THE METABOLIC RESPONSE OF INDIVIDUALS VARYING IN AEROBIC FITNESS TO ACUTE HYPOXIA AND ACCLIMATIZATION TO MODERATE ALTITUDE

by

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B.Sc., The University of British Columbia, 1997

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Zoology

THE UNIVERSITY OF BRITISH COLUMBIA

April 2005

Abstract

The concentrations of high-energy phosphate metabolites and lactate were monitored during and following exercise in ten male subjects varying in endurance fitness status throughout a 3-week moderate altitude acclimatization (3,800m) protocol, in order to determine (1) if metabolic control is altered with altitude acclimatization, and (2) how the metabolic response at altitude varies between fitness groupings. Venous blood lactate and cardiorespiratory measurements were taken during incremental and submaximal (70% relative $\dot{V}_{O_2\ max}$) cycling exercise and recovery at sea-level before (PRE) and after (POST) acclimatization, and at altitude upon immediate exposure (AH) and following acclimatization (ACC). ^{31}Phosphorus nuclear magnetic resonance spectroscopy was used to measure relative intramuscular phosphocreatine (PCr) and inorganic phosphate (P_i) concentrations, and pH during and following incremental plantar flexion exercise to fatigue, under normoxic and hypoxic (F_IO_2 = 13.8%) conditions. In the untrained group, PCr levels tended to decline, the rate of PCr recovery was delayed, and blood lactate concentrations and P_i/PCr (estimate of ADP_{free}) were increased when comparing AH to PRE levels. In ACC, PCr levels, PCr recovery rates, lactate concentrations, and P_i/PCr returned to PRE values, and lactate levels and P_i/PCr decreased further under POST exercise conditions. In the trained subject group, PCr recovery rates, PCr levels and P_i/PCr were unaltered between test conditions, and lowered lactate concentrations did not persist under POST conditions, as observed in the untrained group. Furthermore, the untrained group displayed lower heart rates, higher minute ventilation values, and improvements in the rate of lactate recovery and maximal oxidative capacity during exercise in POST compared to PRE, while all cardiorespiratory and metabolic measurements returned to PRE levels in the trained group. These results suggest that following altitude acclimatization, metabolic control improved in the untrained group, resulting in a lowering of glycolytic flux and lactate production during exercise. In contrast, the exposure to altitude had no effect on the trained subject group, suggesting that this group exhibited an 'optimal' level of metabolic control prior to altitude exposure, or the hypoxic stimulus was insufficient to activate further change. In conclusion, the biochemical and/or physiological adjustments that occurred in the untrained subjects during the acclimatization protocol resulted in a tightening of metabolic control during exercise, normally characteristic of changes seen following endurance training, accounting for the lower perturbation of high-energy phosphate metabolites and reduced lactate levels following altitude acclimatization.

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Δ	change (delta)
δ	chemical shift difference
[ ]	concentration
ACC	acclimatized hypoxia
ADP	adenosine diphosphate
ADP_{free}	free adenosine diphosphate
AH	acute hypoxia
AMP	adenosine monophosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
CaO_2	arterial oxygen content
cm	centimetre(s)
CO_2	carbon dioxide
Cr	creatine
ETC	electron transport chain
F_IO_2	fractional concentration of inspired oxygen
H^+	hydrogen ion
HR	heart rate
ISIS	image selected in-vivo spectroscopy
kg	kilogram(s)
L	litre(s)
LDH	lactate dehydrogenase
m	metre(s)
min	minute(s)
ml	millilitre(s)
mM	millimole(s) per litre
mmHg	millimetre(s) of mercury
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
N_2	nitrogen
NAD^+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NMR	nuclear magnetic resonance
O_2	oxygen

PaO_2	arterial partial pressure of oxygen
P_B	barometric pressure
PCO_2	partial pressure of carbon dioxide
PCr	phosphocreatine
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
pH	negative logarithm of hydrogen ion concentration
P_i	inorganic phosphate
PO_2	partial pressure of oxygen
POST	post acclimatization sea level
PRE	pre acclimatization sea level
RER	respiratory exchange ratio
R*f*	respiratory rate
RPE	ratings of perceived exertion
rpm	revolutions per minute
SaO_2	arterial oxygen saturation
SE	standard error
SL	sea level
S/N	signal to noise
TCA	tricarboxylic acid cycle
TR	repetition time
TV	tidal volume
µL	microlitre(s)
$\dot{V}_{CO_2}$	carbon dioxide production
$\dot{V}_E$	minute ventilation
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_2\,max}$	maximal oxygen consumption
WMRS	White Mountain Research Station

Many individuals and organizations provided extraordinary support for this project, without which it could not have been completed. Dr. Peter Hochachka's enthusiasm for this project, to the very end, was a source of inspiration. A huge thank you is deserved by all the individuals who with great generosity, patience and good humour, dedicated considerable time away from their friends and families to provide the data, or to provide guidance, expertise or an extra set of hands in extracting the data – the volunteers, Dr. Darren Warburton, Dr. Bill Sheel, Dr. Mark Trump and Dr. Don McKenzie. After 5 years of reading and re-reading, analyzing and re-analyzing, this thesis would never have been completed without the insight and careful attention of Dr. Jeff Richards, and the advice of Dr. Trish Schulte and Dr. Bill Milsom. The crew at the University of Alberta, including Dr. Gord Bell, Dr. Stuart Petersen, Katie Petersen, Dr. Peter Allen, and Dr. Chris Hanstock provided invaluable technical assistance and allowed the use of their equipment and facilities. A final thank you is extended to everyone at the WMRS who made the stay comfortable and enjoyable, and to my friends and family who have supported me along the way.

For most of the twentieth century, following the pioneering research of A.V. Hill and Otto Meyerhoff, lactate was thought of as a dead-end waste product of cellular metabolism produced under conditions of limited oxygen availability (Gladden 2004). Its production was implicated as the cause of exercise-induced metabolic acidosis, and the primary culprit leading to muscle fatigue (Roberg 2004). However, over the past 30 years, investigative strides have been made in redefining the role of lactate in energy metabolism. It is now accepted that lactate can be produced under fully aerobic conditions and that dysoxia is only one of several interacting factors that cause increases in muscle and blood lactate during exercise (Gladden 2004). Since the introduction of the lactate shuttle hypothesis (Brooks 1991a), lactate is now recognized as a mobile fuel. Lactate produced by contracting muscle can be transported to neighbouring muscle fibers or other body tissues, such as cardiac muscle, liver and kidney, to be used as a substrate for metabolism or glyconeogenesis. Simple biochemical analysis of the lactate dehydrogenase reaction reveals that lactate production consumes a proton, and is therefore alkalinizing, not acidifying to muscle (Roberg 2004). It is clear that the traditional view of lactate as a simple waste product is no longer valid, but instead lactate is a central player in energy metabolism at the cellular, regional and whole-body level.

Reduced blood lactate accumulation during exercise is uniformly found after endurance training, following acclimatization to hypobaric hypoxia, and in groups indigenous to high altitude. Lower lactate levels are indicative of metabolic adjustments, potentially resulting from an enhanced mitochondrial capacity, a decreased activation of glycolysis, or an improvement in the transport and removal of lactate from the blood. In endurance athletes, metabolic changes observed following training are geared towards improving the maximum aerobic capacity, by increasing muscle capillarization, mitochondrial volume content, and the reliance on fats to fuel exercise. These adjustments in turn reduce glycolytic flux and the production of lactate. While the outcome is the same, similar metabolic adjustments are not observed in altitude acclimatized individuals and high altitude natives, and cannot explain the lower lactate levels compared to unacclimatized lowlander controls. Recent advances in nuclear magnetic resonance (NMR) technology have enabled the measurement of known regulators of oxidative phosphorylation and glycolysis under real-time exercise conditions, providing much needed insight into differences in metabolic control between subject groups varying in training status, and humans born and living at altitude (Matheson 1991). The picture emerging from these studies is that exposure to hypoxia, whether induced through exercise or life-long residence at altitude, results in the reorganization of physiological and biochemical systems in a manner which may differ between various subject

groups, but ultimately is geared toward improving metabolic control and maximizing the contribution of oxidative ATP synthesis to energy balance during exercise. It is expected that short-term altitude acclimatization results in similar changes in metabolic control. If this hypothesis is correct, the extent of lactate production and accumulation during exercise can be considered a graded response, varying with differences in the organization of physiological and biochemical processes that contribute to the overall metabolic control of ATP turnover during exercise.

Metabolism and Energetics of Exercise:

i. Energy Demand And Energy Supply Processes:

A key challenge of physiological systems during exercise is balancing energy supply and energy demand in contracting muscle. The demands for ATP during exercise are set primarily by two tasks. The hydrolysis of ATP generates the energy necessary for actomyosin crossbridge cycling and force generation, and accounts for the greatest energy consumption of contracting muscle (~70-85% ATP supply). Calcium ATPases located in the sarcoplasmic reticulum also require ATP to resequester calcium and allow for muscle relaxation between contractions (~15-30% ATP supply). Energy demand by the ATPases is supported through the synthesis of ATP by high-energy phosphate transfer reactions (creatine kinase and adenylate kinase reactions), glycolysis and oxidative phosphorylation. The rate of ATP synthesis by each metabolic process differs, depending on how the pathway is organized (i.e. maximum enzyme activities, pathway complexity) and on the availability of specific substrates. Ultimately, the metabolic pathway employed to generate ATP and the choice of substrate to support metabolism depend on the intensity of exercise, the availability of oxygen, the pattern of fibre type recruitment, and the level of metabolic control during exercise.

Central to ATP balance in the muscle cell is creatine kinase, which catalyzes the breakdown of phosphocreatine (PCr) to regenerate ATP from ADP. Due to the high activity and near equilibrium nature of the creatine kinase reaction, $PCr + ADP + H^+ \leftrightarrow Cr + ATP$, cellular PCr stores provide an immediate source of high-energy phosphate to maintain ATP supply. If exercise is sustained for more than a minute, oxidative ATP synthesis is activated and balances contractile demand, so that PCr levels plateau. As exercise intensity increases, the creatine kinase reaction functions to buffer any additional changes in ATP, resulting in a further lowering of muscle PCr concentrations. Through the action of the phosphocreatine shuttle, PCr is also believed to be important in the transfer of phosphate groups between the mitochondria and the

actomyosin ATPase during exercise, and as such could be important for the metabolic state of skeletal muscle cells.

Glycolysis mobilizes energy by catabolizing glucose or glycogen to pyruvate. This metabolic pathway yields a carbohydrate substrate, reducing equivalents, electrons and protons necessary for transport into the mitochondria for oxidative phosphorylation, while at the same time generating cytosolic ATP in the absence of oxygen. Compared to fat, the use of glucose as a substrate has the advantage that it can generate ATP at a two-fold higher rate and can be rapidly taken up from the blood or mobilized from glycogen (Saltin 1988). Plus, glucose can directly enter into glycolysis in the cytosol, without the need of diffusing to the mitochondria. Relative to oxidative phosphorylation, the formation of ATP by glycolysis seems inefficient, generating 2-3 ATP/glucose compared to 36 ATP/glucose generated by oxidative metabolism. However, glycolysis occurs rapidly, producing significant quantities of ATP for short periods, making glycolysis an important pathway for the generation of ATP during short bursts of activity or high-intensity exercise.

During low to moderate intensity exercise, the rate of oxidative ATP synthesis primarily meets the rate of contractile ATP demand. This metabolic pathway yields more ATP per mole of substrate compared to glycolysis, and avoids the build up of inhibitory metabolites by using the end products of glycolysis and ATP hydrolysis to generate ATP. However, maximum rates of oxidative ATP synthesis are dependent on the availability and transport of carbon substrates and oxygen. Carbon fuels supplied by intracellular stores (glycogen and triglycerides) or transported from extracellular sources (glucose and free fatty acids) are necessary for the generation of high-energy reduced compounds via glycolysis and the tricarboxylic acid cycle (TCA), which in turn provide electrons and protons (H^+) required by the electron transport chain (ETC) to generate an electrochemical gradient across the inner mitochondrial membrane. This gradient ultimately supplies the energy for mitochondrial ATP synthase to phosphorylate ADP and regenerate ATP. While cellular substrate stores are readily available for entry into glycolysis or diffusion into the mitochondria, blood glucose and free fatty acids require transport mechanisms for delivery to (i.e. fatty acids bind to albumin) or movement into the muscle cell (i.e. glucose transporters), potentially limiting maximal flux rates (Weber 1988). As the 'final electron acceptor', oxygen is essential to mitochondrial ATP production for the maintenance of electron flow along the ETC. Intracellular oxygen stores in the form of oxymyoglobin or dissolved oxygen are small relative to the demand of the muscle. Therefore, oxygen transport to the mitochondria is also critical to maintain oxidative phosphorylation, and depends on a series of steps from the lungs via the circulation to the muscle capillaries. During sustained muscular exercise at low to moderate work

rates, slow-twitch muscle fibres, characterized by a high oxidative capacity and large lipid stores compared to fast-twitch fibres, are primarily recruited. Accordingly, when high rates of ATP regeneration are not required, exercise is fuelled mainly through the oxidation of free fatty acids because of their high energy density (ATP/mol), thereby maintaining blood glucose levels and sparing glycogen stores for prolonged exercise.

At high exercise intensities approaching the maximum aerobic capacity, ATP hydrolysis occurs at a rate that cannot be supported entirely by mitochondrial respiration. This increases the reliance on cytosolic ADP and P_i for ATP regeneration by glycolysis and high-energy phosphate transfer reactions. The proportion of fast-twitch muscle fibres recruited increases at high work rates due to the high glycolytic and PCr buffer capacities, large glycogen stores, and increased rate of calcium and actomyosin cycling observed by these fibre types, relative to slow-twitch muscle fibres. These changes are associated with a switch to the use of carbohydrates, rather than fats, as the main fuel source.

Both PCr breakdown and glycolysis support large ATP flux rates to meet the energy demands of high intensity exercise, but are limited by substrate supply and the generation of by-products that contribute to fatigue. When pyruvate and NADH from glycolysis are produced in excess of mitochondrial capacity, lactate and NAD^+ production increases. Eventually the rate of proton release by ATP hydrolysis and glycolysis will exceed the rate of uptake by the mitochondria or buffering by lactate production, PCr breakdown, and cellular buffer systems. Thus, as exercise intensity increases, calcium and actomyosin ATPases, and the enzymes involved in the regulation of glycolysis and oxidative metabolism become increasingly susceptible to inhibition due to increasing concentrations of H^+. Intracellular PCr and glycogen stores are used almost exclusively to fuel exercise at or above the oxidative capacity. Once this limited substrate supply is depleted, and the rate of ATP supply is unable to match ATP demand, athletic performance cannot be maintained (Saltin 1988). The underlying message is that mitochondrial capacity for acquiring protons and substrates is essential for minimizing the dependence on glycolysis and PCr breakdown for ATP regeneration, and retarding the metabolic acidosis that occurs as ATP hydrolysis exceeds the rate of ATP supply by oxidative metabolism (Robergs 2004).

ii. The Balance of Lactate Production and Removal During Exercise:

Due to the high standard free energy change and equilibrium constant of the lactate dehydrogenase (LDH) reaction, pyruvate that is not immediately consumed by the mitochondria is reduced to form lactate in the cytoplasm according to the following reaction: pyruvate +

$NADH + H^+ \leftrightarrow$ lactate + NAD^+. Traditionally, lactate production was considered to be secondary to exercise-induced hypoxia. However, the current view is that the net formation of lactate or pyruvate depends on the relative glycolytic and mitochondrial activities, not necessarily on the presence of oxygen. For example, if there is insufficient mitochondrial capacity to accept the glycolytic flux, excesses in pyruvate lead to lactate production. Fast-twitch muscle fibres, which possess a higher glycolytic capacity and LDH content and contain relatively few mitochondria compared to slow-twitch fibres, are thus key producers of lactate when recruited during exercise. From a biochemical perspective, the formation of lactate is beneficial for two reasons: (1) the LDH reaction consumes a proton and thus buffers the cell from proton accumulation, and (2) NAD^+ is produced along with lactate and is necessary to maintain the energy state of the cell (Robergs 2003).

As glycolytic flux increases with exercise intensity, attributed to an onset of oxygen lack in contracting muscles, increased recruitment of fast-twitch glycolytic muscle fibres, a greater reliance on carbohydrates for fuel, a change in energy state of the cell, and increased circulating hormones such as epinephrine (Gladden 2004), so does the production of lactate. During steady-state exercise conditions, increases in lactate production are balanced by increases in lactate removal. Lactate generated in working muscle cells can be used for fuel in the cell of origin or be moved across the sarcolemma and into the interstitial space using a monocarboxylate transporter. Neighbouring slow-twitch oxidative muscle fibres take up lactate for use as a substrate for oxidative ATP production, while lactate entering fast-twitch fibres is primarily converted to glycogen for carbohydrate storage (Brooks 2000). Blood lactate constitutes lactate that has entered the circulation to be removed by other skeletal muscles and body tissues such as the heart, liver or kidneys, for subsequent oxidation or glyconeogenesis (Brooks 1991a). At high exercise intensities observed during incremental exercise tests to fatigue, the balance between lactate appearance and removal is shifted, production dominates and lactate accumulation occurs in the muscle and blood.

iii. Regulation of Energy Balance During Exercise:

During exercise, ATP utilization by actomyosin and calcium ATPases must be closely matched to ATP synthesis, or else the modest endogenous ATP supply of the muscle will be quickly depleted and metabolic by-products will accumulate. Changes in metabolite concentrations in skeletal muscle during exercise, especially adenylates, PCr, P_i and lactate, estimate the degree of imbalance between ATPase flux and flux through ATP producing pathways. Current theories of metabolic control predict that changes in the levels of high-energy

phosphate metabolites, particularly ADP_{free} and the phosphorylation potential (ratio of ATP/ADP_{free}), play an important role in adjusting the rate of ATP supply by oxidative phosphorylation and glycolysis to match energy demands (Meyer 1994, Balaban 1990, Chance 1986, Connett 1988).

Muscle contraction, fueled by the hydrolysis of ATP by actomyosin ATPases, leads to increased concentrations of ADP, P_i and H^+, which in turn serve as substrates, positive feedback signals, and enzyme modulators for accelerating ATP supply pathways. Under submaximal exercise conditions, most ADP and P_i are transported back to the mitochondria to be used as substrates for ATP regeneration by oxidative phosphorylation. Pyruvate dehydrogenase (PDH), a key mitochondrial enzyme responsible for catalyzing the conversion of pyruvate to acetyl-CoA for entry into the TCA cycle, is regulated in an on-off fashion by phosphorylation-dephosphorylation mechanisms. The catalytic activity of PDH partly depends on ATP and ADP levels in that PDH is dephosphorylated to its active form when mitochondrial ATP/ADP_{free} decreases, while the inactive form is favoured under energy saturating conditions.

What is often underemphasized is that the same metabolite signal that turns on oxidative metabolism, also signals glycolysis, since ADP, AMP and P_i are substrates and activators of regulatory enzymes of glycolysis such as phosphofructokinase and glycogen phosphorylase. The sensitivity of glycolysis to the phosphorylation state is thought to involve the allosteric inhibition and disinhibition of phosphofructokinase by ATP and ADP, respectively (Connett 1990, Krause 1996). Since ADP is required as a substrate for the phosphoglycerate kinase and pyruvate kinase reactions of the glycolytic pathway, mass action effects can also influence glycolytic flux (Connett 1990). Additionally, free P_i is a key factor in the regulation of glycolysis due to its role as a substrate for phosphorylase a in the mobilization of glycogen (Brooks 2000). Therefore, increases in ADP_{free} and P_i provide a positive feed-back signal to trigger ATP supply mechanisms, directly linking oxidative phosphorylation and glycolysis to the demands of muscle contraction.

Energy Balance During Exercise at High Altitude:

i. Acute Responses and Acclimatization to Hypobaric Hypoxia:

Due to the high ATP yield and lack of inhibitory by-products, energy producing pathways are organized to maximize the utilization of available oxygen during exercise, and the contribution of oxidative phosphorylation to ATP synthesis (Hochachka 1985). To achieve this, oxygen and substrate supplies must meet the oxidative demands of the mitochondria to sustain ATP synthesis and match ATP demand. Exercise at high altitude, where oxygen availability is

compromised, poses additional challenges to muscle metabolic processes. Under these conditions, ATP homeostasis may be maintained using two possible strategies. The cell can accept the reduction in oxygen and limited oxidative phosphorylation, and increase the activation of other metabolic pathways such as the high-energy phosphate transfer system and glycolysis. However, this strategy is limited by the availability of energy stores and the accumulation of inhibitory metabolites. Alternatively, ATP homeostasis may be maintained by enacting physiological and biochemical mechanisms aimed at protecting ATP synthesis rates by oxidative phosphorylation, in spite of a reduction in the oxygen substrate.

In the absence of compensatory adjustments, acute hypoxia results in reductions in arterial oxygen tension (PaO_2), saturation (SaO_2), oxygen content (CaO_2), and the amount of oxygen available to skeletal muscle. To protect oxygen delivery to active muscles under conditions of hypobaric hypoxia, the respiratory and cardiovascular systems employ the latter strategy, and acutely respond by increasing ventilation, cardiac output and muscle blood flow (Rowell, 1986). Carotid body oxygen sensors initiate the hypoxic ventilatory response (Smith 2001), which despite the risk of alkalosis, helps compensate for the oxygen shortage. Additionally, vascular oxygen sensors initiate hypoxic pulmonary vasoconstriction (Reeves 2001) in order to adjust lung perfusion and ventilation-perfusion matching. Essentially, these hypoxia response mechanisms react instantaneously to the environmental change, and are designed to maintain oxygen consumption ($\dot{V}_{O_2}$) during exercise at close to sea level values when oxygen availability is compromised.

With prolonged hypoxic exposure over days and weeks, acclimatization processes occur that may involve the reorganization and restructuring of pre-existing physiological and biochemical machinery to compensate for the oxygen deficit. Vascular endothelial growth factor receptor expression is activated, and thus initiates angiogenesis, especially in the heart and brain (Raichle 2001). Prolonged hypoxia stimulates kidney oxygen sensing mechanisms, activating the release of erythropoietin and upregulating the red blood cell mass (Grover 2001). Increases in hematocrit and hemoglobin concentration further elevate the oxygen content of blood. These adjustments aimed at maintaining $\dot{V}_{O_2}$ and protecting oxidative ATP synthesis rates are fundamental for the preservation of ATP homeostasis during altitude exposure.

ii. Lactate Metabolism at High Altitude and the Lactate Paradox:

While the mechanisms responsible for the protection of oxygen delivery to contracting muscles in hypobaric hypoxia are well-known, less familiar are the adjustments that occur in the

energy producing pathways of muscle cells, both acutely and with acclimatization, to cope with changes in oxygen availability. Alterations in muscle metabolism in response to high altitude exposure are reflected in observations of blood and muscle lactate concentration during exercise. Under acute hypoxia conditions, a relatively large decline in maximum power output and maximum rates of oxygen consumption ($\dot{V}_{O_2\ max}$) are observed in lowlanders (Bushkirk 1967, Ferretti 1990, Hughson 1995), possibly due to oxygen limited cytochrome turnover (Connett 1990). During low intensity, steady-state exercise at moderate altitudes up to 4,000m, whole-body $\dot{V}_{O_2}$ is unaltered by acute hypoxia (Bender 1988, Wolfel 1991). However, at workloads above 60% normoxic $\dot{V}_{O_2\ max}$ during both steady-state and incremental exercise, Hughson et al. (1995) found that $\dot{V}_{O_2}$ is compromised and blood lactate concentration increases more quickly than observed for the same workloads in normoxia. These observations suggest that increased glycolytic ATP synthesis compensates for oxygen-limited mitochondrial respiration in acute hypoxia, allowing total ATP synthesis rates to be comparable to normoxia. At fatigue, further recruitment of glycolysis is prevented or glycolytic rate is inhibited since maximum blood and muscle lactate concentrations between normoxia and acute hypoxia are not different (Hughson 1995).

While oxygen-limited cellular respiration is a logical explanation for the increased blood lactate concentrations observed with exercise upon immediate exposure to high altitude, the explanation is not complete for two reasons. For one, under acute hypoxia conditions, glycolysis appears to be slightly uncoupled from oxidative phosphorylation because for a given $\dot{V}_{O_2}$ during incremental exercise, lactate concentration is higher compared to sea level (Green 1992b). This observation has been attributed to an increase in circulating epinephrine levels stimulating glycogenolysis with acute hypoxia (Brooks 1991b, Mazzeo 1991, Young 1991), and increased glycolytic flux to protect the energy state of the cell (Connett 1990). The second conflicting finding is that upon acclimatization to altitude, maximum blood lactate concentration and blood lactate levels at a given submaximal $\dot{V}_{O_2}$ are lower than subjects exposed to acute hypoxia (Edwards 1936, Bender 1988, Green 1989, Green 1992b, Grassi 1996). What makes this second observation paradoxical is that these decreases in maximum blood lactate concentration occur in the presence of unchanged oxygen delivery compared to acute hypoxia, since hematological adjustments that occur in response to hypobaric hypoxia acclimatization, such as increased hematocrit and hemoglobin concentration, are offset by β-adrenergic downregulation causing a decrease in cardiac output and muscle blood flow (Bender 1988). As a result, whole-body $\dot{V}_{O_2}$

during submaximal exercise and peak $\dot{V}_{O_2}$ during incremental exercise do not change with short-term acclimatization (Wolfel 1991, Bender 1988, Young 1982), and altered tissue oxygenation with acclimatization cannot explain the observed changes in lactate levels. Unlike the unacclimatized state, there is an apparent inability to recruit glycolysis to the same degree to support mitochondrial respiration or to assist in increasing ATP synthesis.

Despite numerous studies into this phenomenon, commonly referred to as the 'lactate paradox' (Hochachka 1988, West 1986), several issues remain unresolved regarding the metabolic adjustments that occur in skeletal muscle with altitude acclimatization and, in particular, the mechanism responsible for decreased blood lactate levels of altitude acclimatized individuals. Tracer studies measuring net lactate appearance and disappearance rates at rest and during exercise (Brooks 1991b, Brooks 1992), indicate that changes in blood lactate concentrations with acclimatization are attributed to a decrease in lactate release, not an increase in removal. At first it would seem evident that the decrease in epinephrine release and blunted adrenergic drive observed with altitude acclimatization compared to acute hypoxia would effectively account for the decreased stimulus for glycolysis and lactate production. However, numerous steady-state exercise studies have shown that β-blockade of unacclimatized subjects in acute hypoxia does not fully abolish the reduction in blood lactate concentration that accompanies altitude acclimatization, though the amplitude was considerably less (Mazzeo 1994, Young 1991). These findings have lead to the conclusion that another mechanism in addition to reduced adrenergic control of glycolysis must exist. Mechanisms tested to date that have not sufficiently accounted for the changes in blood lactate levels observed with acclimatization include: (1) decreased glycolytic enzyme activity (Howald 1990, Green 1992b); (2) decreased glycogen storage and substrate availability (Green 1989, Young 1982); (3) loss of buffering capacity and the inhibition of glycolytic enzymes (Kayser 1993); (4) changes in neuromuscular function and central drive (Green 1989, Savard 1990, Bigland-Ritchie 1988); (5) changes in muscle ultrastructure such as fibre size or fibre capillarization (Green 1992b); and (6) increased muscle oxidative potential (Green 1992b). Another hypothesis requiring greater exploration is the idea that metabolic reorganization occurs with acclimatization simply aimed at improving the coupling between ATP supply by oxidative metabolism and ATP demand of the working muscle (Green 1992b, Matheson 1991, Hochachka 1988), thus blunting glycolysis and lowering lactate release with altitude acclimatization.

iii. Insights from High Altitude Native Studies:

A key insight into unraveling the mechanistic basis for metabolic changes that occur with altitude acclimatization is revealed in the results of comparative studies between lowland residents and indigenous highlanders of the Andes and Himalayas (Matheson 1991, Hochachka 1991b, Allen 1997). In particular, the Andean Quechuas and Nepalese Sherpas are legendary for their endurance exercise performance and characteristically low blood lactate levels in response to exercise. During incremental exercise tests to fatigue under hypoxic conditions, high altitude natives accumulate less lactate at a given power output, and fatigue plasma lactate concentrations are about half (5 to 7 mM) of those seen in lowlanders (Hochachka 1991b). This metabolic response occurs in lowlanders within two to three weeks of altitude acclimatization and vanishes along a similar time course on descent (Grassi 1996). In contrast, low lactate concentrations following exercise are still observed in native highlanders even after six weeks of lowland habitation (Hochachka 1991b), leading to the conclusion that this metabolic characteristic is a fixed feature of genetic origin (Hochachka 1996).

Many of the acute and acclimatory responses to hypobaric hypoxia observed in lowlanders appear to be 'fine-tuned' in the Quechuas and Sherpas over generational time, and describe a high altitude physiological phenotype for hypoxia tolerance (Hochachka 1998). Compared to lowlanders, the hypoxic ventilatory response in native highlanders is blunted to counteract potential alkalosis (Samaja 1997, Niermeyer 2000), and the hypoxic pulmonary vasoconstriction response is reduced to minimize the risk of pulmonary hypertension (Groves 1993). Whole-body oxygen carrying capacity is up-regulated due to an expanded blood volume and red blood cell mass (Leon-Velarde 2000), thereby reducing the requirements of the heart to maintain oxygen delivery to the muscles during exercise. Metabolic organization of high altitude natives favours the aerobic production of ATP (Hochachka 1992b), as indicated by a decreased reliance on glycolysis and lower lactate accumulation compared to lowland controls. These long-term adjustments compensate well for the oxygen limitation caused by hypoxia and protect the skeletal muscle of high altitude natives from the overproduction of lactate; however, the cost of these adaptations include an attenuation of both maximum aerobic and maximum anaerobic metabolic capacities.

Matheson et al. (1991) studied the metabolic response of six Andean Quechuas who were born and lived their whole lives at altitude (3,600 - 4,500m). ^{31}P-magnetic resonance spectroscopy (MRS) techniques were used to determine gastrocnemius muscle metabolite changes during an incremental plantar flexion exercise test to fatigue, under normoxic and normobaric hypoxia conditions. Compared to a group of untrained sea level residents, the high

altitude natives were found to display less of a depression in PCr concentration and pH, and less of an increase in the P_i/PCr ratio (estimate of ADP_{free}) during exercise in normoxia and hypoxia. Compared to a group of endurance trained distance runners, who possessed considerably higher $\dot{V}_{O_2\ max}$ values, the pH and P_i/PCr ratios where essentially the same. In terms of metabolic control, these metabolite measurements would suggest that high altitude natives require less of a stimulus to maintain oxidative phosphorylation, associated with a lower glycolytic rate compared to the untrained subjects, but similar to the response of endurance trained individuals.

The differences in skeletal muscle metabolism between high altitude natives and untrained lowlanders can be considered analogous to the differences in energy balance of cardiac and skeletal muscle (Hochachka 1991a, Hochachka 1997, Matheson 1991), but to a lesser extent. Cardiac muscle is an example of a tightly coupled energy system, where a tight linkage exists between contractile demand and oxidative ATP synthesis. Over the full working range of the heart, there is minimal change in the levels of PCr, P_i and ATP, and no recruitment of glycolytic function (Katz 1988). Tight metabolic control systems are capable of achieving a given increase in mitochondrial respiration with smaller changes in the phosphorylation potential. In contrast to the heart, skeletal muscle possesses a loosely coupled energy supply-demand system. In working skeletal muscle, a change in power output is usually accompanied by changes in high-energy phosphate metabolites (Connett 1989). At the level of the mitochondria, there appears to be a momentary imbalance between contractile ATP demand and ATP supply by oxidative processes, causing a drop in PCr and ATP concentrations, while ADP and P_i concentrations are elevated. These metabolite signals 'drive' oxidative metabolism (Balaban 1990, Meyer 1994), bringing mitochondrial ATP synthase and actomyosin ATPase fluxes back into steady-state as work rate increases. A consequence of loose metabolic control is that glycolytic ATP synthesis and lactate production are simultaneously activated with changes in power output and high-energy phosphate levels, even under fully aerobic conditions.

The significance of metabolic control by the phosphorylation potential during exercise has been challenged on the basis that changes in ATP turnover rates greatly exceed the level of change in these metabolic intermediates (Hochachka 1997, Balaban 1990, Arthur 1992). Regardless, it is clear that ADP concentrations directly reflect changes in ATP demand by muscle ATPases, and facilitate a 'fine-tuning' of energy balance (Hochachka 1997, Allen 1997). In the muscles of high altitude natives, a lower perturbation of high-energy phosphate metabolites and lactate compared to lowlanders during incremental exercise to fatigue, suggests that long-term adaptations of native highlanders involve mechanisms that 'tighten' the coupling between ATP demand and ATP supply (improved ATPase-linked ADP control). The mechanism accounting for

this change is unclear. It has been suggested that a preponderance of slow-twitch muscle fibres found in high altitude native groups compared to lowland controls (Rosser 1993, Kayser 1991), favours a tighter energy coupling, less lactate production and improved endurance performance. However, these studies have employed small sample sizes and inappropriate control groups, so that further research is necessary to verify differences in fibre type composition, mitochondrial content, capillarization, and the enzymatic organization of muscles of high altitude natives compared to equivalent lowland controls. Regardless of the mechanism, tighter metabolic control tends toward maximizing the percent contribution of oxidative versus glycolytic pathways to overall ATP synthesis. However, not being able to rely on significant glycolytic contribution to meeting high ATP demand carries the price of reducing the ceiling on maximum muscle work.

