $\dot{V}O_{2MAX}$- recreational jog, and

(c) 70% $\dot{V}O_{2MAX}$- training run (Gore and Whiters, 1990). All three intensities are common classifications of aerobic exercise, but are solely steady-state exercises. It was determined that the intensity of an exercise can affect the final outcome of EE and EPOC greater than duration. When looking at exercise intensities and durations, interval training should be examined to compare final results to those of steady-state exercise. Gore and Whiters (1990) concluded that intensity accounted for 45% of the systematic variance of EPOC, while duration and the interaction between intensity and duration accounted for only 8.9% and 7.7%. They also mentioned that there could be a different outcome if the exercises completed were done on a consistent basis, where a cumulative effect may be shown.

Warren et al. (2009) continued to support that an increase in EPOC symbolizes an increase in exercise EE. Another important factor is that untrained individuals, who were looking to optimize their weight-loss efforts through exercise, may not be able to complete a constant load exercise of such high intensity for the required duration. Therefore, Warren *et al.* (2009) looked at interspersed interval type training, which included high-intensity bouts of exercise coupled with low-intensity recovery periods. They studied the effects of the three different variations of exercise: duration, intensity, and modality. This particular study compared a long duration (90-minutes) to a short duration (30-minutes). It was discussed that a prolonged exercise, at the required intensity (>75% $\dot{V}O_{2MAX}$), was generally beyond the tolerance level of healthy, non- trained adults; therefore, interval type training could aid in expending the same total amount of energy as continuous exercise bouts.

It has been discussed previously that interval training enhances fat oxidation and also increases EPOC (Warren *et al.*, 2009). They did not find any significant difference between continuous exercise and interval training type exercise on EPOC, but did find a significant difference between

high- and low-intensity exercises. The results determined that an exercise completed at high-intensity for a shorter time period yielded a greater EPOC, compared to a low-intensity exercise completed for a longer duration. The finding were consistent with Gore and Whiters (1990) and Chad *et al.* (1988) that intensity significantly affects EPOC and post-exercise metabolism; however, an understanding of SP involved, post-exercise, needs further investigation.

The SP involved in EPOC has not been studied in great detail, but investigations are beginning to examine the alteration in substrate utilization, and what elements, leading up to EPOC, are important (Kuo *et al*., 2004). As discussed previously, the crossover concept describes how substrates are used during exercise; however, there are no structured concepts developed, to date, which describe how substrates are used post-exercise. Several studies have determined theories as to how the body adjusts post-exercise, but there still remains a debate.

Looking more closely at the EE and SP helps to further evaluate how interval training affects human metabolism. Kuo *et al.* (2004) noticed that during exercise, CHOs were predominantly used when participants completed one hour of exercise at 65% $\dot{V}O_{2MAX}$; and that there was greater lipid contribution during the recovery phase of both exercise trials compared to the non-exercise group. There was an evident difference in substrate utilization between post-exercise and non-exercise groups, but no variance between the two exercise intensities and durations (Kuo *et al*., 2004). The results from Kuo *et al.* (2004) coincide with the theory that the shift to lipid utilization, during recovery post long duration exercise, is due to glycogen depletion and the re-synthesis of glycogen becomes a priority. Overall, it was found that physical activity does raise total EE, resulting in some lipid oxidation during a mild-moderate exercise, and then again post-exercise recovery when CHO are depleted (Kuo *et al.*, 2004).

Melanson, MacLean and Hill (2008) stated that exercise improves fat metabolism in muscle, but does not increase 24-hour fat oxidation. Fat metabolism in muscle is increased in endurance exercise training due to the increased density of mitochondria, the activity of enzymes involved, and O_2 delivery to the muscle (Melanson *et al.*, 2008). Fat oxidation rates are affected by training status, sex, and mode of exercise, but the highest rates of fat oxidation occurs at an exercise intensity of 55-65% $\dot{V}O_{2MAX}$ (Melanson *et al.*, 2008). Melanson *et al.* (2008) noted two key mechanisms of fat oxidation increasing post-exercise; (a) fat oxidation will increase post HIIT if glycogen stores are depleted when the exercise completed, or (b) if the individual regularly partakes in endurance training and is in a state of negative energy balance on training days then it would increase fat oxidation. Therefore, when looking at exercise and 24-hour substrate oxidation it is necessary to look at energy balance within the individual. During positive energy balanceCHO oxidation increases, and during negative energy balance fat oxidation increases (Melanson *et al*., 2008). Melanson *et al.* (2008) concluded that a negative fat balance must be achieved through a negative balance intake, as well as, an altered expenditure to ensure fat oxidation exceeds fat intake. The general consensus when observing SP post-exercise under NOR conditions, is CHO become depleted and fat oxidation becomes the main energy source until the body recovers from the induced exercise. However, research is remains limited and questions remain unanswered. For example, it has been shown that HYP effects SP, while at rest and while completing exercise. Results have varied due to different altitudes, and different intensities. It is during recovery that research is more limited; it has been shown that SP follows similar patterns while under HYP compared to NOR, but the alterations are more severe when a hypoxic environment is introduced. Therefore, it can be said SP shifts from glucose oxidation to fat oxidation quicker under HYP than the NOR counterpart (Kelly *et al.*, 2013). Limited research has been done in the field of the combined conditions. Studies

have been conducted on HYP exposure and post-treatment metabolic response, but there still remains a debate on whether the same effect occurs post-HYP exercise.

Workman and Basset (2012) examined post-metabolic response to passive normobaric HYP exposure in sedentary individuals. Through this research, a decrease in glucose oxidation (31%) and an increase in fat oxidation (44%) from pre- to post-exposure was shown (Workman and Basset, 2012). Workman and Basset 2012) stated that oxidation of lipid is known to contribute significantly to whole body energy turnover, both at rest and during exercise, and is also greatly affected by HYP. This study concluded that acute and short-term normobaric HYP increases metabolic rate and a shift in substrate utilization toward lipid sources (Workman and Basset 2012). Although no exercise was introduced during the protocol, a significant effect on substrate utilization post-HYP exposure was found, leading to future research of post-exercise and HYP metabolism.

2.7: Hypothesis

In order to advance an athlete's performance, to help individuals improve their overall health, and to find the optimal means of exercise to battle the worldwide obesity epidemic, research is constantly evolving. Substrate partitioning and EE have been evaluated through indirect calorimetry of subjects performing exercise under HYP, as well as resting under HYP. Currently, there remains variability in the research conducted on exercise in a HYP condition. The varying factors are: exposure length to HYP and method (sedentary vs. exercise intensity), as well as the intensity of the exercise performed.

Recent research has been progressing toward evaluating all varying factors within the field specific to HYP. Workman and Basset (2012) and Kelly *et al.* (2013) have evaluated HYP exposure and HYP exposure coupled with steady state exercise.

In 2012, Workman and Basset conducted a study on overweight males remaining sedentary under a HYP condition. Two groups were determined: passive acute HYP (3-hours of exposure), and passive short-term HYP (7 days of a single 3-hour exposure). Although no exercise was performed, the results yielded an increase in EE by 16% (during passive acute HYP), and an increase in EE by 12% between day 1-7 (passive short-term HYP). It was also found that fat oxidation increased significantly post-exposure, for both conditions.

