# WHAT EFFECT DOES SHORT-TERM MODERATE HYPOXIA EXPOSURE DURING CONSTANT WORKLOAD EXERCISE HAVE ON POST EXPOSURE MEASUREMENTS OF RESTING SUBSTRATE PARTITIONING?

by

© Liam P Kelly

A Thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements for the degree of

**Master of Science in Kinesiology**

**School of Human Kinetics and Recreation**

**Memorial University of Newfoundland**

**May 2015**

St. John's Newfoundland

# ABSTRACT

Recent studies have shown a potentiation effect of exercise performed under moderate short-term hypoxia on weight loss. From these data, it has been hypothesized that moderate hypoxia exposure affects substrate utilization and that normobaric hypoxia training might be an effective means to induce weight loss. However, limited data is currently available on resting substrate partitioning after a single bout of exercise performed under hypoxia. The purpose of the current study was therefore to asses the viability of moderate short-term hypoxia exposure during constant workload exercise as a means to alter post exposure resting substrate partitioning. Respirometry measurements were recorded at baseline (BMRpre), during 60-min constant workload exercise (under normoxia N-CWE; and hypoxia H-CWE ) and during post exposure resting metabolic measurements; recorded immediately after exercise (PEMR, 0-60-min) and again the next morning (BMRpost). Compared to baseline, lipid oxidation was significantly elevated and carbohydrate oxidation suppressed during PEMR ($p=0.010$ and $p=0.076$, respectively) and BMRpost ($p=0.036$ and $p=0.010$, respectively) after H-CWE. When the same absolute workload was performed under normoxia, no effect on resting substrate partitioning was observed during the post exercise recording periods. An increased reliance on endogenous carbohydrate sources during H-CWE compared to N-CWE is suggested to explain the current findings. In conclusion, a single bout of exercise performed under moderate short-term hypoxia is associated with a shift in resting substrate partitioning toward an increased lipid oxidation up to 22-hr post exposure.

# ACKNOWLEDGEMENTS

I would like to express sincere gratitude to my graduate supervisor and friend Dr. Fabien Basset, who has supported me thought my graduate studies with his patience and knowledge while allowing me the room to work in my own way. Furthermore, I would like to thank Dr. Thamir (Tim) Alkanani for his assistance in the exercise physiology laboratory and encouragement to continue on the path of academia. A special thanks to my parents William and Ann Marie along with the rest of my family, whose support made this possible. Finally, I would like express deepest appreciation to my beloved wife Sarah who is always by my side and makes sacrifices every day to enable me to continue with my studies.

# Table of Contents

# List of Tables

# List of Figures

# List of Abbreviations

Bf: breathing frequency

BMI: body mass index

BMR: basal metabolic rate

BMRpre: baseline basal metabolic rate

BMRpost: basal metabolic rate recorded 22-hr post-exercise

BP: barometric pressure

$CaO_2$: arterial oxygen content

CHO: carbohydrate

EP: energy production

$F_IO_2$: fraction of inspired oxygen

$F_ECO_2$: fraction of expired carbon dioxide

FFA: free fatty acid

Gox: glucose oxidation

HR: heart rate

H-CWE: hypoxia constant workload exercise

HGM: hypoxic gas mixture

IMTG: intramuscular triglycerides

kcal: kilocalorie

Lox: lipid oxidation

MFO: maximal fat oxidation

PEMR: post-exercise metabolic rate recorded 0-60 min post exercise

$P_IO_2$: partial pressure of inspired oxygen

PPTG: post prandial triglyceride

PPO: peak power output

Pox: protein oxidation

RER: respiratory exchange ratio

RMR: resting metabolic rate

$SaO_2$: arterial oxygen saturation

$S_PO_2$: blood oxygen saturation

TG: triglyceride

$\dot{V}CO_2$: carbon dioxide production

$\dot{V}E$: minute ventilation

$\dot{V}O_2$: oxygen uptake

$\dot{V}O_{2peak}$: peak rate of oxygen uptake

VT: tidal volume

# List of Appendices

# Chapter 1 Introduction

## 1.1 Background of study

Recent studies have demonstrated a potentiation effect for normobaric hypoxia exposure during traditional exercise interventions in promoting weight loss in over weigh and obese individuals (27, 57, 76). The precise mechanism for this accelerated weight loss during such normobaric hypoxia training are currently unknown. An elevated energy expenditure, along with a decrease in appetite and poor absorption of nutrients from diet are postulated to explain much of the weight loss experienced during prolonged high altitude exposure (41). However, such mechanisms cannot account for the synergistic relationship between short-term hypoxia exposure (< 90-min / day) and exercise on body composition. In fact, lower workloads are needed under hypoxia in order to achieve the same relative exercise intensity (27, 57, 76). Alternatively, daily substrate balance could be maintained independent of energy balance (18) where the net effect of normobaric hypoxia training is a decrease in adiposity. It has been previously suggested that such training programs lead to enhanced lipid oxidation (76), however, no studies have systematically evaluated whole body substrate oxidation during exercise performed under moderate short-term hypoxia and recovery under normoxia.

Submaximal exercise performed under hypoxia relies preferentially on carbohydrate (CHO) energy sources (glucose, lactate, glycogen). It is clear from respirometry data recorded at altitude and under simulated hypoxia that both the absolute rate of CHO oxidation and its relative contribution to the fuel mixture are elevated compared to

performing the same absolute workload at sea level (48, 58, 71, 75). Such a shift in substrate utilization helps to maintain energy supply in reduced $O_2$ environments as the energy yield per liter of $O_2$ consumed is greater when CHO is the preferred energy substrate (53). This shift in substrate oxidation may also reflect a change in relative exercise intensity given that performing the same absolute workload under both environmental conditions represents a larger fraction of peak oxygen uptake ($\dot{V}O_{2peak}$) under hypoxia (22, 67). Indeed, relative substrate contributions are similar when workloads are matched for relative exercise intensity (8, 48), which has led to the conclusion that a change in relative exercise intensity rather than hypoxia exposure itself causes the observed effect on substrate partitioning (48). However, other studies have reported an increased reliance on CHO oxidation under hypoxia even when workloads are matched for relative exercise intensity under both environmental conditions (21, 40, 58). In either case, an increased reliance on endogenous CHO has been recorded during prolonged exercise performed under hypoxia compared to the same absolute and relative workloads completed under normoxia (58). It is then reasonable to suggest that CHO reserves are depleted to a greater extent after exercise performed under hypoxia compared to normoxia for a given total energy expenditure of exercise (EEE). Glycogen depleting exercise has previously been described to cause an elevated resting lipid oxidation during the post exercise recovery period (42, 73), which may influence daily substrate turnover during normobaric hypoxia training.

However, limited data is currently available on whole body substrate oxidation during the post-exercise recovery period after exercise performed under short-term hypoxia.

Numerous studies have described resting metabolic parameter after exercise performed under normoxia. These studies suggest that although resting energy production (EP) is transiently elevated during the post-exercise recovery period, the greatest influence of prior exercise on body fat mass may be a shift in resting substrate partitioning toward lipid energy sources (28, 29, 43). Accordingly, elevated rates of resting lipid oxidation above time matched resting controls have been reported up to 24-hr after a single bout of exercise (28, 29). Such increments in resting lipid oxidation are associated with energy expenditure of prior exercise (EEE) (28) and the extent to which muscle glycogen depletion occurs (42, 73). Therefore, given the preferential use of endogenous CHO during exercise under hypoxia (58) it is reasoned that exercise performed under hypoxia would lead to an elevated resting lipid oxidation post exposure. However, Katayama et al. (40) reported conflicting findings to this hypothesis displaying an elevated respiratory exchange ratio (RER; $\dot{V}CO_2$ / $\dot{V}O_2$) during 60-min recovery from submaximal exercise performed under hypoxia compared to normoxia. Subjects remained under hypoxia for both exercise and recovery during the hypoxia trial, which could explain the increased reliance on CHO during recovery compared to normoxia. Therefore, studies describing the influence of hypoxia exposure during exercise on post exposure measurements of resting substrate oxidation are needed in order to understand the mechanisms underlying accelerated weight loss observed during normobaric hypoxia training compared to similar or even higher workloads completed under normoxia.

## 1.2 Purpose of study

With the interest in assessing the viability of moderate short-term hypoxia exposure during constant workload exercise as a means to alter post-exposure resting substrate partitioning, the extent of alterations in whole body lipid (Lox) and glucose oxidation (Gox) were assessed after constant absolute workload cycling exercise performed under normoxia and hypoxia. A secondary objective was to describe the effect of short-term moderate hypoxia on fuel selection during prolonged exercise. It was hypothesized that (i) Lox is elevated and Gox depressed during the post-exercise recovery period, although the magnitude of this effect would be greater after exercise performed under hypoxia, and (ii) the amplified Lox observed after hypoxia exposure is associated with an elevated total contribution from Gox during H-CWE.

## 1.3 Significance of study

Obesity has reached pandemic proportions (4) and efficient strategies are desired to reduce body fat mass. Although regular exercise is an important component of a healthy lifestyle, the viability of exercise training to cause weight loss has been questioned (55). Generally, moderate intensity (45-65 % of $\dot{V}O_{2peak}$) and long duration (≥ 60-min) rhythmic exercise is encouraged to promote weight loss in overweight and obese populations (5). Such workloads are understood to maximize the oxidation of lipid substrates during exercise (1, 2, 74) while minimizing biomechanical strain. The post-exercise recovery period is also associated with elevated rates of lipid oxidation, which may have a significant influence on adiposity (29, 43). Recently, EEE and physical fitness

have been described to be the most significant determinants of resting lipid oxidation after a single bout of prolonged exercise (28). However, exercise intensity may have a larger influence under free-living conditions, as high intensity exercise is more effective than moderate intensity exercise for increasing postprandial lipid oxidation (73). Similarly, normobaric hypoxia training leads to accelerated weight loss that cannot be explained by increased EEE. Therefore, understanding the mechanisms that cause an increased weight loss during hypoxic exercise interventions compared to similar or even higher workloads under normoxia may also provide insight into role of exercise in weight loss / maintenance.