Are the adaptive responses to endurance training and altitude acclimatization the same?

Exercise training can create a spectrum of adaptive responses in the muscle cell that depend on the type of activity and duration of training. Of particular interest are the adaptations that occur with endurance exercise training, leading to a lowering of blood and muscle lactate accumulation during exercise, similar to that seen with high altitude acclimatization. In general, physiological and metabolic systems of endurance athletes are geared towards improving oxygen transport and the oxidative capacity of muscle, while limiting glycolytic contributions during exercise (Hochachka 2003). This is achieved through improvements in muscle capillarization and cardiac output, an increase in mitochondrial content, the suppression of epinephrine secretion, and an increased activity of enzymes involved in the TCA cycle (Holloszy 1984, Davies 1981, Brooks 2000). Muscle mass is not particularly high compared to untrained individuals; however, fibre type transformations from fast-twitch to fast-twitch oxidative glycolytic occur with endurance training (Brooks 2000). Oxidative muscle fibres contain greater mitochondrial mass and lower ATPase capacities compared to fast-twitch fibres (Hochachka 1991a), so that actomyosin and calcium ATPase activities are downregulated in the endurance trained compared to untrained individuals.

The increase in mitochondrial content with endurance training has been suggested to increase the sensitivity of respiratory control of skeletal muscle during exercise (Hollozsy 1984, Constable 1987, Karlsson 1972). A greater mitochondrial density results in less oxygen utilization and energy production per mitochondria, so that a given rate of $\dot{V}_{O_2}$ can be achieved with less of a change in the phosphorylation potential. This tightening of metabolic control is thought to decrease the activation of glycolysis, thereby reducing carbohydrate utilization and lactate

production. In part, this would account for the greater use of fats as fuel, and lower blood lactate levels observed during submaximal exercise following endurance training (Holloszy 1984).

Using isotopic tracers to measure lactate turnover, Donovan and Brooks (1984) found that the depression in lactate with endurance training is not entirely due to a reduction in glycolysis, but also the result of an increase in the rate of lactate clearance. These findings are consistent with observations that the number of sarcolemmal lactate transporters increases with exercise training (Brooks 2000), thereby facilitating the transport of lactate from sites of production to sites of uptake for oxidation or glyconeogenesis. However, a recent short-term endurance training study displayed a shift in metabolic control and fuel selection, associated with less lactate production, before any change in mitochondrial content or muscle ultrastructure was observed, calling into question the importance of the effects of increasing oxidative capacity or lactate transport in explaining the lowering of lactate levels with endurance training (Green 1992a).

Similar to the results observed in exercising native highlanders, Matheson et al. (1991) observed that endurance athletes assumed to display lower lactate levels during exercise, also showed less of a perturbation of adenylates, P_i and the phosphorylation potential at fatigue compared to untrained individuals. These findings would suggest that similar metabolic adjustments occur with endurance training and chronic altitude exposure, making skeletal muscle more like the heart in terms of oxidative capacity and respiratory control. Many of the 'adaptable' traits observed in the hypoxia response systems in high altitude natives, are similar to the biochemical and physiological characteristics of endurance trained athletes, including a blunted hypoxic ventilatory response, expanded blood volume, fuel preference adjustments, and enhanced ratio of aerobic/anaerobic contributions to exercise (see Hochachka 1998 and references therein). However, compared to endurance athletes, mitochondrial volume density and muscle mass are decreased in native highlanders (Kayser 1991, Desplanches 1996), so that mitochondrial ATP synthase capacity and ATPase demand is downregulated, generally in step with an observed decline in $\dot{V}_{O_2\,max}$. In endurance athletes, who display a much higher maximum aerobic capacity, these similar traits appear as a high performance version of those found in the phenotype for hypoxia tolerance.

The lactate paradox is a graded, not an all-or-nothing, phenomenon:

Recalling the strategies suggested to maintain ATP homeostasis during exercise in hypobaric hypoxia, mechanisms aimed at protecting ATP synthesis rates by oxidative

phosphorylation in spite of a reduction in the oxygen substrate, seem most likely to be utilized based on the metabolic adjustments observed with endurance training and chronic altitude exposure. Green et al. (1992b) tested acclimatized lowlanders during submaximal exercise at an altitude of 4,300m, and presented evidence that the phosphorylation state of contracting muscle is restored toward sea level values with altitude acclimatization, in conjunction with a decrease in muscle lactate accumulation compared to acute hypoxia levels. Calculations of free adenylates were made using measurements of ADP, AMP and ATP from muscle biopsy samples taken at the end of exercise, and revealed a decrease in ADP_{free} concentrations and an increase in the ATP to ADP_{free} ratio following acclimatization compared to acute hypoxia. Another study compared the energy state of the cell and lactate levels of acclimatized lowlanders following the return to sea level from an expedition to Mt. Denali (Green 2000). With acclimatization, muscle lactate levels during moderate steady-state exercise were lowered upon return to sea level, compared to pre-acclimatization. At the same time, the energy state appeared to improve, indicated by lower muscle IMP (an indicator of ATP levels) and higher PCr levels during exercise. The lower perturbation of adenylates, PCr and lactate observed in these studies suggest that short-term altitude acclimatization also results in a tighter coupling between the processes involved in ATP utilization and the processes involved in ATP synthesis.

With respect to lactate production, the key insight arising is that glycolytic and oxidative pathways are controlled in part by the action of ATPases through the production of high-energy phosphate intermediates (Hochachka 2002). The better the control by the ATPases, the lower the signal for glycolytic activation and thus for lactate production. To test the hypothesis that an improvement in ATPase-linked ADP control underlies the lower than expected lactate concentration of hypoxia-acclimatized lowlanders, we decided to look at the relationship between glycolysis and the creatine kinase reaction, which are linked through ADP_{free} levels of the muscle cell. PCr can be considered a biochemical indicator of the processes that regulate lactate production since PCr levels decrease in response to increases in cytosolic ADP_{free} concentration in an attempt to maintain ATP homeostasis. At the same time, changes in ADP_{free} levels activate the glycolytic pathways of ATP regeneration. If this hypothesis is correct, we would predict an inverse relationship between PCr and lactate concentration, and a direct relationship between ADP_{free} and lactate concentration. Matheson et al. (1991) compared the recovery patterns of PCr and H^+ (used to estimate lactate production) in the gastrocnemius muscle of four subject groups, including Andean Quechuas, sedentary lowlanders, power-trained individuals, and endurance trained subjects, and confirmed these relationships among these groupings under normoxic and hypoxic exercise conditions. In addition to a reduced perturbation of adenylates and PCr levels at

$\dot{V}_{O_2\ max}$, they found the fastest recovery patterns for PCr and H^+ occurred in the endurance trained and Andean groups, followed by power-trained and sedentary individuals. This study, however, did not measure lactate levels directly, and while H^+ accumulation occurs when glycolytic flux and lactate production increases, H^+ concentration is not an accurate measure of lactate concentration.

According to the hypothesized metabolic relationships, the lactate paradox can be considered a graded response, with the relationship between muscle ADP_{free} concentration and glycolytic flux varying with metabolic and physiological state. When comparing hypoxia-acclimatized and non-acclimatized individuals, native highlanders to lowlanders, or subjects of varying training states, the levels of lactate accumulation will depend on the arrangement of ATP synthesis and demand processes organized for 'tight' or 'loose' metabolic control.

Hypothesis:

The key objective of this study was to demonstrate that altitude acclimatization results in improvements in the coupling between ATP supply and ATP demand processes, reducing the perturbation of adenylates and PCr concentration, and lowering the driving force for glycolysis and lactate production during exercise. To test this concept we:

(1) compared whole-body and single limb calf muscle exercise capacities of endurance trained and untrained subject groups before, during and following acclimatization to high altitude;
(2) measured venous blood lactate concentrations during incremental and submaximal exercise tests to confirm the presence of the lactate paradox with altitude acclimatization;
(3) measured relative PCr and P_i concentrations and pH during incremental plantar flexion exercise tests under hypoxic and normoxic conditions, before and following altitude acclimatization. ^{31}P- MRS techniques were used to allow repeated, non-invasive measurements of these key metabolites in the exercising muscle and determine the changes in PCr concentration and phosphorylation potential with training status and altitude acclimatization;
(4) determined rates of PCr recovery and lactate recovery following incremental exercise testing in order to characterize changes in muscle metabolism with training status and altitude acclimatization; and,

(5) presented the relationships between lactate and ADP_{free} concentration (estimated by the ratio of P_i to PCr), and between lactate and PCr concentration of the trained and untrained subject groups to display how lactate production during exercise varies with metabolic and physiological states in a graded, and not an all-or-none, fashion; and finally,

(6) compared the data when combined as whole group, to the results when separated into two groups by training status. This was done to display how differing responses to altitude acclimatization due to individual variability can be lost when subjects differing in their physiology and biochemistry are pooled into a single group.

i. Experimental Subjects:

Ten healthy males (24.3 ± 1.1 years, 74.2 ± 3.3 kg, 178.7 ± 1.5cm) were recruited from Edmonton and Vancouver on a volunteer basis (see Appendix 1 for subject data). Criteria for individual participation included: (1) no previous physical or metabolic ailments such as cardiovascular, respiratory, hepatic or renal problems, (2) $\dot{V}_{O_2\,max}$ between 40 to 70 $ml \cdot min^{-1} \cdot kg^{-1}$, and (3) between the ages of 18 to 40 years old. All subjects were sea level natives, and had not been to altitude within six months of commencement of the study. Prior to collection of data, subjects were informed of the nature and risks of the study and written, informed consent was obtained. The procedures used in these experiments were approved by the Clinical Screening Committee for Research and Other Studies Involving Humans at the Universities of British Columbia and Alberta.

ii. Experimental Protocol Overview:

Following subject selection, the study proceeded in four testing phases; (1) pre-acclimatization normoxia (PRE), (2) acute hypoxia (AH), (3) three weeks acclimatization to 3,800m (ACC), and (4) post-acclimatization normoxia (POST). During each phase, four exercise tests were performed. Incremental exercise to fatigue and steady-state, submaximal exercise tests using a bicycle ergometer were completed during each test period, and blood samples were collected to monitor plasma lactate levels during exercise and 30 minutes of recovery. Physiological data were also collected during exercise, including minute ventilation ($\dot{V}_E$), tidal volume (TV), respiratory rate (R*f*), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), respiratory exchange ratio (RER), heart rate (HR), arterial oxygen saturation (SaO_2), and weight. At each testing phase, an endurance performance exercise test was performed on a bicycle ergometer in which time to fatigue was recorded. In addition, incremental plantar flexion exercise tests using a foot ergometer and ^{31}P-MRS techniques were also completed during each of the four testing phases in order to monitor muscle phosphocreatine, β-ATP, and pH during exercise and 30 minutes of recovery.

iii. Acclimatization Protocol and Testing Phases:

Pre-acclimatization bicycle exercise testing and ^{31}P-MRS foot ergometer exercise tests were completed at the Exercise Physiology Lab and the Biomedical Engineering Lab in

Edmonton (670 m, P_B ~ 700 mmHg) over the course of one week. Upon completion of PRE testing, the subjects were transported to the White Mountain Research Station (WMRS) in California at a moderate altitude of 3,800 m (P_B ~ 480 mmHg). Within four hours of arriving at the research station, each subject completed an incremental cycling exercise test. Within eight hours, a submaximal cycling exercise test was performed, and within 24 hours an endurance performance test was completed. These tests comprised the second phase of bicycle ergometer exercise testing under acute hypoxia conditions (AH). In order to test all subjects, groups of four were transported to the research station on three consecutive days (see Figure 1). After 18 days at altitude, the subjects were considered acclimatized and performed the cycling exercise tests again at altitude under acclimatized hypoxia conditions (ACC). After 21 days of the first group arriving at the WMRS, all subjects returned to Edmonton. Post-acclimatization testing took place in Edmonton within two to four days of leaving the White Mountain Research Station (POST).

As ^{31}P-MRS measurements could not be made at altitude, AH and ACC conditions were created for the foot ergometer exercise tests by having the subjects breathe a hypoxic gas mixture (13.2% O_2 balanced with N_2), such that the inspired O_2 pressure was equivalent to 3,800m. These tests were completed in Edmonton, prior to and following altitude acclimatization.

During the three-week acclimatization period, the subjects remained at the WMRS where all subjects were asked to maintain their normal activity levels and record all exercise activity during this time period. They were permitted to ascend to the peak of the White Mountains, but were asked not to descend lower than 3,000m. Dietary restrictions were not imposed and all subjects maintained a similar diet at altitude and in Edmonton.

iv. Exercise Testing:

Incremental exercise testing ($\dot{V}_{O_2\ max}$): $\dot{V}_{O_2\ max}$ tests were performed initially to identify ten subjects that met the inclusion criteria and fell within a broad fitness spectrum. Following subject screening, $\dot{V}_{O_2\ max}$ tests were performed during each of the four testing phases. A brief, medical questionnaire was completed by each subject prior to exercise testing. Subject weight and height were measured and recorded. $\dot{V}_{O_2\ max}$ was determined using an incremental test to exhaustion. Prior to testing, an indwelling venous catheter was placed in a forearm vein for the withdrawal of blood samples. Subjects pedaled at a cadence of approximately 60 rpm on an electronically braked cycle ergometer (Quinton Excalibur, Lode, Groningen, The Netherlands), while the workload was

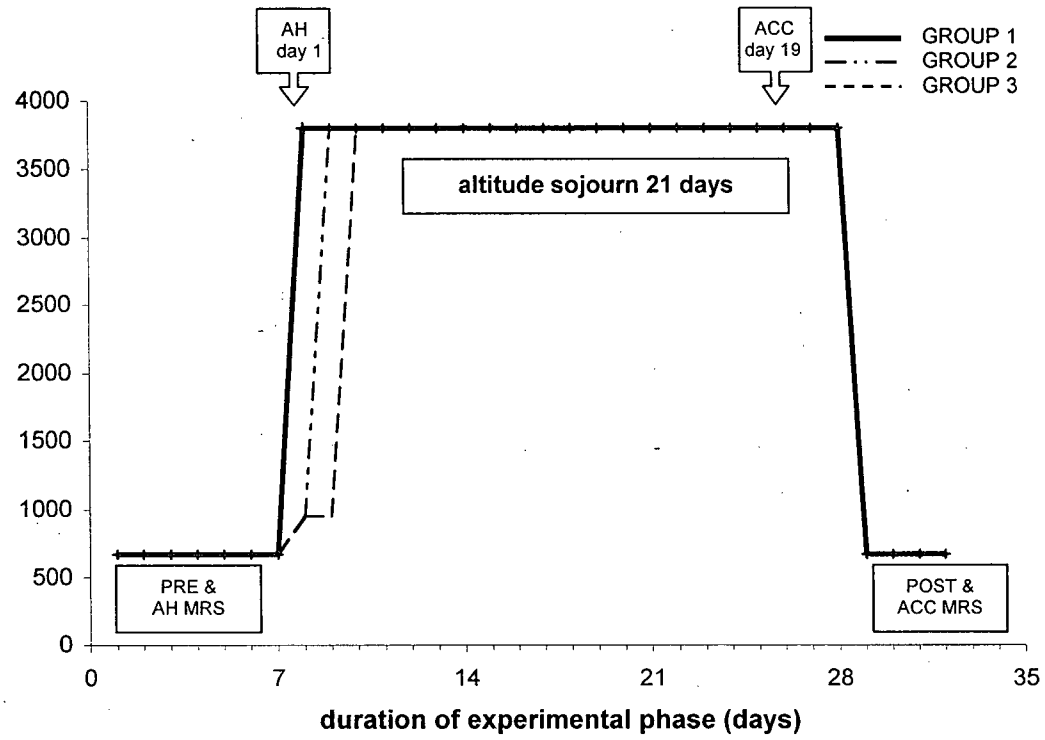

FIGURE 1: Profile of the acclimatization protocol and testing phases. PRE, pre-acclimatization normoxia; AH MRS, pre acclimatization hypoxia ^{31}P-MRS testing; AH, acute hypoxia; ACC, 3 weeks acclimatized hypoxia; POST, post acclimatization normoxia; ACC MRS, post acclimatization hypoxia ^{31}P-MRS testing.

increased by 30 watts/min, until volitional fatigue was reached. Subjects breathed through a Rudolf two-way, non-rebreathing valve (Hans Rudolf Inc, Kansas City, MO), so that throughout exercise. Sea level oxygen utilization ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) measurements were made using a Horizon Metabolic Measurement Cart (SensorMedics, California), while a portable Cosmed K4b(2) metabolic cart (Cosmed, Rome, Italy) was used for all altitude exercise tests. Each subject was assumed to be at $\dot{V}_{O_2\,max}$ when at least three of the following were observed: (1) a plateau in $\dot{V}_{O_2}$ with increasing workload, (2) respiratory exchange ratio (RER) of greater than 1.15, (3) attainment of 90% of age predicted maximal heart rate, and (4) volitional fatigue. All subjects were discouraged from participating in heavy exercise in the 24 hours before testing, and abstained from ingestion of food or fluid for 4 hours, and alcohol and caffeine for 12 hours prior to each exercise.

During $\dot{V}_{O_2\ max}$ testing, venous blood samples (1 ml) were withdrawn at rest and at three-minute intervals until fatigue for lactate determination (see below). Cardiorespiratory data ($\dot{V}_E$, TV, R*f*, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, RER) were collected at rest and continuously during exercise at 15 second intervals. A pulse oximeter (Ohmeda, Louisville, CO) was applied to each subject's ear to record SaO_2 at one minute intervals during the exercise bout. Heart rate was recorded every minute using a portable heart rate monitor (Polar, Kempele, Finland). Following exercise, the subjects were asked to pedal for 5 minutes at a workload of 30 watts. During this period of active recovery, blood samples were taken every minute. Active recovery was followed by 25 minutes of passive recovery where samples were withdrawn at 8, 11, 14, 17, 20, 25 and 30 minutes of total recovery time. A physician or registered nurse was in attendance at all times and was responsible for the safety of the subjects during the study.

Submaximal exercise testing: No less than four hours following $\dot{V}_{O_2\ max}$ testing, a submaximal exercise test was performed. From the maximal exercise data, the workload corresponding to 70% $\dot{V}_{O_2\ max}$ was calculated for each subject under each test condition. This percentage $\dot{V}_{O_2}$ level was chosen as it represented an exercise level that was expected to be below the ventilatory threshold for all subjects. Exercise intensity was intentionally kept below each subject's ventilatory threshold under each condition so that cardiorespiratory and lactate measurements could be made under steady-state conditions. Oxygen consumption was kept constant at 70% $\dot{V}_{O_2\ max}$ throughout the exercise protocol by continually adjusting pedal resistance. According to this study design, subjects would be exercising at the same *relative* exercise intensity under normoxic and hypoxic conditions. Before testing, an indwelling venous catheter was placed in a forearm vein for the withdrawal of blood samples. Subjects were asked to pedal at a cadence of 60 rpm for 15 minutes at the calculated workload. Expired air was collected and sampled during exercise in order to determine O_2 and CO_2 concentrations. During submaximal exercise, physiological measurements ($\dot{V}_E$, TV, R*f*, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, RER, heart rate, SaO_2) were recorded at rest and continuously throughout the exercise. Blood samples (1 ml) were taken at 0 (rest), 4, 8, 12, 15 minutes of exercise for lactate determination (see below). Submaximal exercise was followed by 5 minutes of active recovery at a workload of 30 watts, during which time blood samples were taken every minute. During the final 25 minutes of passive recovery, samples were withdrawn at 8, 11, 14, 17, 20, 25 and 30 minutes.

Lactate concentration determination: During each $\dot{V}_{O_2\ max}$ and submaximal exercise test, blood samples were collected to determine lactate levels during exercise and recovery. No more that 1 ml blood samples were collected into 3 ml gray cap vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) containing a glycolytic inhibitor, sodium fluoride, and an anticoagulant, potassium oxalate. Upon collection, blood samples were immediately centrifuged (IEC Clinical Centrifuge, International Equipment Company, Needham, MA) at 2,000 rpm for 10 minutes. Plasma samples were pipetted into microcentrifuge tubes and placed on ice for immediate lactate concentration analysis using a YSI Sport 1500 Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Each plasma sample was tested in duplicate. Before analysis, plasma samples were vortexed briefly (VWR mini vortexer, IKA Works Inc., Wilmington, NJ). To determine lactate concentration, 25 µl of plasma was pipetted using the YSI Syringepet and placed into a chamber containing a prepared buffer solution (YSI, Yellow Springs, OH). At the beginning of each day and after every 10^{th} sample tested, the lactate analyzer was calibrated manually using a known 5.00 mM lactate solution (YSI, Yellow Springs, OH). Linearity of the analyzer was checked at the beginning of each day and after every 50 samples using known 5.00 and 30.00 mmol/L lactate solutions (YSI, Yellow Springs, OH). When linearity could not be achieved (reading < 28.00 mM or > 32.00 mM), the YSI enzymatic membrane was changed.

To assess the level of lactate efflux from working muscle to blood, the following two variables were determined: (1) peak post-exercise venous lactate concentration, and (2) time to peak venous lactate concentration. To estimate the rate at which lactate was removed from the blood during 30 minutes of recovery, post exercise lactate concentration was expressed as a percentage of maximum lactate and these data were fit with linear regressions.

v. Endurance Performance Exercise Testing:

Twenty-four hours following maximal and submaximal exercise tests, the subjects returned to the Exercise Physiology Lab to perform an endurance performance exercise test. Prior to the onset of the exercise testing, subjects performed a five minute warm-up on a bicycle ergometer at a workload equivalent to 30% $\dot{V}_{O_2\ max}$, according to the $\dot{V}_{O_2\ max}$ value obtained during the pre-acclimatization maximal exercise test. The subjects were then asked to pedal at a workload that elicited 90% of the PRE $\dot{V}_{O_2\ max}$. The subject pedaled until volitional fatigue and cycling time to exhaustion was recorded. Throughout the warm up and exercise test, heart rate,

SaO_2 and perceived exertion values on a scale of 1 to 10 (RPE) were recorded. Subjects were asked to indicate how difficult they perceived their exertion level to be by verbally calling out their rating. This test was repeated under each testing phase using the same absolute workload values from the pre-acclimatization testing period such that subjects would be exercising at the same absolute exercise intensity under normoxic and hypoxic conditions. Due to time constraints, eleven of the twelve recruited subjects were able to complete the endurance performance exercise test.

vi. ^{31}P-MRS Studies:

Plantar flexion exercise protocol: ^{31}P-MRS was used to determine gastrocnemius muscle intracellular PCr and β-ATP concentration and pH during a graded exercise test and during recovery. The right calf muscle was exercised to fatigue in a specifically fabricated non-ferromagnetic ergometer placed in a 3 Tesla magnet. On one end of the ergometer, a foot pedal was mounted, which allowed plantar flexion of the ankle. The right foot was strapped into the foot pedal device using Velcro, so that the axis of rotation of the foot pedal occurred in the same plane as the anatomic axis of plantar flexion of the ankle. The foot pedal was connected via a simple system of rope and a pulley to a weighted basket. Eccentric muscle work was eliminated since the weight attached to the base of the foot pedal served to return it immediately to the neutral position during relaxation.

The exercise protocol was designed as a graduated plantar flexion test to fatigue. The test required the subjects to lift the foot pedal using a constant range of motion at the ankle at a rate of 30 repetitions/min. The rest of the leg and thigh were fixed in position with restraining straps. Each repetition required lifting the weighted basket against gravity for one second, and relaxing for one second. Cadence was maintained by the use of an audible beep. Exercise began with a load of 8 kg, and resistance was increased systematically every minute by adding 1 kg of weight to the basket until the subject was no longer able to maintain the rhythm of displacement.

Each subject performed the plantar flexion exercise test twice at sea level prior to altitude acclimatization, and twice upon return from altitude. In one test, subjects breathed ambient room air (approximately 21% O_2), in the second, altitude conditions (3,800m) were simulated by breathing hypoxic air (13.2% O_2 balanced with N_2). The subjects rested for at least 4 hours between tests, and the order of the test conditions was randomly assigned to prevent any sequencing effects from occurring. Throughout the study, subjects were unaware of the treatment order. Prior to the onset of exercise, subjects "acclimatized" for five minutes, breathing either room or hypoxic air through a mouthpiece and Rudolf two-way non-rebreathing valve (Hans

Rudolf Inc, Kansas City, MO). During acute hypoxia and acclimatized hypoxia tests, hypoxic gas was supplied from a gas cylinder (Praxair, Edmonton, AB) and passed through a Douglas bag to maintain a sufficient volume air for inspiration during exercise. During sea level tests, the Douglas bag was open to room air.

NMR data collection: Subjects were provided with a detailed description of the purpose of the study and the procedures that would be followed, prior to their entry into the study. The University of Alberta In-Vivo NMR Centre volunteer screening form was then reviewed and signed by each subject and the study coordinator, to eliminate any contraindications for the subjects safety in the strong magnetic fields within the magnetic resonance imaging (MRI) unit. All subjects were further screened for the presence of injury in the leg to be studied.

^{31}P-MRS and ^{1}H-MRI were conducted in a 3 Tesla superconducting magnet (Magnex Scientific PCI) interfaced with a Surrey Medical Imaging Systems console and operating system. Subjects were required to lie in a supine position in the magnet, and MR images of the right medial gastrocnemius muscle were taken at rest. The MRI data were acquired using a 25 cm diameter leg birdcage resonator for transmission and signal reception, with the widest point of the calf centred in the circumscribing radio frequency coil. Maximal calf girth was measured by anthropometry before the placement of the subject into the magnet to ensure consistent placement of the leg between testing conditions. Multislice gradient echo imaging in the transverse, coronal and sagittal planes was used to acquire a series of magnetic resonance images (echo time = 20 ms, repetition time (TR) = 1 s, and 5 adjacent slices with slice thickness = 5 mm). The images were used to register the 1D-localized ^{31}P-MRS image-selected in-vivo spectroscopy (ISIS) selected volume to the medial gastrocnemius muscle. This was achieved by orienting the upper edge of the ISIS slice both adjacent to, and parallel with, the fascia separating the gastrocnemius from the soleus muscle.

^{31}P-MRS data were collected by using an 8 cm diameter surface transceiver coil positioned such that the centre of the coil was aligned with the maximal calf girth, and with the gastrocnemius muscle resting directly above. Serial spectral data were acquired, in a continuous fashion, as 10-second data bins (each the sum of 10 averages) by using the 1D-ISIS sequence and with a TR of 1 second. Initially, subjects remained in a resting position for 2 minutes while baseline measurements were acquired, and then completed a graded exercise test to voluntary fatigue using the plantar flexion ergometer. Following exercise, collection of spectra was continued during first active (5 minutes at 30% maximum work capacity) and then resting recovery (25 minutes).

^{31}P-MRS data analysis: All data were analyzed using a ^{31}P-MRS analysis program designed by Dr. Christopher C. Hanstock at the University of Alberta using the Matlab programming environment (MATLAB®, The Mathworks Inc., Natick, MA). To obtain adequate signal-to-noise (S/N), three adjacent 10 second spectroscopy data bins were summed to give 30 second temporal resolution. The MRS time domain data were first filtered, by multiplying with a 5-Hz exponential function, to further improve the S/N. The data were then Fourier transformed and zero and first order phase corrected. The resulting frequency domain spectra were baseline corrected and the spectra were analyzed to provide estimates of the peak areas for P_i, PCr and β-ATP. The levels of PCr resulting from this analysis were normalized to 100% by using the average value obtained during the first two minutes of rest. Muscle intracellular pH was calculated from the chemical shift difference (δ) of the P_i peak in parts per million, relative to the position of the PCr peak, by using the following equation: $pH = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$ (Taylor, 1983). Recognizing that creatine and P_i contents in skeletal muscle are nearly equal, P_i/PCr was used as an estimate for free ADP concentration: $[Pi]/[PCr] = [ADP]\, k_{CK}\, [H^+]/[ATP]$ (Meyer 1994, Matheson 1991).

To analyze the rate of PCr resynthesis following exhaustive exercise, mono-exponential curves were fit to the PCr recovery data: $y = a[1 - \exp(-bx)] + c$, where y represents the PCr value at any given time x, a is the change in PCr during recovery, b is the rate constant, and c is the initial PCr value at the onset of recovery (Meyer, 1988). PCr recovery starts at c, rises to a + c, with a time constant of 1/b.

vii. Statistical Analysis:

The data were organized into three groups for analysis: (1) the data were pooled to include all subjects (n=10), and (2) the data were divided into trained (n=5) and untrained (n=5) groups based on $\dot{V}_{O_2\,max}$ values and training status (see results selection criteria). Within each grouping, peak and exercise work capacity, cardiorespiratory values, lactate concentration, and ^{31}P-MRS values were analyzed.

Pooled data: To compare peak values *between* test conditions within the pooled data, one-way ANOVA with repeated measures across the test condition was used to identify peak values that differed significantly. Two-way ANOVA (test condition x time) with repeated measures across both independent factors, was used : (1) to determine if there were any differences in samples taken during exercise *between* test conditions, and (2) to identify any time points that differed significantly during exercise *within* a condition.

Fitness group data: Comparisons of the two subject groups were made using simple descriptive statistics. To determine differences in peak values *between* groups under each test condition, two-way ANOVA (test x grouping) with repeated measures across the test condition was used. Three-way ANOVA (test x time x grouping), with repeated measures across the test condition and time, was applied to the group data: (1) to determine differences *between* groups in samples taken at a particular workload, (2) to determine changes in dependent variables during exercise *between* test conditions *within* each grouping, and (2) to identify any time points that differed significantly during exercise *within* each test condition and grouping.

Any significant F ratios were tested for statistical significance using Student Newman-Keuls post hoc test. For all statistical tests employed, values were considered to be significantly different at the $p \leq 0.05$ level. Significant main effects for test condition were focused on comparisons between normoxic and hypoxic conditions (PRE versus AH and ACC), and tests for effects of acclimatization (AH versus ACC, PRE versus POST). Statistical analysis was done using a statistical software program (STATISTICA for Windows, version 5.1 (1997). StatSoft, Inc., Tulsa, OK)

A. Metabolic and cardiorespiratory responses to exercise in acute hypoxia and following altitude acclimatization

I. Incremental Exercise Testing:

i. $\dot{V}_{O_2\ max}$ and Workload:

Compared to PRE levels, peak workload and $\dot{V}_{O_2\ max}$ decreased by 11.0% and 3.4%, respectively upon arrival at altitude (AH; Figure 2A and B; see appendix 2 for individual data). After 3 weeks of altitude acclimatization (ACC), $\dot{V}_{O_2\ max}$ did not change from AH levels, while peak workload increased by 6.1% compared to AH. Upon return to Edmonton following acclimatization (POST), $\dot{V}_{O_2\ max}$ increased by 7.2% and workload increased by 5.6% compared to PRE.

ii. Cardiorespiratory Measurements:

Under all test conditions, with increases in workload beyond 90 watts, minute ventilation ($\dot{V}_E$) increased continuously until fatigue (Figure 3). Compared to PRE, $\dot{V}_E$ response curves were shifted upwards in AH and ACC, indicating a more rapid rise in $\dot{V}_E$ during hypoxic exercise. At fatigue, $\dot{V}_E$ increased by 9.9% in AH, and by 16.9% in ACC (Table 1; Appendix 3 for individual data). Despite a further increase in $\dot{V}_E$ with acclimatization from AH levels, the change was not significant. In POST, $\dot{V}_E$ during exercise decreased from AH and ACC levels, but remained significantly elevated compared to PRE levels at workloads between 90 to 270 watts.

TABLE 1: Alterations in mean (± SE) peak cardiorespiratory values at fatigue from incremental cycling exercise at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

test period	$\dot{V}_E$ (L/min)	TV (L/breath)	Rf (breaths/min)	RER	HR (beats/min)
PRE	168.2 ± 12.1	3.456 ± 0224	54.7 ± 2.4	1.27 ± 0.02	186.6 ± 2.3
AH	184.8 ± 13.2 [a]	3.543 ± 0.328	61.2 ± 3.1 [a]	1.02 ± 0.03	179.8 ± 2.5 [a]
ACC	196.6 ± 9.1 [a]	3.325 ± 0.242	64.8 ± 3.0 [a]	1.17 ± 0.03	175.8 ± 2.6 [a]
POST	177.6 ± 11.6	3.417 ± 0.229	56.0 ± 1.8	1.18 ± 0.02	182.8 ± 2.4

$\dot{V}_E$, minute ventilation; TV, tidal volume; Rf, respiratory rate; HR, maximum heart rate; RER, respiratory exchange ratio, at fatigue.
a, significantly different from PRE values.