Additional studies have been published to observe the effects of moderate HYP at a constant load exercise. Kelly *et al*. (2013) conducted a study that involved seven active males, who completed two 60-minutes cycle ergometer exercises under NOR and HYP conditions. The subjects performed the exercise at 50% of their peak power output, in both conditions. Substrate partitioning was evaluated during exercise, as well as post-exercise. It was concluded that lipid usage had increased, while muscle glycogen depletion played a role in the substrate utilization shift.

The hypothesis of the current study was derived from the studies noted above. Gore *et al.* (1990) concluded that as intensity of exercise is increased, for the same duration, EPOC is increased, as well. Workman and Basset (2012) did not show EPOC, but examined any post-hypoxic exposure metabolic rate alterations. It was found that hypoxic exposure did affect EE and SP, in that fat oxidation increased and glucose oxidation decreased. Kelly *et al.* (2013) reported that at similar constant workload intensity, the post-exercise MR was significantly higher than those performed under a normoxic condition (see Table 4 below). Therefore, an assumption can be made that, if an individual is performing a HIIT exercise protocol under HYP, the post-treatment metabolic rate will be significantly higher than that intensity performed under NOR.

In conclusion, there are three hypotheses that are discussed in this current study. (A) There will be a difference in SP between NOR and HYP conditions; (b) there will be an increase in EE in HYP compared to NOR; and finally, (c) there will be a fat oxidation shift during the post-exercise MR in both conditions (with greater effect in HYP compared to NOR). These questions will be addressed by comparing post-exercise metabolic rates of the same workload exercise in a NOR and HYP condition. Overall, it will be shown that exercise performed at the same workload will yield a greater effect on SP and EE under the HYP condition compared to the NOR counterpart.

Chapter 3: Manuscript

Post-Exercise Metabolic Response to Acute Hypoxic Interval Bouts

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3.1: Introduction

Research has shown that HIIT elevates EE compared to steady-state exercise (Gore and Whiters, 1990), and predominately uses CHO as an energy source during exercise, and lipids as an energy source post-exercise (Brooks, 1997). Understanding substrate utilization is very useful in exercise physiology. Although extensive research has been conducted on substrate utilization during a given exercise, the understanding of substrate utilization during the post-exercise recovery period is still vague.

Recovery, post-exercise, is important for several reasons; such as energy and nutritional replenishment, injury prevention, dehydration, as well as, muscle growth and health. Gore and Whiters (1990) compared the intensity and duration of exercise, and its effect on EE. It was concluded that intensity accounted for 45% of the systematic variance of EPOC, while duration was not as significant in the alteration of EE post-exercise. It has been shown that, an increase in EE post-exercise can promote lipid metabolism. Kuo *et al.* (2004) stated that, during exercise, CHOs were the predominant energy source when participants exercised for one-hour at 65% VO_{2MAX}. It was found that lipid oxidation was predominant during the recovery period of the exercise trials compared to recovery period of the non-exercise group (Kuo *et al.*, 2004).

Melanson *et al.* (2008) hypothesized two key mechanisms of fat oxidation, post-exercise: (a) fat oxidation will increase following high-intensity training if glycogen stores are depleted, or (b) if the individual regularly partakes in endurance training and is in a state of negative energy balance on training days then it would increase fat oxidation. It was concluded that, lipids can become the main energy source during post-exercise recovery when depletion of glucose occurs, during moderate to

intense exercise at sea-level. Exercising under these conditions utilizes, predominately, CHO stores. This is also where depletion of these stores occurs.

There are conflicting variations in the literature as to what degree altitude training affects SP and EE. Bernadi *et al.* (2001) defines interval HYP training as repeated short periods (5-7min) of steady or progressive HYP, interrupted by similar periods of rest/recovery (similar to this current study's protocol). This research reported that, in order to trigger positive responses from HYP, a sequence of HYP/NOR is necessary. It was also shown that HYP exposure positively affects SP and EE. Energy expenditure has been shown to increase when under HYP, creating a negative energy balance, and in turn, promoting weight-loss (Bernardi *et al*., 2001; Tschop *et al*., 2001). Katayama *et al.* (2010) stated that a change in the regulation of metabolic pathways to favour greater dependence on CHO utilization at altitude would aid in maintaining homeostasis by optimizing the energy yield per unit O_2; therefore, altering substrate utilization.

Substrate partitioning and EE, during recovery from HYP exposure, remains under review. The process of recovery, post-treatment (altitude training), is still not as clear as the cross-over concept established during exercise. Recent research has shown a decrease in glucose oxidation (31%) and an increase in fat oxidation (44%) from pre- to post-exposure (passive normobaric HYP exposure in sedentary individuals) (Workman and Basset, 2012). Kelly *et al.* (2013) stated that lipid usage increased post-exercise, due to muscle glycogen depletion. This is in line with previous research, by Millet *et al.* (2010), that has concluded that the body becomes glycogen depleted post-HYP exposure.

Research is limited when looking at the effects of HIIT coupled with HYP. It has been shown that HIIT affects SP and EE in a positive way to benefit athletic performance/or weight loss. Hypoxia has

also been found to have an effect on these parts of human metabolism. Therefore, coupling HYP and HIIT are of high interest in this current study.

An overwhelming number of investigations has examined the effect of low arterial partial pressure of oxygen (PaO_2) on cardiorespiratory response and athletic performance. Less attention has been given to the effect of hypoxic interval exercise on post-exposure substrate oxidation. The study, therefore, examines the effects of hypoxic interval exercise on post-exercise SP and EE. It was hypothesized that at same exercise workload, compared to NOR, (a) there will be greater EE, (b) there will be a shift towards fat after exercising in HYP.

3.2: Methods

3.2.1: Participants

Ten highly active men were recruited for the study; however only seven completed all testing requirements. Each participant was competitive in their respected endurance sport (running, cycling, or swimming) and trained an average of ten hours a week. The participants were verbally informed of the study's requirements and procedures. They were provided written and informed consent, which was approved by the Health Research Ethics Authority. Participants were required to complete a Physical Activity Readiness Questionnaire (PAR-Q), as well as a physical activity questionnaire, generated by the researchers prior to any testing being conducted. Table 1 summarizes the subject characteristics.

Table 1: Characteristics of the participants

Age	Body Mass	Height	$\dot{V}O_{2max}$	RER	HR_{max}	PPO
(years)	(kg)	(cm)	($ml \cdot min^{-1} \cdot kg^{-1}$)	(AU)	(bpm)	(watt)
26.8±5.5	76.2±5.0	178±7	57.4±6.9	1.12±0.3	178±7	331±30

Participants averaged 4.0 ± 2.5 sessions of training per week, ranging from endurance training to specific strength training. All individuals underwent a $\dot{V}O_{2MAX}$ ramp protocol to retrieve their peak power output (PPO) and their absolute $\dot{V}O_{2MAX}$ (Table 1). Table 2 displays their athletic profiles.