# Chapter 2 Review of Literature

## 2.1 Short-term moderate hypoxia

HYPOXIA, or decreased availability of oxygen, is characteristic of exposure to natural and simulated altitude. The terms hypoxia and altitude are therefore used interchangeably to describe a reduction in oxygen availability (12). This differs from ischemia that can lead to local hypoxia or complete absence of oxygen (anoxia). The amount of oxygen available per volume of air inspired decreases with increasing altitude due to decreasing atmospheric pressure, which is referred to as hypobaric hypoxia. Partial pressure of inspired oxygen ($P_IO_2$) is reduced in such hypobaric environments, which results in low arterial oxygen saturation ($SaO_2$), and thus, a decreased arterial $O_2$ content ($CaO_2$). Decreased $CaO_2$ influences muscle hemoglobin $O_2$ saturation (31) , which can alter fuel selection (14, 31) and impede endurance performance (22, 53, 67). Hypobaric hypoxia can also be simulated at sea-level in specialized rooms called hypobaric chambers, which are used to decrease air pressure. Another technique commonly employed to simulate hypoxia is to reduce the fraction of inspired $O_2$ ($F_IO_2$), which is described as normobaric hypoxia due to the fact that barometric pressure remains at sea level values. For example, standard sea level atmospheric pressure is 101.33 kpa and decreases to 72.40 kpa at 2750 m (66), therefore, given that $F_IO_2$ is 0.2093 at both elevations, $P_IO_2$ drops from 21.21 kpa to 15.15 kpa. Similarly, lowering $F_IO_2$ from 0.2093 to 0.15 reduces $P_IO_2$ to 15.20 kpa (i.e. 101.33 kpa * 0.15). Hypoxicators are often used during short-term hypoxia interventions due to the relatively short exposure periods and there are several cost effective models on

the market. Hypoxicators produce hypoxic gas mixtures ($F_IO_2 < 0.2093$) either by infusing supply air with nitrogen or through a deoxygenating processes, such as filters that restrict $O_2$ supply.

Similar to any physiological stimulus, hypoxia is typically defined in terms of duration and intensity of exposure. Duration has been defined to include a wide range exposures lasting minutes to generations, where short-term hypoxia describes continuous exposure lasting minutes to hours (33). Level of hypoxia exposure is often described in terms of distance above sea level, which ranges from low altitude (500 to 2000 m) to extreme altitudes (> 5500 m). The most common altitudes used to study physiological responses are moderate (2000 to 3000 m) and high altitudes (3000 to 5500 m) (6), which corresponds to a $F_IO_2$ between 0.15 and 0.11, respectively. Endurance exercise performed under hypoxia poses significant challenges to the oxygen delivery and metabolic pathways. Reduced $SaO_2$ experienced at altitude is further compromised during exercise because of increased pulmonary blood flow that limits gas exchange at the alveoli (6). A graded response in decreasing $SaO_2$ is therefore observed for increments in altitude and workload (16). Breathing frequency (Bf), minute ventilation ($\dot{V}_E$), and heart rate (HR) are all elevated above similar workloads performed at sea level in order to maintain oxygen transport (53). These mechanisms are limited however and are unable to maintain oxygen supply during maximal exercise. Accordingly, $\dot{V}O_{2peak}$ decreases with increments in altitude at a rate of approximately 7 % for each 1000 meter increase in elevation (22, 67). From this perspective, hypoxia exposure during endurance training protocols can be viewed as an additional metabolic stress that the body must overcome in order to maintain energy supply within exercising muscle.

For several decades athletes have availed of altitude training for the purpose of improving sea level performance. Those that encourage these performance enhancing strategies propose that the body acclimates to such $O_2$ deprived environments through mechanisms that i) enhance $O_2$ delivery capacity through processes such as erythropoiesis that increase red blood cell mass (45) and/or ii) improve muscle metabolism through a variety of pathways including angiogenesis, glucose transport, glycolysis and pH regulation (24). However, the severity and duration of exposure can greatly influence the acclimatory responses of cardiovascular and metabolic systems to hypoxia (15). Accordingly, three different strategies for altitude exposure are described and evaluated in the scientific literature: 1) live high train high (LHTH), where athletes primarily live and train under conditions of hypoxia; 2) live low train high (LLTH) or intermittent hypoxia training, where athletes live under normoxia but undertake intermittent, or short-term, training under hypoxia; and 3) live high train low (LHTL), where athletes live under hypoxia but train under normoxia. With respect to improving sea level performance, the LHTL protocol has been given the most attention in recent scientific literature (13, 34, 63). This training paradigm avoids the detrimental effects of decreased absolute training loads on the neuromuscular system during training at altitude while at the same time providing a metabolic stress that is proposed to improve $O_2$ delivery capacity (45). Although it seems clear that altitude training is an important strategy to improve performance at altitude (32), its influence on sea level performance in elite athletes has been questioned (7, 61, 69).

Exercise training under hypoxia has also been described to provide a superior metabolic stress in individuals with risk factors for metabolic syndrome. Normobaric hypoxia

training is a type of LLTH protocol where exercise training is performed under simulated altitude while the recovery period between exercise bouts occurs under typical sea level conditions. Moderate short-term hypoxia exposure has been described during these exercise sessions lasting between 60 and 90-min three times per week for 4 (27, 76) and 8 weeks (57). As during traditional sea level training, exercise intensity is typically maintained between 60-65% of $\dot{V}O_{2peak}$ recorded under hypoxia. Using this protocol, Wiesner et al. (2010) demonstrated that training under hypoxia elicits a similar or even better response in terms of physical fitness, metabolic risk markers, and body composition in obese subjects at lower mechanical workloads compared to normoxia (76). Lower mechanical workloads are used under hypoxia in order to match relative exercise intensity, which did not negatively influence the outcomes. Similarly, Netzer et al (2007) described accelerated weight loss during exercise training under hypoxia compared to the same relative workloads completed under sham hypoxia (57). These outcomes suggest that normobaric hypoxia training could be beneficial in the treatment of metabolic syndrome, especially in patents with orthopedic limitations (27, 57, 76). The mechanisms underlying the synergistic relationship between short-term hypoxia and exercise on body composition are currently unknown. A decrease in energy consumption, which was associated with changes in basal plasma leptin levels, along with a increase in basal metabolic rate (BMR) was described to cause weight loss in subjects with metabolic syndrome during a 7-day stay at 2650 m (47). However, these results are difficult to apply in normobaric hypoxia training studies because subjects were continuously exposed to hypoxia throughout the study period. Although not discussed in this study, a lower mean resting RER after the 7-day stay under moderate hypoxia was reported in addition to a

significantly elevated BMR (47), indicating that the absolute rate of resting Lox was also elevated after the stay at altitude. Also, Workman and Basset (2012) demonstrated that 7-days of short-term passive hypoxia exposure was sufficient to shift resting substrate partitioning toward lipid oxidation up to 24-hr in overweight sedentary male subjects (77). Accordingly, altered substrate oxidation has been speculated to account for the greater reductions in body fat after training under hypoxia compared to normoxia (76). Decreased blood triglyceride levels have also been recorded after normobaric hypoxia training (27, 57), which could also reflect altered energy metabolism (29, 43). However, no studies have evaluated whole body substrate partitioning during the post-exercise recovery period under normoxia after exercise performed under short-term moderate hypoxia. Therefore, more studies are necessary to fully elucidate the effects of hypoxia exposure during exercise on changes in fuel selection.

### 2.2 Energy production during prolonged exercise under short-term hypoxia

Two different methods are described in the scientific literature for evaluating the effect of short-term hypoxia exposure on metabolic responses during exercise. Accordingly, workloads are either assigned to a given absolute power output or relative exercise intensity is matched under hypoxia and normoxia. Low-to-moderate intensity sea level exercise (i.e. 40 to 55 % of $\dot{V}O_{2peak}$) is typical of studies that evaluate the effect of hypoxia exposure on metabolic responses at a given absolute workload (10, 39, 48, 58). This is due to the fact that $\dot{V}O_{2peak}$ decrease at a rate of approximately 7 % for every 1000 m increase in elevation (22, 67) and, therefore, such workloads approach the limit that can be sustained at moderate to high altitudes. Alternatively, workloads are also assigned to a

given fraction of $\dot{V}O_{2peak}$ (8, 31, 48, 58, 76, 78) ventilatory threshold (21), and/or blood lactate accumulation (27, 49) determined during a maximal incremental exercise test recorded under each respective environmental condition.

Constant absolute workload exercise performed under hypoxia relies preferentially on CHO substrate sources. The absolute rate of Gox and its relative contribution to EP are elevated during short-term simulated high altitude exposure compared to similar exercise performed under normoxia (48, 58). This was demonstrated during constant load cycling under hypoxia (4100 – 4300 m; ~12.5 % $O_2$) lasting 60-min (48) and 80-min (58) at 45 and 54 % of sea-level $\dot{V}O_{2peak}$, respectively. Conversely, Jones et al. (39) reported increased FFA mobilization and utilization during 30-min cycling exercise at 25 % and 50 % of sea-level $\dot{V}O_{2peak}$ under short-term hypoxia (10 - 13 % $O_2$). However, the elevated RER and blood lactate concentration recorded under hypoxia in this study may also be interpreted to suggest an increased dependence on CHO energy sources. More recent studies describing the effect of short-term and more prolonged high altitude exposures on metabolic responses also found elevated arterial glycerol and FFA concentrations, however, they actually reported increased glucose uptake and decreased fatty acid disappearance during constant load exercise under hypoxia (10, 62). Information on whole body substrate partitioning was not provided in these studies as substrate disappearance was calculated from arteriovenous differences taken in the exercising leg. Peronnet et al. (2006) also reported increased FFA concentration during hypoxia exposure, however, using $^{13}C$-labelled glucose and indirect calorimetry they were able to demonstrate that lipid oxidation was reduced while blood glucose uptake was similar under hypoxia compared to normoxia and that an increased contribution from endogenous

CHO was required to complete the given workload under hypoxia (58). In fact, these authors concluded that the higher Gox recorded under hypoxia for a given absolute workload was entirely due to a larger utilization of endogenous CHO than under normoxia (58). The discrepancies between this and previous studies reporting elevated rates of blood glucose uptake under hypoxia (10, 62) may be explained by differences in relative workloads under hypoxia (78 % vs. 65 % of $\dot{V}O_{2peak}$, respectively) given that the contribution from muscle glycogen increases at workloads beyond 65 % of $\dot{V}O_{2peak}$ (64). Taken together, these studies can be interpreted to suggest that performing constant workload exercise under relatively high levels of hypoxia increases mobilization of energy substrates, where the net effect is an increased utilization of CHO that is supplied proportionally more from endogenous CHO reserves. It is uncertain if this pattern of substrate utilization is maintained during constant load exercise under more moderate levels of hypoxia as limited data is currently available on whole body substrate oxidation at this altitude.

Hypoxia influences circulating hormones and metabolites in terms of substrate metabolism during prolonged exercise. An altered sympathoadrenal system response to constant load exercise has been repeatedly reported under hypoxia (9, 10, 48, 58, 62). Plasma concentrations of norepinephrine and epinephrine are significantly elevated during submaximal cycling exercise at high altitude (4300 m) compared to sea level (62). A 2-fold increase in plasma epinephrine concentration recorded during exercise under hypoxia is associated with similar increases in blood lactate (9) along with elevated rates of appearance and disappearance of blood glucose (10). Although arterial lactate concentration is elevated under short-term hypoxia, lactate production is matched by

clearance most likely via oxidation in the liver, heart, red skeletal muscles and other oxidative tissues (9, 39). Therefore, increased lactate flux during steady state exercise under hypoxia may help to distribute fuel and provide gluconeogenic substrate (9). Increased arterial catecholamine concentrations recorded during exercise under hypoxia is also associated with elevated plasma glycerol and FFA concentrations (48). Thus, the interaction between hormones and metabolites may be altered under hypoxia such that an increased reliance on CHO substrate predominates (40) even when FFA supply is elevated. In fact, accelerated CHO metabolism has previously been described to inhibit lipid oxidation possibly by limiting fatty acid uptake into the mitochondria (68). An increased contribution from Gox toward EP improves the ATP equivalent (ATP / $\dot{V}O_2$) (17) and thus, would help to maintain EP during times of limited oxygen availability (10, 53) .