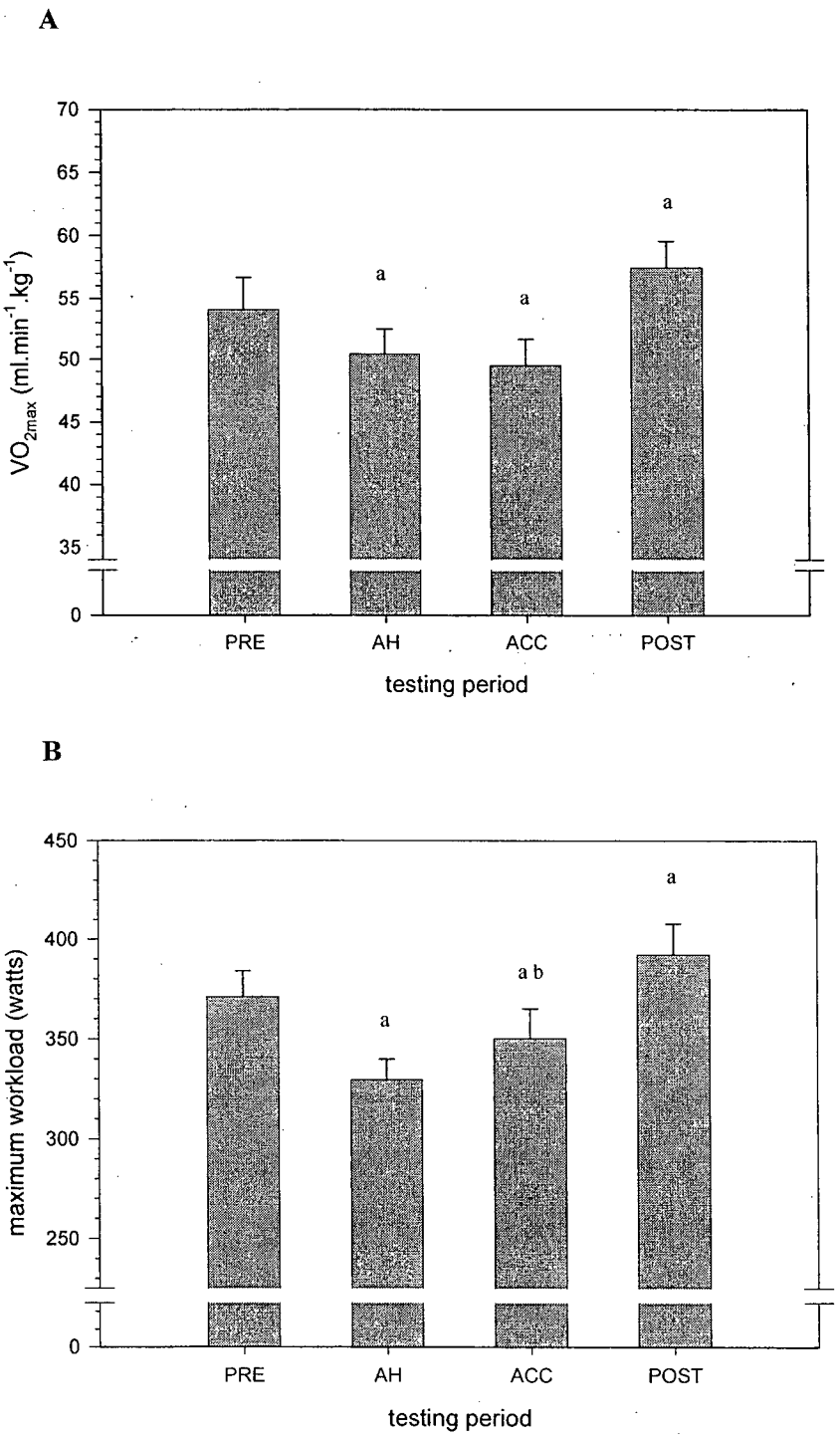

FIGURE 2: (**A**) Mean maximal oxygen consumption ($\dot{V}_{O_2\ max}$), and (**B**) mean workload at fatigue from incremental cycling exercise, at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Graphs display data where n=10 at all test periods. Values are means ± SE. a, significantly different from PRE values; b, significantly different from AH values.

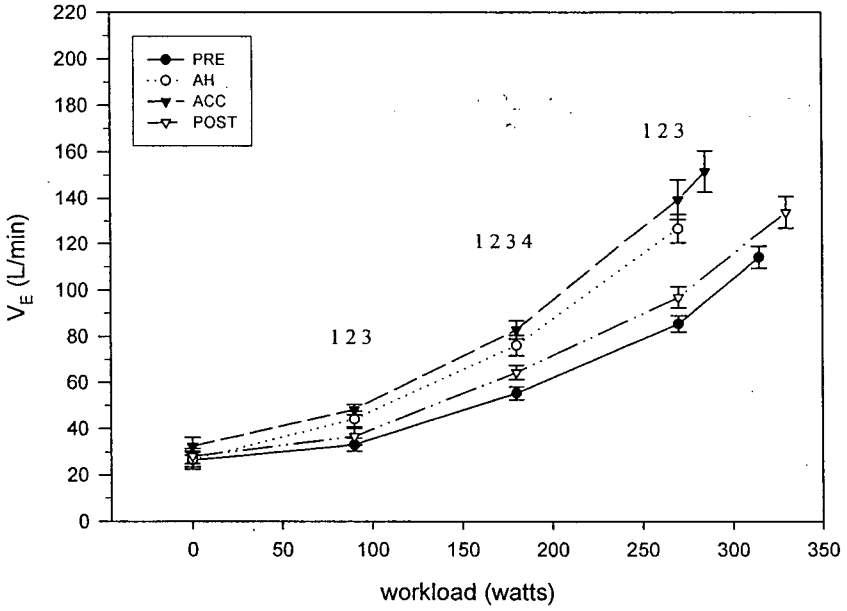

FIGURE 3: Mean (± SE) minute ventilation ($\dot{V}_E$) during incremental cycling exercise performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 10. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 3, PRE significantly different from POST; 4, AH significantly different from ACC.

$\dot{V}_E$ is calculated by multiplying tidal volume (TV) by respiratory rate (R*f*). Within each test condition, TV increased significantly throughout the course of incremental exercise (Figure 4), reaching a plateau before fatigue (Table 1; Appendix 3 for individual data). Two-way ANOVA testing (test condition x time) revealed that the main effect for test condition is not significant, suggesting that peak and exercise TV did not differ between the test conditions.

Therefore, changes in ventilation at altitude can be attributed to changes in R*f*. Similar to the trends observed in $\dot{V}_E$ under each test condition, R*f* significantly increased at workloads greater than 90 watts (Figure 5), and continued to rise until fatigue (Table 1; Appendix 3 for individual data). At workloads greater than 90 watts, AH and ACC values were significantly higher than PRE levels and indicated a significant hyperventilatory response in hypoxia; however, there was no difference between AH and ACC. In POST, R*f* remained significantly elevated compared to PRE levels.

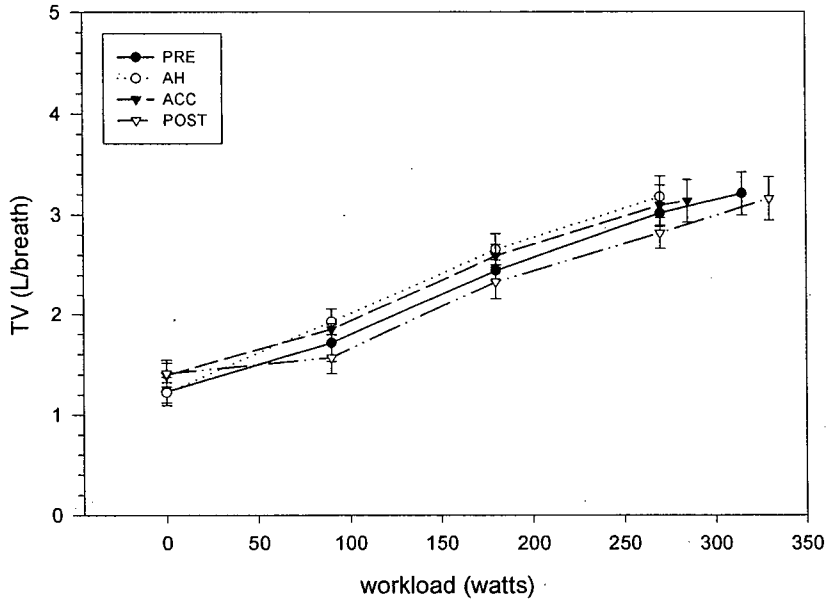

FIGURE 4: Mean (± SE) tidal volume (TV) during incremental cycling exercise performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 10.

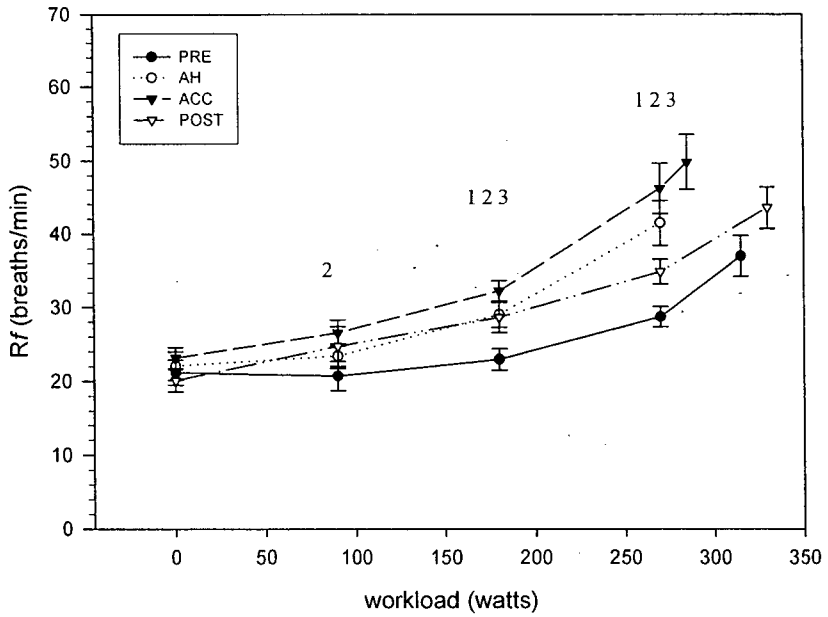

FIGURE 5: Mean (± SE) respiratory rate (R*f*) during incremental cycling exercise performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 10. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 3, PRE significantly different from POST; 4, AH significantly different from ACC.

Respiratory exchange ratios (RER) during incremental exercise were determined in PRE and POST subjects (Figure 6). At 270 watts, RER was significantly lower in POST compared to PRE.

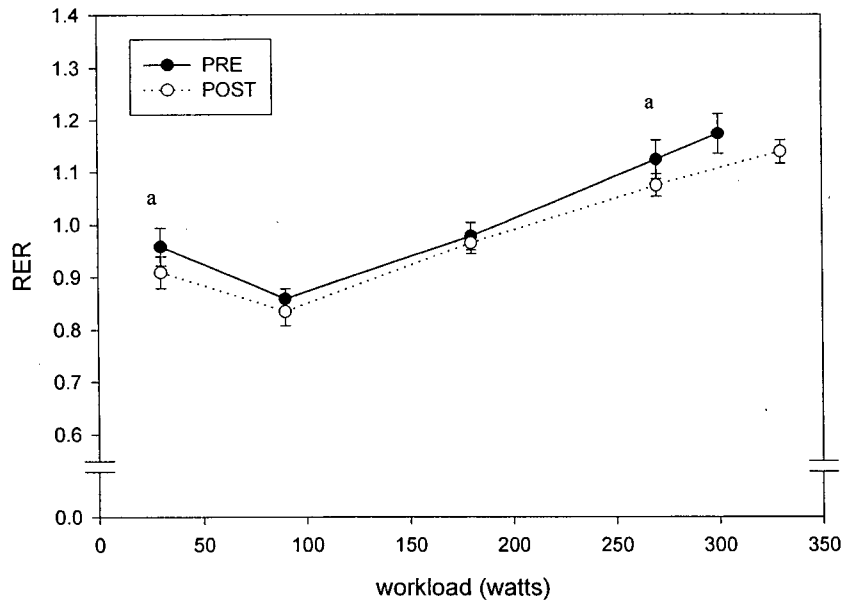

FIGURE 6: Mean (± SE) respiratory exchange ratio (RER) during incremental cycling exercise performed at sea level before (PRE) and following (POST) altitude acclimatization. Displayed are workloads where n = 10. a, significantly different from PRE.

Under all test conditions, mean heart rate increased immediately upon the onset of exercise, and continued to rise linearly with increasing workload until fatigue (Figure 7; Table 1; Appendix 3 for individual data). Exercise in AH elicited a significant increase in heart rate compared to PRE at workloads of 90 watts and higher. Heart rate decreased toward sea level values in ACC, and was significantly lower than AH levels at workloads of 180 and 270 watts. As shown in Table 1, despite raised heart rates during exercise, maximum heart rates decreased significantly by 3.6% in AH and by 5.8% in ACC, compared to values obtained under PRE conditions. These decreases in maximum heart rate at altitude occurred in conjunction with decreases in exercise performance (Figure 2). Finally, in the POST group, heart rate significantly decreased at all workloads compared to PRE levels, but maximum heart rate levels did not change.

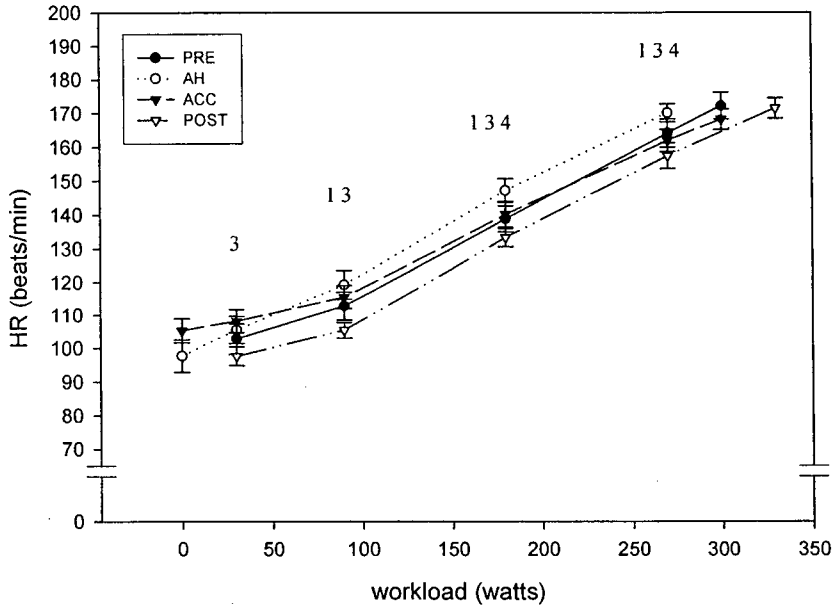

FIGURE 7: Mean (± SE) heart rate (HR) during incremental cycling exercise performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 10. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 3, PRE significantly different from POST.

iii. Exercise Plasma Lactate Concentration:

In all test conditions, mean lactate concentration increased significantly at a workload of 180 watts, and continued to rise with increasing workload, until fatigue (Figure 8; see Appendix 5 for individual data). In subjects exposed to AH, the lactate response curve is shifted slightly to the left compared to all test conditions, indicating an increase in lactate concentration at a given workload in AH. At a workload of 270 watts, a workload reached by all subjects during incremental exercise testing AH, lactate concentration increased by approximately 21.0% from PRE levels. In ACC, lactate levels decreased from the AH levels by 14.6%, and decreased further in POST, reaching values approximately 13.4% lower than PRE levels.

It is well known that lactate levels at high intensity exercise increase with both exercise duration and intensity. When exhaustion from incremental exercise is determined by the subject, exhaustion may occur at different times and work rates on any given day depending on energy levels, motivation, and other factors. This makes analyzing lactate data at fatigue from $\dot{V}_{O_2\,max}$ exercise very difficult. For this reason, lactate levels at a submaximal workload of 270 watts were chosen for the primary analysis of between-test condition differences, rather than levels at fatigue.

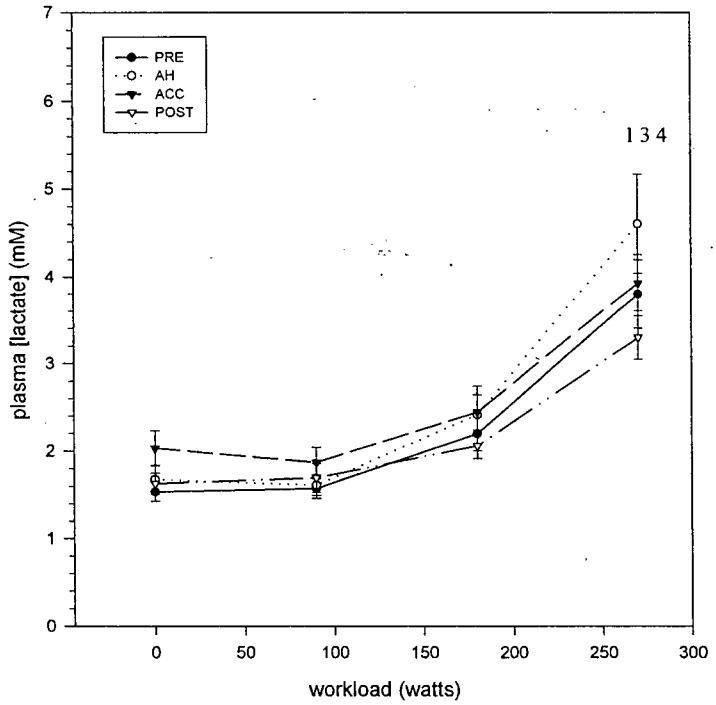

FIGURE 8: Mean (± SE) plasma lactate concentration during 9 minutes (up to 270 watts) of incremental cycling exercise. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 10. 1, PRE significantly different from AH; 3, PRE significantly different from POST; 4, AH significantly different from ACC.

iv. Exercise Intramuscular PCr and pH:

The time course of intramuscular phosphocreatine (PCr) decline during incremental plantar flexion exercise, expressed as a percentage of resting PCr levels, is shown in Figure 9. This figure displays a continuous decrease in PCr levels in all conditions up to workloads of 12 kg, with a significant time effect between successive samples from 0 to 10 kg. Statistical analysis did not reveal any significant between-condition differences in PCr levels at the workloads displayed. In comparing pH levels between test conditions, the only significant differences were found between AH and PRE conditions, with a greater decrease in pH occurring at 9 and 10 kg workloads in AH (Figure 10). PCr and pH levels at fatigue are presented in Table 2. One-way ANOVA did not reveal any significant condition effect for end-exercise PCr or pH. And while PCr and pH appeared to decrease to lower end-exercise levels in AH, the decreases were not found to be significant.

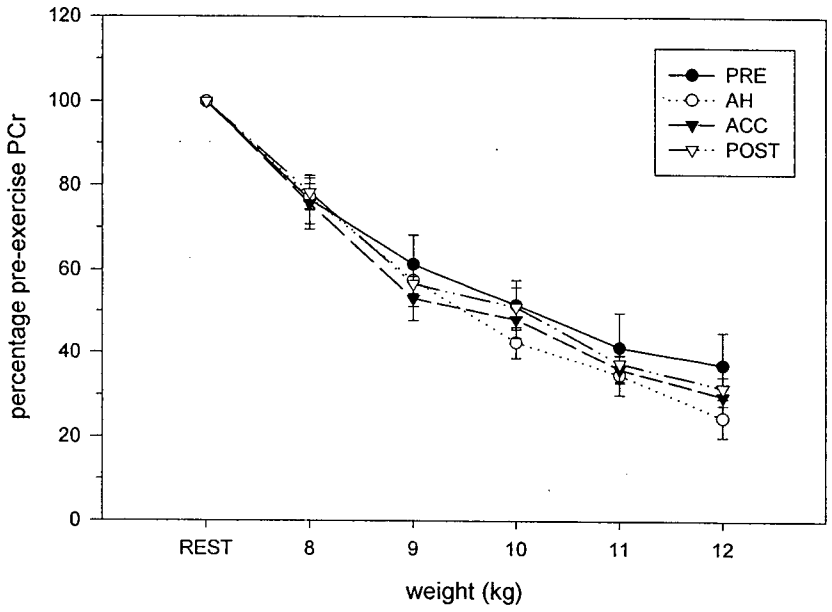

FIGURE 9: Mean (± SE) intramuscular phosphocreatine (percentage pre-exercise concentration) during incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 10.

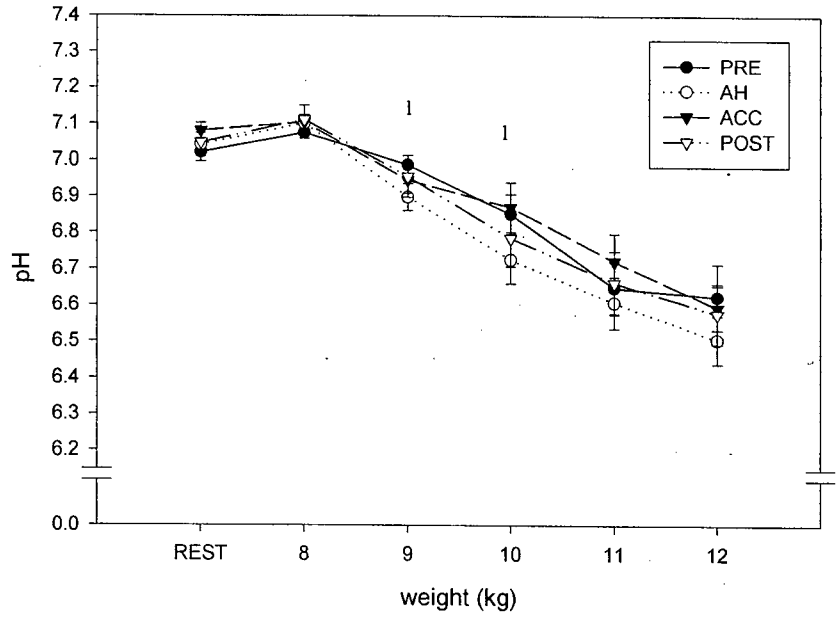

FIGURE 10: Mean (± SE) intramuscular pH during incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 10. 1, AH significantly different from PRE values.

TABLE 2: Mean (± SE) intramuscular PCr and pH levels at the end of incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

testing period	PCr (% rest)	pH
PRE	25.5 ± 8.3	6.503 ± 0.066
AH	20.7 ± 3.2	6.440 ± 0.082
ACC	25.4 ± 3.9	6.591 ± 0.085
POST	25.6 ± 2.9	6.547 ± 0.061

II. Incremental Exercise Recovery:

i. Recovery Plasma Lactate Concentration:

During recovery from incremental exercise, all subjects showed the classic biphasic pattern, with plasma lactate concentration increasing following exercise, reaching a peak within 4 to 8 minutes, and decreasing progressively toward pre-exercise levels (Figure 11A; Appendix 8 for individual data). To reflect the level of lactate efflux from muscle to blood, peak post-exercise lactate concentration and time to peak lactate concentration were determined. As shown in Table 3, no condition main effect was found for these variables (see Appendix 9 for individual data).

The decline in lactate concentration, when expressed as a percentage of maximum lactate, followed a linear trend during the first 30 minutes of recovery. Accordingly, linear regressions were fit to the mean data ($r^2 = 0.985 - 0.993$) as shown in Figure 11B. The slope of the linear regression was used to estimate the rate of lactate removal from blood (Table 3; Appendix 9 for individual data). One-way ANOVA revealed a condition main effect for lactate removal rate. The condition effect, which was found with acclimatization, indicated an increase in recovery rate from AH to ACC, and from PRE to POST test conditions.

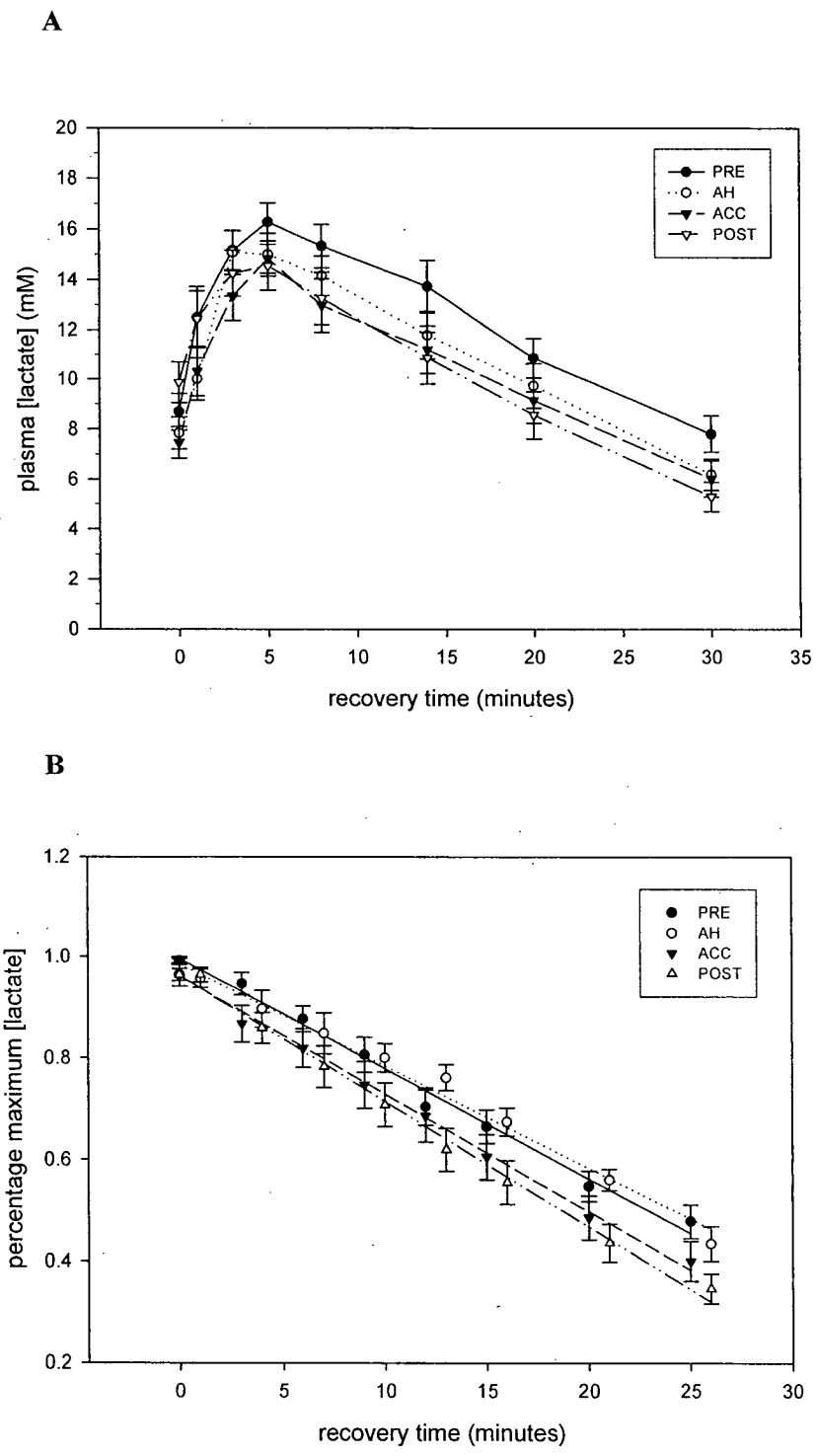

FIGURE 11: (**A**) Mean plasma lactate concentration during recovery from incremental cycling exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery) and (**B**) percentage maximum lactate during recovery with linear regression lines. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Values are means ± SE.

TABLE 3: Mean (± SE) recovery lactate data (time to peak, peak lactate concentration, and slope of linear regression) during 30 minutes of recovery from incremental cycling exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery), at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

test period	peak lactate time (min)	peak [la⁻] (mM)	slope (%Δ/min)
PRE	5.0 ± 0.5	15.99 ± 0.68	-2.14 ± 0.13
AH	4.1 ± 0.5	14.78 ± 0.94	-2.00 ± 0.18
ACC	5.3 ± 0.7	14.85 ± 0.56	-2.32 ± 0.11 [b]
POST	4.0 ± 0.9	15.03 ± 0.90	-2.46 ± 0.06 [a]

a, significantly different from PRE; b, significantly different from AH.

ii. Recovery PCr, pH and P_i/PCr:

Figure 12 displays PCr recovery data fitted with mono-exponential curves ($r^2 = 0.972 - 0.998$; see Appendix 10 for individual data). In AH, the PCr recovery curve is shifted to the right relative to all other test conditions, indicating a slower recovery rate for PCr in AH. The rate constant for PCr recovery in AH (Table 4, Appendix 11 for individual data) indicates a decrease in the rate of PCr recovery in acute hypoxia, which tended to return toward PRE levels in ACC; however, no significant difference in rate constants were found between test conditions. In all groups, intramuscular pH initially remained low, or transiently decreased from end-exercise levels (Figure 13; Appendix 12 for individual data). Thereafter, pH gradually recovered toward pre-exercise levels, with a significant increase observed at 6 minutes and complete recovery within 10 minutes. No significant differences in pH between test conditions were observed. At fatigue and during the first minutes of recovery, P_i/PCr tended to increase in AH and decrease in POST conditions compared to PRE; however, these changes were not significant (Figure 14).

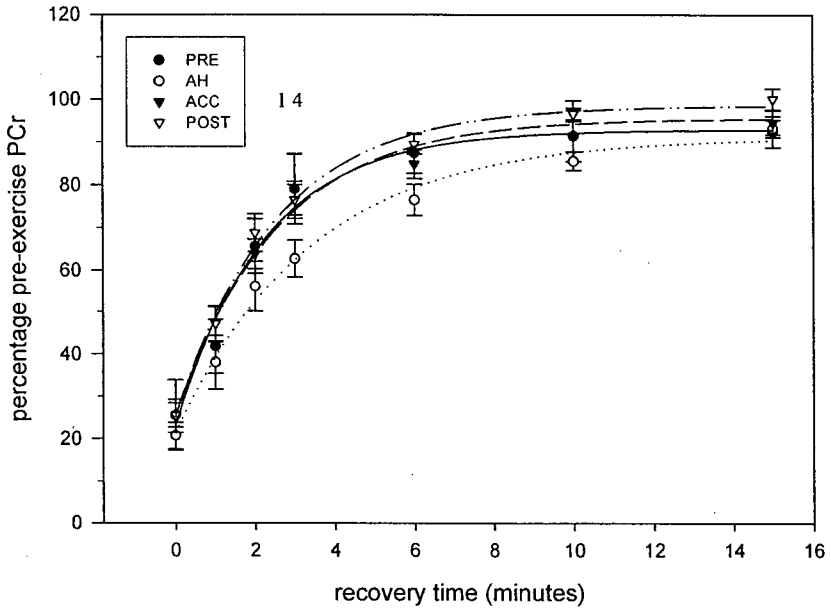

FIGURE 12: Mean (± SE) intramuscular phosphocreatine (expressed as percentage of resting) during recovery (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery) from incremental plantar flexion exercise. Mono-exponential regression lines fitted for each testing period data set. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

TABLE 4: Mean (± SE) rate constants for phosphocreatine (PCr) recovery from incremental plantar flexion exercise performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

test period	PCr recovery rate constant (unitΔ/min)
PRE	0.4250 ± 0.0300
AH	0.2874 ± 0.0219
ACC	0.3150 ± 0.0255
POST	0.3955 ± 0.0247

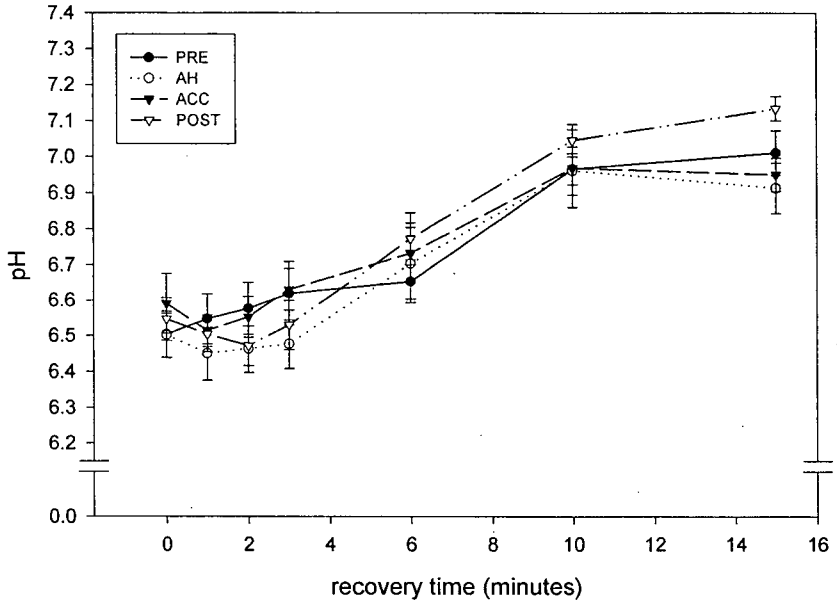

FIGURE 13: Mean (± SE) intramuscular pH during recovery from incremental plantar flexion exercise (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery). Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

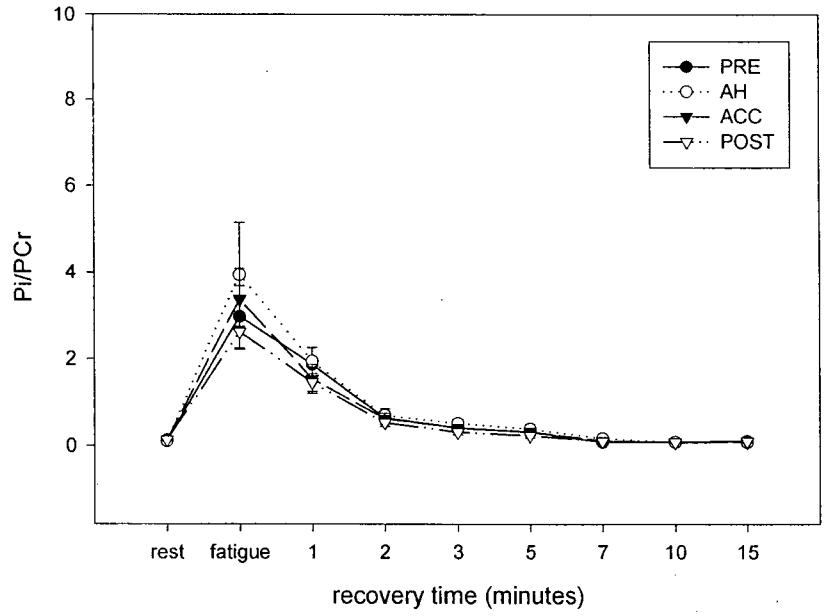

FIGURE 14: Mean (± SE) P_i/PCr at fatigue and during recovery from incremental plantar flexion exercise (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery). Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

III. Submaximal Exercise Testing:

i. Submaximal Workload:

Workload during submaximal exercise (Table 5) was set at a level corresponding to the workload achieved at 70% $\dot{V}_{O_2\ max}$ for a particular testing period. Submaximal workloads were significantly lower in AH and ACC compared to PRE. If the same submaximal workload level under PRE conditions was used at altitude, this workload would correspond to a higher percentage of $\dot{V}_{O_2\ max}$, and exercise would be more difficult, substrate utilization proportions would change, energy production requirements would increase, and steady state conditions would not be maintained. With POST acclimatization, exercise performance was improved, and submaximal workloads increased significantly when comparing AH to ACC and PRE to POST levels.