Table 2: Training profile of the participants

Training experience	Training Sessions	Interval Training (>75% $\dot{V}O_{2max}$)	Training load
(years)	(N•week^{-1})	(N•week^{-1})	(km•week^{-1})
7.4±6.5	4.3±2.6	1.4±0.6	54.0±7.1

3.2.2: Experimental Approach

The study consisted of a cross-over design with two conditions (treatment: HYP and control: NOR) that both underwent an exercise regime. Measurements of interest (SP and EE) were observed pre- and post-exercise; while measurements of physiological stress (Heart rate (HR), SpO_2, blood pressure (BP), and RPE were measured for the entire testing segment.

3.2.3: Experimental condition

Environmental Parameters

Room temperature was kept at a constant value of 22ºC throughout the study. Barometric pressure and humidity were internally controlled from within the SABLE system (Sable System International, North Las Vegas, USA).

Familiarization Session

The purpose of the first lab session was to obtain baseline measurements which included resting BP and HR, anthropometric measurements, cycle ergometer measurements, as well as a $\dot{V}O_{2MAX}$ test on a cycle ergometer in the NOR condition. The $\dot{V}O_{2MAX}$ test was standard ramp protocol that commenced at a 50-W baseline and increased 1-W every 3-secs. Cadence was freely chosen by the

participants, and was allowed as long as it remained above 60-rpm. Peak power output was the final wattage recorded on the Veltron cycle system (Velotron, Racer Mate, Seattle Washington, USA), once the participant reached their peak$\dot{V}O_2$, at the end of testing. At the completion of the ramp protocol, a 5-min period of rest was given. The rest period was immediately followed by a verification phase which consisted of the participant pedalling at 105% of the PPO they had just completed, while maintaining an RPM of 60, or higher.

Familiarization to the HYP environment was also introduced, during this first session, to ensure the participant did not feel any acute mountain sickness symptoms. The acute mountain sickness symptoms were derived from the Lake Louise Mountain Sickness test. Symptoms include: nausea, dizziness, light-headedness, feeling faint, and headache. Qualitative recordings of symptoms were collected throughout familiarization. All participants identified themselves as asymptomatic, scoring 0-1 on the Lake Louise Mountain Sickness test. Oxygen saturation was also monitored during this time to give researchers a physiological measurement to support qualitative findings.

2.3.4 Treatment Session (control and hypoxia)

Hypoxic Condition

The experimental condition for this study was the HYP environment. This required an O_2 concentration (concentration- Douglas bags) of 15%, compared to the NOR condition of 20.94% (sea level). The HYP environment simulated during this study is considered to be an equivalent to moderate altitude of 2200-2500m (Levine, 2000). The HYP environment was simulated through the GO_2 Altitude System (Biomedtech, Melbourne, Australia). The HYP environment was solely introduced during the exercise protocol of the testing session. The remainder of the testing was completed in a NOR environment.

Basal Metabolic Rate (BMR)

A BMR was recorded at the beginning of each testing session. As per BMR standards, all sessions took place first thing in the morning, 30-minutes after the participant woke up. All participants were required to have vehicular transportation to the testing session, to minimize any alterations to their EE levels and basal rates. The BMR consisted of the participant lying still and relaxed on a bed, under a canopy, while connected to a metabolic cart. The participant was asked to refrain from falling asleep. During this rest period, participants were supplied with ear plugs and noise cancelling headphones to eliminate any possible distractions. At this time, the participant was connected to both a HR and SpO_2 monitor, for the duration of the study. These protocols were followed consistently to minimize any alterations in BMR recordings. The BMR was recorded for a 45-min period.

Cycle Protocol

After the BMR was completed, participants were transferred to the cycle ergometer to begin the exercise protocol. A 5-minute warm-up @ 35% PPO was given prior to each exercise session. Once the warm-up was completed, the 60-minute interval training commenced, at a work interval of 70% PPO. Each interval session lasted for 3-minutes of 70% PPO and 4.5-minutes at 35% PPO (active rest interval). Preceding each interval (work and active rest), for the duration of the exercise protocol, the participant's RPE rating (Borg Scale, 1962) was recorded. Heart rate and SpO_2 were also recorded.

Post-exercise Metabolic Rate (PEMR)

Immediately after the exercise protocol was completed, PEMR was conducted to measure SP through gas analysis, during the recovery phase. The same restrictions applied to the PEMR protocol

as the previously conducted BMR (pg. 45). The PEMR took place for 60-min. Heart rate, SpO_2, and gas analysis were recorded throughout the recovery phase.

Determinants of Termination

If participants felt or recorded any of the following conditions, the experiment would cease (a) RPE of 20, (b) SpO_2 below 80%, (c) the participant did not feel well enough to continue, and/or (d) could not maintain a cadence above 60 rpm. High altitude mountain sickness was monitored throughout the entire testing protocol using the symptoms stated on the Lake Louise Mountain Sickness Scale. It was ensured that participants were asymptomatic and in good health, prior to continuing the testing and prior to leaving the researchers supervision.

3.2.5: Techniques

Hypoxic Environment

The hypoxic environment was introduced by using the GO_2 Altitude System (Biomedtech, Melbourne, Australia). The GO_2 Altitude System simulates the hypoxic condition through a semi-permeable nitrogen filtration membrane, which decreases the oxygen concentration. Once the air is circulated through the GO_2 altitude air pump, it flows through tubing connected to a series of Douglas Bags. Two oxygen sensors were used to monitor oxygen levels (a) an oxygen sensor (Cambridge Sensotech, Cambus, UK) connected to the Douglas bags and (b) an additional oxygen sensor that was encrypted within the GO_2 altitude system, this ensured the O_2 concentration remained constant. The Douglas Bags were directly connected to the mask system, which was hooked up to the participant for the duration of the exercise protocol. This system emits a continuous airflow of 200 $L \bullet min^{-1}$ through the tubing and is connected directly to the three valve oro-nasal mask the participant wore. A pulse oximeter (Radical 7 SET, Massimo, Irvine, CA) was placed on the

participants' forehead to monitor SpO_2. A SpO_2 level of 80%, or above, was required. (Workman & Basset, 2012).

Metabolic Cart ($\dot{V}O_{2MAX}$, BMR, PEMR)

The metabolic cart used for this study (Sable Systems International, Las Vegas, NV) collected $\dot{V}O_2$ and $\dot{V}CO_2$ simultaneously through a two-way valve mouthpiece (during $\dot{V}O_{2MAX}$) and through a canopy (during BMR and PEMR). This negative pressure system recorded both atmospheric temperature and the temperature inside the mixing chamber. It corrected for barometric pressure, keeping all recordings constant. During each testing session, the subsample flow rate was set to 200 $ml \bullet min^{-1}$, this remained constant throughout the testing protocols, to guarantee proper airflow through the system. The O_2 and CO_2 monitors were turned on at least one day prior to testing to ensure sensors were warmed up and calibrated correctly. Once the researcher arrived at the laboratory, the remainder of the system was turned on to the appropriate settings.

During the $\dot{V}O_{2MAX}$ testing, the flow-kit (which generates the high mass flow rates needed for respirometry) was set to 400 $L \bullet min^{-1}$. This ensured the correct volume of air was provided to the participant throughout the testing protocol. The metabolic cart was connected to the individual's mask by the standardized tubing. This lead from the flow-kit of the sable system to the three-way valve attached to the mask.