A shift in substrate partitioning toward Gox also occurs at sea level when workloads are increased from moderate to high intensity exercise. Under typical dietary conditions, energy demands of light-to-moderate intensity and long duration exercise primarily stems from lipid oxidation while the relative contribution of Gox becomes increasingly important at higher exercise intensities (11). Upon initiation of exercise an increased sympathoadrenal response is observed, which acts to increase the rate of lipolysis and glycogenolysis in order to supply substrate for ATP production within working muscles (37). There is a graded response between exercise intensity and catecholamine release where low intensity exercise results in minimal increases in plasma epinephrine and norepinephrine concentrations while maximal rates are recorded during high intensity exercise (64). Although catecholamine release continues to rise, maximal rates of

lipolysis are achieved during low intensity exercise (37, 64) and decreased adipose tissue blood flow may limit delivery of FFA to exercising muscle during high intensity exercise (20, 72). Adipose tissue derived FFA provides the main fuel for exercising muscles during low intensity and prolonged exercise (64). The absolute rate of lipid oxidation continues to rise until a maximum (MFO), which occurs somewhere between 48 and 63 % of $\dot{V}O_{2peak}$ (1, 74) at which point intramuscular triglycerides (IMTG) are proposed to make a significant contribution (64). Further increments in workload results in diminished lipid oxidation and increased CHO oxidation. At workloads beyond MFO, uptake of glucose continues to increase such that the contribution from plasma derived substrate is maintained over a large range of exercise intensities (64). As workloads increase beyond 85 % of $\dot{V}O_{2peak}$, muscle glycogen provides the primary substrate for ATP production within contracting skeletal muscles (64). Achten and Jeukendrup (2004) demonstrated that this pattern of substrate utilization corresponds with measurements of blood lactate concentrations, where increases in blood lactate above baseline are recorded at intensities near MFO and negligible rates of lipid oxidation are recorded at lactate threshold (3). Although a clear relationship exists between exercise intensity and fuel selection, the mechanisms underlying this diminished capacity for Lox at exercise intensities beyond 50 – 65 % of $VO_{2peak}$ are not understood. A decrease in FFA supply (20, 72) and an inability of muscle to transport them into the mitochondria (68) has been described to cause a reduction in lipid oxidation. However, FFA supply is maintained and even elevated for a given workload under hypoxia which indicates, at least under hypoxia, that mitochondrial uptake is likely the limiting factor.

The shift toward CHO utilization observed during prolonged exercise at high altitude might also reflect a change in relative exercise intensity. No significant differences in relative fuel selection between normoxia and hypoxia were reported during 60-min of cycling exercise at 60% of $\dot{V}O_{2peak}$ in the respective condition at simulated moderate (8) or high altitude (48). This has led to the conclusion that the shift in substrate partitioning observed when performing a given absolute workload at high altitude is due to the increase in relative exercise intensity rather than an effect of hypoxia per say (48). However, inconsistent findings are reported on whole body substrate partitioning when implementing the same relative workload under normoxia and hypoxia. Increased reliance on Gox under moderate (2,500; ~15% $O_2$) and more sever hypoxia (4,100 m; 12.4% $O_2$) has been demonstrated at 82 – 83 % (21) and 77 % of $\dot{V}O_{2peak}$ in each environmental condition (58). It has been suggested that these diverse findings stem from differences in experimental design, where substrate partitioning is not affected if relative workload and/or the degree of hypoxia remain moderate (58). Although, 30 minutes of cycling exercise at 50% of $\dot{V}O_{2peak}$ was sufficient to cause significant elevations in RER during exercise under simulated moderate hypoxia compared to the normoxia condition (40). In either case, matching workloads for relative exercise intensity at altitudes grater than 2000 m results in diminished $\dot{V}O_2$ (8, 21, 31, 40, 48, 58) because the absolute workload and, hence, the energy required to complete the activity are lower when compared to the same relative workload completed at sea level. It should also be recognized that total substrate oxidation is lower under hypoxia when workloads are decreased to match relative exercise intensity and, therefore, hypoxia exposure affects substrate oxidation regardless of the model used. Perhaps the most significant influence

of hypoxia exposure is a shift from exogenous glucose (plasma) to endogenous CHO (muscle glycogen) even when relative workloads are used (58). In terms of daily energy balance, the distinction between exogenous and endogenous energy sources is not obvious, however, substrate availability may be regulated independent of daily EP and is likely an important consideration in the amount of adiposity maintained under a given set of circumstances (18).

### 2.3 Substrate partitioning after exercise performed under short-term hypoxia

Historically, gas exchange measurements recorded during the post-exercise recovery period were taken to describe elevations in resting metabolic rate due to prior exercise. Increased resting EP was originally thought to result from an $O_2$ debt incurred during exercise, that is proportionate to anaerobic energy production during the prior activity (23). However, several other factors such as changes in circulating hormones and body temperature are also associated with elevated $\dot{V}O_2$ and therefore the term "excess post-exercise oxygen consumption" or EPOC is now used to avoid mechanistic implications of the $O_2$ debt hypothesis (23). The magnitude of EPOC is related to intensity and duration of the prior bout of exercise and although contributing to the thermic effect of exercise it does not make a significant contribution to daily energy balance (44). More recently, investigators have shifted their efforts toward describing the influence of prior exercise on resting substrate partitioning (28, 29, 43, 51, 55, 73). Given that resting metabolic rate comprises the largest fraction of daily EP in most individuals (46), the contribution of lipid oxidation toward EP at rest is of interest to those looking to decrease body fat mass. Therefore, the influence of prior exercise on 24-hr lipid oxidation should also be

considered when describing the influence of exercise training on weight loss / maintenance.

Although the post-exercise recovery period is generally associated with elevated rates of resting lipid oxidation, there is currently little data describing the effect of prior exercise performed under hypoxia on post exposure resting substrate partitioning. One such study that recorded whole body respirometry measurements during and after exercise under short-term moderate hypoxia actually reported an elevated RER at rest (40). However, subjects remained under hypoxia for both the exercise and recovery periods during the hypoxia trial, which could explain the increased reliance on Gox during recovery compared to normoxia. Several reports have displayed elevated rates of resting Lox after endurance exercise performed under normoxia compared to time matched resting controls (28, 29, 42, 43, 51, 73). Magkos et al. (2006) reported a 40 % increase in resting Lox compared to non-exercise controls the morning after an evening bout of exercise lasting 2-hr at 60 % of $\dot{V}O_{2peak}$ (51) Kuo et al (2004) and later Henderson et al (2007) also demonstrated that submaximal exercise performed at 45 and 65% of $\dot{V}O_{2peak}$ for 90 and 60-min, respectfully, resulted in a significantly elevated resting lipid oxidation above time matched resting controls (29, 43). Such elevations in resting lipid oxidation persists throughout the post-exercise recording periods lasting from 3-hr (43) up to 24-hr (29) after the single bout of exercise. A recent meta analysis of 18 studies that used time matched resting controls clearly demonstrated an effect of prior exercise on 24-hr resting lipid oxidation. The authors of this study concluded that EEE and individual $\dot{V}O_{2peak}$ were the main determinants of resting Lox, where the magnitude of their effect is related to the time since the last bout of exercise (28). However, the range of exercise intensities

examined in the studies discussed above is relatively narrow (40 – 65 % of $\dot{V}O_{2peak}$) and using higher exercise intensities (i.e. ≥ 80 % of $\dot{V}O_{2peak}$) may result in greater increases in resting lipid oxidation. Indeed, Trombold et al. (2013) reported that high intensity exercise (alternating 2 min at 25% and 2 min at 90% $\dot{V}O_{2peak}$) was more effective than moderated intensity exercise (50 % of $\dot{V}O_{2peak}$ for 60-min) for attenuating postprandial triglycerides (PPTG) and increasing resting lipid oxidation when matched for EEE (73). The observed decrease in PPTG was described to result from increased resting Lox the morning after an evening bout of exercise, which was proportionally greater after high intensity compared to low intensity exercise (73). Although muscle glycogen content was not measured in that study, the authors reasoned that an increased endogenous Gox during high intensity exercise may explain the greater shift in resting substrate oxidation (73). Skeletal muscle glycogen content is relatively small and under tight regulation (18), for this reason dietary CHO consumed after high intensity and long duration exercise is directed toward storage while plasma FFA and TGs are important fuels for oxidative metabolism (35, 42). Although plasma derived Gox increases with exercise intensity (11, 36) workloads beyond 65 % of $\dot{V}O_{2peak}$ and / or long duration (i.e. > 2hr) are needed to significantly deplete muscle glycogen content (64). This may explain why exercise intensity does not influence resting substrate partitioning when workloads are matched for EEE below 65% of $\dot{V}O_{2peak}$ as similar levels of muscle glycogen depletion are expected to occur. Similar to high intensity exercise, increased reliance on endogenous glucose is also observed during prolonged exercise under short-term hypoxia (58). Therefore, although limited data is presently available, it is reasonable to expect that post exposure resting

substrate partitioning would be shifted toward Lox up to 24-hr after exercise performed under short-term moderate hypoxia.

Increased 24-hr Lox may also explain why individuals experience accelerated weight loss during normobaric hypoxia training compared to similar or even higher workloads performed under normoxia. Contrary to the glucose / fatty acid cycle, the intracellular availability of glucose is proposed to determine the relative contribution of substrates to EP (68) and perhaps the most significant effect of exercise training on fat mass is to reduce the extent to which the bodies glycogen stores are habitually maintained (18). Therefore, workloads that rely heavily on endogenous glucose may be an effective strategy to increase 24-hr lipid oxidation and thus induce decreases in body fat mass. This is in contrast to current recommendations for prolonged moderate intensity exercise for weight loss, which focuses on increasing EEE and Lox during the actual activity. In fact, EEE is a relatively minor component of daily EP in most individuals and shifting resting substrate partitioning would have a larger influence on 24-hr lipid oxidation. The challenge in evaluating the effect of exercise on resting substrate partitioning using workloads that rely heavily on endogenous CHO sources (e.g. > 80 % $\dot{V}O_{2peak}$) is that such activities are not sustainable in untrained individuals. However, moderate normobaric hypoxia exposure during submaximal exercise may be an effective paradigm to evaluate the influence of exercise intensity on resting Lox. Peak workloads decrease at a rate of approximately 7 % for every 1000 m increase in elevation above sea level and, hence, the addition of moderate normobaric hypoxia to a given workload will increase the relative effort by 20 %. In addition, because the absolute amount of work performed is the same under normoxia and hypoxia in this paradigm EEE is matched between the two

conditions. Although it seems logical that prolonged exercise performed under moderate normobaric hypoxia would lead to similar increases in resting Lox as high intensity exercise, more studies are needed to describe its effect of whole body substrate partitioning.