TABLE 5: Mean (± SE) steady-state cardiorespiratory values (12 and 15 minute average) during submaximal cycling exercise corresponding to 70% relative $\dot{V}_{O_2\ max}$. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

test period	workload (watts)	$\dot{V}_E$ (L/min)	TV (L/breath)	R*f* (breath/min)	RER	HR (beats/min)
PRE	192.0 ± 11.4	78.0 ± 5.6	2.463 ± 0.157	32.0 ± 1.8	0.99 ± 0.01	157.4 ± 4.2
AH	135.0 ± 8.8 [a]	78.3 ± 5.9	2.425 ± 0.237	32.9 ± 1.5	0.75 ± 0.03	151.8 ± 3.3
ACC	149.0 ± 10.4 [ab]	86.7 ± 5.4 [ab]	2.316 ± 0.169	37.8 ± 0.9 [ab]	0.91 ± 0.03	148.4 ± 2.0 [a]
POST	212.5 ± 14.5 [a]	88.8 ± 5.8 [a]	2.492 ± 0.187	36.2 ± 1.6 [a]	0.99 ± 0.01	155.5 ± 2.0

$\dot{V}_E$, minute ventilation; TV, tidal volume; R*f*, respiratory rate; HR, heart rate; RER, respiratory exchange ratio, at fatigue. a, significantly different from PRE values; b, significantly different from AH values. a, significantly different from PRE.

ii. Cardiorespiratory Measurements:

With the onset of submaximal exercise, mean $\dot{V}_E$ values under all conditions showed a rapid initial rise followed by a plateau (time course data not shown). Steady-state conditions were achieved between 8 to 15 minutes of exercise, indicated by no significant change in $\dot{V}_E$ between sample times. Mean steady-state cardiorespiratory data, calculated as the average over the last three minutes of steady-state exercise, are displayed in Table 5 (Appendix 4 for individual data). During submaximal exercise in AH, no significant change in steady-state $\dot{V}_E$ from PRE levels was seen. Statistical analysis did reveal a significant increase in steady-state $\dot{V}_E$ in ACC,

increasing by 11.2% from PRE values and 10.7% from AH values. Under these conditions, submaximal workload was set at a level significantly higher than AH, but still below PRE levels. In the POST group, submaximal workload increased significantly with improvements in exercise performance, and steady-state $\dot{V}_E$ remained significantly elevated by approximately 13.9% from PRE levels.

Similar to the results observed with incremental exercise, steady-state TV did not differ between PRE and AH or ACC conditions during submaximal exercise (Table 5; Appendix 4 for individual data). Increases in ventilation between test conditions are attributed to changes in R*f*. Compared to PRE values, steady-state R*f* did not change significantly in AH (Table 5; Appendix 4 for individual data). This accounts for the lack of change in $\dot{V}_E$ during AH submaximal exercise. With altitude acclimatization, steady-state R*f* significantly increased in ACC from AH levels, and remained elevated upon return to sea level.

Respiratory exchange ratios (RER) during submaximal exercise were determined in PRE and POST subjects. No significant change in RER was found during submaximal exercise when comparing PRE and POST conditions (Table 5; Appendix 4 for individual data).

During submaximal exercise, heart rate increased upon the onset of exercise, reaching constant levels between 8 to 15 minutes in PRE, AH and ACC conditions (Table 5). Under POST testing conditions, heart rate gradually increased until 12 minutes of exercise, such that steady-state conditions were only observed between 12 to 15 minutes. Similar to the respiratory data, no significant change in steady-state heart rate was found when comparing AH to PRE (Table 5; Appendix 4 for individual data). This lack of response in steady-state heart rate in acute hypoxia may be attributed to the large decrease in submaximal workload (Table 5) under these conditions to maintain $\dot{V}_{O_2}$ levels at 70% maximum. A condition main effect was found for steady-state heart rate between AH and ACC conditions, indicating a significant drop in heart rate with acclimatization. It is interesting to note that while heart rate decreased in ACC, ventilation increased.

iii. Exercise plasma lactate concentration:

During submaximal exercise testing, lactate concentration increased during the first 8 minutes of exercise, with concentrations increasing at a faster rate in PRE and POST compared to AH and ACC (Figure 15; Appendix 7). Lactate concentration reached steady state levels between 8 to 15 minutes of exercise in PRE, ACC and POST conditions, shown by no significant change

in concentration between sample times. In AH, steady-state conditions were reached between 12 to 15 minutes.

Due to a large decrease in exercise performance under AH conditions, workload levels corresponding to 70% $\dot{V}_{O_2\ max}$ were substantially lower than pre-acclimatization levels. At a mean workload of 135.0 ± 8.8 watts during submaximal exercise, steady-state lactate concentration did not change significantly in AH compared to PRE levels (Figure 15; Appendix 6 for steady-state values). These results agree with the incremental exercise data, in which no change in lactate concentration was observed at workloads less than 180 watts (Figure 8). Similar to the trends observed with lactate during $\dot{V}_{O_2\ max}$ testing, steady-state lactate concentration was significantly lower during submaximal exercise in ACC compared to AH. Lactate concentration decreased by approximately 18% following acclimatization, despite a significant increase in submaximal workload from AH levels. Post-acclimatization normoxia results do not reveal any acclimatization effect on submaximal exercise lactate concentration, as observed with lactate during incremental exercise. Under these conditions, no change in lactate concentration was observed during submaximal exercise compared to PRE levels, despite a significant increase in submaximal workload.

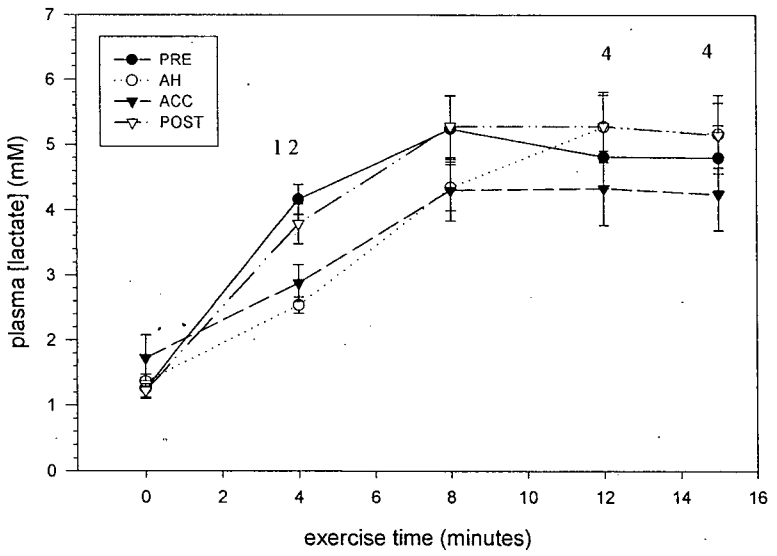

FIGURE 15: Mean (± SE) plasma lactate concentration during 15 minutes of submaximal cycling exercise, corresponding to 70% relative $\dot{V}_{O_2\ max}$. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 4, AH significantly different from ACC.

IV. Endurance Exercise Testing:

When the subjects exercised to exhaustion at a constant power output corresponding to the workload achieved at 90% PRE $\dot{V}_{O_2\ max}$, the mean times to fatigue shown in Figure 16 were calculated. These results indicate that under acute hypoxia conditions, exercise performance declined significantly. Upon return to sea level following altitude acclimatization, exercise performance improved compared to pre-acclimatization, shown as a significant increase in exercise time.

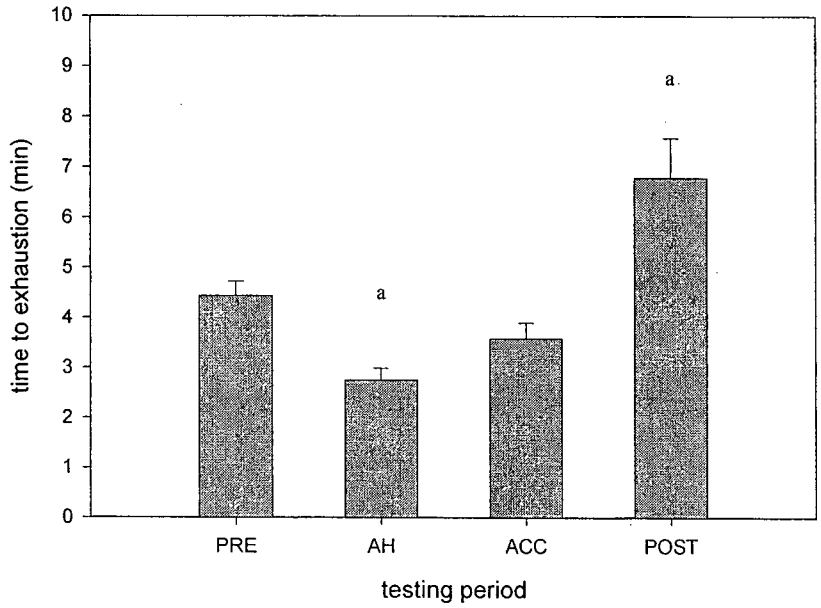

FIGURE 16: Mean (± SE) time to exhaustion from cycling exercise performed at a workload corresponding to 90% of pre-acclimatization sea level $\dot{V}_{O_2\ max}$. Endurance tests were performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). a, significantly different from PRE values.

B. Effects of training status on metabolic and cardiorespiratory responses to acute hypoxia and altitude acclimatization.

I. Subject group determination and descriptive data:

For further analysis, the subjects were divided into two groups based on the results of PRE $\dot{V}_{O_2\ max}$ and levels of regular exercise activity. The endurance trained group was selected based on a minimum $\dot{V}_{O_2\ max}$ of 58 $ml \cdot min^{-1} \cdot kg^{-1}$, and was comprised of competitive distance runners, multi-sport athletes and competitive cyclists. Subjects selected for the untrained group displayed a maximum $\dot{V}_{O_2\ max}$ of 52 $ml \cdot min^{-1} \cdot kg^{-1}$, and participated in power sports such as rugby and weight lifting, or exercised at a recreational level less than 3 days a week. There were no differences between trained and untrained subject groups in age, height, or weight (Table 6). Results of PRE $\dot{V}_{O_2\ max}$ tests (Figure 17) indicate that the trained subject group displayed significantly higher $\dot{V}_{O_2\ max}$ and maximum workload values, confirming a difference in aerobic fitness levels between the two groups.

TABLE 6: Descriptive data for trained and untrained subject groupings.

subject group	age (years)	height (cm)	weight (kg)
UNTRAINED	23.2 ± 1.5	178.5 ± 2.0	76.3 ± 6.8
TRAINED	25.4 ± 1.5	179.0 ± 2.5	72.0 ± 3.2

II. Incremental Exercise Testing:

i. $\dot{V}_{O_2\ max}$ ***and Workload:***

Overall, the trained group displayed significantly higher $\dot{V}_{O_2\ max}$ and maximum workload values under all experimental testing conditions compared to the untrained group (Figure 17). In both fitness groups, exposure to AH decreased maximum workload; however, only the trained group displayed a decrease in $\dot{V}_{O_2\ max}$ (9.6%) under these conditions. In ACC, exercise performance at altitude of the trained group improved significantly, shown by an 8.4% increase in maximum workload compared to AH levels; however, $\dot{V}_{O_2\ max}$ remained unchanged and significantly lower than PRE $\dot{V}_{O_2\ max}$ values. While the untrained group did not show any

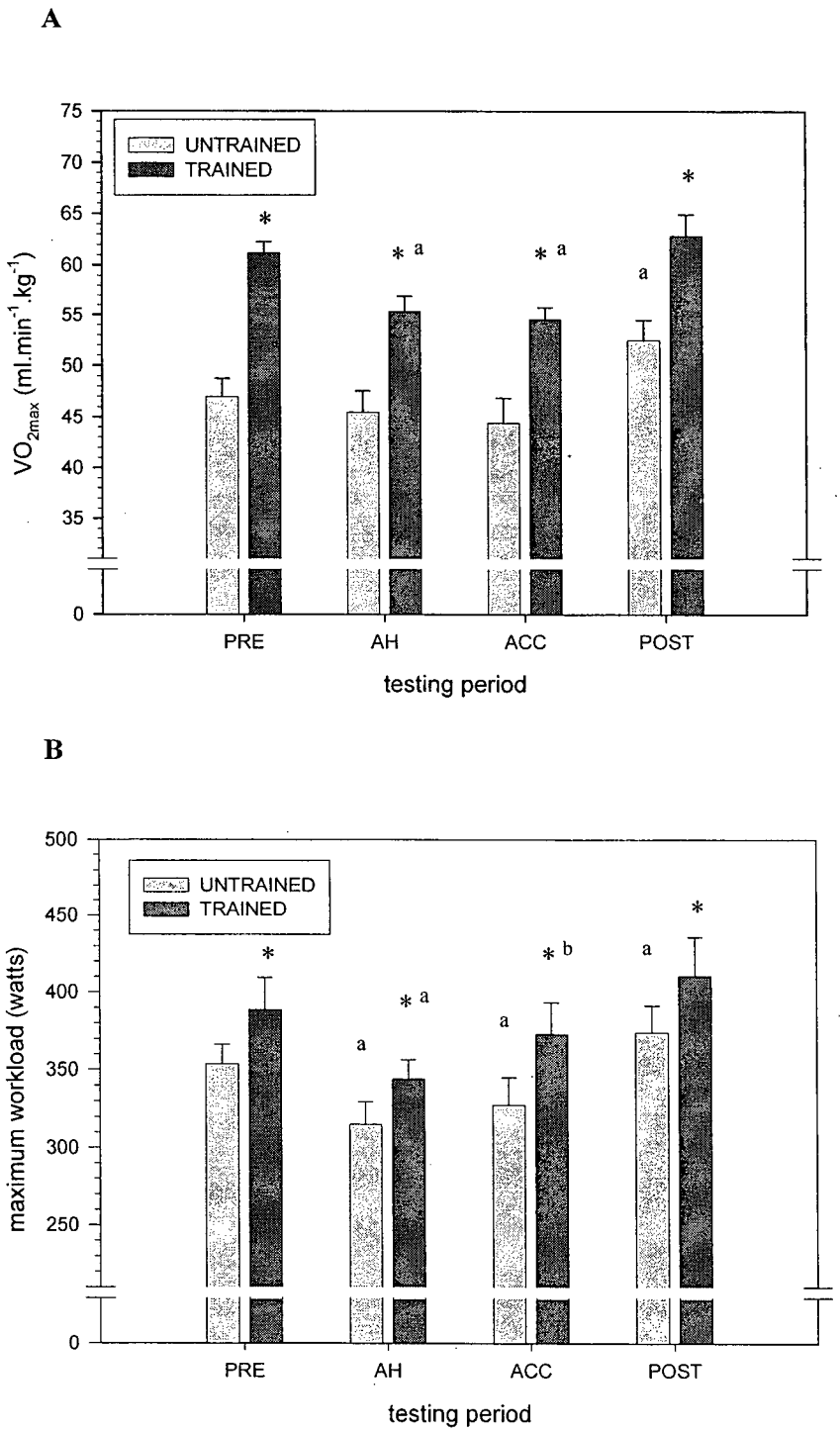

FIGURE 17: Mean (± SE) (**A**) maximal oxygen consumption ($\dot{V}_{O_2\ max}$) and (**B**) maximum workload of trained and untrained subject groups during incremental cycling exercise. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). *, significant differences between TR and UT groups; a, significantly different from PRE values; b, significantly different from AH values.

significant changes in $\dot{V}_{O_2\ max}$ under AH or ACC conditions, maximum workload increased by 5.8%, and $\dot{V}_{O_2\ max}$ increased by 11.7% in POST compared to PRE levels.

ii. Cardiorespiratory Measurements:

In comparing the time course of $\dot{V}_E$ during incremental exercise between-groups (Figure 18), no significant differences were found between trained and untrained subjects at a given workload during any of the experimental conditions. Similarly, no grouping effect was observed with $\dot{V}_E$ values at fatigue (Table 7); however, the trained subject group tended to reach higher maximum $\dot{V}_E$ values under all test conditions, associated with higher $\dot{V}_{O_2\ max}$ levels.

Both groups displayed similar trends in the $\dot{V}_E$ response to incremental exercise at altitude, with $\dot{V}_E$ values during exercise increasing significantly in both AH and ACC, and $\dot{V}_E$ at fatigue increasing significantly in ACC compared to PRE levels. At a workload of 270 watts during exercise in ACC, the untrained group displayed an additional increase in $\dot{V}_E$ from AH levels, which was not seen in the trained group. Upon return to sea level (POST), $\dot{V}_E$ remained significantly elevated above PRE levels during exercise in the untrained group; however, $\dot{V}_{E\ max}$ did not change significantly.

TABLE 7: Changes in mean (± SE) cardiorespiratory measurements of trained (TR) and untrained (UT) subject groups at fatigue from incremental cycling exercise. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

subject group	test condition	$\dot{V}_E$ (L/min)	TV (L/breath)	R*f* (breath/min)	RER	HR (beats/min)
UT	PRE	157.9 ± 18.8	3.163 ± 0.263 *	54.5 ± 2.9	1.29 ± 0.01	187.4 ± 1.4
	AH	178.4 ± 21.6	3.188 ± 0.262 *	62.3 ± 4.5 [a]	1.05 ± 0.02	180.8 ± 3.3
	ACC	187.0 ± 13.1 [a]	3.060 ± 0.161 *	66.6 ± 1.9 [a]	1.12 ± 0.04	180.2 ± 3.2 *
	POST	170.2 ± 17.3	3.212 ± 0.252 *	54.9 ± 2.8	1.20 ± 0.03	185.2 ± 2.1
TR	PRE	178.4 ± 16.1	3.749 ± 0.337 *	54.8 ± 4.1	1.24 ± 0.02	185.8 ± 4.6
	AH	191.3 ± 17.1	3.897 ± 0.594 *	60.1 ± 4.9	0.99 ± 0.06	178.8 ± 4.0
	ACC	206.3 ± 12.4 [a]	3.591 ± 0.451 *	63.0 ± 6.0 [a]	1.22 ± 0.03	171.4 ± 3.2 *[ab]
	POST	185.1 ± 16.6	3.621 ± 0.388 *	57.1 ± 2.6	1.15 ± 0.01	180.4 ± 4.4

$\dot{V}_E$, minute ventilation; TV, tidal volume; R*f*, respiratory rate (breaths/min); RER, respiratory exchange ratio; HR, heart rate (beats/min); * significant differences between trained and untrained groups. a, significantly different from PRE levels; b, significantly different from AH levels.

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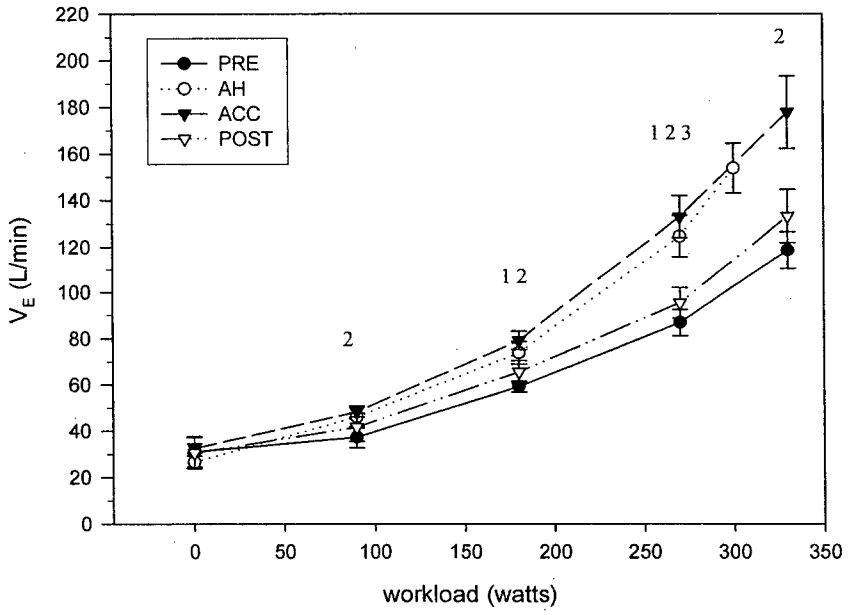

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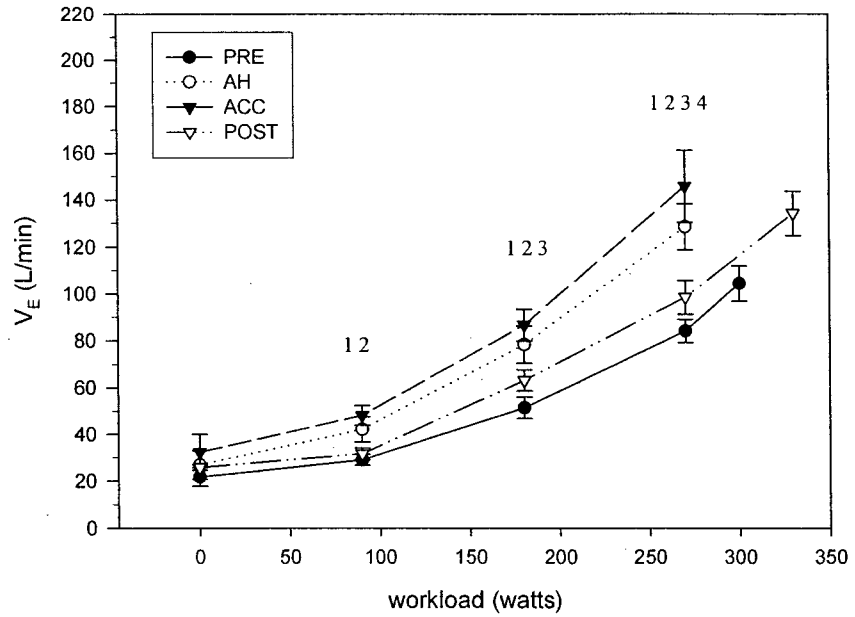


FIGURE 18: Mean (± SE) minute ventilation ($\dot{V}_E$) during incremental exercise testing of (**A**) trained and (**B**) untrained subjects. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 5 for each fitness group. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 3, PRE significantly different from POST; 4, AH significantly different from ACC.

In both groups, changes in $\dot{V}_E$ in AH and ACC could be attributed mainly to changes in R*f*, as TV during exercise (Figure 19) did not significantly differ between-groups or between-conditions. It is interesting to note that TV at fatigue (Table 7) from incremental exercise was significantly higher in the trained group under all test conditions compared to the untrained group. However, analysis of the individual data (Appendix 3, subject 9) indicates that one of the trained subjects displayed substantially higher tidal volumes under each test condition, compared to all other subjects, resulting in an unexpectedly high mean TV for the trained group.

Similar to the trends observed with $\dot{V}_E$ during incremental exercise, both trained and untrained subjects displayed a significant increase in R*f* in AH and ACC, compared to PRE levels (Figure 20). In comparing between-group differences in R*f* at altitude, the untrained group displayed higher absolute R*f* values. In this group, increases in R*f* in AH and ACC were observed at lower workloads during exercise, compared to the trained group. At a workload of 270 watts, the untrained group displayed significantly higher R*f* levels in AH and ACC than the trained group. In addition, R*f* at fatigue increased significantly in the untrained group in AH, while trained subjects did not exhibit a significant increase (Table 7). In ACC, R*f* remained elevated in the untrained group, while R*f* of the trained group increased significantly compared to PRE.

RER during incremental exercise was compared between PRE and POST test conditions. Compared to PRE, the untrained subject group displayed a significant decrease in POST RER at workloads of 270 watts and higher, which was not observed in the trained group (Figure 21).

The untrained group did not exhibit an effect of altitude exposure on heart rate during exercise, and displayed higher heart rate values during PRE and ACC exercise compared to the trained subject group (Figure 22). In contrast, the trained group revealed a significant response in heart rate to exercise in AH, shown by an upward shift in the AH heart rate response curve toward values displayed by the untrained group. In ACC, heart rate values of the trained group during exercise returned to PRE levels, with maximum values decreasing compared to PRE. Under these conditions, maximum heart rate of the trained group was significantly lower than that of the untrained group (Table 7). Under POST exercise conditions, the untrained individuals showed a nonsignificant decrease in heart rate during exercise, not seen in the trained subjects, so that heart rate levels no longer differed between subject groups.

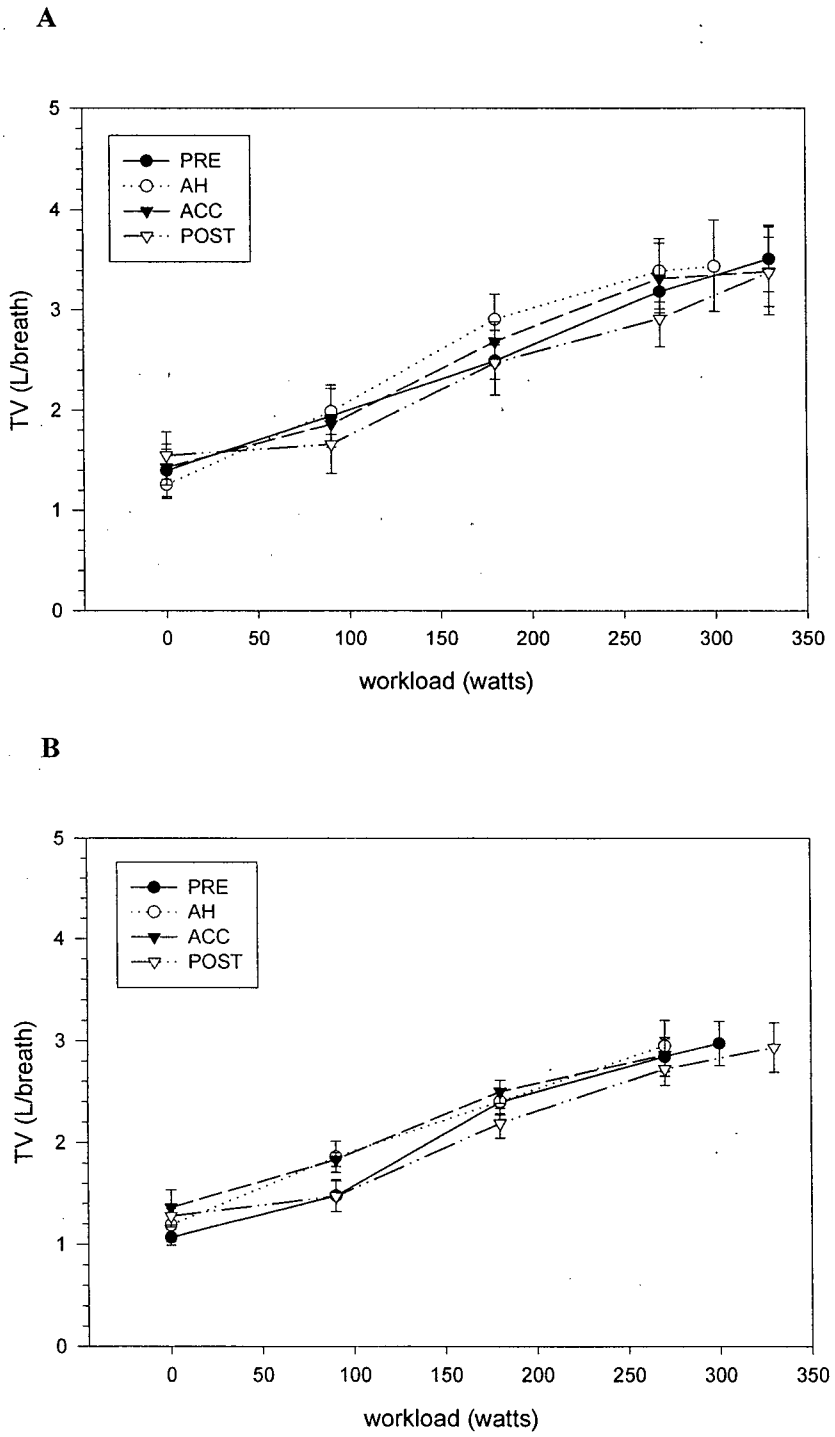


FIGURE 19: Mean (± SE) tidal volume (TV) during incremental cycling exercise of (**A**) trained and (**B**) untrained subject groups. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 5 for each fitness group.

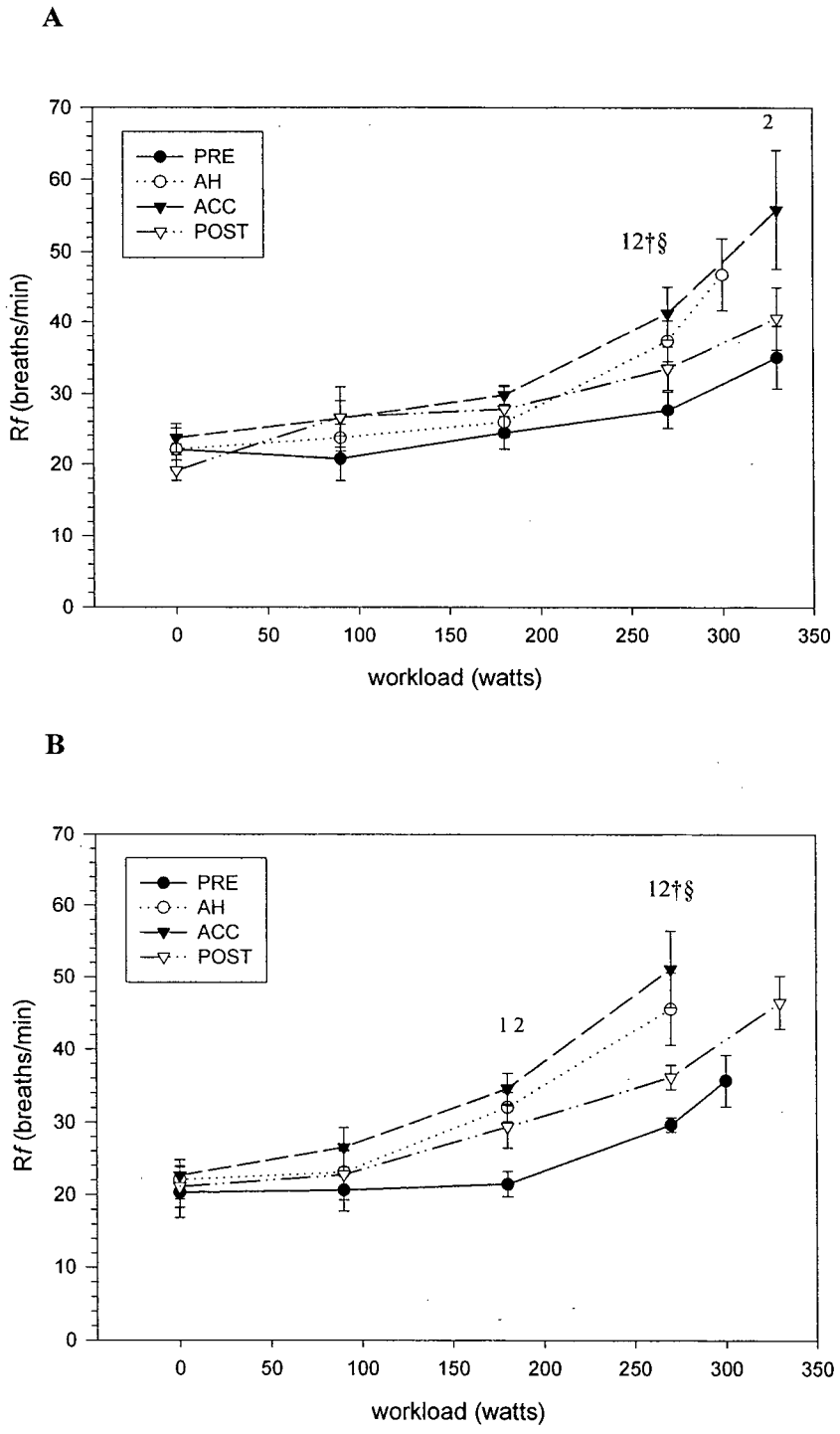


FIGURE 20: Mean (± SE) respiratory rate (R*f*) during incremental cycling exercise of (**A**) trained and (**B**) untrained subject groups. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 5 for each fitness group. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; †, TR significantly different from UT in AH; §, TR significantly different from UT in ACC.