During the BMR, the flow-kit was set to 75 $L \bullet min^{-1}$. During the PEMR it was increased to100 $L \bullet min^{-1}$. This increase is due to the higher flow rate of O_2 and CO_2 during recovery, post-exercise compared to basal rates.

Substrate partitioning

Oxidation rates were calculated using $\dot{V}O_2$ and $\dot{V}CO_2$ values from the metabolic rates. Through these measurements, the researchers were able to calculate the substrate partitioning for CHO and lipids, using the following equations (Simonson *et al.*, 1990; Workman and Basser, 2012):

(Eq.1) $CHO_{ox}\ (g \bullet min^{-1}) = 4.57 \bullet \dot{V}CO_2\ (l \bullet min^{-1}) - 3.23 \bullet \dot{V}O_2\ (l \bullet min^{-1})$

(Eq.2) $LIPID_{ox}\ (g \bullet min^{-1}) = 1.69 \bullet \dot{V}O_2\ (l \bullet min^{-1}) - 1.69 \bullet \dot{V}CO_2\ (l \bullet min^{-1})$

For the purpose of this study, $\dot{V}O_2\ (l \bullet min^{-1})$ and $\dot{V}CO_2\ (l \bullet min^{-1})$ were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \bullet g^{-1}$, respectively). Relative contribution of the substrates protein oxidation rates were estimated at 66 $mg \bullet min^{-1}$, which was based from research conducted on urinary urea excretion measurements, post-exercise (Haman *et al.*, 2004).

3.2.6: Control Variables

Rate of Perceived Exertion

Rate of perceived exertion, derived from the Borg Scale (1962), was taken throughout the exercise protocol. The participant indicated his RPE score after each interval (work and active rest) for both conditions. This rating gave researchers a quantitative perspective of the amount of stress the participant was feeling through each stage of the exercise protocol. This measurement was able to be cross-compared between the two cycle protocol conditions (HYP and NOR).

Heart Rate & SpO_2

Heart rate and SpO_2 were taken throughout the entire testing protocol. Heart rate and SpO_2 were monitored for medical safety reasons. They also gave researchers a physiological measurement for

stress. As previously stated, HR and SpO_2 were measured through the oximeter (Radical 7 SET, Massimo, Irvine, CA) that was connected to the participant's forehead. The measurements were recorded online for the full duration of the protocol (BMR-Exercise-PEMR). Measurements were manually recorded in five-minute segments for the metabolic rates (BMR and PEMR) and after each interval of the exercise protocol.

3.2.7: Data Reduction

All metabolic data was collected through the Sable System (Sable System International, North Las Vegas, USA). For all metabolic rates, the first five-minutes and the last five-minutes were discarded in order to nullify any fluctuation due to familiarisation with the air-tight canopy and the expected termination of data collection. All baseline measurements, which were recorded every 15-minutes, were also discarded from the data. The remaining data was normalized over time, and a Macro processing was run to correct for any O_2 drift. Finally, all metabolic data was converted in and expressed as EE in kilocalories (Kcal) and as oxidation ($mg \bullet min^{-1}$). Deltas of all these values were calculated for comparison purposes within each of the respected conditions. All other measurements, HR, SpO_2, and RPE were averaged over time and compared to the respected measurements, in the opposing condition, at the same moment in time.

3.2.8: Statistical Analysis

Quantitative statistics for this study include descriptive statistics and two-way analysis of variance (ANOVA). For the physiological measurements (RPE, HR, and SpO_2) a two-way ANOVA was completed in SPSS for Windows (SPSS, Version 17.0, Polar Engineering and Consulting). The two-way ANOVA consisted of both conditions (NOR and HYP) during exercise. All data was deemed

significant if $p < 0.05$. Descriptive statistics (means, standard deviations, delta values) were calculated for all parameters, including SP, EE, HR, and SpO_2.

3.3: Results

3.3.1: BMR

All participants underwent two BMR recordings (one per condition). This gave researchers a baseline measurement for the current day. It was shown through the t-test that the difference between the individual BMR values for each participant, were not significant.

The SpO_2 recorded during each BMR was an average at 97.0% ± 0.7 (NOR: 97.5% ± 1.9; HYP: 97.0% ± 0.7). During each Resting HR was 54 ± 10 bpm, while mean resting BP was 118/72 mmHg. Heart rate remained constant while at rest, with an average of 50 ± 2 bpm (NOR: 50 ± 3 bpm; HYP: 51 ± 2 bpm). A t-test was conducted to confirm that there was no significant difference between HR and SpO_2 values between BMR sessions.

3.3.2: Exercise

Heart rate did not significantly differ between conditions. However, there was a significant difference between HR values during exercise and recovery. It was shown that there was a significant alteration on HR recovery between conditions.