## 2.4 Hypothesis

With the interest in assessing the viability of moderate shot-term hypoxia exposure during constant workload exercise as a means to alter post exposure resting substrate partitioning, the extent of alterations in whole body Lox and Gox were assessed after constant absolute workload cycling exercise performed under normoxia and hypoxia. A secondary objective was to describe the effect of short-term moderate hypoxia on fuel selection during prolonged exercise. It was hypothesized that (i) Lox is elevated and Gox depressed during the post-exercise recovery period, although the magnitude of this effect is greater after exercise performed under hypoxia, and (ii) the amplified Lox observed after hypoxia exposure is associated with an elevated total contribution from Gox during exercise.

# Chapter 3 Material and Methods

## 3.1 Study participants

Seven active healthy male participants were recruited from Memorial University of Newfoundland St. John's campus and regional community. Participants filled in a Physical Activity Readiness Questionnaire (PAR-Q) to determine level of activity, and to screen for a history of any health condition including smoking history, hypertension, cardiorespiratory disease, diabetes, musculoskeletal injuries or family history of any of the above-mentioned conditions in addition to known previous mountain sickness or altitude symptoms. They were excluded from the study if they took prescribed medication of any kind, were smokers or diagnosed as having; respiratory problems, heart disease, hypertension, chronic or acute illness, anxiety disorders, and drug or alcohol abuse. Screened participants, attended an orientation session in which they were given information about equipment used in the study and the experimental design, in addition to undergoing anthropometrics measurement. Finally, each participant signed a written informed consent in compliance with the declaration of Helsinki and with Memorial University's ethics committee regulations (reference number: 08.92). Anthropometric and physical fitness characteristics of the study participant are reported in Table 1.

Table 3-1. Anthropometric and fitness characteristics of participants

| Parameter | Score |
|---|---|
| Age (yr) | 26 ± 4.0 |
| Height (cm) | 175.5 ± 4.0 |
| Weight (kg) | 77.5 ± 9.7 |
| BMI (kg $m^{-2}$) | 25.2 ± 3.1 |
| $\dot{V}O_{2peak}$ (L $min^{-1}$ $kg^{-1}$) | 4.01 ± 0.31 |
| PPO (W) | 314 ± 26 |

### 3.2 Experimental design

A crossover study design, with a 7-day washout period, was used to evaluate the effect of moderate short-term hypoxia exposure during submaximal exercise on post-exposure measurements of substrate oxidation via indirect calorimetry. As depicted on figure 3-1, participants visited the laboratory on five separate occasions over a three-week period. Participants were first subjected to a maximal graded exercise test (GXT) on the cycle ergometer in order to determine $\dot{V}O_{2peak}$ and to assign a workload corresponding to 50% of sea level peak power output (PPO), which was maintained during constant workload exercise (CWE) performed under normoxia (N-CWE) and hypoxia (H-CWE). Sessions two and three occurred within a 25-hr time frame, which included a baseline basal metabolic rate ($BMR_{pre}$) measurement, followed by 60-min of CWE, immediately followed by a 60-min post-exercise metabolic rate (PEMR) measurement, and concluding with a second basal metabolic rate measurement the next morning ($BMR_{post}$).

Sessions four and five mirrored the two previous sessions except that CWE was performed in the opposite environmental condition (i.e. N-CWE or H-CWE).

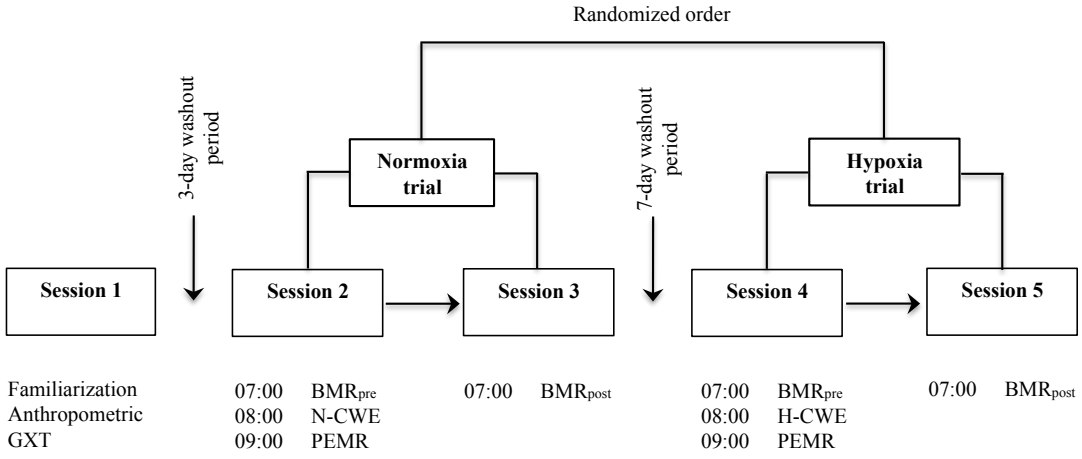


Figure 3-1. Experimental design

In order to control for the thermic effect of food and within subject variability for substrate partitioning, participants consumed standardized meals (780 Kcal; 26 g fat, 98 g carbohydrate, and 28 g protein) at 19:00 and fasted 12-hr prior to sessions 2 through 5. Participants were also requested to maintain a diet log throughout the experimental period and match food intake patterns between experimental trials. In addition, moderate-to-vigorous intensity and long duration exercise was avoided 48-hr prior to each testing session.

### 3.3 Environmental conditions

A simulated moderate altitude of approximately 2750 meters above sea-level corresponding to a $F_IO_2 = 0.15$ was used during H-CWE, while all other measurements were recorded under typical sea-level conditions ($F_IO_2 = 0.2093$). The HGM was supplied using a generator equipped with semi-permeable filtration membrane (GO2Altitude,

Biomedtech Melbourne Australia) that continuously pumped (120 L $min^{-1}$) air into five 120-liter Douglas bags. Gas concentration within the Douglas bags was continuously monitored using an oxygen sensor (Rapidox $O_2$, Sensotec, Cambridge, UK), ensuring that the target $F_IO_2$ was maintained at 15 ± 0.2 %. Participants were interfaced with the hypoxicator using a two-way non-rebreathing valve (2700, Hans Rudolph, Kansas, USA) and tubing. The same interface was used during N-CWE except the tubing was left open to room air. Temperature, barometric pressure and humidity were also monitored over the course of the study.

### 3.4 Respirometry measurements

Expired air was continuously analyzed throughout experimental protocols using an automated indirect calorimetry system (Jaeger Oxycon Pro, Cardinal Health, Hochberg, Germany) to recorde $\dot{V}O_2$, $\dot{V}CO_2$, VT, $B_f$, and $\dot{V}E$. The system was configured in push and pull set-ups for exercise and resting measurements, respectfully. During exercise, participants expired air was analyzed breath-by-breath using a mouthpiece (Reusable Series 9060, Hans Rudolph, Kansas, USA), nose clip (Reusable Series 9015, Hans Rudolph, Kansas, USA ) and the systems triple-v volume transducer, sample line and housing connected directly to the mouthpiece. This experimental set-up was chosen during exercise in order to obtain real time respirometric measurements and the mouthpiece could be quickly removed and replaced if necessary. Resting measurements were recorded in the supine position while the systems ventilator pulled air through a canopy, which covered participants head, into a mixing chamber from which subsamples were drawn. The ventilated hood technique was used during all resting measurements to

improve measurement accuracy and for participant comfort. Flow rate was manipulated to maintain a fraction of expired carbon dioxide ($FeCO_2$) between 0.7 - 1.0 within the canopy, as described elsewhere (70). During both experimental setups, $\dot{V}E$ was corrected to BTPS and water vapor was removed from subsample via nephron sample tube prior to reaching the infrared $CO_2$ analyzer and fuel cell $O_2$ analyzers. Prior to testing each day, the metabolic cart was given appropriate time to warm-up (≥ 2-hrs), after which, gas analyzers and volume transducer were calibrated with medically certified calibration gases (15% $O_2$ and 5% $CO_2$) and the systems built-in calibration functions, according to manufacture instructions. In addition, to insure accurate performance of the metabolic system, a propane gas verification was performed with a gas mass flow meter set at 200, 300 and 400 ml $min^{-1}$ and measured $\dot{V}O_2$ and $\dot{V}CO_2$ were compared against empirical values.

### 3.5 Cycling exercise

Participants were first subjected to a GXT on a magnetic break cycle ergometer (Velotron, Racer Mate, Seattle Washington, USA) to determine $\dot{V}O_{2peak}$ and PPO. A ramp protocol was implemented using self-selected cadence above 60 revolutions per minute (RPM) starting at 50 watts and increased by 1 watt every 3 seconds until participants could no longer maintain the minimum 60 RPM. After 5 minutes of recovery, a verification phase was implemented to confirm that a true VO2max was achieved, as described elsewhere (65) .

Submaximal cycling during N-CWE and H-CWE was performed at the same absolute workload corresponding to 50% PPO determined in normoxia (157±5 W) and RPM was

matched between conditions. The same absolute workload was used in both environmental conditions in order to match the EEE between environmental conditions and, therefore, control for the effect of energy deficit imposed by prior exercise on 24-hr substrate partitioning. Pulse oximeter (MasimoSET, Masimo Corporation, California, USA) analysis of blood oxygen saturation and heart rate were recorded inline with breath-by-breath respirometry measurements for later analysis. A cutoff score of 80% blood saturation was set to control for excessive metabolic stress during H-CWE. In addition, the Lake Louise Acute Mountain Sickness questionnaire was administered at the end of the H-CWE. Participants did not report symptoms of acute mountain sickness.

### 3.6 Basal and post-exercise metabolic rate

Basal metabolic rate was recorded prior to N-CWE and H-CWE and again 22-hr post-exercise in each environmental condition. In addition, PEMR was recorded up to 60-min after N-CWE and H-CWE for a total of 6 resting metabolic rate measurements over the study period. Standardized protocols were followed prior to and during all resting metabolic rate recordings; participants were instructed not to consume food or energy-containing beverage for 12-hrs prior to BMRpre and BMRpost but could consume water *ad libitum*, measurements lasted 45-min starting 07:00, measurements were recorded in the supine position with participants head supported by a single pillow, room temperature was maintained at 22°C and lights were dimmed, participants were instructed to lie motionless but awake and not to talk. Immediately following exercise participants were transferred from the cycle ergometer to the bed where they lay again in the supine position for 60-min while PEMR was recorded.