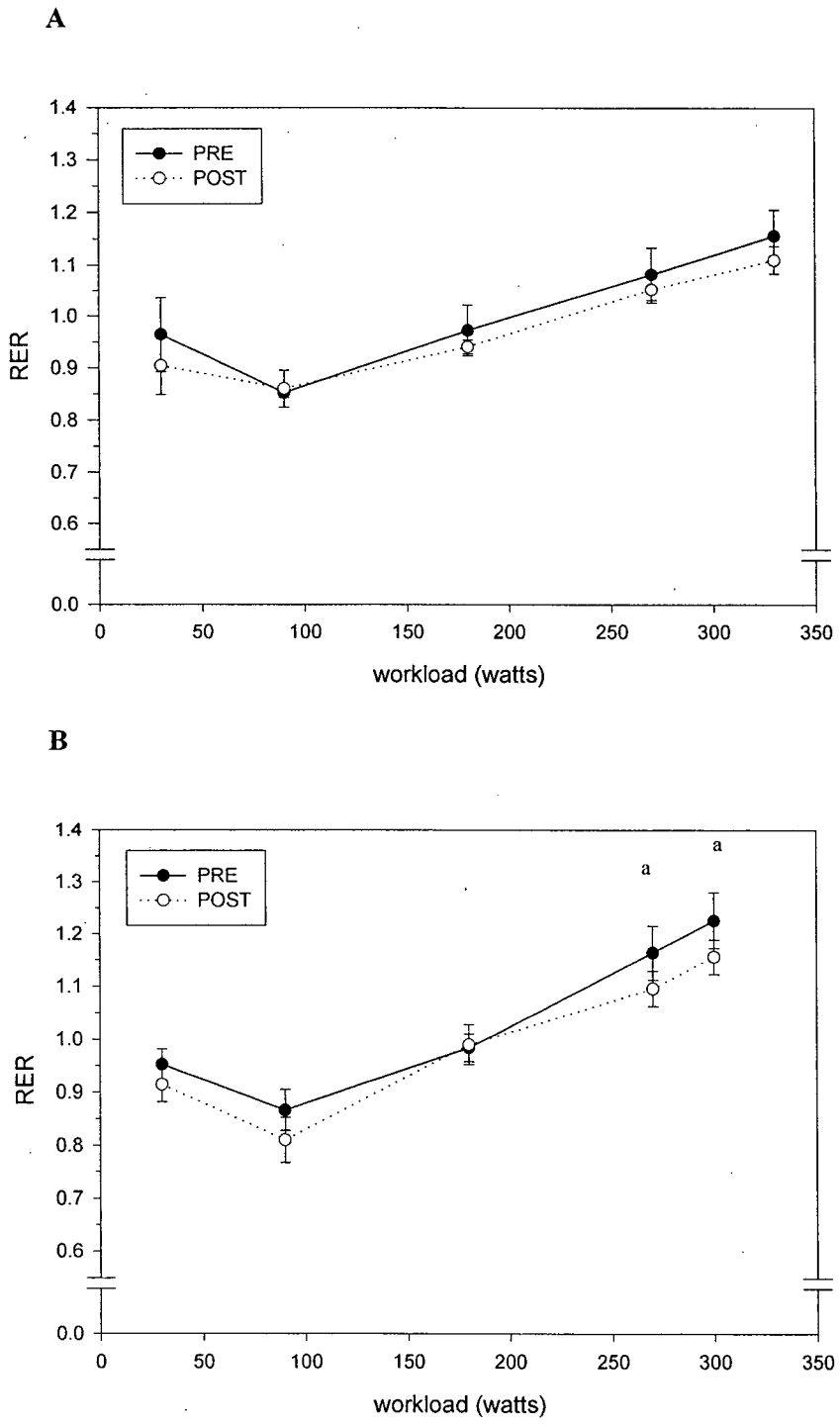


FIGURE 21: Mean (± SE) respiratory exchange ratio (RER) during incremental cycling exercise of (**A**) trained and (**B**) untrained subject groups, at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 5 for each fitness group. a, significantly different from PRE.

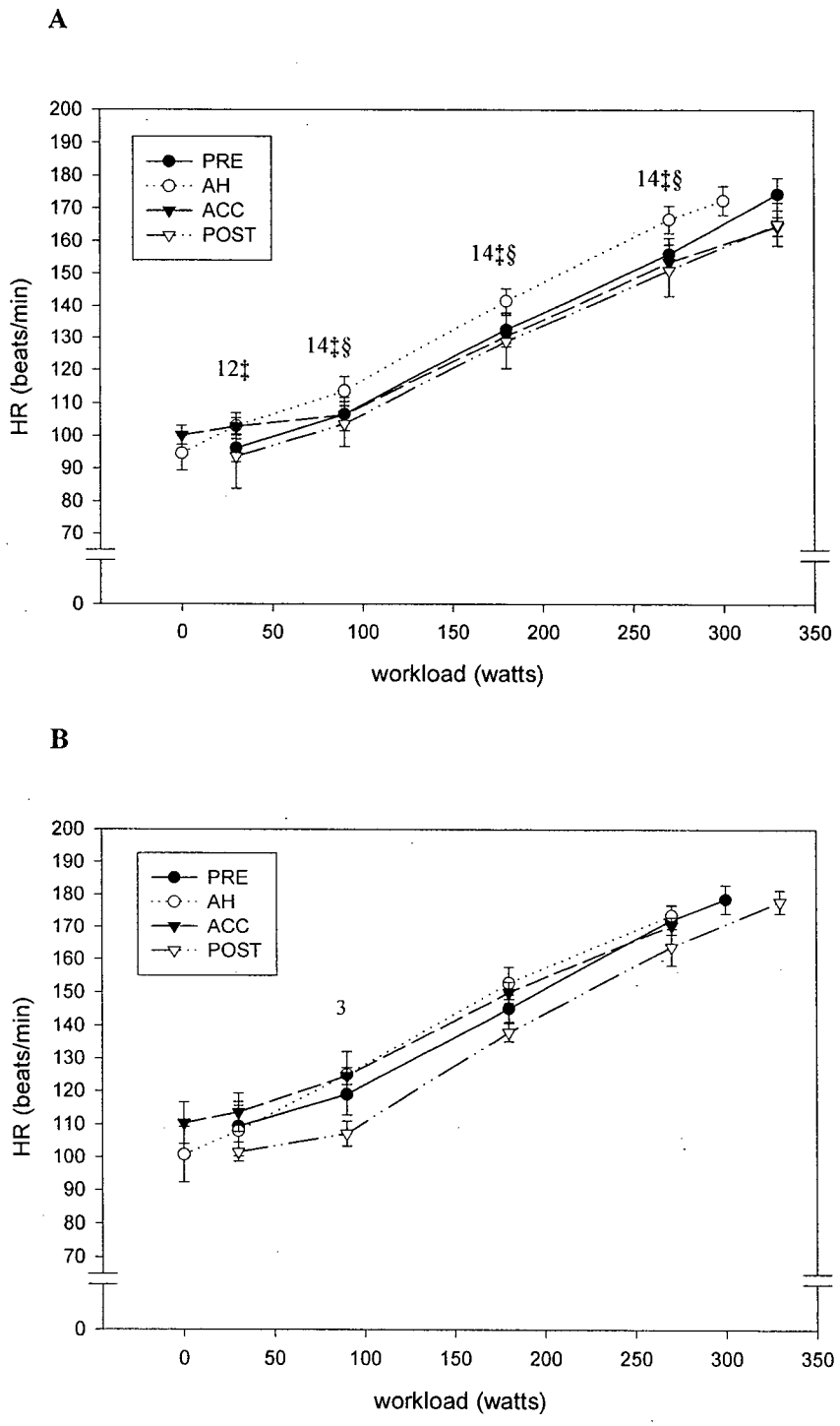


FIGURE 22: Mean (± SE) heart rate during incremental exercise testing of (**A**) trained and (**B**) untrained subject groups, at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n = 5 for each fitness group. 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 3, PRE significantly different from POST; 4, AH significantly different from ACC; ‡, TR significantly different from UT in PRE; §, TR significantly different from UT in ACC.

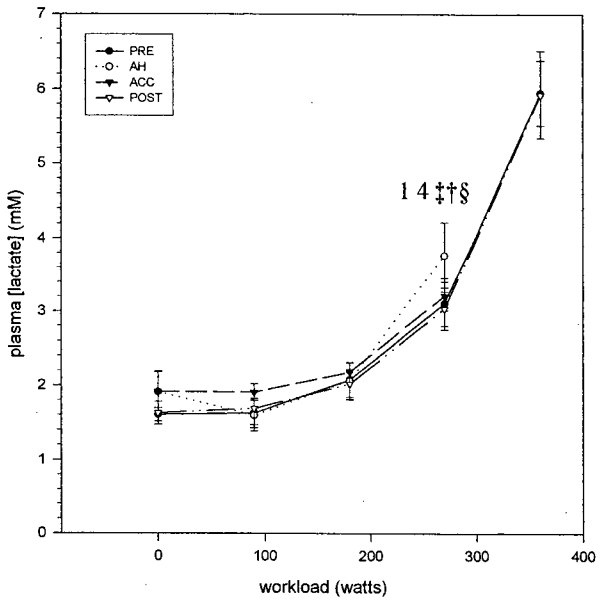
iii. Exercise Plasma Lactate Concentration:

In comparing the lactate response between subject groups, the untrained group displayed a greater rate of lactate accumulation at higher workloads, such that at 270 watts, the lactate levels of the untrained group were significantly greater than those of trained subjects under PRE, AH and ACC conditions (Figure 23). Despite these differences in absolute lactate levels, the percentage change in lactate concentration during exercise in hypoxia was similar in the two groups. In AH, lactate concentration at 270 watts increased significantly by 21.2% in the untrained group, and by 21.6% in the trained group. In ACC, lactate levels at 270 watts decreased significantly from AH levels by 14.7% in the untrained group, and by 14.8% in the trained group. Upon return to sea level (POST), lactate concentration during exercise in the trained group returned to pre-acclimatization levels. In contrast, the untrained group displayed a significant decrease in lactate concentration below PRE values, approaching levels seen in the trained group.

iv. Exercise Intramuscular PCr and pH:

In comparing PCr levels between-groups, at exercise workloads between 10 to 12 kg, the trained group displayed significantly higher PCr levels compared to the untrained group under PRE and AH conditions (Figure 24). Fatigue PCr levels tended to be higher in the trained group under all test conditions, except ACC; however, the differences were not significant. In comparing PCr levels between-conditions, fatigue and exercise PCr levels were unaltered in the trained group despite altitude exposure and acclimatization. In contrast, the untrained group displayed a significant decline in PCr levels at workloads greater than 10 kg in AH compared to PRE, which returned toward PRE levels with acclimatization (ACC). In addition, POST PCr levels of the untrained group tended to increase compared to PRE; however, the change was not significant. As shown in Figure 25, intramuscular pH during incremental exercise decreased in both trained and untrained groups; however, neither group displayed a significant change between-conditions in pH during exercise or at fatigue between. While not significant, exercise and fatigue pH appeared to decrease to a greater extent in the untrained subject group under AH conditions compared to the trained group.

A



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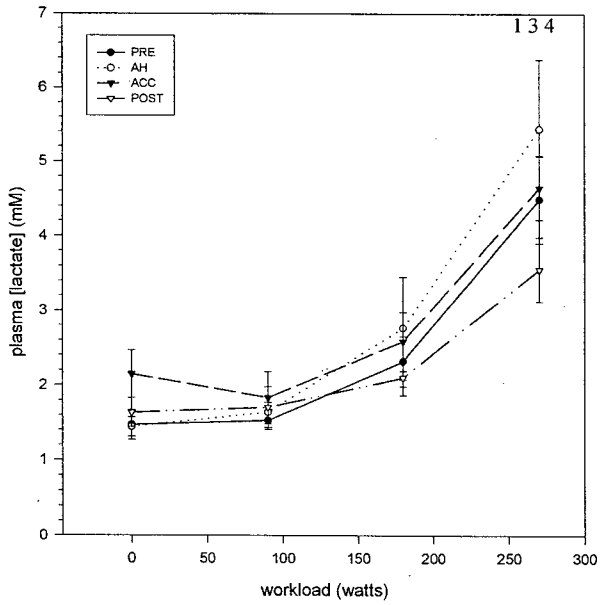

FIGURE 23: Mean (± SE) plasma lactate concentration during incremental cycling exercise of (**A**) trained and (**B**) untrained subject groups. Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). Displayed are workloads where n=5 for each fitness group. 1, PRE significantly different from AH; 3, PRE significantly different from POST; 4, AH significantly different from ACC; ‡, TR significantly different from UT in PRE; †, TR significantly different from UT in AH; §, TR significantly different from UT in ACC.

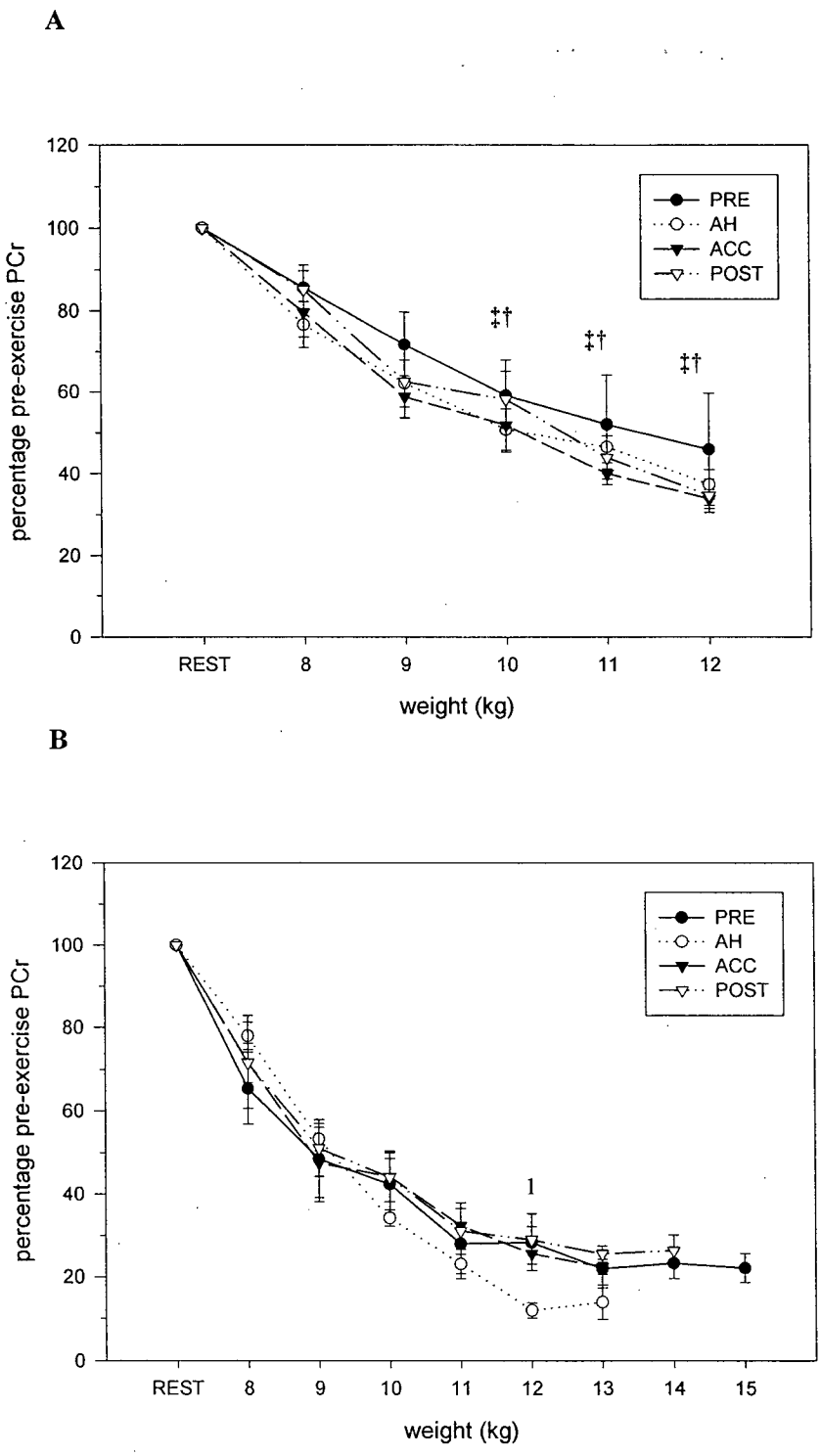

FIGURE 24: Mean (± SE) intramuscular phosphocreatine (percentage of pre-exercise concentration) of (**A**) trained and (**B**) untrained subject groups during incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 5 for each subject group, except PRE PCr untrained where data is missing for subject 2. 1, PRE significantly different from AH; ‡, TR significantly different from UT in PRE; †, TR significantly different from UT in AH.

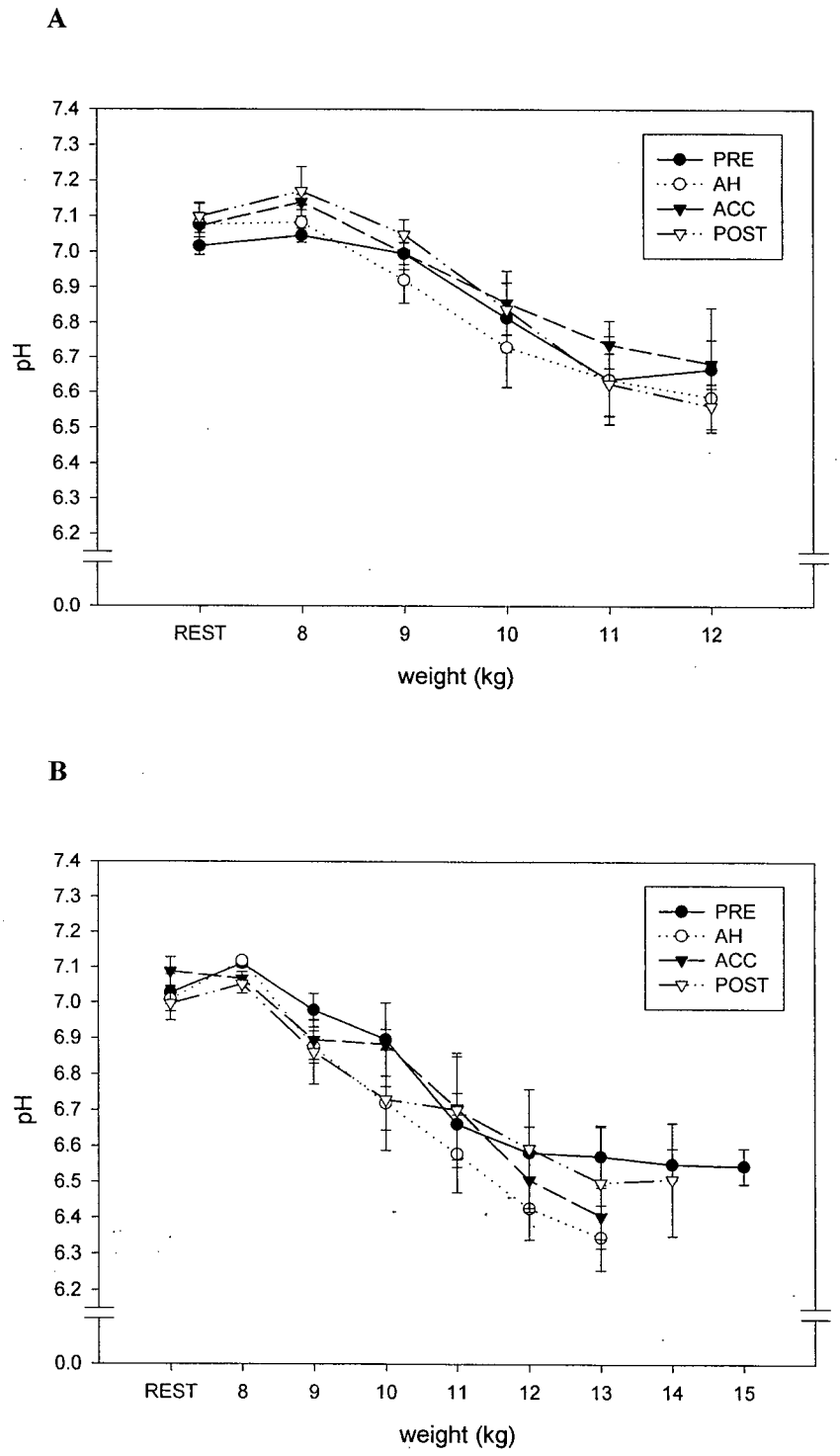

FIGURE 25: Mean (± SE.) intramuscular pH of (**A**) trained and (**B**) untrained subject groups during incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 5 for each subject group.

TABLE 8: Mean (± SE) intramuscular PCr and pH at the end of incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization.

subject group	test condition	PCr (% rest)	pH
UNTRAINED	PRE	16.2 ± 4.3	6.49 ± 0.02
	AH	14.7 ± 3.7	6.43 ± 0.08
	ACC	27.2 ± 5.5 [b]	6.52 ± 0.16
	POST	20.8 ± 2.6	6.50 ± 0.09
TRAINED	PRE	33.0 ± 14.4	6.51 ± 0.12
	AH	26.6 ± 3.7	6.45 ± 0.15
	ACC	23.5 ± 6.2	6.66 ± 0.06
	POST	30.3 ± 4.3	6.59 ± 0.09

III. Incremental Exercise Recovery:

i. Recovery Plasma Lactate Concentration:

For each test condition, lactate concentration increased immediately following the cessation of exercise, reaching peak levels within 5 minutes, in both groups (Figure 26). Statistical analysis of the time to peak lactate and peak post-exercise lactate concentrations (Table 9) did not reveal any significant differences between-conditions or between-groups. However, visual analysis of the lactate curves during this phase of recovery reveals two interesting observations: (1) lactate concentration increased more slowly in AH in the trained group, and (2) lactate concentration increased more slowly in ACC in the untrained group, compared to the other test conditions.

After lactate reached peak levels, lactate concentration during the subsequent 25 minutes of recovery gradually decreased in a linear fashion toward pre-exercise levels. When analyzing the absolute lactate values shown in Figure 26, it is interesting to note that in the trained group, no difference was seen in lactate concentration between test conditions, for a given time point during recovery. The untrained group displayed higher lactate values during PRE recovery compared to all other test conditions; however, lactate values were not found to be significantly different.

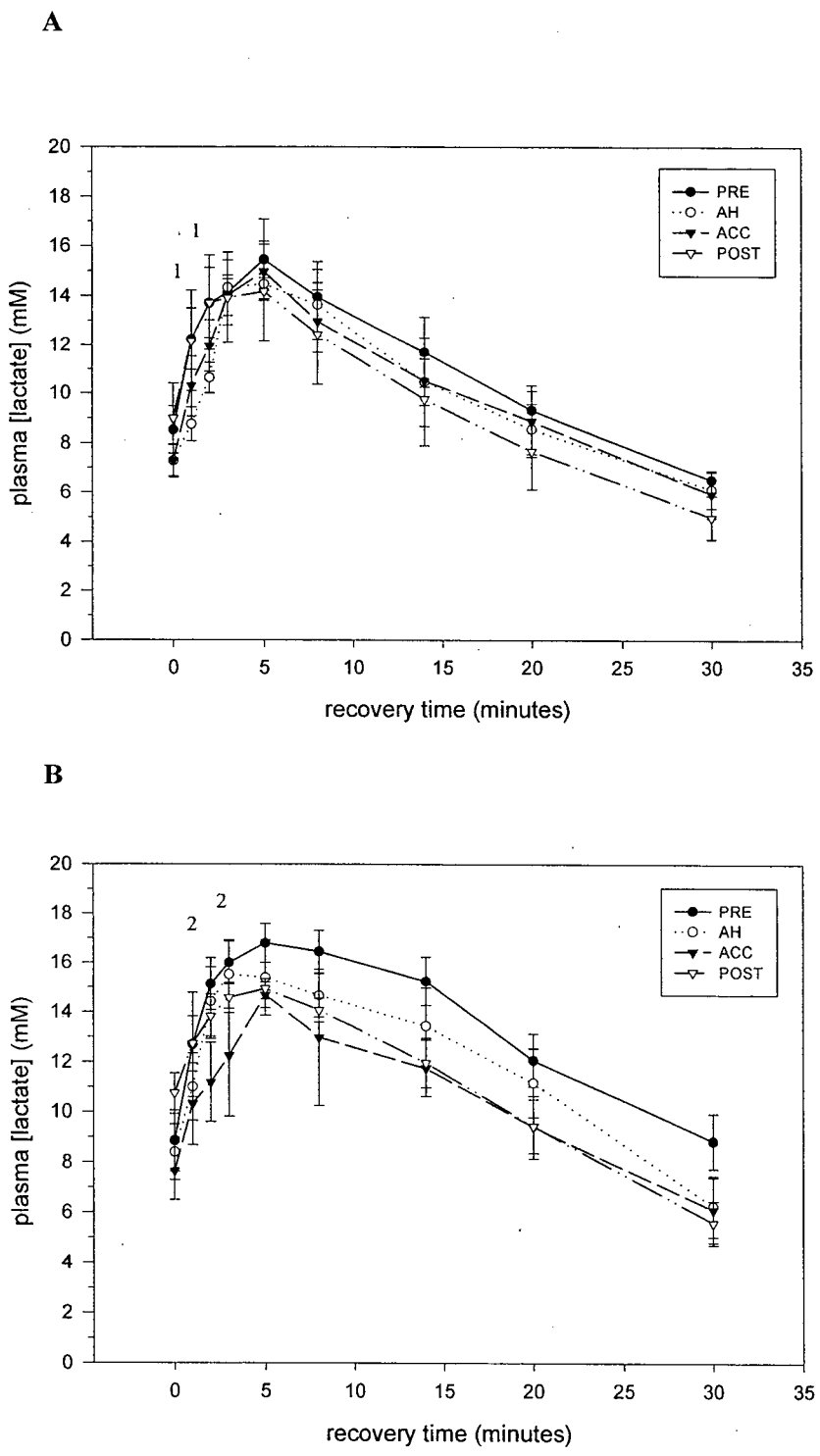

FIGURE 26: Mean (± SE) plasma lactate concentration of (**A**) trained and (**B**) untrained subject groups during recovery from incremental cycling exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery), at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). 1, PRE significantly different from AH; 2, PRE significantly different from ACC.

Table 9: Rate of lactate recovery and peak lactate values (mean ± SE) of trained and untrained subjects following incremental cycling exercise. Exercise tests were performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

subject group	test period	time to peak (min)	peak [lactate] (mM)	slope ($\%\Delta.min^{-1}$)
UNTRAINED	PRE	5.4 ± 0.7	17.05 ± 0.82	-2.08 ± 0.21
	AH	4.2 ± 0.4	16.05 ± 1.29	-2.01 ± 0.30
	ACC	6.4 ± 1.2	14.61 ± 0.45	-2.29 ± 0.17 [b]
	POST	4.6 ± 1.6	15.43 ± 0.39	-2.40 ± 0.08 [a]
TRAINED	PRE	4.6 ± 0.7	14.93 ± 0.92	-2.25 ± 0.09
	AH	4.0 ± 1.0	13.50 ± 1.22	-2.15 ± 0.14
	ACC	4.2 ± 0.2	15.10 ± 1.09	-2.42 ± 0.16
	POST	3.4 ± 0.7	14.63 ± 1.84	-2.54 ± 0.08

a, significantly different from PRE; b, significantly different from AH.

In comparing the rates of lactate recovery between-groups (Table 9, Figure 27), no significant differences were observed; however, the trained group tended to display greater rates under all test conditions. Analysis of between-condition effects for each group indicated that changes in lactate recovery rate were only observed in the untrained group, in which the rate of recovery was significantly improved with acclimatization. Compared to PRE levels, this group displayed a 10.1% rate increase in ACC, and a 15.4% increase in POST.

ii. Recovery PCr, pH and P_i/PCr:

When PCr recovery data are fitted with mono-exponential curves, differences between the two fitness groups can be seen clearly (Figure 28). While the trained subjects did not show any change in PCr, pH or P_i/PCr recovery between test conditions (Figures 28 to 30), the untrained group displayed a significant change in the rate of PCr recovery and P_i/PCr levels in response to AH and ACC conditions. Mean rate constants for PCr recovery (Table 10) clearly indicate a significant decrease the PCr recovery rate in the untrained group in AH compared to PRE, to a level significantly lower than the trained group. This was associated with an increase in P_i/PCr at fatigue and during the initial minutes of recovery (Figure 30). In ACC, PCr recovery rates and P_i/PCr levels of the untrained group returned to PRE values. Following return to sea level (POST), PCr recovery rates were unaltered from PRE levels; however, P_i/PCr ratios were lower at fatigue and during the initial minutes of recovery.

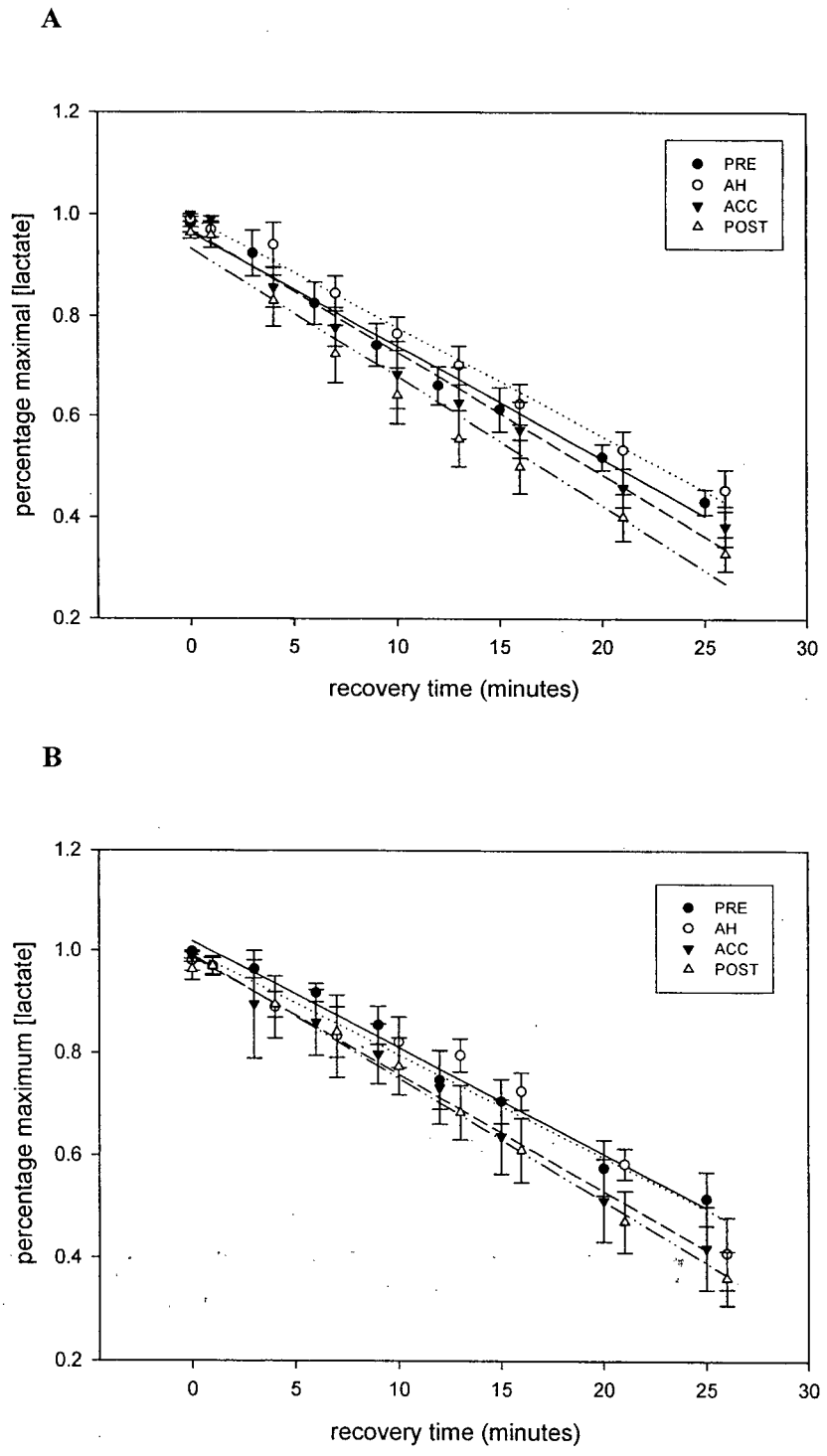

FIGURE 27: Mean (± SE) percentage maximum lactate concentration (with linear regressions) of (**A**) trained and (**B**) untrained subject groups during recovery from incremental cycling exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery). Exercise was performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

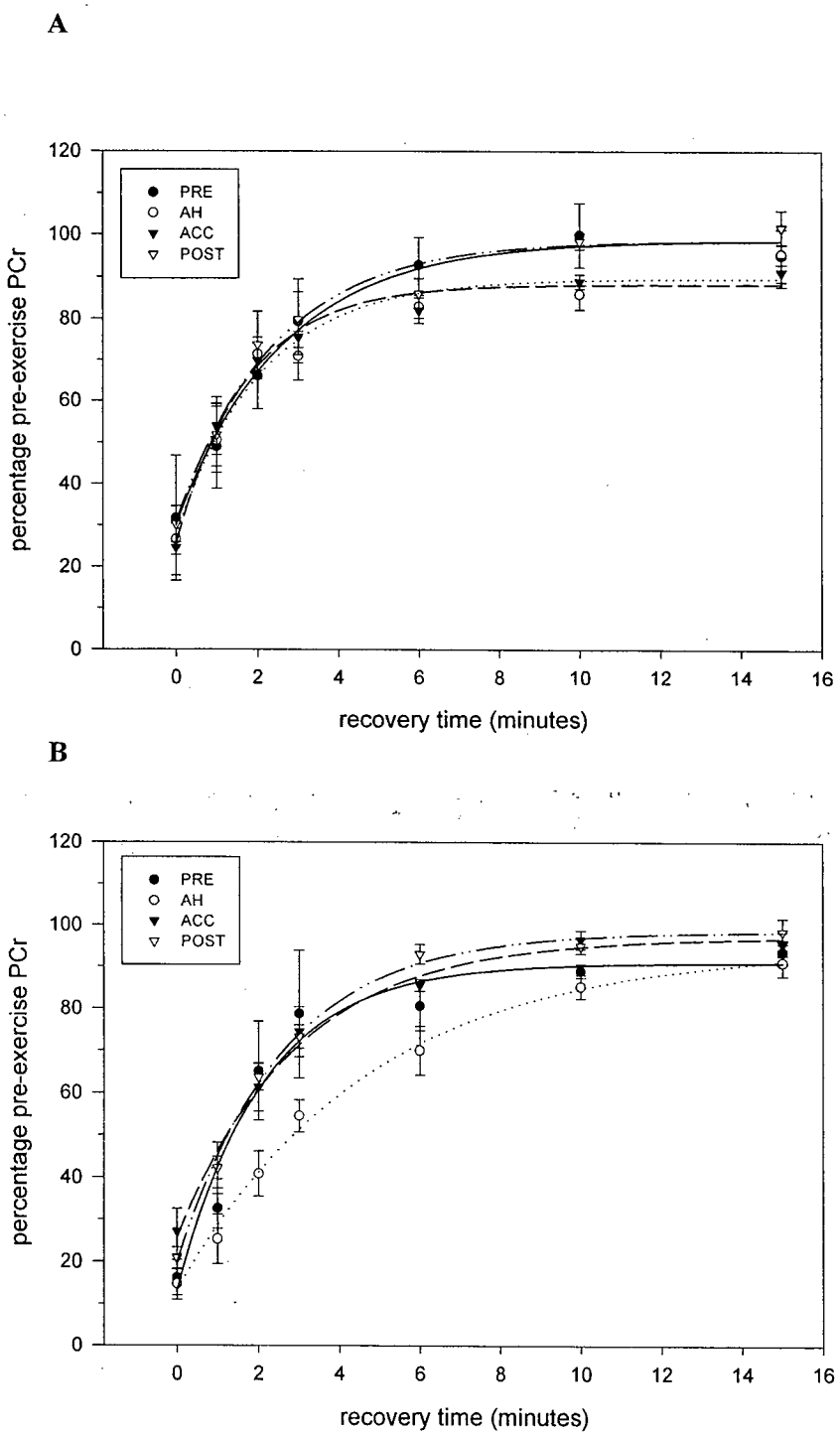

FIGURE 28: Mean (± SE) intramuscular phosphocreatine (percentage of pre-exercise concentration) of (**A**) trained and (**B**) untrained subject groups during recovery (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery) from incremental plantar flexion exercise. Mono-exponential regression lines plotted for each testing period data set. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 5 for each subject group, except PRE PCr untrained where data is missing for subject 2.