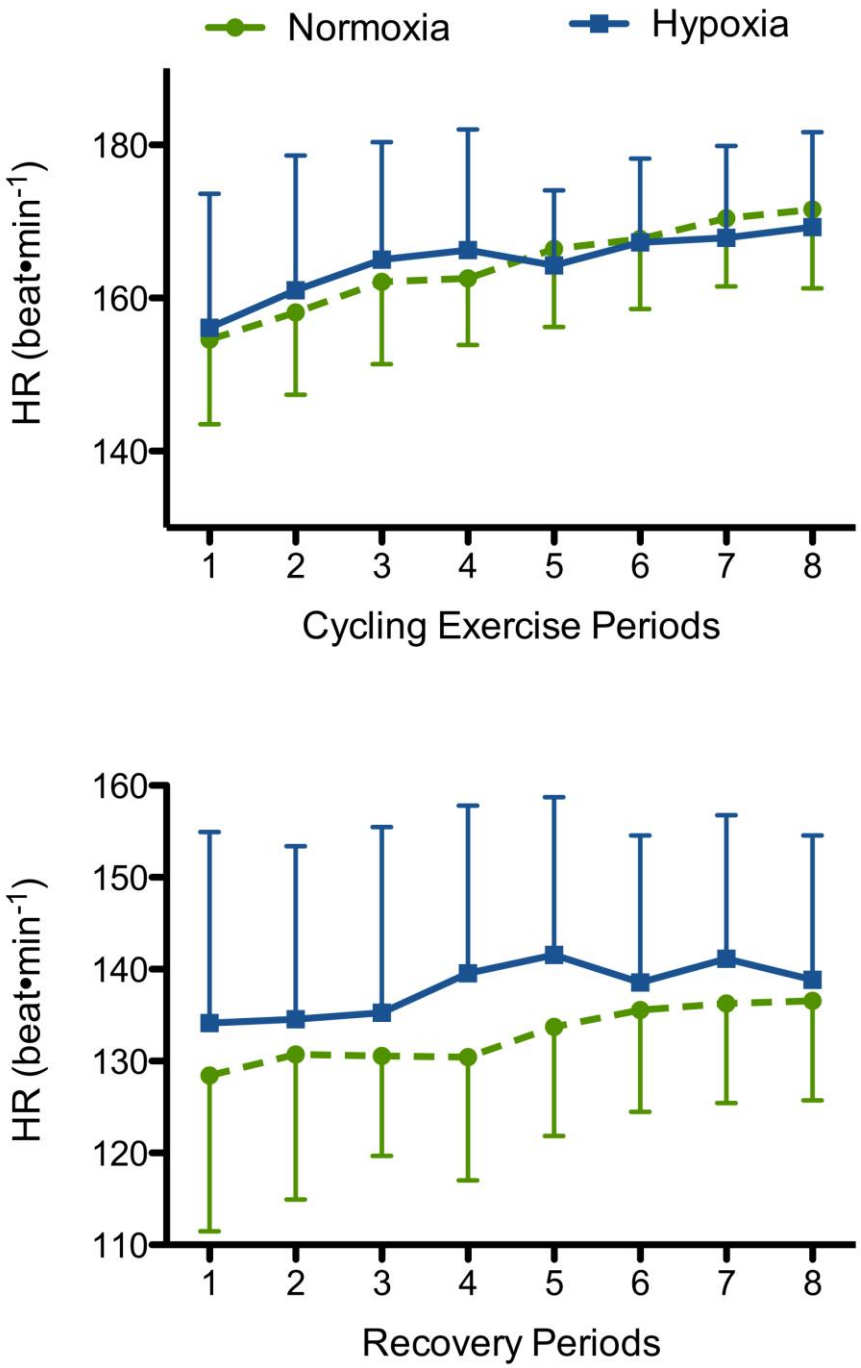

Figure 1: Heart rate (bpm) during exercise (upper panel) and recovery (lower panel) periods.

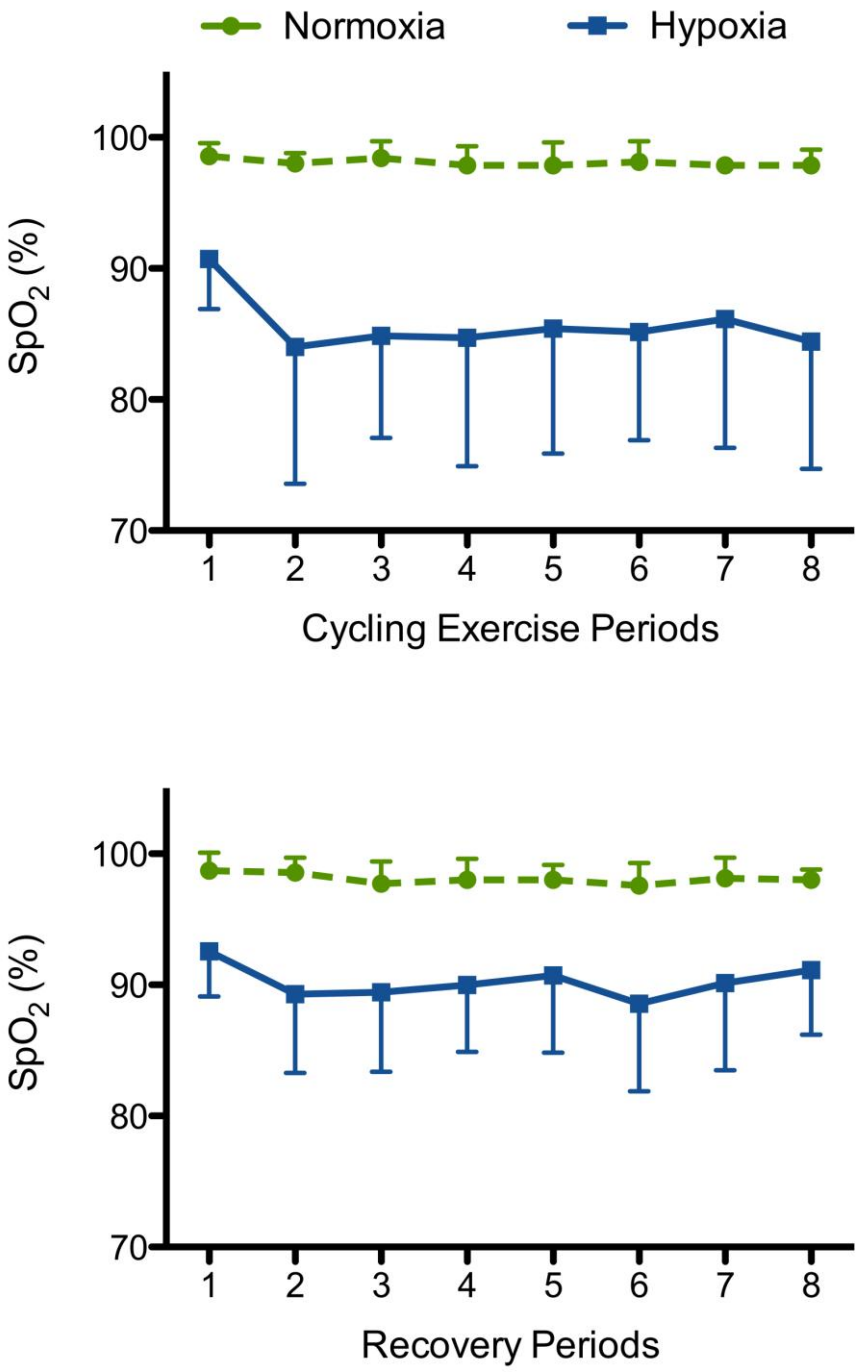

Figure 2: Blood oxygen saturation (%) during exercise (upper panel) and recovery (lower panel) periods both in hypoxic and normoxic conditions.