## 3.7 Calculations

Glucose (Gox) and lipid (Lox) oxidation rates were calculate according to the following equations derived from published data (38, 59), see appendix B for workings.

Equation 3-1

$Gox\ (g\ min^{-1}) = -\ 3.226\ \dot{V}O_2 + 4.585\ \dot{V}CO_2 - 0.461\ Pox$

Equation 3-2

$Lox\ (g\ min^{-1}) = 1.695\ \dot{V}O_2 - 1.701\ \dot{V}CO_2 - 0.319\ Pox$

Where $\dot{V}O_2$ and $\dot{V}CO_2$ are expressed in $l\ min^{-1}$ and protein oxidation rate (Pox) was estimated at $0.066\ g\ min^{-1}$ based on previously published urinary urea excretion measurements made on 12-h post-absorptive men with normal CHO reserves (25, 26). Energy production (EP) was then calculated from individual contributions of each substrate to the fuel mixture as follows (38, 59):

Equation 3-3

$EP\ (kcal\ min^{-1}) = 3.868\ Gox + 9.746\ Lox + 4.09\ Pox$

## 3.8 Data reduction and statistical analysis

Pre- and post basal metabolic rate measurements were truncated by 20-min out of 45-min data collection. The procedure discarded the first 15-min and last 10-min in order to nullify any metabolic rate fluctuation due to familiarization with the ventilated hood and the expected termination of data collection. Respirometry data was, then, integrated, normalized over time, and used for calculations of substrate oxidation and EP. Data

collected during exercise measurements were integrated over time and a mean value representing the entire 60-min recording period was recorded and used for calculations of substrate oxidation. In order minimize the influence of exercise on depleting bicarbonate stores and subsequent $CO_2$ retention during recovery (30) the first 40-min of PEMR was discarded and the final 20-min were integrated over time and a mean value was recorded and used for calculations. Paired-sample t-tests were used to identify differences in metabolic, respirometry, and cardiovascular data. Data is reported as means with their standard deviation except otherwise stated. Statistical significant was set at $P < 0.05$. Statistical Package for the Social Sciences for Windows (version 18.0; Inc., Chicago, IL, USA) was used for data evaluation.

# Chapter 4 Results

## 4.1 Cycling exercise

Inline with previously reported data (6, 75) exercise performed under moderate hypoxia resulted in significantly lower $SpO_2$ compared to similar exercise in normoxia ($p = 0.001$). The decreased $O_2$ availability lead to a characteristic hypoxic ventilatory response (HVR) where $\dot{V}E$ ($p = 0.01$) and Bf ($p = 0.009$) were significantly elevated above N-CWE, while VT was similar between the two conditions ($p = 0.56$). Although statistical significance was not reached, HR was also elevated in hypoxia compared to normoxia ($\Delta$ 5 ± 2 beat $min^{-1}$; $p = 0.087$). As displayed on Table 4-1, $\dot{V}O_2$ and, hence, EP were significantly lower under hypoxia compared to normoxia ($p = 0.029$ and $p = 0.035$, respectfully) during cycling exercise performed at a constant absolute workload (157 ± 5 W). This workload corresponded to 69 % $\dot{V}O_{2max}$ under normoxia and approximately 80 % of $\dot{V}O_{2max}$ under hypoxia given that aerobic capacity decreases at a rate of 7 % for each 1000 m increase in elevation (22, 67). It therefore appears as though exercise economy ($VO_2$ / W) was improved during H-CWE. However, the diminished $\dot{V}O_2$ recorded under hypoxia may actually reflect an increased contribution from anaerobic metabolism during H-CWE (31). In fact, as displayed on Table 4-1, Lox decreased by approximately 26% while Gox increased by only 0.8 % during H-CWE compared to N-CWE. Assuming that this non-oxidative energy production comes from endogenous CHO (58) and 3 mol of ATP are produced per mol glycosyl unit from fast glycolysis (17) then approximately 280 g of muscle glycogen would be required to make up the 122 Kcal deficit in hypoxia.

Table 4-1. Characteristics of exercise bouts

| | Normoxia | Hypoxia |
|---|---|---|
| $\dot{V}O_2$ (ml $min^{-1}$) | 2759 ± 168 | 2546 ± 175* |
| $\dot{V}CO_2$ (ml $min^{-1}$) | 2481 ± 135 | 2337 ± 102 |
| RER | 0.90 ± 0.02 | 0.92 ± 0.03 |
| $SpO_2$ (%) | 98.9 ± 1.4 | 87.7 ± 3.4* |
| Bf (breath $min^{-1}$) | 33.3 ± 4.9 | 39.6 ± 7.0* |
| VT (L) | 2.16 ± 0.12 | 2.19 ± 0.16 |
| $\dot{V}E$ (L $min^{-1}$) | 70.4 ± 8.3 | 82.9 ± 6.7* |
| HR (beat $min^{-1}$) | 158 ± 10 | 163 ± 7 |
| EP (kcal) | 821.5 ± 83.9 | 699.8 ± 57.7* |
| Total Gox (kcal) | 567.5 ± 143.2 | 573.1 ± 125.8 |
| Total Lox (kcal) | 254.5 ± 106.0 | 187.1 ±120.6 |

* Significantly different from N-CWE ($p < 0.05$)

## 4.2 Resting metabolic rate

Baseline BMR recorded prior to N-CWE and H-CWE were not significantly different from each other for EP, Lox or Gox. As displayed on figure 4-1, EP was significantly elevated above BMRpre after N-CWE ($p = 0.037$) and H-CWE ($p = 0.000$) during the last 20-min of PEMR. However, substrate partitioning was only affected after H-CWE (figures 4-2 and 4-3) displaying a higher Lox ($p=0.010$) and a trend toward a lower Gox ($p=0.076$). In addition, $\dot{V}O_2$, $\dot{V}CO_2$, and HR remained significantly elevated, while $SpO_2$ returned to baseline values, during the last 20-min of PEMR compared to baseline in each environmental condition. The statistical analysis also revealed a significant carry over

effect the next morning after H-CWE. In fact, Lox remained elevated while CHO oxidation was significantly reduced 22-hr after H-CWE.

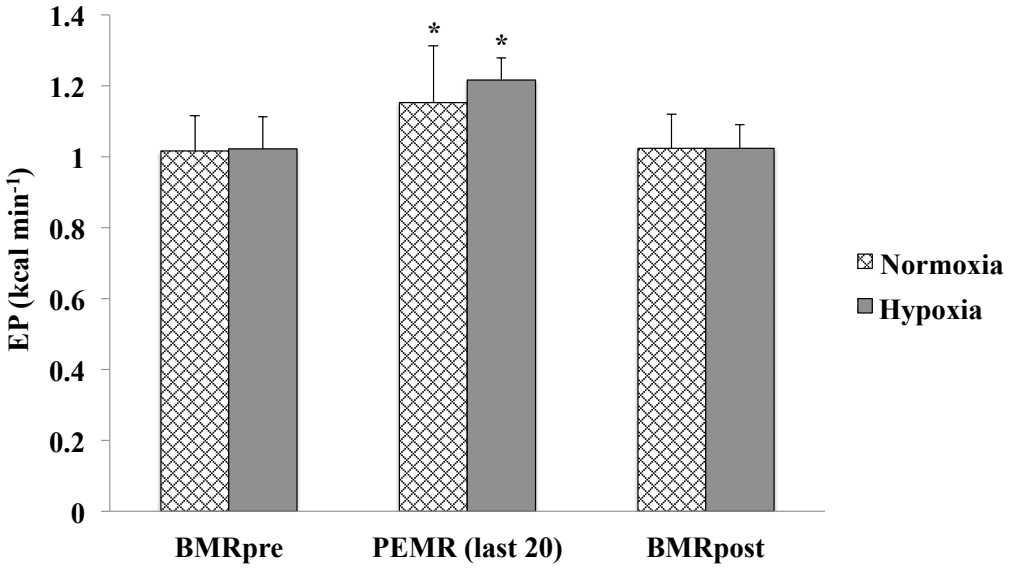


Figure 4-1. Metabolic rate recorded at baseline (BMRpre), 40-60 min post-exercise (PEMR), and 22-hr post-exercise (BMRpost). * total energy production (EP) significantly different from BMRpre in the respective environmental condition ($p < 0.05$).

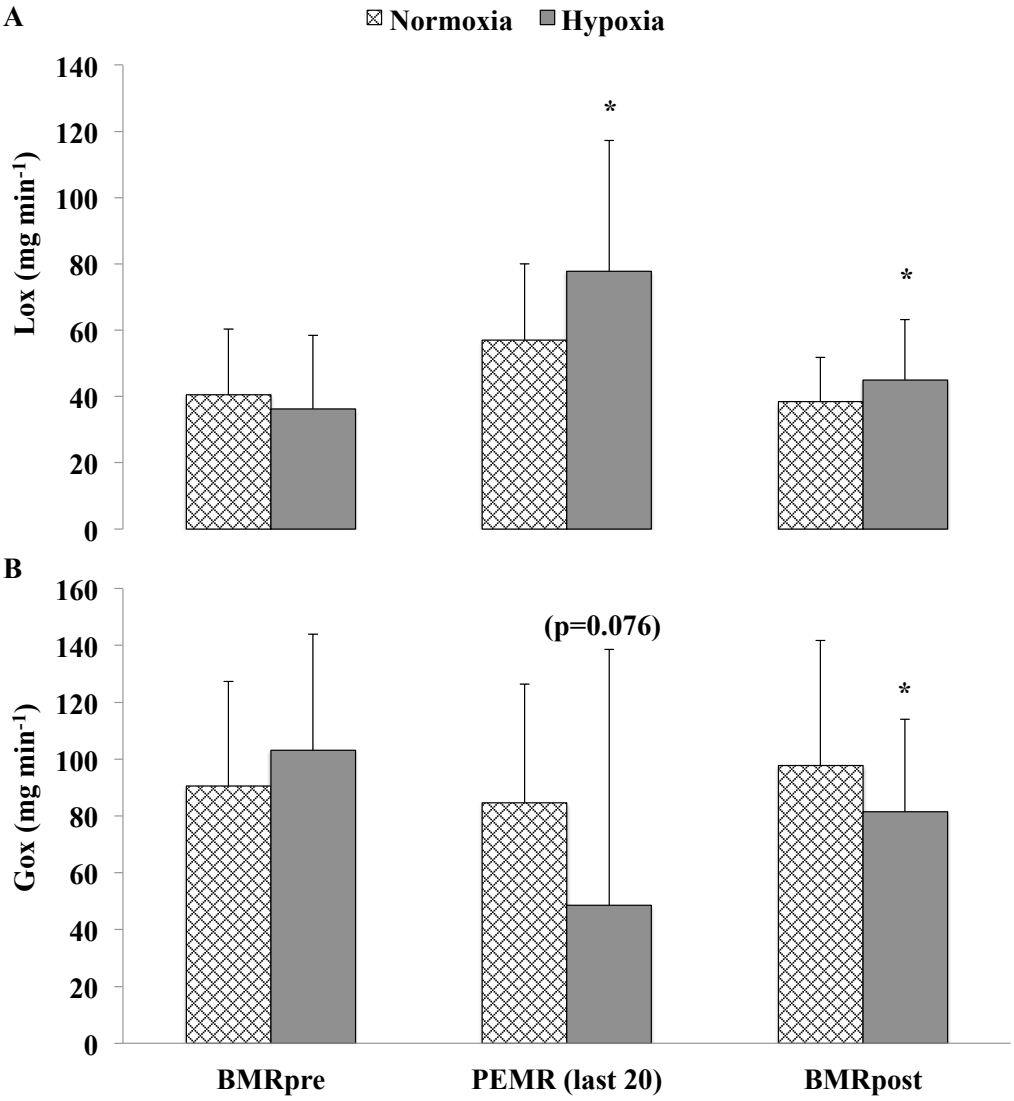


Figure 4-2. Total lipid oxidation (Lox) (A) and glucose oxidation (Gox) (B) at baseline (BMRpre), 40-60 min post-exercise (PEMR), and 22-hr post-exercise (BMRpost). * Significantly different from BMRpre in the respective environmental condition ($p < 0.05$).