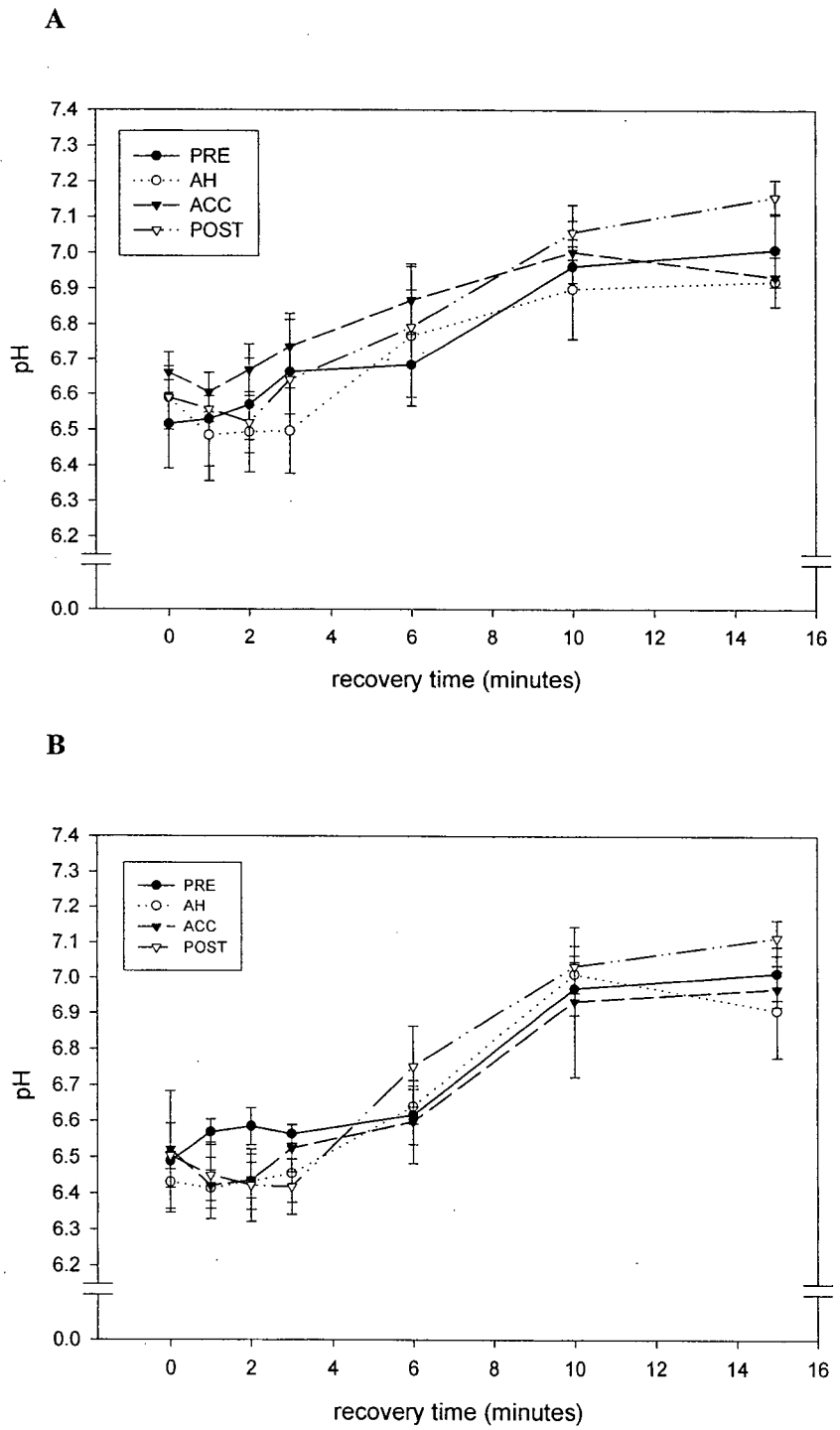

FIGURE 29: Mean (± SE) intramuscular pH of (**A**) untrained and (**B**) trained subject groups during recovery from incremental plantar flexion exercise (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery). Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air acclimatization.

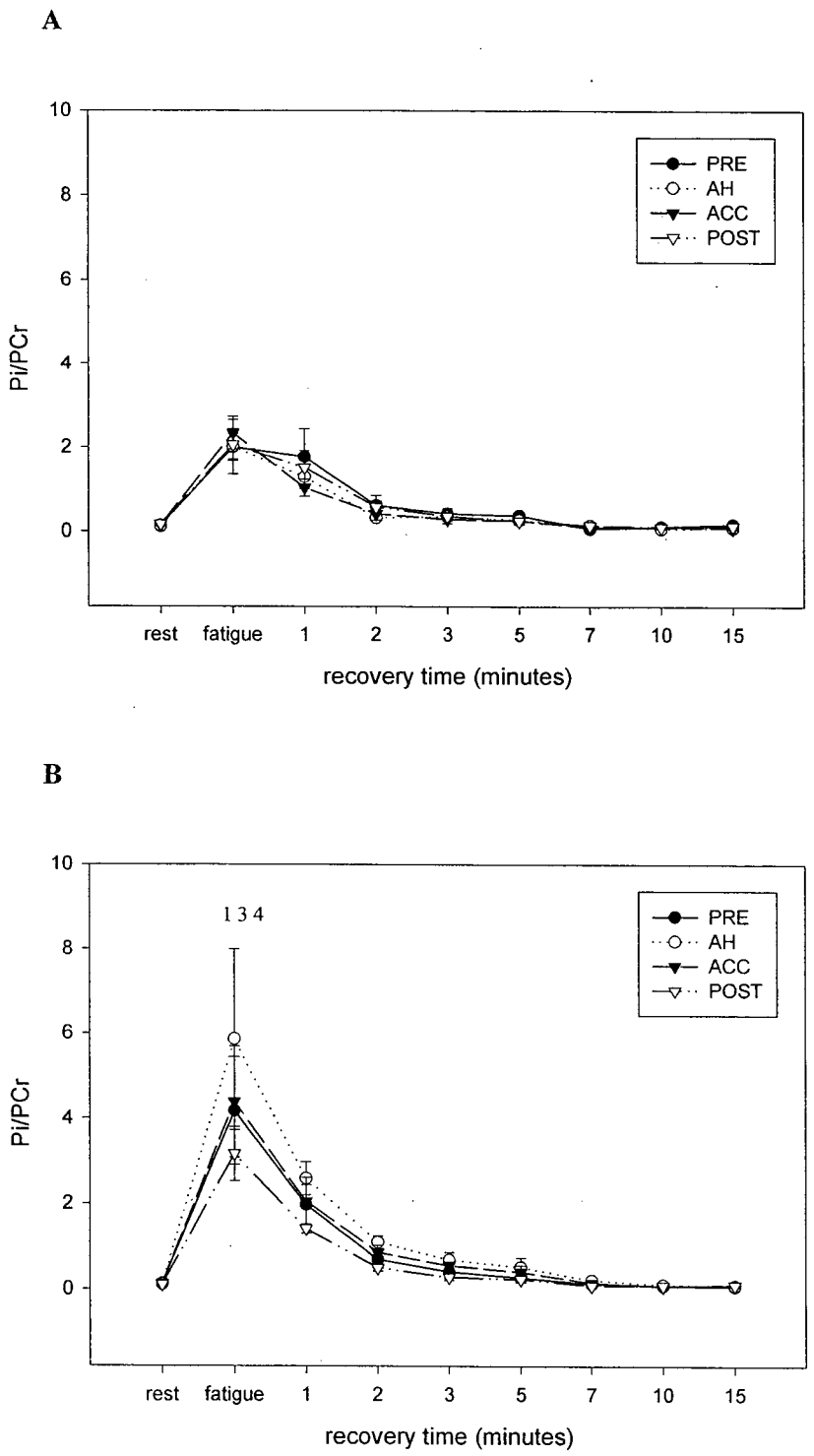

FIGURE 30: Mean (± SE.) intramuscular P_i/PCr of (**A**) trained and (**B**) untrained subject groups at fatigue and during recovery from incremental plantar flexion exercise. Exercise was performed while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization, and while breathing hypoxic (ACC) and normoxic (POST) air following altitude acclimatization. Displayed are workloads where n = 5 for each subject group.

TABLE 10: Mean (± SE) phosphocreatine recovery rate constants of trained and untrained subject groups while breathing normoxic (PRE) and hypoxic (AH) air before altitude acclimatization at 3,800m, and while breathing hypoxic (ACC) and normoxic (POST) air after acclimatization.

subject group	PCr recovery rate constant (unit Δ/min)			
	PRE	AH	ACC	POST
UNTRAINED	0.4540 ± 0.0492	0.2255 ± 0.0176 *[a]	0.3081 ± 0.0215	0.3994 ± 0.0214
TRAINED	0.4014 ± 0.0348	0.4339 ± 0.0466 *	0.4008 ± 0.0431	0.3909 ± 0.0351

PCr, phosphocreatine; *, TR significantly different from UT; [a], significantly different from PRE ($P<0.05$)

III. Incremental Exercise Metabolite Relationships:

When plasma lactate concentration (at 270 watts) is plotted against fatigue P_i/PCr for each fitness group, a direct relationship is observed between lactate and P_i/PCr (Figure 31). Lower plasma lactate concentrations were associated with lower P_i/PCr values in the trained group. When comparing PRE to POST, or AH to ACC, it can be seen that the relationship did not change between test conditions for the trained group. The untrained group displayed higher plasma lactate concentrations, associated with higher fatigue P_i/PCr values compared to the trained group. With acclimatization, the metabolite relationships displayed by the untrained group shifted to approach those of the trained group.

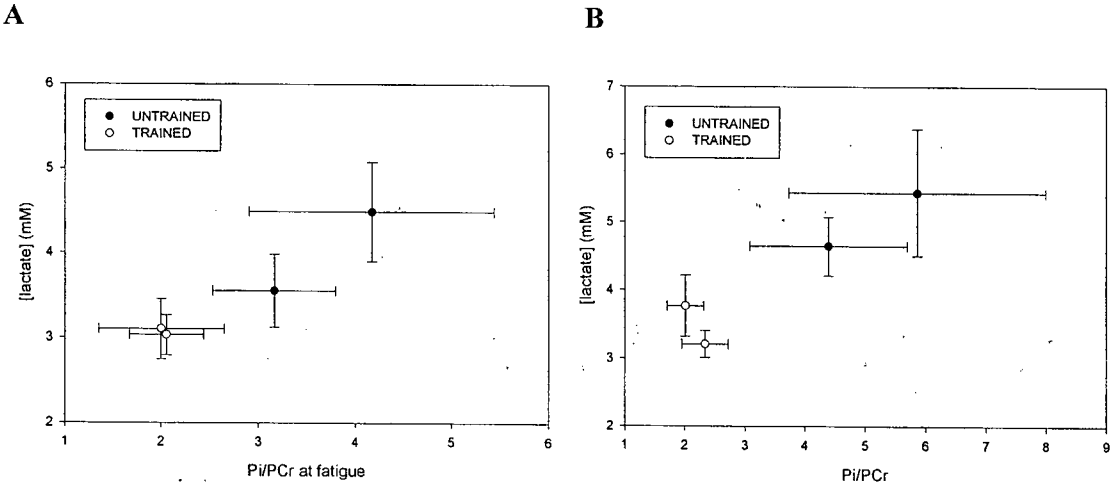

FIGURE 31: Relationship between fatigue P_i/PCr and plasma lactate concentration (at 270 watts) of trained and untrained subject groups, (**A**) comparing pre- and post- acclimatization (PRE and POST), and (**B**) comparing acute and acclimatized hypoxia (AH and ACC).

When plasma lactate concentration (at 270 watts) is plotted against fatigue PCr, an inverse relationship is observed between lactate and PCr (Figure 32). The trained group displayed lower plasma lactate concentrations and higher end-exercise PCr levels, while the higher lactate levels of the untrained group were associated with lower PCr levels at fatigue. When comparing PRE to POST, or AH to ACC, it can be seen that the relationship did not change between test conditions for the trained group. With acclimatization, the metabolite relationships displayed by the untrained group shifted towards those of the trained group.

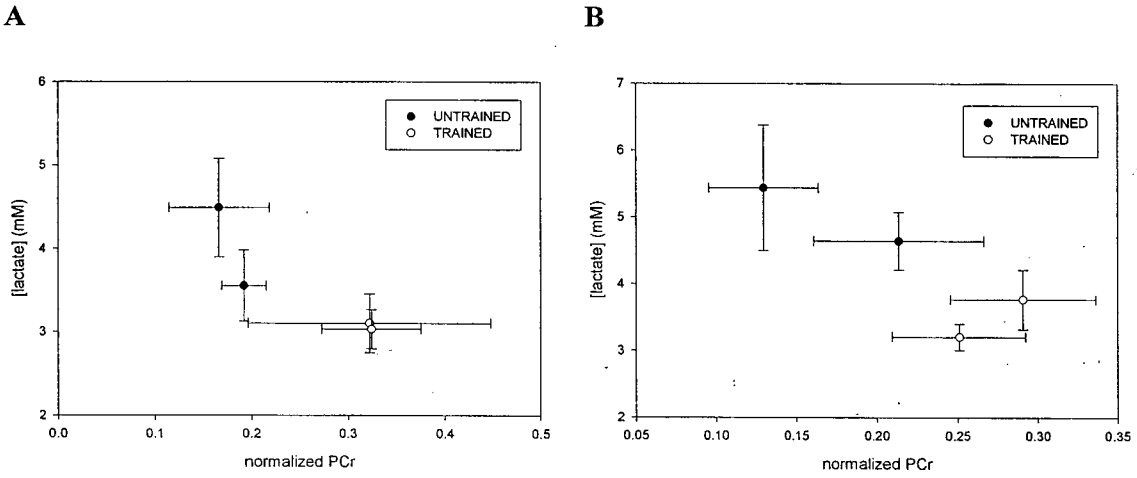

FIGURE 32: Relationship between fatigue PCr and plasma lactate concentration (at 270 watts) of trained and untrained subject groups, (**A**) comparing pre- and post- acclimatization (PRE and POST), and (**B**) comparing acute and acclimatized hypoxia (AH and ACC).

IV: Submaximal Exercise Testing:

i. Submaximal Cardiorespiratory Measurements:

In both fitness groups, steady-state exercise was maintained between 8 to 15 minutes of submaximal exercise under all test conditions, except in POST where R*f* displayed a continuous increase in the untrained group. The changes in steady-state cardiorespiratory values between experimental conditions, calculated as the average over the last three minutes of steady-state exercise, are presented in Table 11.

TABLE 11: Changes in mean (± SE) steady-state (mean of 12 and 15 minute data) exercise capacity and cardiorespiratory measurements of trained (TR) and untrained (UT) subject groups during submaximal cycling exercise at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST).

group	test	workload (watts)	$\dot{V}_E$ (L/min)	TV (L/breath)	R*f* (breath/min)	RER	HR (beats/min)
UT	PRE	167.0 ± 9.9 *	67.4 ± 6.1 *	2.283 ± 0.184	29.6 ± 1.8 *	0.97 ± 0.01	154.1 ± 4.6
	AH	128.0 ± 12.1 [a]	76.1 ± 7.9 [a]	2.104 ± 0.198 *	36.3 ± 2.2 *[a]	0.77 ± 0.05	155.5 ± 5.0
	ACC	129.5 ± 10.3 *[a]	77.4 ± 5.1 *[a]	1.983 ± 0.133 *	39.2 ± 1.0 [a]	0.84 ± 0.02	149.9 ± 2.9
	POST	187.0 ± 18.0 *[a]	82.9 ± 7.7 *[a†]	2.338 ± 0.241	35.6 ± 1.1 [a†]	1.01 ± 0.01	155.1 ± 3.6
TR	PRE	217.0 ± 13.0 *	88.6 ± 6.8 *	2.643 ± 0.248	34.3 ± 2.0 *	1.00 ± 0.01	160.6 ± 7.4
	AH	143.8 ± 13.3 [a]	80.0 ± 9.2 [a]	2.681 ± 0.376 *	30.2 ± 0.9 *[a]	0.74 ± 0.05	148.8 ± 4.3
	ACC	168.5 ± 13.7 *[ab]	95.9 ± 8.0 *[b]	2.649 ± 0.235 *	36.4 ± 1.3 [b]	0.98 ± 0.03	146.9 ± 2.9 [a]
	POST	238.0 ± 17.2 *[a]	94.8 ± 8.7 *	2.645 ± 0.297	36.8 ± 3.2	0.97 ± 0.02	155.8 ± 2.5

Workload, cycling workload; HR, heart rate; $\dot{V}_E$, ventilatory rate; R*f*, respiratory rate; TV, tidal volume; RER, respiratory exchange ratio. *, significant differences between TR and UT; a, significantly different from PRE, b, significantly different from AH. †, not in steady-state.

During incremental exercise in AH, the trained group displayed a greater percentage decrease in exercise performance, compared to the untrained group (Figure 17). Consequently, workload at 70% $\dot{V}_{O_2\,max}$ dropped significantly in the trained group from PRE conditions, so that submaximal workload in AH did not differ between the two groups. Despite the large change in workload level, submaximal $\dot{V}_E$ of the trained group did not change from PRE levels. In comparison, the untrained group displayed an increase in $\dot{V}_E$, to a level significantly higher than that of the trained subjects.

Following ACC, submaximal $\dot{V}_E$ of the trained group increased compared to AH levels, associated with improvements in exercise performance at altitude. Under these conditions, submaximal $\dot{V}_E$ of the trained group increased significantly compared to AH levels. In contrast, submaximal workload and $\dot{V}_E$ of the untrained group did not change between AH and ACC.

In POST, with increases in exercise performance, submaximal workloads significantly increased from PRE levels in both groups. Only the untrained group displayed a significant change in submaximal $\dot{V}_E$, exhibiting a higher level of ventilation upon return to sea level. However, because steady-state conditions were not observed with R*f* during POST exercise tests, submaximal $\dot{V}_E$ may not be accurately compared between POST and the other test conditions.

Similar to the respiratory results observed during incremental exercise, no significant between-condition changes in TV during submaximal exercise were found in either the trained or untrained groups. Therefore, increases in submaximal $\dot{V}_E$ observed in AH and ACC by the untrained group can be attributed to an increase in R*f* during submaximal exercise under these test conditions. In comparison, the trained group displayed a significant decrease in R*f* during submaximal exercise in AH, to a level significantly lower than the untrained group. With acclimatization, workload and respiratory rate significantly increased in the trained group, returning to PRE levels. Respiratory rate in the untrained group did not reach steady-state levels during POST testing in order to make an accurate comparison between PRE and POST experimental conditions.

RER during submaximal exercise was compared under PRE and POST conditions. No significant change in RER was found in either group.

With changes in workload levels to match workloads at 70% $\dot{V}_{O_2\ max}$ for each test condition, the untrained group revealed no significant change in submaximal heart rate between conditions. In comparison, the trained group displayed significantly lower steady-state heart rate levels during submaximal exercise in AH, despite increases observed during incremental exercise under these test conditions. In POST and ACC, heart rate remained significantly decreased compared to PRE conditions.

ii. Submaximal Plasma Lactate Concentration:

During a given submaximal exercise test, lactate increased during the first 8 minutes of exercise, generally reaching steady-state levels between 12 to 15 minutes under all test conditions (Figure 33). It is interesting to note that the pattern of lactate increase varied between test conditions, especially in the trained group where lactate accumulation occurred more slowly in AH and ACC.

In comparing submaximal lactate concentrations between-groups, statistical analysis did not reveal any differences under any of the test conditions. Analysis of between-condition effects for each fitness group indicated that changes in submaximal lactate concentration were only observed in the untrained group. The condition effect, only observed between AH and ACC, resulted in a significant decrease in submaximal lactate concentration in ACC compared to AH levels, despite no change in submaximal workloads between these two test conditions.

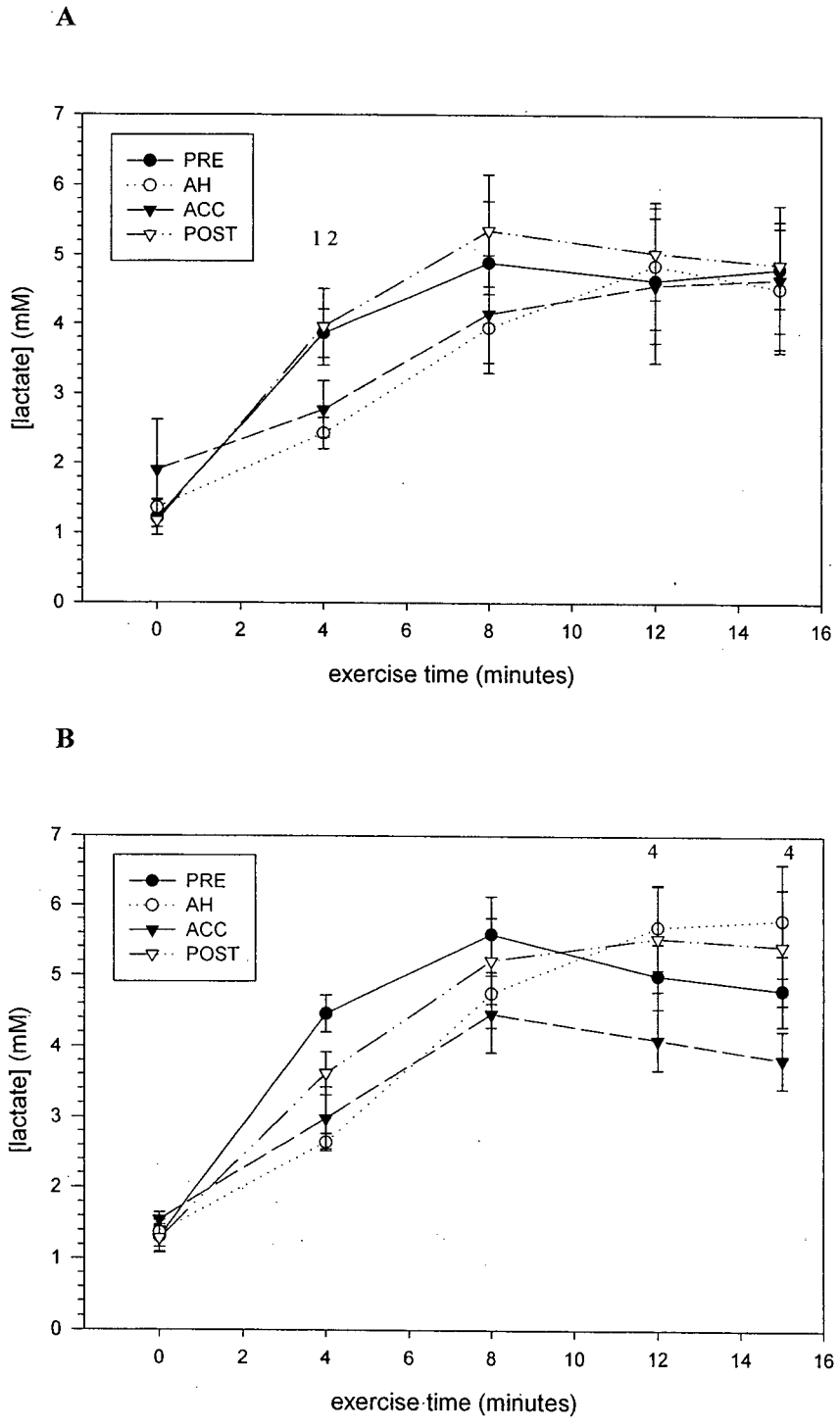

FIGURE 33: Mean (± SE) plasma lactate concentration of (**A**) trained and (**B**) untrained subject groups during 15 minutes of submaximal cycling exercise, corresponding to 70% relative $\dot{V}_{O_2\,max}$, at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). 1, PRE significantly different from AH; 2, PRE significantly different from ACC; 4, AH significantly different from ACC.

IV. Endurance Exercise Testing:

When endurance exercise performance was evaluated by having the subjects exercise until fatigue at a workload corresponding to 90% PRE $\dot{V}_{O_2\ max}$, the exhaustion times shown in Figure 37 were observed. These results indicate that exercise performance declined significantly in both groups during exercise in AH compared to PRE. During exercise tests under ACC conditions, exercise times did not change compared to AH levels, in either group. Upon return to sea level following altitude acclimatization (POST), both groups displayed a significant improvement in exercise performance. However, the change in exhaustion times observed in the untrained group increased to a greater extent, compared to the trained group, so that exhaustion times of the untrained subjects were significantly higher than the trained.

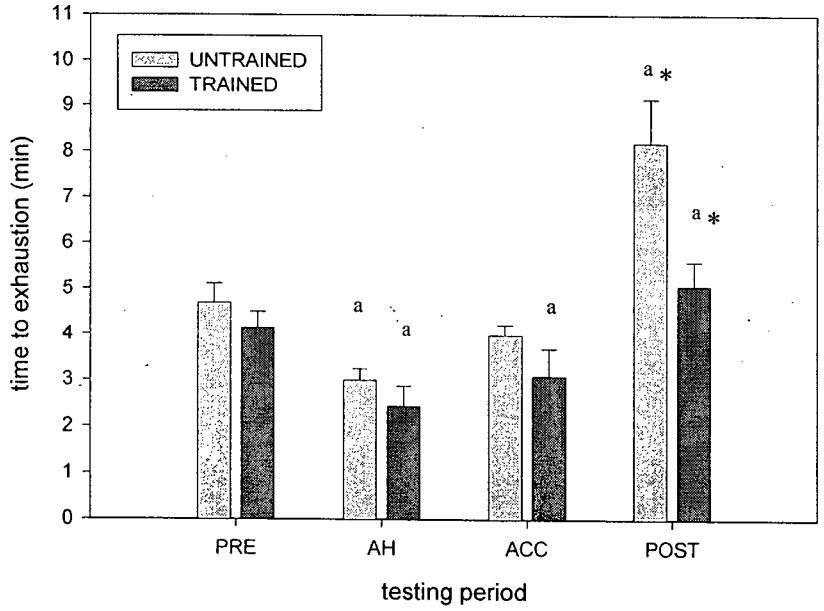

FIGURE 34: Mean (± SE) exhaustion times of untrained and trained subject groups following exercise performed at a workload corresponding to 90% of pre-acclimatization sea level $\dot{V}_{O_2\ max}$. Endurance tests were performed at sea level before altitude acclimatization (PRE), in acute hypoxia (AH), after 3 weeks at 3,800m (ACC), and at sea level following acclimatization (POST). a, significantly different from PRE; *, significant difference between trained and untrained.

A lowering of exercise-induced blood lactate concentration was observed in all subjects following acclimatization to hypobaric hypoxia; a phenomenon known as the lactate paradox. Furthermore, this reduced accumulation was maintained following return to sea level, and was not associated with any change in energy balance or oxidative control. However, when analyzing the results of trained and untrained subjects separately, a markedly different metabolic response was observed between the two groups. The key finding was that untrained individuals displayed an improved coupling between the processes involved in ATP utilization and ATP synthesis following hypobaric hypoxia acclimatization, while similar metabolic changes were not observed in the endurance trained group in response to hypoxic exposure. Evidence that an improved ATP coupling had occurred is indicated by alterations in the high-energy phosphate transfer system and lactate accumulation. Following acclimatization, higher PCr concentrations and a lower P_i to PCr ratio at fatigue from incremental exercise was found in the untrained group when comparing acute to acclimatized hypoxia, and pre- to post-acclimatization conditions. These metabolic changes were associated with reduced blood lactate levels, suggesting that acclimatization resulted in a lowering of glycolytic flux, secondary to an improvement in oxidative control. Following acclimatization, the untrained subject group more closely resembled the trained group in terms of metabolic control and the biochemical and physiological responses to exercise. These findings draw to attention the importance of assessing the variability in responses of subjects differing in training status, and as will be discussed, the importance in monitoring physical activity and training effects when studying metabolic adjustments to hypobaric hypoxia.

Lactate production and removal at high altitude and the lactate paradox:

i. The lactate response in acute hypoxia:

Under acute hypoxia conditions, all subjects displayed an increase in blood lactate concentration during incremental cycling exercise compared to sea level. In the untrained group, this change in lactate was associated with a greater breakdown of PCr during exercise and an increase in fatigue P_i/PCr (estimate of ADP_{free} concentration), suggesting an increase in PCr hydrolysis and glycolytic activation in response to hypoxia and changes in the energy state of the cell. Increased glycolysis with acute exposure to hypoxia would initially seem important in maintaining ATP synthesis rates at close to normoxic levels, thus compensating for the impairment in oxidative phosphorylation under oxygen-limited conditions. What is interesting is that the same metabolite changes that provide for an increased glycolytic flux, particularly the elevated P_i/PCr levels observed by the untrained subject group, simultaneously stimulate

mitochondrial respiration (Connett 1990, Honig 1992). The increase in glycolytic flux helps maintain a redox ($NADH/NAD^+$) gradient between the cytosol and mitochondria, necessary for the transport of reducing equivalents into the mitochondria via the redox shuttle. In turn, lactate production plays a pivotal role in supplying NAD^+, needed to maintain the cytosolic redox potential and substrate flux through glycolysis (Connett 1990, Robergs 2004). Therefore, increased glycolytic flux and lactate production indirectly support $\dot{V}_{O_2}$ by contributing to the redox drive of oxidative phosphorylation, which provides for compensation to hypoxia (Connett 1990). Even though these compensatory adjustments are initially successful in maintaining the level of oxidative phosphorylation, glycogen depletion occurs more quickly, and by-products of glycolysis accumulate.