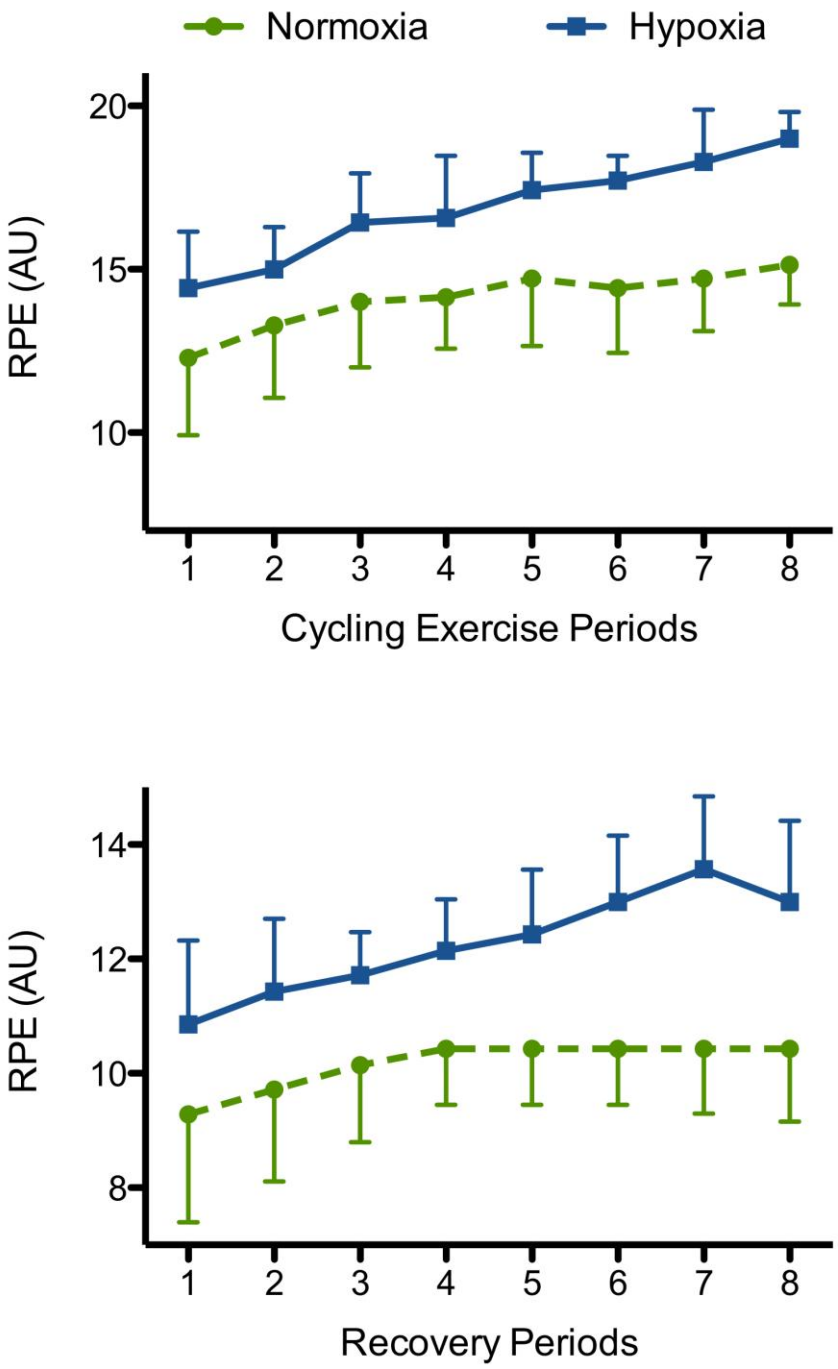

Figure 3: Rate of perceived exertion (RPE) during exercise (upper panel) and recovery (lower panel) periods both in hypoxic and normoxic conditions.

Heart rate had a condition effect, as well as a time effect. As shown in Figure 1, HR did not alter greatly between conditions during the work intervals, but was significantly different between conditions in the rest intervals ($p< 0.05$). The average HR during the rest interval during NOR was 81 ± 16 bpm and in HYP was 87 ± 9 bpm. It can be concluded that the heart recovers faster under NOR than HYP.

Oxygen saturation varied greatly between exercise conditions. The average SpO_2 during the NOR exercise was 98 ± 0.30, whereas during HYP the SpO_2 varied between rest (89 ± 1.0) and work (85 ± 2.8) intervals. Figure 2 shows that SpO2 was significantly lower when under the HYP condition when compared to the NOR condition. Therefore, it was concluded that there was a significant condition effect ($p<0.05$).

Rate of perceived exertion increased linearly with the duration of the exercise, in both HYP and NOR (see Figure 3). As expected, RPE decreased from the work intervals to rest intervals, as well as, between HYP and NOR conditions. It was determined that RPE had a condition effect, as well as a time effect and overall, RPE was higher while under the HYP condition. The average RPE value was 14 ± 1 (work) and 10 ± 0.5 (active rest) in NOR, while RPE was 17 ± 1 (work) and 12 ± 1 (active rest) in HYP. All three-control measurements (RPE, HR, SpO_2) were taken in order to ensure the subject was experiencing a certain level of stress during the testing.

3.3.3: PEMR

The overall average SpO_2 during the PEMRs was 98 % ± 0.1, not differing greatly from SpO_2 levels during the BMRs (0.8 differences). It should also be mentioned that the differences between SpO_2 levels between conditions during PEMR were minuscule.

The average HR was 68 ± 5 bpm during the HYP recovery (PEMR), whereas the average HR during the NOR recovery (PEMR) was 66 ± 4 bpm. It is noteworthy that both conditions had a linear

decrease, but HR was higher post-HYP exercise (HYP slope: y= -1.35x + 77.4; NOR slope: y=-1.02 + 72.52).

Delta energy production differences between BMR and PEMR were insignificant with 0.14 kJ/min and 0.13 kJ/min for NOR and HYP conditions, respectively (Figure 4).

Substrate partitioning was significantly altered during PEMR for both conditions. Delta glucose oxidation was found insignificant (pre-exercise: 0.18 ± 0.09 kJ/min and post-exercise: 0.18 ± 0.07 kJ/min) under the NOR condition (difference of 0.02). However, delta glucose oxidation was significantly ($p < 0.05$) affected when HYP was introduced. Glucose oxidation decreased by 0.14 from pre-HYP (0.26 ± 0.06 kJ/min) to post-HYP (0.12 ± 0.11 kJ/min). These results support that glycogen depletion occurs when exercise is completed under HYP. This is further supported through the lipid oxidation results, where a shift in lipid oxidation occurred for both conditions. Delta lipid oxidation was significant under HYP ($p<0.05$), with an increase from 0.03 ± 0.02 kJ/min to 0.10 ± 0.06 kJ/min. Results derived from delta lipid oxidation under NOR were deemed insignificant. It is clear that HIIT positively affects lipid oxidation, however coupling the exercise protocol with HYP was shown to triple lipid oxidation (difference: 0.07). These results are shown clearly in Figure 5 where delta fat oxidation and delta CHO oxidation are shown between conditions.