# Chapter 5 Discussion

The novelty of the current study lays in its paradigm – that is, constant absolute workload cycling exercise performed under normoxia and hypoxia while recovering in normoxia for both environmental conditions. Normobaric hypoxia training has been described to provide a superior metabolic stress for weight loss at lower mechanical workloads compared to traditional exercise interventions (27, 57, 76). It has been speculated that enhanced lipid metabolism could explain such acclimations (76, 77). Although increased reliance on Gox is observed during prolonged exercise under hypoxia (40, 58, 75) the post exposure time periods between bouts of exercise are likely important considerations during normobaric hypoxia training. However, limited data is currently available on the post-exercise recovery period after exercise performed under hypoxia. Therefore, the aim of this study was to evaluate the effect of moderate short-term hypoxia exposure during constant workload cycling exercise on post exposure measurements of whole body substrate oxidation. The primary finding of the current study was that resting substrate partitioning was shifted toward Lox and away from Gox after submaximal exercise performed under hypoxia. However, no significant effect on resting substrate partitioning was observed after submaximal exercise performed at the same absolute workload under normoxia.

Elevated Lox was recorded immediately following H-CWE and again the next morning 22-hr after the single bout of exercise performed under short-term moderate hypoxia compared to BMRpre ($p = 0.01$ and $p = 0.036$, respectively). Accordingly, Gox was suppressed throughout the post-exercise recovery period while EP was initially elevated

during PEMR (1.22 vs. 1.02 kcal $min^{-1}$; p = 0.00) but returned to baseline values the next morning (p = 0.91). Elevated rates of resting lipid oxidation have previously been reported in healthy male subjects after similar workloads completed in the current study (29, 43, 52) However, these studies were conducted completely in normoxia and it is unclear if hypoxia itself had an effect on substrate partitioning. Katayama et al. (2010) recorded whole body respirometry during 30-min of cycling exercise and 60-min post exercise recovery under moderate hypoxia (40). This group actually reported conflicting finding to the current study displaying elevated RER's during resting recovery (40). However, subjects remained under hypoxia for both the bout of exercise and the recovery period in their study, which could explain the discrepancies with the current study. To best of the authors knowledge, the current study is the first to evaluate resting substrate partitioning under typical sea level conditions after a single bout of constant workload exercise performed under moderate short-term hypoxia.

Surprisingly, resting substrate oxidation was not different from baseline during post-exercise measurements when the same absolute workload was performed under normoxia. Aside from an elevated EP recorded immediately following N-CWE (1.15 vs. 1.02 kcal $min^{-1}$; p = 0.037), no significant differences in metabolic parameters were recorded during PEMR or BMRpost compared to BMRpre in the normoxia condition. Using similar workloads to the current study (i.e. 60-min at 60 % of $\dot{V}O_{2peak}$), Magkos et al. (2007) also failed to report a significant effect on resting substrate oxidation the morning after an evening bout of exercise (50). In contrast, Kuo et al. (2005) and Henderson et al. (2007) reported elevated resting Lox compared to time matched sedentary controls that lasted from 3 to 24-hr after performing 60-min of cycling at 65 %

of $\dot{V}O_{2peak}$ (29, 43). Differences in dietary controls used during the study period may explain theses discrepancies. Kuo et al. (2005) and Henderson et al. (2007) controlled for 24-hr nutrient intake ensuring that exercise and non-exercise trials consumed the same diet while Magkos et al. (2007) did not. In fact, Melanson et al. (2002) reported that 24-hr lipid oxidation was not different between exercise days (30-min at 40 and 70% of $\dot{V}O_{2peak}$) and non-exercise days when energy balance was maintained through consumption of additional calories to replace energy expended during exercise (56). This group reproduced these findings in obese subjects, older adults and endurance trained individuals (54) and later concluded that exercise has little effect on daily Lox in non-fasted individuals (55). However, this experimental design has been criticized for having a relatively high dietary CHO intake and increasing energy consumption beyond what is expended during the actual activity (28). In the current study, subjects performed the same total amount of work during N-CWE and H-CWE (i.e. matched for EEE) and stringent diets were not imposed. Therefore, a larger energy deficit imposed during H-CWE cannot explain why performing exercise under hypoxia shifted resting substrate partitioning toward Lox up to 22-hr post-exposure while no effect was observed after performing the same absolute workload under typical sea level conditions.

Increased FFA mobilization and/or muscle glycogen depletion during prolonged exercise under moderate hypoxia are possible mechanisms to explain the elevated Lox after H-CWE while no effect was observed after N-CWE. Sympathoadrenal activity, as indicated by plasma levels of epinephrine and norepinephrine, act to increase the rate of lipolysis while lactate and insulin have an inhibitory effect (37). Although blood samples were not taken during the current study, submaximal exercise performed under short-term hypoxia

is associated with elevated plasma epinephrine and lactate concentrations (40, 48) along with elevations in glycerol and FFA. Increased mobilization of FFA combined with elevated adipose tissue blood flow during the post-exercise period (72) would make lipid substrate readily available for either re-esterification or oxidation. Increased availability of FFA promotes Lox and inhibits Gox (60), which would contribute to the elevated Lox experienced during PEMR but is unlikely to have an effect 22-hr into recovery. Plasma insulin concentrations have also been reported to be suppressed after hypoxia exposure (49), which would likely contribute to maintaining elevated rates of lipolysis during the post exposure recording periods. Accordingly, hypoxia exposure during constant workload exercise may lead to increased resting Lox through increased availability of FFA. Alternatively, decreased availability of endogenous glucose after H-CWE may account for the shift in resting substrate partitioning toward Lox (68). Increased reliance on endogenous glucose has been described during prolonged exercise under hypoxia compared to performing the same absolute or relative workload under normoxia (58). Correspondingly, lipid oxidation was 26% lower during H-CWE compared to N-CWE in the current study. However, calculated rates of Gox were very similar under normoxia and hypoxia, which led to a significantly lower EP during H-CWE as determined through indirect calorimetry. Given that the total amount of work performed was maintained under both environmental conditions, it is reasoned that an increased contribution from non-oxidative energy pathways occurred during H-CWE. Although muscle glycogen content was not monitored in the current study, taken together these data can be interpreted to suggest that endogenous CHO reserves were depleted to a greater extent after H-CWE compared to N-CWE. Glycogen depleting exercise has previously been

described to increase resting Lox during the post-exercise recovery period when glycogen resynthesizes is a priority and plasma as IMTG are likely to be important fuel sources for aerobic energy (42). From this perspective, elevated rates of Lox recorded after H-CWE could be explained by an increased contribution from endogenous glucose utilization during the prior exercise.

A couple of limitations are inherent in the current study design. Firstly, calculations of substrate oxidation using respirometry measurements are based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ recorded at the mouth reflect that at the tissue level (17). During intense exercise, hyperventilation could increase $\dot{V}CO_2$ at the mouth above $\dot{V}CO_2$ in tissues and lead to an inflated RER (38). Although $\dot{V}E$ was significantly elevated under hypoxia compared to normoxia, $\dot{V}CO_2$ was similar during exercise under both environmental conditions and $\dot{V}E$ remained steady. Likewise, $CO_2$ retention is transiently increased for the first hour of recovery following high intensity exercise in order to replenish bicarbonate pools (30), which would be reflected in a low RER and over estimation of Lox. Hence, the large variability in substrate oxidation during PEMR after H-CWE may partially be explained by $CO_2$ retention, however, the observed elevation in resting Lox persisted 22-hr post-exercise when bicarbonate stores would have been restored. Therefore, calculations of substrate oxidation used in the current study can be take to reflect changes occurring at the tissue level. Secondly, stringent diets were not imposed throughout the current study and therefore we cannot exclude the possibility that energy intake may have been lower during the hypoxia session compared to normoxia. However, participants maintained a diet log throughout the experimental period and matched food intake patterns between experimental trials. Previous studies have imposed strict dietary

controls throughout the study period ensuring that a net energy deficit was maintained during the exercise trials (29, 43). It has been argued that such dietary controls lead to increased resting Lox because of the energy deficit on exercise days rather than prior exercise its self. Therefore, keeping participants on their routine food and using a crossover study design seemed the natural choice in order to uninfluenced resting substrate partitioning by a special diet.

The current findings support previous assumptions of elevated Lox after exercise performed under hypoxia and may help to explain why subjects experience accelerated weight loss during normobaric hypoxia training at lower mechanical workloads compared to training under normoxia. The intracellular availability of glucose is proposed to determine the relative contribution of substrates to EP (68) and perhaps the most significant effect of exercise training on fat mass is to reduce the extent to which the bodies glycogen stores are habitually maintained (18). Therefore, workloads that rely heavily on endogenous glucose may be an effective strategy to increase 24-hr lipid oxidation and thus induce decreases in body fat mass. This is in contrast to current recommendations for prolonged moderate intensity exercise for weight loss, which focuses on increasing EEE and Lox during the actual activity. In fact, EEE is a relatively minor component of daily EP and shifting resting substrate partitioning would have a larger influence on 24-hr lipid oxidation. Although the total amount of work performed was maintained under both environmental conditions in the current study, relative exercise intensity was approximately 20 % higher during H-CWE. However, it cannot be determined if the shift in resting substrate partitioning reflects the higher relative exercise intensity or if its characteristic of the hypoxia exposure itself. More studies are therefore

need to fully understand the affect of hypoxia exposure during submaximal exercise on resting substrate partitioning.