In the endurance-trained group, elevated lactate levels were not associated with any change in PCr concentration or P_i/PCr, as observed in the untrained group. This suggests that metabolic control of trained subjects was not altered in response to acute exposure to hypoxia, and other mechanisms must contribute to the observed increase in exercise lactate. It has been proposed that elevated lactate levels in acute hypoxia are related to a rise in circulating epinephrine levels (Mazzeo 1991, Young 1991, Brooks 1992, Hughson 1995). Through the stimulation of β-adrenergic receptors found on skeletal muscle, epinephrine activates glycogenolysis by facilitating the transformation of phosphorylase b to its active form. At a given absolute $\dot{V}_{O_2}$ and substrate flux through oxidative phosphorylation, acute hypoxia increases epinephrine release compared to sea level, which would stimulate glycogenolysis and glycolysis, leading to an increase in lactate release (Brooks 1992). It seems likely that multiple factors influence changes in lactate production at altitude, and that an epinephrine-induced increase in glycolysis would occur in both subject groups. It is interesting to note that the trained subject group also displayed a significant increase in heart rate during incremental exercise in acute hypoxia that was not observed in the untrained group. The heart rate response is also mediated in part by increased circulating epinephrine levels.

ii. The lactate response following altitude acclimatization:

Following three weeks of altitude acclimatization, all subjects displayed a decrease in exercise lactate concentration from acute hypoxia levels, as observed in previous studies (Edwards 1936, Bender 1989, Green 1992b). When separating the lactate results into the fitness groupings, an interesting difference is observed between trained and untrained subjects. While both trained and untrained groups displayed similar changes in exercise lactate levels under acute

hypoxia and acclimatized hypoxia test conditions, the persistence of the lactate paradox upon return to sea level was not observed in trained individuals. The lower lactate levels of untrained subjects following return from altitude indicates that metabolic adjustments in response to hypobaric hypoxia persisted in the absence of the hypoxic stimulus. In contrast, the changes in lactate levels at altitude of the trained group were a temporary response to hypoxia. Under post-acclimatization conditions, exercise lactate concentrations between fitness groups no longer differed. In effect, the untrained group displayed a lowering of glycolytic activation and lactate production, which is characteristic of endurance training.

Earlier studies measured lactate concentrations between test conditions during steady-state submaximal exercise at a given absolute workload (Green 1992b). Compared to incremental exercise tests, which assess the response of muscle metabolism to a progressive increase in exercise intensity, submaximal exercise tests provide different insight into how the working muscle responds to changes in oxygen availability at altitude by keeping the workload constant. The effects of hypoxia on lactate production and clearance are often difficult to interpret during incremental exercise where fuel selection, muscle recruitment and fibre type activation change with exercise intensity. Most often submaximal exercise is performed at the same absolute exercise intensity during both normoxia and hypoxia. The difficulty or confusion in using this model to examine muscle metabolism is that exercise performance decreases under hypoxia, so that a given absolute workload represents a higher percentage of $\dot{V}_{O_2\ max}$.

To keep exercise intensity constant between test conditions and avoid differential fuel selection, epinephrine release, and muscle fibre recruitment changes, we measured lactate concentration during submaximal exercise at workloads corresponding to 70% $\dot{V}_{O_2\ max}$ for that particular test condition. The trained subject group did not display any significant change in lactate concentration during submaximal exercise between test conditions. However, because this group displayed a considerable decrease in exercise performance under acute and acclimatized hypoxia conditions, the submaximal workloads at altitude were substantially lower compared to sea level. Therefore, the effect of decreased workload or factors affecting $\dot{V}_{O_2\ max}$, such as motivation, energy levels and perceived exertion, cannot be excluded in explaining the lack of change in lactate levels in the trained group under acute and acclimatized hypoxia test conditions. The unexpected decrease in minute ventilation and heart rate during submaximal exercise under acute hypoxia in the trained group suggests that the workload settings were lowered to such a degree that substrate flux and lactate levels would be lower than expected. In contrast, the untrained group displayed less of a change in $\dot{V}_{O_2\ max}$ between test conditions, and while

submaximal workloads were unaltered between acute and acclimatized hypoxia, plasma lactate concentrations did display a significant decrease, characteristic of the lactate paradox.

iii. Lactate recovery following incremental exercise:

As muscle returns to a pre-exercise state following exercise, two phases of recovery are observed. During the initial fast phase of recovery, lasting up to five minutes depending on the type and intensity of exercise, tissue oxygen stores are quickly replenished and most of the ATP and PCr depleted in the muscle are restored. $\dot{V}_{O_2}$ remains elevated above resting levels because the rate of PCr resynthesis depends on the supply of ATP by oxidative metabolism (Quistorff 1992, Harris 1976). The following slow recovery phase involves the removal of lactate and accumulated protons. Lactate recovery can take an hour or longer and depends on the capacity to exchange lactate between the muscle and blood, and factors affecting tissue uptake of lactate and subsequent use as a substrate for oxidation and glyconeogenesis.

The initial phase of lactate recovery from exhaustive exercise is characterized by a rapid increase in blood lactate levels owing to the efflux of lactate from previously active muscle. This is followed by a gradual decrease as lactate is removed from the body tissues to be used as a substrate for oxidation or glyconeogenesis. Although measured during recovery, the time to peak lactate and the rate of decrease provide indirect information on the lactate exchange and removal abilities during the previously performed exercise. The results of the present study indicate that altitude exposure and acclimatization did not have a significant effect on lactate efflux following incremental exercise, shown by no change in the time to peak post exercise lactate concentration from fatigue in either fitness group. In terms of lactate removal, a significant improvement in the rate of lactate decrease during the recovery phase was observed with altitude acclimatization when comparing acute to acclimatized hypoxia, and pre- to post- sea level conditions.

During exercise and recovery, lactate is transported out of the muscle cell using monocarboxylate transporters or by diffusion driven by the muscle-to-blood lactate gradient. Thus, the rate of efflux during the initial phase of recovery depends on blood flow, the transport capacity of the muscle cell and the lactate gradient. With acute altitude exposure, lactate efflux was unaltered, most likely because structural changes affecting the lactate transport capacity (i.e. increased capillarization or increased transporter recruitment) cannot occur within the time frame of acute hypoxia exposure. Also, fatigue lactate levels in acute hypoxia were unchanged compared to sea level values, so that the lactate gradient was maintained during recovery.

Several studies monitoring lactate recovery kinetics have shown that endurance training improves lactate efflux rates from the muscle (Fukuba 1999). This improvement is thought to be

the result of greater muscle capillarization with endurance training (Saltin 1983), which provides an increased surface area and decreased diffusion distance for the movement of lactate from the muscle to capillary. While not significant, the trained subject group in the present study tended to display faster efflux rates (shown by the time to peak concentration) under all conditions. This adaptation to endurance training is beneficial during exercise because the removal of each lactate molecule from the working muscle is always coupled with a single proton (Juel 1997), and as such, assists in delaying the onset of fatigue. Increased capillarization and improved lactate efflux would also appear to be a beneficial adaptation to altitude. However, muscle capillary density when related to fibre size has previously been shown not to change with acclimatization to moderate and high altitudes (Green 1992), which could account for the observed lack of effect of acclimatization on lactate efflux in this and previous studies (Bender 1989).

The introduction of the cell-to-cell lactate shuttle (Brooks 1991a) has called to attention the important role of lactate as a mobile fuel released from active muscle for subsequent uptake by other muscle fibres, the heart, liver and brain. Following altitude acclimatization, an enhanced rate of lactate removal from the blood was observed when comparing acute and acclimatized hypoxia, and pre- and post-acclimatization sea level recovery rates. These findings suggest that the capacity for lactate uptake was improved with acclimatization, and potentially the contribution of lactate oxidation to fuel exercise, or the use of lactate to maintain glycogen stores may be increased during exercise. A similar improvement in lactate clearance is observed with exercise training (Fukuba 1999). It has been suggested that that the greater percentage of slow-twitch muscle fibres observed in endurance athletes, which are more efficient for lactate uptake and oxidation than fast-twitch fibres (Juel 1999, Pilegaard 1999, Van Hall 2000), may account for the improved removal ability and lower blood lactate levels observed in endurance athletes compared to sedentary individuals (Fukuba 1999, Messonnier 2002). It is interesting to note that high altitude natives also display a higher proportion of slow-twitch muscle fibres (Kayser 1991), which may possibly be related to the lower lactate levels observed in this group. These adaptations that ultimately improve exercise performance in endurance athletes and high altitude natives; however, are not part of the skeletal muscle fibre adjustments to hypobaric hypoxia. Fibre type distribution, cross sectional fibre areas, and mitochondrial and capillary volume densities of acclimatized lowlanders returning from moderate altitude (Green 1992b), elite mountaineers who have scaled 8,500m peaks (Hoppeler 1990), and lowlanders decompressed to simulated altitude (Green 1989), are either unchanged from sedentary lowlander values or somewhat reduced.

In fact, the observation of increased lactate clearance with acclimatization contradicts the findings of previous studies that attributed lower lactate levels during steady-state exercise to a decrease in lactate production, and not improved clearance (Bender 1989, Brooks 1991b). When the lactate recovery data of the present study were analyzed in trained and untrained subjects separately, it was shown that the improved lactate recovery rate could be attributed primarily to changes observed with the untrained subject group. These results suggest that an effect of training, rather than hypoxia alone, may account in part for some of the changes observed in the untrained group following the three weeks at altitude.

Potential mechanisms accounting for the lactate paradox:

During exercise, blood lactate levels depend on several factors including the availability of oxygen and mitochondrial capacity, the choice of substrate to fuel exercise, the proportion of fast-twitch and slow-twitch muscle fibres recruited, the energy state of the cell, and hormonal influences (i.e. epinephrine and insulin release). These multiple factors make accounting for the changes in exercise lactate levels with exposure to altitude complicated and confusing. Due to the decrease in oxygen availability at altitude, it has been tempting to attribute changes in lactate concentration to the effects of oxygen availability on mitochondrial function. With acute exposure to hypobaric hypoxia, the role of oxygen in modulating muscle metabolism resulting in lactate production appears to be important, yet compensatory mechanisms affecting oxygen transport cannot account for changes in lactate production with altitude acclimatization.

Three weeks of altitude acclimatization resulted in a decrease in plasma lactate concentration during incremental cycling exercise compared to the levels observed under acute hypoxia conditions. Similar acclimatization studies at moderate altitudes (4,300m) have demonstrated comparable changes in lactate levels (Green 1992b), a lower glycolytic rate and glycogen sparing (Brooks 1991b), and an increased dependence on blood glucose (Brooks 1991c) during submaximal exercise at a constant $\dot{V}_{O_2}$, and have indicated that changes in blood lactate levels are the result of a decrease in production and not an increase in removal (Brooks 1992). Because improvements in oxygen delivery do not occur with acclimatization (Wolfel 1991) and reduced adrenergic control of glycolysis (Mazzeo 1994) cannot completely account for the changes observed in lactate production, other compensatory adjustments must take place to account for the metabolic changes observed. Green et al. (1992b) explored the possibility that the decrease in lactate production is secondary to an improvement in oxidative potential. However, this was considered unlikely because the decrease in muscle lactate concentration was not

associated with any change in whole-body $\dot{V}_{O_2}$ or in the maximal activity levels of representative enzymes of oxidative phosphorylation (succinic dehydrogenase) or β-oxidation (3-hydroxyacyl CoA dehydrogenase) following acclimatization. In fact, the enzymatic data from several studies conducted at similar or higher altitudes (Young 1984, Howald 1990) or under simulated hypobaric hypoxia conditions mimicking an expedition to Everest (Green 1989), displayed either no change or a decrease in the activity of representative oxidative enzymes.

Since the decrease in blood lactate concentration following acclimatization has been attributed to a reduced glycolytic flux (Brooks 1991b) and not an increase in lactate clearance, many studies have explored the effect of altitude exposure on fuel availability to explain this apparent blunting of the glycolytic rate (Green 1992b, Brooks 1991c). Lactate production requires a source of glucose or glycogen, so that a decrease in substrate availability or a switch in fuel preference to protect glycogen stores could potentially affect lactate production during exercise at altitude. Regardless of the altitude or acclimatization state, it has been previously shown that relative exercise intensity determines fuel selection during exercise (McClelland 1998, Lundby 2002). Because $\dot{V}_{O_2\,max}$ was unchanged between acute and acclimatized hypoxia, both trained and untrained subjects were assumed to be working at the same relative exercise intensity under each condition, so that fuel selection was expected to be unaltered and could not account for a change in lactate production. Through circulatory changes, improved mitochondrial content, and in some cases genetic endowment with a high percentage of oxidative muscle fibres, endurance athletes have an enhanced ability to utilize fats as a fuel for oxidative phosphorylation, thus saving glycogen stores for when they are required to fuel high-intensity exercise and during the rest-to-work transition. These changes in mitochondrial content or muscle fibre profile, however, are not observed with altitude acclimatization (Green 1992b). A lack of change in the respiratory exchange ratio during submaximal exercise at the same relative workload after acclimatization further supports these findings that fuel selection is not altered during prolonged hypoxia exposure. In terms of substrate availability for glycolysis, earlier studies have also shown that glycogen levels are preserved between acute and acclimatized hypoxia (Green 1992b, Brooks 1992c), and glycogen depletion during exercise has never been reported. This muscle glycogen sparing effect with altitude acclimatization has been attributed to a lesser β-adrenergic stimulation of glycogenolysis and increased dependence on blood glucose during exercise compared to acute hypoxia.

Other hypotheses examined have suggested that a reduced buffering capacity at altitude, resulting from the compensation to hyperventilation and associated reduction in $PaCO_2$,

contributes to an increase in H^+ concentration during exercise at altitude and the consequent inhibition of glycolysis, most likely at the level of phosphofructokinase (Kayser 1993, West 1986). It has also been proposed that hypoxia impairs skeletal muscle contractile function either through a disturbance in neuromuscular transmission (Green 1989) or central motor drive (Bigland-Ritchie 1988). These topics have been extensively reviewed (Cerretelli 2003, Kayser 1996), concluding that neither hypothesis could adequately explain the lactate paradox at moderate to high altitudes.

A key finding of the present study was that trained and untrained subjects responded differently to altitude acclimatization. In particular, several metabolic and physiological adjustments were maintained in the untrained group upon return to sea level following acclimatization, while the trained subject group returned to their pre-acclimatization state. This poses the question, can these different responses of individuals varying in training status account for the variability in the lactate response?

Can cardiorespiratory adjustments account for differences in lactate concentration between fitness groupings?

i. Cardiorespiratory compensation to acute hypoxia:

A key strategy of physiological systems during exercise when exposed to acute hypoxia conditions is to undergo adjustments aimed at maintaining oxidative ATP supply, despite reductions in the arterial oxygen content. In response to this strategy, several compensatory adjustments of the respiratory and cardiovascular systems must occur at altitude to preserve oxygen transport to the working muscle. Oxygen transport from the inspired air to the site of oxidation in the mitochondria depends on a series of steps, including alveolar ventilation, alveoli-pulmonary capillary diffusion, cardiovascular blood transport, and tissue capillary-mitochondria diffusion. Alterations of these steps may compensate for the reduction in oxygen availability, or conversely, they may contribute to the limitation in exercise performance observed at altitude.

Compared to sea level, much greater volumes of air for a given rate of work are necessary to supply enough oxygen to the body from atmospheric air, in which the level of oxygen is reduced. Accordingly, all subjects displayed an increase in minute ventilation and respiratory rate at a given power output during incremental exercise upon immediate hypoxia exposure. Hypoxemic stimulation of the carotid body chemoreceptors for the most part accounts for these respiratory changes (Smith 2001). On the plus side, the oxygen content of blood at altitude is initially improved by the hyperventilatory response by keeping alveolar PO_2 sufficiently high for oxygen diffusion down its pressure gradient from the alveoli to capillary

blood. Additionally, the respiratory alkalosis caused by hyperventilation results in an increased affinity of hemoglobin for oxygen (West 1988). However, improving the oxygen content of blood by hyperventilation has a down side of increasing the muscular work and oxygen cost of breathing, 'stealing' blood from the working locomotor muscles, and contributing to a mechanical limitation to exercise at altitude (Cibella 1999). Maximum minute ventilation did not change in either group compared to sea level values, indicating that sufficient muscular capacity exists to meet the increased requirements of ventilatory work during exercise at high altitude.

The extent of the ventilatory increase during exercise at altitude is partly dependent on the inherent hypoxic ventilatory response of an individual (Schoene 1984). A large ventilatory response conveys a greater respiratory alkalosis and higher arterial oxygen saturation, and may confer a better exercise performance at high altitude (Schoene 1984). In this study, the untrained subject group displayed a greater ventilatory response to acute and acclimatized hypoxia compared to the trained subject group. This was not unexpected since highly trained individuals often exhibit a blunted ventilatory response and a tendency to desaturate (Dempsey 1984) during exercise at sea level. It is interesting to note that the trained group also displayed a greater decrease in exercise performance under acute hypoxia conditions, shown by a greater percentage change in $\dot{V}_{O_2\,max}$ and maximum power output when compared to the untrained group. That endurance trained athletes and high altitude natives are shown to display a blunted hypoxic ventilatory response indicates a possible adaptation aimed at alleviating the metabolic cost of breathing and the sensation of breathlessness during exercise. Although this would be an advantage for the endurance athlete at sea level, it is a weakness at altitude. Native highlanders are not disadvantaged because they exhibit a much larger pulmonary diffusing capacity, both at rest and during exercise, compared to lowlanders (Smith 2001).

Also important to the acute compensatory responses to high altitude exposure are the cardiovascular adjustments aimed at maintaining oxygen delivery to the working muscle to support the oxygen demands of mitochondrial metabolism. During incremental exercise upon arrival at altitude, the trained subject group displayed an increase in heart rate at a given workload compared to sea level, not shown by the untrained group. Because stroke volume plays only a minor role in the acute cardiovascular response (Rowell 1987), the change in heart rate is essential in increasing cardiac output. To compensate for the decrease in CaO_2 under acute hypoxia conditions, changes in cardiac output coupled with the vasodilation of muscle vascular beds are key adaptive mechanisms employed to supply more blood to the active muscles and maintain $\dot{V}_{O_2}$ at a given level during exercise (Rowell 1986). The beneficial effects of increasing

cardiac output have an upper limit, especially at high intensity workloads where a high cardiac output will reduce capillary transit time and interfere with the time needed for equilibration of the pulmonary blood. Similar to the results of previous studies (Stenberg 1966), maximum heart rate was not significantly changed from sea level values in either group, but it was attained at a lower maximum workload. This would indicate that heart rate and cardiac output were able to reach a similar maximal capacity as observed under normoxic conditions, but this maximum could not be exceeded to further improve oxygen transport.

At sea level before travel to altitude, the trained group displayed lower heart rate values during incremental exercise, compared to the untrained group. This is because endurance training improves the efficiency of the heart to pump blood by increasing the contribution of stroke volume to the maintenance of cardiac output, while heart rate is decreased through mechanisms involving an increase in parasympathetic vagal tone (Brooks 2000). This adaptation reduces myocardial oxygen consumption, thus decreasing the heart's requirements for blood during submaximal exercise. With acute hypoxia, cardiac vagal activity is inhibited in response to increased central inspiratory neural activity (Wolfel 2001), partly contributing to the increase in heart rate of trained subjects toward values exhibited by the untrained group during incremental exercise. Under these conditions, heart rate did not differ between the two subject groups. That the untrained group did not display a similar significant increase in heart rate during exercise was surprising. Possibly the greater ventilatory response to acute hypoxia observed by the untrained group, in combination with an expected vasodilation of muscle blood vessels and an inherently higher heart rate, may have been sufficient in maintaining the oxygen content of blood and delivery of oxygen to the active muscles.

At maximal workloads, compensatory mechanisms cannot maintain oxygen transport at sea level values (Stenberg 1966), contributing to the decrease in exercise performance seen especially by the trained subject group and the increase in lactate production (Cerretelli 1976, West 1983). Despite the differing cardiorespiratory responses and changes in maximal oxidative capacity exhibited by the two fitness groups in acute hypoxia, both displayed a similar percentage increase in lactate concentration from sea level values. This would suggest that oxygen delivery did not differ between the two groups, or other factors influenced the metabolic changes observed in acute hypoxia.

ii. Cardiorespiratory adjustments with altitude acclimatization:

Oxygen delivery to the working muscle following altitude acclimatization was enhanced by increases in ventilation and hemoglobin concentration, which function to improve the oxygen

content of blood. Following three weeks of altitude acclimatization, the ventilatory response at a given workload during incremental exercise was shown to increase further compared to acute hypoxia. This response during exercise was more evident in the untrained group, while both groups displayed a higher maximum minute ventilation compared to sea level.

With acclimatization, the cardiovascular system tends to respond to adjustments by the respiratory and hematological systems, rather than initiate changes in oxygen availability (Wolfel 2001). In the endurance trained group, heart rate during incremental exercise returned toward pre-acclimatization sea level values under acclimatized hypoxia conditions, attributed in part to a down-regulation of cardiac β-receptors in response to persistent sympathetic hyperactivity with prolonged time spent at altitude (Richalet 1988). At maximum workloads, the trained group displayed a significant decrease in maximum heart rate below sea level values. These observations are commonly observed (West 1983, Cerretelli 1976, Wolfel 1991, Vogel 1974) and occur along with a more substantial decrease in stroke volume, suggesting that acclimatization produces a 'cardiac sparing' effect. These changes in heart rate, and consequently cardiac output, may be adaptive in reducing the metabolic demands of the heart and improving the diffusive time for extraction of oxygen at the level of the lungs and skeletal muscle. However, the changes in heart function, along with a decrease in peripheral blood flow, tend to offset the increase in the oxygen carrying capacity of blood in response to ventilatory and hematological changes with altitude acclimatization, so that mass oxygen transport (whole-body $\dot{V}_{O_2}$) during submaximal exercise at a given power output or at fatigue does not change from acute hypoxia levels (Wolfel 1991, Bender 1988).

While differences exist between trained and untrained subjects in terms of the cardiorespiratory response to altitude, with the trained subjects displaying a greater effect on cardiac function and the untrained group showing a more significant ventilatory response, the changes are unable to explain the observed decrease in blood lactate accumulation under acclimatized hypoxia exercise conditions. Upon return to sea level, the untrained group maintained a higher ventilatory rate compared to pre-acclimatization, and heart rate during exercise decreased toward values observed by the trained subjects. In contrast, cardiorespiratory measurements of the endurance-trained group were unaltered from pre-acclimatization levels. Therefore, cardiorespiratory mechanisms aimed at improving the oxygen carrying capacity of blood and oxygen delivery to the working muscle were maintained in the untrained group, and may in part account for the increase in $\dot{V}_{O_2\,\max}$ and lowering of lactate production upon return to

sea level. The reduction in heart rate during exercise also indicates a 'training effect' in the untrained group in response to the 3-week stay at altitude.

Does an improved coupling between ATP supply and ATP demand occur with altitude acclimatization?

i. Estimation of energy balance by PCr and P_i/PCr levels:

We hypothesized that a decrease in glycolytic flux is secondary to a tighter integration of ATP supply and ATP demand pathways with altitude acclimatization, accounting for the observed decrease in lactate concentration. To test this hypothesis, the changes in exercise lactate concentration shown by all subjects were compared to the levels of PCr and P_i/PCr during the plantar flexion exercise tests to fatigue. In acute hypoxia, higher lactate concentrations were associated with lower PCr levels, compared to sea level values. Under acclimatized hypoxia conditions, both lactate and PCr returned toward sea levels values. These changes, however, could not necessarily be attributed to an improvement in energy coupling or metabolic control since P_i/PCr, used to estimate ADP_{free} concentrations, was unaltered between test conditions.

When analyzing the results of trained and untrained subjects separately, a clear trend is observed in the untrained group. Compared to acute hypoxia, the decrease in lactate concentration toward sea level values in acclimatized hypobaric hypoxia appears to be associated with less of a perturbation of PCr and a lowering of P_i/PCr at fatigue under similar simulated-hypoxia conditions. Additionally, upon return from altitude, plasma lactate concentration during exercise in the untrained group was found to be decreased compared to pre-acclimatization concentrations. Similarly, this finding was associated with a lowering of P_i/PCr, though the levels of PCr were unchanged compared to sea level before acclimatization. These results are supported by similar findings of metabolite levels at the end of exercise in acclimatized hypobaric hypoxia (Green 1992b) and during submaximal exercise following return to sea level (Green 2000). Submaximal plantar flexion exercise tests were not conducted in conjunction with submaximal cycling exercise tests; however, we can assume from the results of earlier submaximal exercise studies (Green 1992b) that the decrease in lactate concentration observed during submaximal cycling exercise in the untrained group following acclimatization would be associated with less of a perturbation of PCr and the phosphorylation potential.

In contrast to the untrained group, the endurance-trained subjects did not appear to display any change in metabolic control with acclimatization. PCr and P_i/PCr ratios were unaltered between test conditions, and the persistence of lower lactate levels upon return to sea level following acclimatization was not observed. Throughout the study, this group tended to

exhibit lower plasma lactate concentrations during exercise, higher fatigue PCr levels, and lower P_i/PCr ratios compared to the untrained group. These findings suggest that metabolic control in the trained subject group was 'optimal', and could not be changed with altitude acclimatization or required a greater hypoxic stimulus. Following return to sea level, the differences in metabolite levels between the two fitness groups lessened, suggesting that altitude acclimatization resulted in an improved metabolic state in untrained individuals, normally observed following endurance training. It is also noteworthy to mention that the untrained subject group also appeared to display an improved physiological state upon return to sea level, as indicated by a lowering of heart rate toward the levels observed in the trained subject group during incremental exercise.

ii. Mechanisms accounting for improved energy balance and decreased glycolytic flux with altitude acclimatization:

As previously discussed, oxidative phosphorylation is protected after ascent and acclimatization to 3,800m, in part by well known adjustments of the cardiovascular, ventilatory and hematological systems. The observation by the present study and others (Green 1992b, Green 2000) that less of a perturbation of PCr and ADP_{free} (estimated by P_i/PCr) occurred during exercise following acclimatization suggests that peripheral adjustments also take place in response to prolonged hypobaric hypoxia that are aimed at improving the balance between the rate of ATP synthesis and ATP utililization, and in the process maintaining lower ADP_{free} concentrations. Because ADP_{free} has been implicated in the activation of phosphorylase and phosphofructokinase (Connett 1990), the enzymes controlling glycogenolysis and glycolysis, a lower level of ADP_{free} would be expected to lower glycolytic flux. An earlier study by Green et al. (1992b) displayed a decrease in phosphofructokinase activity, and thus glycolytic flux, at the end of submaximal exercise following 3-weeks of acclimatization compared to sea level, associated with lower levels of ADP_{free}. Accordingly, the observed decrease in lactate concentration with acclimatization would be considered secondary to the metabolic changes resulting in a tighter coupling between ATP supply and demand.

To date, the mechanism accounting for these changes in exercise metabolite levels, especially with altitude acclimatization, is uncertain. From current concepts, the findings of a reduced perturbation of adenylates and PCr concentrations can be attributed to: (1) a change in fuel preference from carbohydrates to fats, (2) an increased proportion of slow-twitch fibres recruited during exercise, (3) a tighter coupling between ATP supply and ATP demand pathways, and (4) an increased sensitivity to the regulators of respiratory control. Because fuel selection and fibre type recruitment profiles do not change with altitude acclimatization, these results suggest

that metabolic changes occurring in the untrained group are aimed at improving the coupling between ATP supply and demand. An improvement in ATP coupling with acclimatization could result from an increased rate of ATP synthesis by PCr breakdown, glycolysis, or oxidative phosphorylation. However, as previously mentioned, PCr levels were unaltered, lactate concentrations were reduced and $\dot{V}_{O_2}$ measured during exercise was unchanged between pre- and post-acclimatization conditions.

The improvement in energy balance that occurs with endurance training has been attributed to two possible mechanisms: (1) more responsive blood flow adjustments during rest-work transitions (Shoemaker 1996), and (2) an increase in the mitochondrial content (number of active mitochondrial ATP synthases) of muscle (Hollozsy 1984). The former mechanism would allow for a more rapid ATP production by oxidative phosphorylation at the onset of exercise and during non-steady state conditions, thereby reducing the imbalance between ATP supply and demand and the activation of glycolysis. The second mechanism suggests that a greater mitochondrial density results in less oxygen utilization and energy production per mitochondria, and thus less of a stimulus (i.e. increase in ADP_{free}) is required for respiration to attain the same rate of $\dot{V}_{O_2}$ in trained athletes compared to untrained individuals (Hollozsy 1984). Accordingly, at a given work rate, ATP and PCr concentrations are expected to decrease less and ADP_{free} would increase less in the trained individual. This proposed increase in mitochondrial sensitivity to the regulators of respiration through an increase in mitochondrial content, cannot be extended to the acclimatized lowlander since it has been shown that mitochondrial density does not change with altitude acclimatization (Green 1992b). Furthermore, this concept has been recently challenged by the findings of Green et al. (1992a, Shoemaker 1996), which indicated that changes in mitochondrial control with short-term exercise training occur early in the training protocol, well before changes in muscle structure and mitochondrial content are able to take place.

Because the cellular changes seen with exercise training, such as increases in capillary density or mitochondrial content, are not exhibited by altitude acclimatized individuals, other mechanisms need to be examined to explain the metabolic changes that occur with acclimatization. Potential mechanisms could involve the redistribution of cellular structures to allow for improved oxygen diffusion, alterations at the level of the mitochondrial membrane to facilitate adenylate transport, increased mechanical efficiency, or changes at the level of enzymatic pathways. An improvement in metabolic efficiency through a downregulation of ATPase activity, and thus ATP demand, as an adaptive strategy in defending against oxygen limitation and restoring a tighter ATP coupling was first suggested in response to studies of high

altitude natives who displayed persistently lower $\dot{V}_{O_2}$ levels for a given amount of mechanical work compared to lowlander controls (Hochachka 1991b). Differences in $\dot{V}_{O_2}$ during exercise between test conditions, however, were not observed in the present or previous acclimatization studies (Wolfel 1991, Bender 1988), or in high altitude natives when equated to lowlander controls for body mass. It has also been proposed that metabolic reorganization occurs with altitude acclimatization, resulting in an improved aerobic metabolic control characteristic of tightly coupled systems (Hochachka 1988, Matheson 1991). This mechanism is thought to involve an increase in the number of active enzymes recruited (i.e. mitochondrial ATP synthase) to match the catalytic capacity of actomyosin ATPase, or by allosterically or covalently activating the enzymes of mitochondrial metabolism in step with the increase in work rate. The major advantage of this type of regulation is that it would allow for a large magnitude of change in ATP turnover, with minimal or no change in substrate and product concentrations.

It has also been proposed that a decrease in the recruitment of mitochondria with adequate oxygen supply in acute hypoxia, may in effect reduce mitochondrial content and loosen metabolic control, so that a greater change in concentration of metabolic regulators is necessary to maintain $\dot{V}_{O_2}$ (Haseler 1999). Richardson et al. (1995), utilizing NMR spectroscopy to measure myoglobin saturation in the working human quadriceps muscle, have shown that at similar workloads and rates of muscle respiration, intracellular myoglobin saturation (and thereby intracellular PO_2) is significantly reduced when breathing a hypoxic gas mixture during exercise, and increased with a hyperoxic gas mixture. When this finding was related to changes in PCr, P_i, and ADP, it was suggested that the level of oxygen available to the mitochondria, even if sufficient for the required level of $\dot{V}_{O_2}$, influences the concentration of cellular metabolites and the metabolic state of the cell (Haseler 1999). Thereby, any change in intracellular PO_2 with acute hypoxia or acclimatization, or changes in oxygen availability with exercise training, may change the quantity of functional mitochondria, and in effect, lead to a loosening or tightening of metabolic control. While a reduced dependence on blood flow and an increased oxygen extraction across the working limb following altitude acclimatization indicate a shift in the strategy for preserving $\dot{V}_{O_2}$ (Wolfel 1991, Bender 1988), it is unclear whether cellular PO_2 is affected.

iii. Muscle metabolite relationships:

The main functional advantages of the lactate paradox during exercise are clear. Metabolite homeostasis at fatigue is improved, recovery is quicker, and over-activation of the energetically inefficient glycolytic pathway is avoided. Less clear are the underlying mechanisms

explaining the decrease in lactate production following acclimatization. A key insight is that when comparing individuals of varying biochemistry or physiology, each displays different metabolic control patterns, depending on the organization of the steps involved in ATP supply (oxygen delivery and metabolic pathways) and ATP demand (actomyosin and calcium ATPases). Each of these steps contributes to the control of an overall physiological or metabolic process, such as maximum ATP turnover, but to varying degrees depending on the individual. For example, compared to untrained individuals, the key processes in energy supply are all upregulated in endurance-trained athletes. At $\dot{V}_{O_2\,max}$, cardiac output and muscle citrate synthase may be two fold higher in the endurance trained than in the untrained individual (Brooks 2000). Energy demand processes in contrast are downregulated since muscle fibres following endurance training are predominantly oxidative (slow-twitch and fast-twitch oxidative glycolytic) and contain lower actomyosin and calcium ATPase activities compared to fast-twitch fibres (Hochachka 1991a). In terms of metabolic control, a greater contribution to the control of maximum ATP turnover is exerted by the ATP demand steps in the endurance trained compared to the untrained (see Hochachka 2002 and 2003 for explanation of multiple control analysis). A similar analysis of high altitude natives compared to normoxic lowlanders, and acclimatized to non-acclimatized individuals, deduces similar trends. Though the individual contributors to metabolic control may vary, the overall trend reveals that more control strength is focused on tissue level ATP supply and ATP demand processes, and less on ventilatory and cardiovascular processes (Hochachka 2003).

When the relationship between muscle ADP_{free} concentration and glycolytic flux was examined, by comparing P_i/PCr ratios to lactate concentrations at the end of incremental exercise, a direct relationship was observed with untrained subjects displaying higher P_i/PCr ratios and lactate levels, compared to the trained subject group. When this relationship was evaluated during sea level exercise before and after altitude acclimatization, or between acute and acclimatized hypoxia, the metabolic and physiological adjustments with acclimatization moved the P_i/PCr to lactate relationship of the untrained group toward those displayed by the trained group. A similar trend was observed when comparing PCr to lactate concentration, however the relationship was inverse. We would predict that when comparing different kinds of subjects in these kind of plots (normoxic lowlanders to high altitude natives, endurance to power trained athletes, altitude acclimatized to non-acclimatized individuals), their differing biochemistry and physiology should move peak lactate values up or down the general relationship. Therefore, lactate production can be considered a function of how control features are organized, and the changes in lactate

concentration with altitude exposure would consequently represent a graded response, not an all-or-nothing phenomenon.