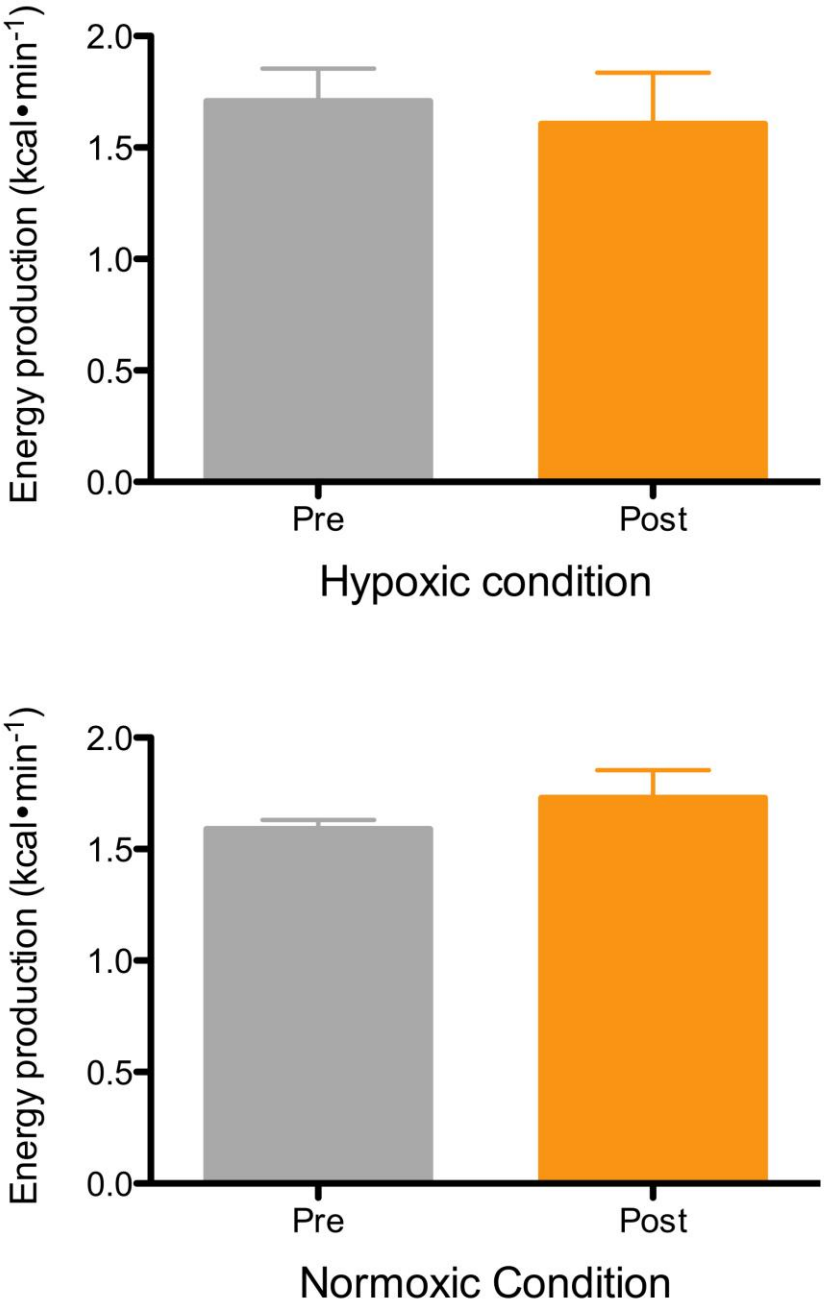

Figure 4: Pre- and post-exercise energy production (kcal·min^{-1}) both in hypoxic (upper panel) and normoxic (lower panel) conditions

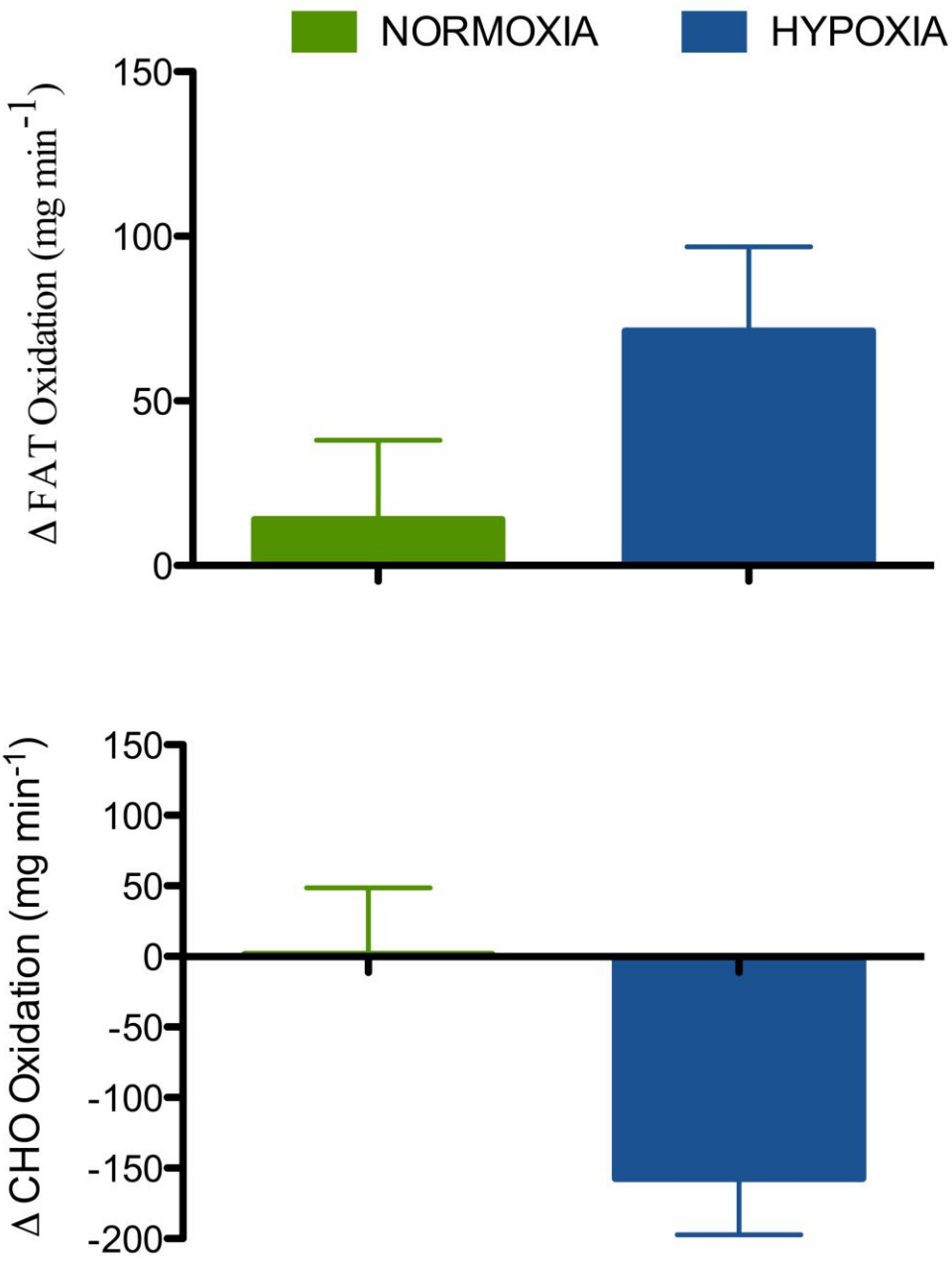

Figure 5: Difference (mg min^{-1}) from baseline in fat oxidation (upper panel) and CHO oxidation (lower panel) both in hypoxic and normoxic conditions.

3.4 Discussion

The purpose of this study was to examine the effects of HYP, coupled with HIIT, on the human metabolic responses, during exercise recovery. Few studies have been conducted using 1-hr interval training that incorporates 3-minutes of 70% PPO and 4.5-minutes at 35% PPO at HYP. It was hypothesized that there would be a shift in fuel utilization towards lipid oxidation, post-exercise, in both conditions, as well as a greater difference between pre- and post- in HYP compared to NOR. It was concluded that there was a shift towards lipid oxidation post-exercise, as well as a greater shift post-intervention. It was also hypothesized that there would be greater EE during post-exercise in both conditions, as well as a greater EE under HYP compared to NOR. Although previous research suggested a greater EE post-exercise for both conditions, as well as a greater overall EE post-HYP (compared to NOR), current findings suggest otherwise. The margin of change within the delta energy productions for both conditions was deemed to be insignificant. The overall assumptions have been confirmed. There is an effect on human metabolism (SP) when exercise and HYP are coupled.