# Chapter 6 Conclusion

As hypothesized, moderate shot-term hypoxia exposure during constant workload cycling exercise is an effective means to alter post exposure substrate partitioning. The observed increase in resting Lox persisted 22-hr after the single bout of exercise performed under hypoxia while resting EP returned to baseline values. However, the constant absolute workload used in the current study was not sufficient to alter resting substrate partitioning after exercise performed under normoxia. Given that EEE and nutritional intake were matched between environmental conditions, these results cannot be explained by a larger energy deficit during H-CWE. The current data support previous findings of increased endogenous glucose utilization for a given absolute workload performed under hypoxia compared to normoxia (58). This is reflected in the significantly lower EP and Lox recorded via indirect calorimetry during constant workload cycling exercise performed under hypoxia in the current study. It is reasoned that the lower EP reflects a larger contribution of ATP produced through non-oxidative pathways during H-CWE. Theoretically, this would result in a larger glycogen depletion during H-CWE, which could explain the increased Lox observed after hypoxia exposure. These results need to be reproduced using additional techniques that discriminate between exogenous and endogenous substrate utilization during exercise along with measurements of glycogen replenishment post hypoxia exposure. However, the current findings may help to explain the seemingly paradoxical observation of accelerated weight loss during normobaric hypoxia training when EEE is actually lower compared to normoxia. This is supported by

Flatt's theory of weight maintenance (18, 19), which indicates that substrate balance may be regulated independent of energy balance. From this perspective, the accelerated weight loss observed during normobaric hypoxia training results from a preferential use of endogenous CHO during exercise that leads to an increased 24-hr lipid oxidation. Perhaps the most significant effect of exercise training on fat mass is to reduce the extent to which the bodies glycogen stores are habitually maintained and, thus, exercise that derives a large contribution of EP from endogenous glucose should be encouraged. However, it is unclear if the current findings are due to the relatively higher exercise intensity experienced during H-CWE or the result of prior hypoxia exposure itself.

# Bibliography

1. **Achten J, Gleeson M and Jeukendrup AE.** Determination of the exercise intensity that elicits maximal fat oxidation. *Med.Sci.Sports Exerc*. 34: 1: 92-97, 2002.

2. **Achten J and Jeukendrup AE.** Optimizing fat oxidation through exercise and diet. *Nutrition* 20: 7-8: 716-727, 2004.

3. **Achten J and Jeukendrup AE.** Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int.J.Sports Med*. 25: 1: 32-37, 2004.

4. **Alwan A.** *Global status report on noncommunicable diseases 2010*. World Health Organization, 2011.

5. **American College of Sports Medicine.** Preparticipation Health Screening and Risk Straification. In: *ACSM's Guidlines for Exercise Testing and Prescription*, edited by Thompson WR, Gordon NF and Pescatello LS. Philadelphia USA: Lippincott Williams &Wilkins, 2010, p. 18.

6. **Bartsch P and Saltin B.** General introduction to altitude adaptation and mountain sickness. *Scand.J.Med.Sci.Sports* 18 Suppl 1: 1-10, 2008.

7. **Basset FA, Joanisse DR, Boivin F, St-Onge J, Billaut F, Dore J, Chouinard R, Falgairette G, Richard D and Boulay MR.** Effects of short-term normobaric hypoxia on haematology, muscle phenotypes and physical performance in highly trained athletes. *Exp.Physiol*. 91: 2: 391-402, 2006.

8. **Bouissou P, Guezennec CY, Defer G and Pesquies P.** Oxygen consumption, lactate accumulation, and sympathetic response during prolonged exercise under hypoxia. *Int.J.Sports Med*. 8: 4: 266-269, 1987.

9. **Brooks GA, Butterfield GE, Wolfe RR, Groves BM, Mazzeo RS, Sutton JR, Wolfel EE and Reeves JT.** Decreased reliance on lactate during exercise after acclimatization to 4,300 m. *J.Appl.Physiol.(1985)* 71: 1: 333-341, 1991.

10. **Brooks GA, Butterfield GE, Wolfe RR, Groves BM, Mazzeo RS, Sutton JR, Wolfel EE and Reeves JT.** Increased dependence on blood glucose after acclimatization to 4,300 m. *J.Appl.Physiol.(1985)* 70: 2: 919-927, 1991.

11. **Brooks GA and Mercier J.** Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J.Appl.Physiol.(1985)* 76: 6: 2253-2261, 1994.

12. **Burtscher M, Mairer K, Wille M, Gatterer H, Ruedl G, Faulhaber M and Sumann G.** Short-term exposure to hypoxia for work and leisure activities in health and disease: which level of hypoxia is safe? *Sleep Breath* 16: 2: 435-442, 2012.

13. **Chapman RF, Karlsen T, Resaland GK, Ge RL, Harber MP, Witkowski S, Stray-Gundersen J and Levine BD.** Defining the "dose" of altitude training: how high to live for optimal sea level performance enhancement. *J.Appl.Physiol.(1985)* 116: 6: 595-603, 2014.

14. **Clanton TL, Hogan MC and Gladden LB.** Regulation of cellular gas exchange, oxygen sensing, and metabolic control. *Compr.Physiol*. 3: 3: 1135-1190, 2013.

15. **Clanton TL and Klawitter PF.** Invited review: Adaptive responses of skeletal muscle to intermittent hypoxia: the known and the unknown. *J.Appl.Physiol*. 90: 6: 2476-2487, 2001.

16. **Clark SA, Bourdon PC, Schmidt W, Singh B, Cable G, Onus KJ, Woolford SM, Stanef T, Gore CJ and Aughey RJ.** The effect of acute simulated moderate altitude on power, performance and pacing strategies in well-trained cyclists. *Eur.J.Appl.Physiol*. 102: 1: 45-55, 2007.

17. **Ferrannini E.** The theoretical basis of indirect calorimetry: a review. 37: 3: 287-301, 1988.

18. **Flatt JP.** Misconceptions in body weight regulation: implications for the obesity pandemic. *Crit.Rev.Clin.Lab.Sci*. 49: 4: 150-165, 2012.

19. **Flatt JP.** Issues and misconceptions about obesity. *Obesity (Silver Spring)* 19: 4: 676-686, 2011.

20. **Frayn KN.** Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. *Acta Physiol.(Oxf)* 199: 4: 509-518, 2010.

21. **Friedmann B, Bauer T, Menold E and Bartsch P.** Exercise with the intensity of the individual anaerobic threshold in acute hypoxia. *Med.Sci.Sports Exerc*. 36: 10: 1737-1742, 2004.

22. **Fulco CS, Rock PB and Cymerman A.** Maximal and submaximal exercise performance at altitude. *Aviat.Space Environ.Med*. 69: 8: 793-801, 1998.

23. **Gaesser GA and Brooks GA.** Metabolic bases of excess post-exercise oxygen consumption: a review. *Med.Sci.Sports Exerc*. 16: 1: 29-43, 1984.

24. **Gore CJ, Clark SA and Saunders PU.** Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Med.Sci.Sports Exerc*. 39: 9: 1600-1609, 2007.

25. **Haman F, Legault SR and Weber JM.** Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J.Physiol*. 556: Pt 1: 305-313, 2004.

26. **Haman F, Peronnet F, Kenny GP, Massicotte D, Lavoie C, Scott C and Weber JM.** Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids. *J.Appl.Physiol*. 93: 1: 77-84, 2002.

27. **Haufe S, Wiesner S, Engeli S, Luft FC and Jordan J.** Influences of normobaric hypoxia training on metabolic risk markers in human subjects. *Med.Sci.Sports Exerc*. 40: 11: 1939-1944, 2008.

28. **Henderson GC and Alderman BL.** Determinants of resting lipid oxidation in response to a prior bout of endurance exercise. *J.Appl.Physiol.(1985)* 116: 1: 95-103, 2014.

29. **Henderson GC, Fattor JA, Horning MA, Faghihnia N, Johnson ML, Mau TL, Luke-Zeitoun M and Brooks GA.** Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *J.Physiol*. 584: Pt 3: 963-981, 2007.

30. **Henderson GC, Fattor JA, Horning MA, Faghihnia N, Luke-Zeitoun M and Brooks GA.** Retention of intravenously infused [13C]bicarbonate is transiently increased during recovery from hard exercise. *J.Appl.Physiol.(1985)* 103: 5: 1604-1612, 2007.

31. **Heubert RA, Quaresima V, Laffite LP, Koralsztein JP and Billat VL.** Acute moderate hypoxia affects the oxygen desaturation and the performance but not the oxygen uptake response. *Int.J.Sports Med*. 26: 7: 542-551, 2005.

32. **Hoppeler H and Vogt M.** Hypoxia training for sea-level performance. Training high-living low. *Adv.Exp.Med.Biol*. 502: 61-73, 2001.

33. **Hoppeler H, Vogt M, Weibel ER and Fluck M.** Response of skeletal muscle mitochondria to hypoxia. *Exp.Physiol*. 88: 1: 109-119, 2003.

34. **Humberstone-Gough CE, Saunders PU, Bonetti DL, Stephens S, Bullock N, Anson JM and Gore CJ.** Comparison of live high: train low altitude and intermittent hypoxic exposure. *J.Sports Sci.Med*. 12: 3: 394-401, 2013.

35. **Jentjens R and Jeukendrup A.** Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med*. 33: 2: 117-144, 2003.

36. **Jeukendrup AE and Jentjens R.** Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. *Sports Med*. 29: 6: 407-424, 2000.

37. **Jeukendrup AE, Saris WH and Wagenmakers AJ.** Fat metabolism during exercise: a review. Part I: fatty acid mobilization and muscle metabolism. *Int.J.Sports Med*. 19: 4: 231-244, 1998.

38. **Jeukendrup AE and Wallis GA.** measurement of substrate oxidation during exercise by means of gas exchange measurements. 26: suppl1: s28-37, 2005.

39. **Jones NL, Robertson DG, Kane JW and Hart RA.** Effect of hypoxia on free fatty acid metabolism during exercise. *J.Appl.Physiol*. 33: 6: 733-738, 1972.

40. **Katayama K, Goto K, Ishida K and Ogita F.** Substrate utilization during exercise and recovery at moderate altitude. 59: 959-966, 2010.

41. **Kayser B and Verges S.** Hypoxia, energy balance and obesity: from pathophysiological mechanisms to new treatment strategies. *Obes.Rev*. 14: 7: 579-592, 2013.

42. **Kimber NE, Heigenhauser GJ, Spriet LL and Dyck DJ.** Skeletal muscle fat and carbohydrate metabolism during recovery from glycogen-depleting exercise in humans. *J.Physiol*. 548: Pt 3: 919-927, 2003.

43. **Kuo C,C, Fattor J,A, Henderson G,C and Brooks G,A.** Lipid oxidation in fit young adults during postexercise recovery. 99: 349-356, 2005.

44. **LaForgia J, Withers RT and Gore CJ.** Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *J.Sports Sci*. 24: 12: 1247-1264, 2006.