3.4.2: Exercise

Previous research has shown that SpO_2, HR, and RPE are greatly affected by high altitude (Scott *et al.*, 2014). Oxygen saturation was significantly different between the two conditions, which did not differ from previous research (Scott *et al.*, 2014). Oxygen saturation stayed constant throughout exercise in NOR (see Figure 2), while it fluctuated between work and active rest intervals during HYP. It was concluded that there was a condition effect; SpO_2 remained lower during the HYP condition compared to the NOR condition. A lowered SpO_2 during HYP was not surprising. The less O_2 supply available to the body, the less O_2 there is available to transport, therefore creating a lowered level of availability.

Heart rate was not significantly different.. Although, overall, HR was not altered greatly, HR recovery did fluctuate between conditions. Heart rate recovery was significantly higher in the HYP condition than the NOR. This was due to a lower partial pressure of O_2, where the tissue relies more on the glycolytic system and; therefore, releases more metabolites into circulation (Bailey *et al.*, 1998). The higher HR in recovery phase during HYP, mirrors the higher blood flow needed to washout all these by-products. Figure 1 shows that the HR and HR recovery remains elevated throughout the entire protocol. It can be concluded that there was a condition effect and time effect on HR and HR recovery. It is worth noting that additional research closely monitoring HR and HR recovery would be beneficial to understand HR recovery and max HR during hypoxic exercise.

Rate of perceived exertion (Borg Scale) is a widely used measurement to determine an individual's perceived fatigue. During both conditions (HYP and NOR), there was a common trend in the fluctuation of RPE between the work and rest interval (rest interval was always lower). This result was expected, since the workload was altered during these times. Through the RPE readings, it was shown that the subjects were fatigued after the work intervals, but had enough time during the rest interval to recover and continue on with the next work interval. It has been shown in previous studies, that RPE increases when an individual is introduced to high altitude (Tschopp *et al.*, 2001) . This is most likely due to the decreased level of O_2 available to the individual. A decrease in O_2 availability has been shown to fatigue the body more quickly, as well as to create symptoms of uneasiness and nausea (Tschop *et al.*, 2001; Basset *et al.*, 2006). Therefore, it was expected that greater RPE readings would be recorded during a HYP condition when coupled with exercise.

3.4.3: PEMR

Energy expenditure is widely known to increase post-exercise stimulation. This current study is congruent with the literature in showing that exercise has a positive effect on EE, no matter the environmental condition. Previous studies have discussed and evaluated the increase in EE when high altitude is exposed to human subjects (Bernardi *et al.*, 2001; Clanton *et al.*, 2001; Levine, 2000). Tschop *et al.* (2001) stated that high altitude is accompanied with an increase in EE, which creates a negative energy balance. This could also be due to feeling nauseous and/or full, which are two common symptoms felt at high altitude. Generally, these symptoms do not occur until the individual is under HYP for a significant period of time. For this study, there were no significant findings when delta energy production (BMR/PEMR) was calculated during the current study; therefore, short intermittent HYP did have an increased effect on delta energy production compared to the NOR counterpart.

The main purpose of this study was to evaluate the effects of exercising under HYP on SP. It was concluded that HYP, coupled with exercise (HIIT), had a greater effect on SP compared to the NOR condition. In both conditions, there was a shift towards lipid oxidation, which is congruent with previous findings. The literature has shown that interval training (HIIT) results in a shift towards lipid oxidation post-activity (Kuo *et al.*, 2010; Melanson *et al.*, 2009), further supporting the results found in this current study (shift towards lipid oxidation in both conditions). However, as stated previously there was a significantly higher shift towards lipid oxidation when the exercise was coupled with a HYP environment. Past studies have shown that HYP can affect SP, in that there is a shift towards lipid oxidation post-exposure (Katayama *et al.*, 2010; Workman and Basset, 2012). This phenomena has been noted while participants are at rest, as well as while performing different

intensities and durations of a given activity, under HYP (Kelly *et al*., 2013; Workman and Basset, 2012).

The major theory behind this substrate utilization shift is the depletion of glycogen. Multiple studies have discussed that CHOs can become depleted after exercise, and; therefore, while the body recovering from exercise, lipids are used as a primary fuel source to supply ATP for vital functions and glycogen replenishment. Although there was an increase in lipid oxidation in both conditions, glucose oxidation was not affected the same way for both. It was shown that glucose oxidation was significantly decreased post-exercise under HYP, while there was little to no alteration post-exercise under NOR. Therefore, it appears HYP, too, has an effect on glucose oxidation. Tschop *et al.* (2001) stated that high altitude requires more energy from the body, creating a negative energy balance, leading the body into utilizing more lipid oxidation for energy than glucose oxidation. Having this decrease in glucose oxidation, post-exercise under HYP, could be due to the glycogen depletion phenomena. It was shown through the stress measurements (HR, SpO_2, RPE) that exercise under HYP had a higher exertion level, which could cause the body to rely on a greater amount of CHOs during the session compared to the NOR. It is still uncertain if the body is truly under glycogen depletion post-HYP exercise, therefore no conclusion can be drawn to this hypothesis.

3.4.4: Study limitations

The major limitation of this study is the small sample size, which could have affected the statistical power. It was clearly shown that there was a significant difference between the two conditions in SP and EE. The researchers are confident in saying that most individuals who did not complete the testing stated that the breathing in HYP was the main reason for stopping early.

3.4.5: Future studies

Further research is needed to give additional information as to how long this rise of lipid utilization is sustained. For future studies, it would be recommended to have a longer PEMR, to

determine how long the elevation in lipid utilization occurs. Finally, it would be interesting to look at different interval training programs that involve a 1:1 work-rest ratio, or rest period that is complete rest. These insights would further clarify previous conclusions made regarding SP and EE during recovery, and could allow a more complete picture of how RPE, HR, and SpO_2 are affected. .

Chapter 4: Conclusion

4.1: Responses to the Research Hypothesis

This research project had three hypotheses which stated the following; (a) There will be a difference in SP between NOR and HYP conditions; (b) there will be an increase in EE in HYP compared to NOR; and finally, (c) there will be a fat oxidation shift during EPOC in both conditions (greater in HYP compared to NOR). The first statement is significant and accepted. There was a shift in substrate utilization between both conditions. There was slight increase in EE in HYP compared to NOR, but the results were found to be insignificant. Energy expenditure did increase between both conditions but it was did not have a significant effect. Finally, the third statement was also accepted. There was a shift toward fat oxidation in both conditions, and a greater shift happened within the HYP condition. Overall, there was a positive affect among the participants.

4.2: Summary

Overall the current study was a success in examining the effects of HYP and HIIT on human metabolism. The experiment allowed researchers to evaluate the cardiovascular and psychological measurements during the exercise protocol, as well as the post-exercise/exposure metabolic rates used to calculate SP and EE. Measuring cardiovascular and psychological parameters allowed researchers to understand how HYP, as well as the exercise affected the participants. All measurements (HR, SpO2, and RPE) were recorded to allow researchers to understand the stress placed on the body pre-, during, and post- exercise. Recording these parameters throughout the experiment helped researchers. The final conclusions drawn from this study are that a significant increase in lipid oxidation post-exposure and exercise occur, and that the effect on EE in hypoxic-exercise still requires further evaluation.

Chapter 5: Overall References

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