45. **Levine BD and Stray-Gundersen J.** Point: positive effects of intermittent hypoxia (live high:train low) on exercise performance are mediated primarily by augmented red cell volume. *J.Appl.Physiol.(1985)* 99: 5: 2053-2055, 2005.

46. **Levine JA, McCrady SK, Boyne S, Smith J, Cargill K and Forrester T.** Non-exercise physical activity in agricultural and urban people. *Urban Stud*. 48: 11: 2417-2427, 2011.

47. **Lippl FJ.** Hypobaric Hypoxia Causes Body Weight Reduction in Obese Subjects. 18: 4: 675-681, 2010.

48. **Lundby C and Van Hall G.** Substrate utilization in sea level residents during exercise in acute hypoxia and after 4 weeks of acclimatization to 4100 m. *Acta Physiol.Scand*. 176: 3: 195-201, 2002.

49. **Mackenzie R, Maxwell N, Castle P, Brickley G and Watt P.** Acute hypoxia and exercise improve insulin sensitivity (S(I) (2*)) in individuals with type 2 diabetes. *Diabetes Metab.Res.Rev*. 27: 1: 94-101, 2011.

50. **Magkos F, Patterson BW, Mohammed BS and Mittendorfer B.** A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men. *Am.J.Physiol.Endocrinol.Metab*. 292: 6: E1568-74, 2007.

51. **Magkos F, Wright DC, Patterson BW, Mohammed BS and Mittendorfer B.** Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *Am.J.Physiol.Endocrinol.Metab*. 290: 2: E355-62, 2006.

52. **Malatesta D, Werlen C, Bulfaro S, Cheneviere X and Borrani F.** Effect of high-intensity interval exercise on lipid oxidation during postexercise recovery. *Med.Sci.Sports Exerc*. 41: 2: 364-374, 2009.

53. **Mazzeo RS.** Physiological responses to exercise at altitude : an update. *Sports Med*. 38: 1: 1-8, 2008.

54. **Melanson EL, Gozansky WS, Barry DW, Maclean PS, Grunwald GK and Hill JO.** When energy balance is maintained, exercise does not induce negative fat balance in lean sedentary, obese sedentary, or lean endurance-trained individuals. *J.Appl.Physiol*. 107: 6: 1847-1856, 2009.

55. **Melanson EL, MacLean PS and Hill JO.** Exercise improves fat metabolism in muscle but does not increase 24-h fat oxidation. *Exerc.Sport Sci.Rev*. 37: 2: 93-101, 2009.

56. **Melanson EL, Sharp TA, Seagle HM, Horton TJ, Donahoo WT, Grunwald GK, Hamilton JT and Hill JO.** Effect of exercise intensity on 24-h energy expenditure and nutrient oxidation. *J.Appl.Physiol.(1985)* 92: 3: 1045-1052, 2002.

57. **Netzer, Nikolaus. C., Chytra, Roland., Kupper, Thomas.** Low intense physical exercise in normobaric hypoxia leads to more weight loss in obese people than low intense physical exercise in normobaric sham hypoxia. 12: 129-134, 2007.

58. **Péronnet F.** Substrate utilization during prolonged exercise with ingestion of 13C-glucose in acute hypobaric hypoxia (4,300 m). *Eur.J.Appl.Physiol*. 97: 5: 527-534, 2006.

59. **Peronnet F and Massicotte D.** Table of nonprotein respiratory quotient: an update. *Can.J.Sport Sci*. 16: 1: 23-29, 1991.

60. **Randle PJ.** Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab.Rev*. 14: 4: 263-283, 1998.

61. **Robach P and Lundby C.** Is live high-train low altitude training relevant for elite athletes with already high total hemoglobin mass? *Scand.J.Med.Sci.Sports* 22: 3: 303-305, 2012.

62. **Roberts AC, Butterfield GE, Cymerman A, Reeves JT, Wolfel EE and Brooks GA.** Acclimatization to 4,300-m altitude decreases reliance on fat as a substrate. *J.Appl.Physiol.(1985)* 81: 4: 1762-1771, 1996.

63. **Robertson EY, Saunders PU, Pyne DB, Aughey RJ, Anson JM and Gore CJ.** Reproducibility of performance changes to simulated live high/train low altitude. *Med.Sci.Sports Exerc*. 42: 2: 394-401, 2010.

64. **Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E and Wolfe RR.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am.J.Physiol*. 265: 3 Pt 1: E380-91, 1993.

65. **Rossiter HB, Kowalchuk JM and Whipp BJ.** A test to establish maximum O2 uptake despite no plateau in the O2 uptake response to ramp incremental exercise. *J.Appl.Physiol*. 100: 3: 764-770, 2006.

66. **Sabel Systems International.** Altitude & Barometric Pressure [Online]. http://sablesys.com/support/technical-library/barometric-pressure-vs-altitude-table/ [11/2014 2014].

67. **Saunders PU, Pyne DB and Gore CJ.** Endurance training at altitude. *High Alt.Med.Biol*. 10: 2: 135-148, 2009.

68. **Sidossis LS and Wolfe RR.** Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose-fatty acid cycle reversed. *Am.J.Physiol*. 270: 4 Pt 1: E733-8, 1996.

69. **Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M and Lundby C.** "Live high-train low" using normobaric hypoxia: a double-blinded, placebo-controlled study. *J.Appl.Physiol.(1985)* 112: 1: 106-117, 2012.

70. **Simonson DC and DeFronzo RA.** Indirect calorimetry: methodological and interpretative problems. *Am.J.Physiol*. 258: 3 Pt 1: E399-412, 1990.

71. **Sutton JR, Reeves JT, Wagner PD, Groves BM, Cymerman A, Malconian MK, Rock PB, Young PM, Walter SD and Houston CS.** Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *J.Appl.Physiol.(1985)* 64: 4: 1309-1321, 1988.

72. **Thompson D, Karpe F, Lafontan M and Frayn K.** Physical activity and exercise in the regulation of human adipose tissue physiology. *Physiol.Rev*. 92: 1: 157-191, 2012.

73. **Trombold JR, Christmas KM, Machin DR, Kim IY and Coyle EF.** Acute high-intensity endurance exercise is more effective than moderate-intensity exercise for attenuation of postprandial triglyceride elevation. *J.Appl.Physiol*. 114: 6: 792-800, 2013.

74. **Venables MC, Achten J and Jeukendrup AE.** Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J.Appl.Physiol.(1985)* 98: 1: 160-167, 2005.

75. **Wagner PD, Gale GE, Moon RE, Torre-Bueno JR, Stolp BW and Saltzman HA.** Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J.Appl.Physiol.(1985)* 61: 1: 260-270, 1986.

76. **Wiesner S.** Influences of Normobaric Hypoxia Training on Physical Fitness and Metabolic Risk Markers in Overweight to Obese Subjects. 18: 1: 116-120, 2010.

77. **Workman C and Basset FA.** Post-metabolic response to passive normobaric hypoxic exposure in sedendary overweight males: a pilot study. *Nutr.Metab.(Lond)* 9: 1: 103-7075-9-103, 2012.

78. **Young AJ, Evans WJ, Cymerman A, Pandolf KB, Knapik JJ and Maher JT.** Sparing effect of chronic high-altitude exposure on muscle glycogen utilization. *J.Appl.Physiol.Respir.Environ.Exerc.Physiol*. 52: 4: 857-862, 1982.

# Appendices

## Appendix A: Lake Louise Score for the Diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
1. A rise in altitude within the last 4 days
2. Presence of a headache

**PLUS**

3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

**SELF-REPORT QUESTIONNAIRE**

Add together the individual scores for each symptom to get the **total score.**

| | | | |
|---|---|---|---|
| **Headache** | No headache | **0** | |
| | Mild headache | **1** | |
| | Moderate headache | **2** | |
| | Severe headache, incapacitating | **3** | |
| | | | |
| **Gastrointestinal symptoms** | None | **0** | |
| | Poor appetite or nausea | **1** | |
| | Moderate nausea &/or vomiting | **2** | |
| | Severe nausea &/or vomiting | **3** | |
| | | | |
| **Fatigue &/or weakness** | Not tired or weak | **0** | |
| | Mild fatigue/ weakness | **1** | |
| | Moderate fatigue/ weakness | **2** | |
| | Severe fatigue/ weakness | **3** | |
| | | | |
| **Dizziness/lightheadedness** | Not dizzy | **0** | |
| | Mild dizziness | **1** | |
| | Moderate dizziness | **2** | |
| | Severe dizziness, incapacitating | **3** | |
| | | | |
| **Difficulty sleeping** | Slept as well as usual | **0** | |
| | Did not sleep as well as usual | **1** | |
| | Woke many times, poor sleep | **2** | |
| | Could not sleep at all | **3** | |
| | **TOTAL SCORE**: | | |

**Total score** of:

- 3 to 5 = mild AMS
- 6 or more = severe AMS

Note:

- Do not ascend with symptoms of AMS
- Descend if symptoms are not improving or getting worse
- Descend if symptoms of HACE or HAPE develop

## Appendix B: Equations for Lipid and Carbohydrate Oxidation

Biochemical data used in computation (38, 59) :

| | MW | Energy (kcal/g) | $O_2$ required (l/g) | $CO_2$ produced (l/g) | RQ | Equivalent of $O_2$ (kcal/l) |
|---|---|---|---|---|---|---|
| Glucose | 180.158 | 3.8683 | 0.7455 | 0.7426 | 0.996 | 5.189 |
| Average fatty acid | 272.4051 | 9.7460 | 2.0092 | 1.4136 | 0.704 | 4.851 |
| Average amino acid | 116 | 4.09 | 0.9842 | 0.7931 | 0.807 | 4.16 |

From knowledge of $\dot{V}O_2$, $\dot{V}CO_2$ (l $min^{-1}$), calculations for glucose (Gox) and lipid (Lox) oxidation rates (g $min^{-1}$) can be derived as follows:

$\dot{V}O_2 = 0.7455\ Gox + 2.0092\ Lox + 0.9842\ Pox$

$\dot{V}CO_2 = 0.7426\ Gox + 1.4136 Lox + 0.7931\ Pox$

By appropriate mathematical substitution, the above equations can be solve for Gox and Lox as follows:

$Lox = (0.4977\ \dot{V}O_2) – (0.3710\ Gox) – (0.4898\ Pox) = (0.7074\ \dot{V}CO_2) – (0.5253\ Gox) – (0.561\ Pox)$

**$Gox = - 3.2255\ \dot{V}O_2 + 4.5846\ \dot{V}CO_2 - 0.4614\ Pox$**

$Lox = (0.4977\ \dot{V}O_2) – (0.3710\ Gox) – (0.4898\ Pox)$

$Lox = (0.4977\ \dot{V}O_2) – 0.3710\ (-3.2255\ \dot{V}O_2 + 4.5846\ \dot{V}CO_2 - 0.461\ Pox) - 0.4898\ Pox$

**$Lox = 1.6944\ \dot{V}O_2 – 1.7009\ \dot{V}CO_2 – 0.3186\ Pox$**