Can metabolic adjustments be explained by activity level at altitude?

Physical activity is a major confounding variable when trying to ascertain the independent effects of prolonged hypobaric hypoxia exposure on muscle adaptability. For example, reduced activity levels of well-trained individuals at altitude may result in adjustments that counter or lessen the effects of hypoxia. Untrained individuals pose a different challenge because an increase in activity, which often occurs during a prolonged period of acclimatization when there are more activity options and available time, would make separating the specific effects of hypoxia and training extremely difficult. In normoxia, even a modest increase of activity over short periods can alter muscle composition and function (Saltin 1983). During the acclimatization period, all subjects reported that daily activity levels in terms of frequency or duration of exercise did not differ from their normal routine at sea level. However, the type of activities performed by the untrained group at altitude (i.e. hiking and mountain biking) differed from those performed at sea level (i.e. weight-lifting, recreational rugby and basketball), possibly stimulating muscle adaptations and improving exercise performance independent of the hypoxic stimulus.

Upon return to sea level, the untrained group displayed a significant increase in $\dot{V}_{O_2\,max}$ and endurance exercise time. Several hematological, cardiovascular, ventilatory and metabolic adjustments occurring with prolonged altitude exposure can account for this improvement in performance. It is expected that the rise in hemoglobin concentration that accompanies altitude acclimatization contributed significantly in enhancing exercise performance upon return to sea level (Ferretti 1992), by increasing the oxygen content of blood and alleviating the myocardial workload required to maintain oxygen delivery to the working muscle. This was observed in the untrained group as a lowering of heart rate during sea level exercise. By reducing heart rate and cardiac output, peripheral diffusion time and oxygen extraction by the muscle cell is improved. While most likely short-lived, the untrained group also displayed a continuation of the high ventilatory response during sea level exercise, contributing to an increase in alveolar oxygenation following acclimatization. If the hypothesis of this study is correct, then we would expect that 'tighter' metabolic control, shown by less of a perturbation of lactate, PCr and the P_i/PCr ratio in the untrained group, would also contribute to the increase in endurance performance upon return to sea level. Because many of the adjustments that occur with endurance training, such as a

lowering of lactate, improved energy balance, and increased exercise performance, are also observed with altitude acclimatization, we cannot rule out the possibility that the improvement in metabolite homeostasis in the untrained group was the result of a training effect (i.e. increased mitochondrial density, increased capillarization, improved lactate removal, fibre type or enzymatic changes), rather than an independent outcome of prolonged hypoxic exposure. Or possibly a change in the type of activity potentiated the effects of hypoxia within the untrained group. Concurrent measurement of muscle ultrastructure, fibre type profiles, substrate use, and other changes that are known to occur with exercise training alone would be required to help differentiate between the effects of hypoxia acclimatization and exercise training.

Under acute and acclimatized hypoxia conditions, the endurance-trained group displayed a significant decrease in exercise performance. This was not unexpected since highly trained athletes often exhibit a blunted ventilatory response (Schoene 1984) or large decreases in SaO_2 at high work rates under normoxic conditions (Dempsey 1984), making them more susceptible to a reduction in $\dot{V}_{O_2\ max}$ at altitude (Chapman 1984). Because of this decrease in oxygen transport, even though this group maintained their normal training routine at altitude, they most likely were unable to sustain the same training intensity as at sea level. This reduction in training intensity at altitude has been suggested to explain why living and training at altitude generally does not further improve sea level $\dot{V}_{O_2\ max}$ in highly trained athletes (Levine 1997, Chapman 1998), as observed with the endurance trained group in the present study. In contrast to the untrained subject group, physiological and metabolic adjustments for exercise performance were not sufficiently stimulated by prolonged hypoxia exposure or through exercise training, or these systems were already maximized.

An interesting finding was that endurance time of both groups improved with acclimatization, while $\dot{V}_{O_2\ max}$ did not change in the trained subject group. This increase in endurance exercise time, independent of changes in $\dot{V}_{O_2\ max}$, has been observed previously by Maher et al. (1974) who reported a 45% increase in endurance time at 75% $\dot{V}_{O_2\ max}$ between day 2 to 12 at 4,300m, while Horstman et al. (1980) found a 59% increase in treadmill running time at 85% $\dot{V}_{O_2\ max}$ following 16 days at the same altitude. It is important to remember that decrements in muscular performance during endurance exercise can occur well before the maximal aerobic capacity of the muscle is reached. Thus, factors suggested to regulate $\dot{V}_{O_2\ max}$, such as oxygen availability and the mitochondrial potential, are different from those affecting fatigue from endurance exercise. In healthy systems, fatigue occurs when there is an imbalance between ATP

supply and ATP demand, and results from a number of factors which are synergistic in their actions, including substrate depletions, energetic limitations, end-product accumulations, or muscle damage (Brooks 2000). The different responses of $\dot{V}_{O_2\ max}$ and endurance performance with acclimatization suggest that adaptations affecting muscular work following exposure to prolonged hypoxia may occur independently of changes in the determinants of maximal aerobic capacity.

Can we learn anything from recovery metabolism?

Whole-body $\dot{V}_{O_2}$ during maximal work has been used as the standard method of determining oxidative capacity, yet it does not provide information about the specific muscles fueling exercise. Alternatively, because oxidative metabolism dominates ATP regeneration during the initial recovery phase, the rate of PCr resynthesis has been accepted as an index of muscle oxidative capacity or mitochondrial function (Kemp 1993, Paganini 1997). PCr resynthesis rates determined in this study revealed that the oxidative capacity of the gastrocnemius muscle decreased during exercise upon ascent to altitude, returning to sea level values with altitude acclimatization. These changes could be entirely attributed to the untrained subject group, since PCr recovery rates of trained subjects were unaltered throughout the study.

The decreased rate of PCr recovery observed in the untrained subject group with acute exposure to hypoxia can be explained by a change in the metabolic state of the muscle at fatigue or by an effect of oxygen availability on mitochondrial function (Haseler 1999, Haseler 2004). Takahaski et al. (1995) measured PCr recovery rates in the quadriceps muscles of endurance trained runners and untrained controls, and determined that the recovery rate depends on exercise intensity. Exhaustive exercise that results in a greater decrease in PCr concentration, lower pH and increased ADP_{free} levels when compared to low or moderate intensity exercise was shown to delay PCr recovery. Theoretically, an increase in H^+ and ADP concentration would inhibit PCr resynthesis due to the creatine kinase equilibrium. Therefore, PCr recovery following exhaustive exercise may not be an accurate reflection of oxidative capacity, but may instead reveal the metabolic state of the cell. The acute hypoxia recovery data for the untrained group in our study was associated with lower end-exercise PCr concentrations, a slight decrease in pH, and elevated ADP_{free} levels (indicated by P_i/PCr) compared to sea level. With acclimatization, end-exercise and recovery P_i/PCr and PCr recovery rates returned to normoxia levels, suggesting that the observed change in PCr recovery with immediate and prolonged hypoxia exposure is a result a change in the cellular environment at fatigue and during the initial stage of recovery.

Haseler et al. (1999, 2004) studied the role of oxygen availability on the rate of PCr recovery in endurance trained and sedentary individuals by manipulating the fraction of inspired oxygen (normoxic, hypoxic, hyperoxic) while performing a submaximal plantar flexion exercise test. The findings of this study revealed that PCr recovery was impaired under hypoxic conditions, with the sedentary group displaying a slower rate of recovery compared to the trained subjects. These results agree with the decreased PCr recovery rate observed in the untrained group of the present study in response to acute hypoxia. Because a change in oxidative capacity in terms of mitochondrial content or structural changes cannot occur with acute hypoxia, Haseler proposed that under simulated-hypoxia conditions, PCr recovery is either limited by an alteration in the number of mitochondria recruited with an adequate supply of oxygen during recovery (discussed above in *Mechanisms accounting for improved oxidative control and reduced glycolytic flux with altitude acclimatization*), or as a result of an impairment in mitochondrial function due to a decrease in oxygen supply (ie. impaired oxygen delivery or diffusion limitation of oxygen from the blood to mitochondria). The similarity in results between experiments would suggest that the untrained group examined in our study displayed a decrease in mitochondrial function with exposure to acute hypoxia that improved with acclimatization. However, that the trained group did not display a similar decrease in the rate of PCr recovery as would be expected suggests that the exercise challenge used in the present investigation resulted in PCr recovery rates that do not accurately reflect the oxidative capacity, and instead the recovery rates reflect the metabolic state of the cell.

Several studies have shown faster PCr recovery rates in elite endurance athletes compared to sprint trained and sedentary individuals (McCully 1992, Yoshida 2002, Haseler 1999), consistent with the increased mitochondrial content and activities of enzymes associated with oxidative metabolism allowing a greater capacity for oxidative metabolism. Unexpectedly, we did not observe a difference in PCr recovery rates between the two fitness groups tested in our study, except under acute hypoxia conditions when recovery rates decreased in the untrained group. Similar experiments using high intensity exercise protocols (Cooke 1997, Takahashi 1995) also failed to reveal a difference in recovery rates between trained and untrained subject groups. Several of the untrained subjects participated in occasional recreational or power-based activities, so that individual variability in oxidative capacity or an insufficient distinction between the two fitness groups may also account for the lack of a significant difference in recovery rates between the trained and untrained groups of this study.

The key finding of this study was that the metabolic and physiological responses of trained and untrained subjects differed following a 3-week stay at 3,800 m, possibly accounting for differences in the lactate response with altitude acclimatization. Untrained individuals displayed an improvement in energy balance associated with lower lactate levels during exercise when comparing pre- to post- acclimatization, and acute to acclimatized hypoxia conditions. While the mechanism for this change is uncertain, the benefits clearly involve an improvement in metabolite homeostasis at fatigue, quicker exercise recovery, and a decrease in the activation of the energetically inefficient glycolytic pathway. In contrast, the levels of high-energy phosphates and indicators of oxidative control did not change in the trained subject group throughout the study, and the persistence of the lactate paradox was not observed upon return to sea level. In this group, changes in lactate production were a temporary response to exercise at altitude, and not indicative of metabolic adaptation. These findings suggest that the pre-existing metabolic organization of endurance-trained individuals, along with known compensatory mechanisms that maintain oxygen delivery and oxidative phosphorylation, were sufficient to cope with the imposed oxygen limitation of moderate altitude, or that a greater hypoxic stimulus was required to activate a metabolic change. In addition to the changes in energy balance and exercise lactate concentration, the untrained group displayed several significant adjustments, normally seen following endurance training, and not with altitude acclimatization. In particular, the increased rate of lactate recovery and lower heart rate during exercise, to similar levels observed by the trained subject group following acclimatization, suggest that an effect of training, either alone or in addition to the hypoxic stimulus, may be responsible for the metabolic changes observed in the untrained subject group. These findings draw to attention the importance of assessing individual variability and monitoring physical activity when studying the effects of hypobaric hypoxia on muscle metabolism. While a moderate altitude of acclimatization was chosen for the current study to minimize complications associated with decreased muscle mass, loss of appetite, acute mountain sickness, or an inability to maintain regular activity, future studies using highly trained individuals may require a greater hypoxic stimulus when studying metabolic changes with acclimatization. Furthermore, additional testing of groups clearly representing different physiological and biochemical states (power trained, endurance trained, sedentary, high altitude natives) is required to confirm the direct relationship between lactate and ADP_{free} concentration during exercise, and the dependence of lactate production on the organization of metabolic control contributors.

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APPENDIX 1: *Individual descriptive data for all subjects.*

subject	age (years)	height (cm)	weight (kg)			
			PRE	AH	ACC	POST
1	20	178	94.9	93.2	90.9	93.0
2	20	186	74.9	75.0	74.5	77.8
3	24	174.5	87.9	83.0	85.4	87.3
4	24	177	57.9	60.0	58.2	58.8
5	28	177	65.8	64.9	61.8	64.3
6	27	182	65.0	63.2	64.1	64.7
7	29	183	77.2	77.3	77.3	80.3
8	27	173	65.1	65.3	64.5	66.0
9	23	184	81.1	81.4	80.9	82.7
10	21	173	71.8	72.3	70.0	72.8

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 2: *Individual whole-body work capacity values during incremental cycling exercise at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

	test period	individual data									
		1	2	3	4	5	6	7	8	9	10
work (watts)	PRE	370	372.5	380	322.5	322.5	375	450	330	420	367.5
	AH	340	345	330	275	285	320	390	330	350	330
	ACC	312.5	385	350	290	300	332.5	435	337.5	407.5	352.5
	POST	382.5	420	400	332.5	335	380	480	345	457.5	390
$\dot{V}_{O_2\ max}$ ($L.min^{-1}.kg^{-1}$)	PRE	41.7	50.9	48.0	50.2	44.0	64.2	59.9	59.2	63.3	59.4
	AH	39.0		45.0	47.5	44.2	59.8	50.5	55.4		53.7
	ACC	39.4	51.6	39.3	48.4	43.3	54.2	57.0	53.6	57.3	50.7
	POST	47.1	57.5	51.3	56.8	49.6	66.9	63.9	56.1	62.9	62.4

Work, maximum cycling workload; $\dot{V}_{O_2\ max}$, relative maximal O_2 uptake (graded cycle exercise). The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 3: *Individual peak cardiorespiratory values during incremental cycling exercise. Exercise was performed at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

	test period	individual data									
		1	2	3	4	5	6	7	8	9	10
TV (L/breath)	PRE	3.803	3.537	3.396	2.584	2.497	3.311	3.979	2.957	4.919	3.581
	AH	3.944	3.419	3.184	3.061	2.334	3.398	3.343	2.847	6.213	3.685
	ACC	3.378	3.302	3.135	3.016	2.468	3.034	3.492	2.896	5.35	3.182
	POST	3.726	3.880	3.051	2.796	2.608	3.236	3.907	2.706	4.973	3.282
Rf (brth/min)	PRE	56.4	51.7	61.2	58.4	44.9	60.0	47.7	45.8	52.7	68.0
	AH	65.0	72.9	69.3	55.1	49.0	69.0	59.6	59.2	42.7	69.8
	ACC	66.4	65.4	68.3	72.1	60.7	74.1	55.5	64.7	44.3	76.5
	POST	55.6	50.0	63.6	57.5	47.8	55.3	56.0	53.1	53.8	67.1
RER	PRE	1.26	1.30	1.26	1.32	1.32	1.22	1.25	1.19	1.22	1.33
	AH	1.14	1.00	1.04	1.04	1.04	0.85	1.09	1.00	0.87	1.14
	ACC	1.02	1.12	1.05	1.19	1.20	1.24	1.15	1.14	1.28	1.27
	POST	1.11	1.24	1.17	1.20	1.27	1.16	1.19	1.10	1.16	1.15
HR (beats/min)	PRE	189	182	188	188	190	192	171	185	198	183
	AH	181	185	186	168	184	175	167	178	183	191
	ACC	172	188	184	173	184	162	170	168	178	179
	POST	185	187	185	178	191	181	177	168	195	181

TV, tidal volume; Rf, respiratory rate; HR, maximum heart rate; RER, respiratory exchange ratio at fatigue. The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 4: *Individual steady-state (12 and 15 minute average) cardiorespiratory values during submaximal cycling exercise corresponding to 70% relative $\dot{V}_{O_2\,max}$. Exercise was performed at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

	test period	Individual data									
		1	2	3	4	5	6	7	8	9	10
TV (L/breath)	PRE	2.871	2.459	2.297	1.897	1.893	3.120	2.137	2.214	3.348	2.394
	AH	2.671	2.063	1.907		1.775	2.218	2.107	2.160	4.094	2.827
	ACC	2.308	2.239	2.001	1.713	1.654	2.343	2.403	2.697	3.543	2.258
	POST	2.720	3.021	2.318	1.861	1.770	2.906	2.825	1.748	3.486	2.261
Rf (breath/min)	PRE	26.7	30.2	34.3	32.6	24.4	23.8	34.5	39.5	32.9	41.0
	AH	35.4	35.4	42.3		31.9	28.6	31.7	32.5	27.8	30.5
	ACC	38.6	37.7	41.1	41.7	36.7	34.7	34.0	36.3	35.8	41.2
	POST	34.3	33.2	39.4	37.0	34.3	29.6	42.6	30.6	35.8	45.7
RER	PRE	0.98	0.96	0.98	0.96	1.01	1.00	1.03	1.03	0.96	0.98
	AH	0.90	0.69	0.73		0.76	0.64	0.86	0.75	0.63	0.83
	ACC	0.79	0.84	0.88	0.84	0.87	1.00	1.00	0.94	1.06	0.90
	POST	1.03	1.05	1.00	0.98	1.00	0.98	1.01	1.00	0.97	0.91
HR (beats/min)	PRE	142.5	155.5	145.0	166.0	161.5	170.0	134.0	160.0	178.0	161.0
	AH	152.5	163.5	142.5		163.5	145.5	133.5	156.5	156.0	152.5
	ACC	146.0	156.5	149.0	141.5	156.5	141.0	143.0	143.5	157.0	150.0
	POST	152.5	167.0	147.5	149.5	159.0	159.0	151.5	152.0	164.0	152.5

TV, tidal volume; R*f*, respiratory rate; HR, maximum heart rate; RER, respiratory exchange ratio at fatigue. The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 5: *Individual plasma lactate concentration during nine minutes (0, 90, 180 and 270 watts) of incremental cycling exercise, up to a workload of 270 watts. Exercise was performed at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

testing period	work (watts)	individual plasma [lactate] (mM)									
		1	2	3	4	5	6	7	8	9	10
PRE	0	1.53	1.12	1.85	1.94	0.90	1.64	1.48	1.93	1.56	1.40
	90	1.66	1.07	1.48	1.74	1.68	2.32	1.54	1.09	1.62	1.54
	180	2.09	1.56	1.83	2.66	3.44	2.52	1.66	2.74	1.64	1.81
	270	4.32	2.68	3.90	5.81	5.74	3.40	2.20	4.25	2.57	3.10
AH	0	1.52	0.96	1.52	1.44	1.76	1.56	2.40	1.56	2.76	1.26
	90	1.59	1.14	1.82	1.89	1.74	1.75	2.02	1.80	0.80	1.58
	180	2.38	1.53	2.40	2.44	5.07	1.95	2.23	2.64	1.13	2.35
	270	5.71	3.52	4.00	5.10	8.86	3.78	2.40	5.17	3.45	4.04
ACC	0	2.00	1.22	3.03	2.62	1.86	1.36	2.52	1.24	2.01	2.44
	90	0.73	1.40	2.60	2.49	1.94	1.78	2.22	1.82	2.10	1.61
	180	1.27	2.14	3.07	2.96	3.48	2.19	2.80	2.18	2.17	2.17
	270	3.76	4.68	5.00	3.73	6.05	3.79	2.68	2.89	3.24	3.44
POST	0	1.70	0.96	2.05	1.48	1.98	1.54	1.92	1.18	1.46	2.02
	90	1.29	1.29	2.66	1.98	1.30	1.62	2.27	0.93	1.73	1.87
	180	1.76	1.60	2.15	2.94	2.04	1.96	2.42	1.28	2.11	2.32
	270	2.49	3.02	3.22	4.90	4.14	3.14	2.56	2.44	3.34	3.68

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 6: *Individual plasma lactate concentration at fatigue from maximal exercise and during steady state submaximal (70% relative $\dot{V}_{O_2\ max}$) exercise at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

lactate (mM)	test period	individual plasma [lactate] (mM)									
		1	2	3	4	5	6	7	8	9	10
fatigue	PRE	10.46	4.87	11.71	9.72	7.48	7.85	11.00	6.27	10.50	7.00
	AH	9.12	6.76	9.71	5.10	11.29	5.14	7.18	6.94	7.88	9.25
	ACC	5.52	11.29	9.00	5.00	7.43	6.46	6.77	5.82	8.00	9.40
	POST	12.00	9.74	13.34	9.41	9.2	7.33	9.68	4.95	13.47	9.50
submax	PRE	5.33	4.98	3.08	5.87	5.22	5.66	1.88	4.50	4.18	7.39
	AH	6.63	6.01	3.75	4.66	7.67	3.57	1.75	6.56	5.61	5.95
	ACC	3.32	4.80	2.76	3.99	4.94	3.54	3.02	4.80	8.77	3.02
	POST	5.86	7.97	3.33	4.36	5.85	5.67	2.75	4.33	6.35	5.66

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 7: *Individual plasma lactate concentration during 15 minutes of submaximal (70% relative $\dot{V}_{O_2\,max}$) cycling exercise, at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

testing period	time (mins)	individual plasma [lactate] (mM)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
PRE	0	1.26	0.84	1.26	2.18	1.00	1.14	1.44	0.86	0.59	2.04
	4	3.98	3.86	4.74	5.28	4.44	4.33	2.68	3.72	3.78	4.80
	8	5.71	5.00	4.29	7.53	5.41	6.10	2.24	4.61	4.08	7.42
	12	5.44	5.09	3.26	5.95	5.22	5.92	1.82	4.30	4.00	7.14
	15	5.22	4.86	2.89	5.78	5.22	5.40	1.94	4.70	4.36	7.63
AH	0	1.50	0.94	2.10	1.14	1.16	1.16	1.84	1.18	1.32	1.30
	4	2.44	2.52	3.06	2.79	2.38	2.54	2.36	2.08	3.24	1.94
	8	4.07	6.12	4.08	3.78	5.68	3.59	2.44	4.82	3.60	5.26
	12	6.13	6.32	3.84	4.84	7.32	3.48	1.94	6.77	6.05	6.00
	15	7.12	5.70	3.65	4.48	8.01	3.65	1.56	6.35	5.17	5.89
ACC	0	1.86	1.34	1.52	1.35	1.68	1.08	4.74	1.04	1.76	0.88
	4	2.16	4.52	2.38	2.44	3.40	2.08	4.29	2.38	2.88	2.24
	8	3.08	5.72		4.42	4.62	3.26	3.26	3.36	6.71	
	12	3.42	4.90	2.82	4.28	5.08	3.64	2.88	4.68	8.85	2.78
	15	3.21	4.69	2.69	3.70	4.80	3.44	3.16	4.92	8.69	3.08
POST	0	1.08	1.12	0.80	1.54	1.88	1.12	1.50	1.18	0.90	1.16
	4	3.74	3.64	2.80	4.64	3.25	2.70	3.44	3.49	5.98	4.18
	8	6.18	6.76	3.21	4.88	5.02	5.56	2.86	4.33	6.80	7.20
	12	5.96	7.84	3.30	4.64	5.91	5.74	2.77	4.37	6.48	5.79
	15	5.76	8.10	3.36	4.08	5.78	5.60	2.73	4.28	6.21	5.53

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 8: *Individual plasma lactate concentrations during 30 minutes of recovery from maximal exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery), at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

testing period	time (mins)	individual plasma [lactate] (mM)									
		1	2	3	4	5	6	7	8	9	10
PRE	0	10.46	4.87	11.71	9.72	7.48	7.85	11.00	6.27	10.50	7.00
	1	17.24	5.24	15.74	11.52	13.71	11.28	11.81	9.96	15.85	7.68
	2	17.06	13.46	17.22	11.89	16.00	12.88	12.54	11.52	17.88	13.53
	3	16.52	13.24	18.04	14.92	17.23	13.18	13.62	11.82	17.88	13.64
	5	16.58	14.04	18.51	16.58	18.16	14.98	12.88		18.49	
	8	15.10	13.80	18.35	17.87	17.03	14.16	10.78	13.17	17.67	10.95
	14	12.31		16.48	16.02	16.17	12.12	9.00	10.18	13.97	13.10
	20	9.02	10.10	13.02	14.20	14.03	9.20	7.21	10.20	10.64	12.39
	30	5.30	7.49	9.52	11.10	10.80	6.54	5.58	6.92	7.01	9.73
AH	0	9.12	6.76	9.71	5.10	11.29	5.14	7.18	6.94	7.88	9.25
	1	13.99	7.26	11.28	8.64	13.79	8.13	7.96	8.15		10.76
	2	15.40	12.22	18.28	10.34	15.82	11.10	9.39	9.83	12.18	8.38
	3	15.02	14.86	18.88	10.90	17.94	10.87	8.86	14.72	14.64	13.62
	5	15.36	15.52	19.08	11.58	16.13	11.39	8.04	14.05	14.42	14.92
	8	15.26	15.79	16.58	12.93	13.03	11.88	7.40	12.57	10.90	16.44
	14	11.63	14.49	17.28	10.30	11.90	9.84	7.82	10.10	10.74	13.80
	20	9.88	11.96		8.23	14.61	7.16	6.27	8.20	9.07	12.10
	30	3.16	8.31		5.45	8.08	6.22	4.23	5.21	6.18	8.76
ACC	0	5.52	11.29	9.00	5.00	7.43	6.46	6.77	5.82	8.00	9.40
	1	5.69	14.76	11.19	7.62	12.31	8.00	10.40	9.13	13.66	15.40
	2	6.32	15.03	13.07	8.82	12.66	10.20	12.93	10.17	16.72	14.50
	3	7.46	15.35	13.92		11.92	11.90	14.50	12.36	16.06	15.24
	5	12.96	15.45	14.69	14.30	12.72	12.12	15.04	12.95	17.19	17.55
	8	10.23	15.71	16.70	10.16	12.74	11.17	11.22	10.34	16.30	15.78
	14	8.00	14.04	11.07	11.48	14.14	8.36		6.49	13.76	13.36
	20	5.47	11.08	9.68	8.10	12.66	5.86	8.75	5.64	12.83	11.28
	30	3.12		6.02	5.84	9.45	4.12	6.09	3.44	8.46	7.52
POST	0	12.00	9.74	13.34	9.41	9.2	7.33	9.68	4.95	13.47	9.50
	1	14.28	14.74	14.48	10.43	9.66	9.12	12.20	6.16	16.30	16.98
	2	14.90	15.10	15.72	12.10	11.24	10.35	13.27	8.92	18.74	17.29
	3	14.56	15.68	16.18	13.42	13.04	11.78	13.17	8.72	18.71	17.21
	5	13.80	15.14	15.94	14.18	15.62	13.69	13.00	7.73	19.74	16.68
	8	13.18	13.60	13.84	13.72	15.95	12.55	10.82	5.88	18.52	14.42
	14	9.94	11.86	10.88	11.44	15.69	9.44	8.36	3.90	14.86	12.15
	20	7.22	8.80	8.36	9.33	13.46	7.50	6.72	2.66	12.09	9.42
	30	4.00	4.46	4.81	5.82	8.84	5.21	5.02	1.76	6.88	6.04

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 9: *Individual recovery lactate data (time to peak, peak lactate concentration, and slope of linear regression) during 30 minutes of recovery from incremental exercise (5 minutes active recovery at 30 watts, 25 minutes passive recovery), at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC). N=10 for all testing periods.*

	test period	individual data									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
peak lactate time (min)	PRE	5	5	5	8	4	5	3	7	5	3
	AH	4	4	5	5	3	4	2	3	3	8
	ACC	5	7	5	4	11	5	4	4	4	4
	POST	2	3	3	4	11	5	3	2	5	2
peak [lactate] (mM)	PRE	16.58	14.04	18.51	17.87	18.26	14.98	13.62	13.94	18.49	13.64
	AH	15.53	16.14	19.08	11.58	17.94	12.30	9.39	14.72	14.64	16.44
	ACC	12.96	15.71	14.69	14.81	14.87	12.12	15.07	13.28	17.48	17.55
	POST	14.90	15.68	16.18	14.18	16.20	13.69	13.53	8.92	19.74	17.29
slope (%Δ per min)	PRE	-2.83	-2.02	-2.08	-1.74	-1.60	-2.35	-2.25	-2.21	-2.62	
	AH	-2.90	-1.90	-0.92	-2.25	-1.40	-2.18	-1.60	-2.44	-2.25	-2.04
	ACC	-2.88	-2.66	-2.44	-2.42	-1.85	-2.76	-1.94	-2.72	-2.10	-2.25
	POST	-2.81	-2.61	-2.71	-2.37	-2.44	-2.40	-2.43	-2.85	-2.69	-2.49

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 10: *Individual phosphocreatine (relative to resting PCr concentration) during recovery (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery) from incremental exercise using a foot ergometer. Exercise was performed while breathing normoxic air before (PRE) and after (POST) altitude acclimatization, and while breathing hypoxic air before (AH) and after (ACC) acclimatization.*

testing period	time (min)	individual recovery PCr (relative to resting PCr concentration)									
		1	2	3	4	5	6	7	8	9	10
PRE	0	0.157		0.058	0.266	0.166	0.224	0.859	0.151	0.383	0.218
	1	0.271		0.243	0.331	0.460	0.192	0.784	0.355	0.556	0.562
	2	0.452		0.536	0.985	0.632	0.433	0.825	0.504	0.737	0.802
	3	0.485		0.689	1.206	0.767	0.501	1.075	0.641	0.823	0.925
	6	0.643		0.842	0.928	0.811	0.758	1.089	0.803	0.943	1.045
	10	0.788		0.923	0.614	0.910	0.790	1.096	0.915	0.960	1.234
	15	0.837		0.864	0.985	0.881	0.859	0.934	0.822	0.958	1.181
AH	0	0.146	0.029	0.264	0.155	0.141	0.350	0.361	0.234	0.188	0.200
	1	0.191	0.062	0.397	0.327	0.290	0.608	0.768	0.354	0.421	0.378
	2	0.309	0.268	0.491	0.551	0.422	0.756	0.799	0.557	0.739	0.717
	3	0.401	0.565	0.628	0.550	0.580	0.889	0.801	0.557	0.633	0.664
	6	0.535	0.667	0.881	0.664	0.751	0.823	0.923	0.809	0.752	0.835
	10	0.843	0.763	0.936	0.856	0.862	0.860	0.853	0.920	0.934	0.726
	15	0.830	0.950	1.014	0.872	0.883	0.967	0.934	0.926	0.900	1.042
ACC	0	0.181	0.362	0.103	0.339	0.372	0.203	0.313	0.006	0.327	0.329
	1	0.441	0.488	0.186	0.453	0.537	0.490	0.730	0.510	0.478	0.388
	2	0.547	0.693	0.421	0.665	0.741	0.753	0.760	0.650	0.607	0.544
	3	0.695	0.945	0.586	0.786	0.708	0.770	1.048	0.747	0.649	0.706
	6	0.842	0.867	0.830	0.833	0.905	0.627	0.978	0.834	1.005	0.762
	10	0.986	1.026	0.936	0.980	0.897	0.940	1.040	0.871	1.131	0.925
	15	1.017	0.912	0.933	0.885	1.031	0.846	1.015	0.780	1.063	0.978
POST	0	0.256	0.140	0.167	0.281	0.199	0.454	0.289	0.260	0.319	0.192
	1	0.388	0.482	0.492	0.376	0.372	0.615	0.619	0.572	0.564	0.218
	2	0.693	0.642	0.714	0.601	0.537	0.934	0.891	0.712	0.651	0.498
	3	0.739	0.790	0.762	0.626	0.741	1.004	0.878	0.764	0.725	0.613
	6	0.916	0.939	1.013	0.869	0.911	0.807	0.981	0.901	0.832	0.779
	10	0.917	1.013	0.964	0.941	0.912	1.057	0.953	0.973	0.965	0.971
	15	0.995	0.984	1.001	1.069	0.878	1.164	0.947	1.051	0.969	0.955

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 11: *Rate constants for PCr recovery from incremental plantar flexion exercise performed while breathing normoxic air before (PRE) and after (POST) altitude acclimatization, and while breathing hypoxic air before (AH) and after (ACC) acclimatization.*

test period	individual rate constant (min)									
	1	2	3	4	5	6	7	8	9	10
PRE	0.1798		0.4033	0.5627	0.4908	0.2473	0.8088	0.3608	0.4482	0.5537
AH	0.1093	0.2472	0.2883	0.1998	0.3462	0.7160	0.6648	0.3447	0.3457	0.4178
ACC	0.1565	0.4277	0.3248	0.3773	0.4118	0.4177	0.8400	0.5813	0.1948	0.1583
POST	0.4268	0.6058	0.4728	0.2258	0.3908	0.2633	0.7388	0.4412	0.3207	0.2777

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 12: *Individual pH during recovery (5 minutes active recovery at 30% maximum workload, 10 minutes passive recovery) from incremental plantar flexion exercise. Exercise was performed while breathing normoxic air before (PRE) and after (POST) altitude acclimatization, and while breathing hypoxic air before (AH) and after (ACC) acclimatization.*

test period	time (min)	individual pH									
		1	2	3	4	5	6	7	8	9	10
PRE	0	6.43		6.50	6.52	6.50	6.27	6.96	6.54	6.52	6.29
	1	6.50		6.58	6.66	6.53	6.27	7.03	6.39	6.44	6.52
	2	6.46		6.54	6.70	6.64	6.27	6.99	6.34	6.48	6.43
	3	6.57		6.54	6.63	6.93	6.35	6.60	6.34	6.52	6.91
	6	6.57		6.61	6.63	6.63	6.51	6.63	6.50	7.01	6.55
	10	6.95		6.56	6.77	7.48	7.33	6.96	6.78	6.92	6.56
	15	6.89		7.23	6.94	7.21	7.33	6.99	7.03	7.01	6.27
AH	0	6.64	6.25	6.62	6.34	6.30	6.40	6.57	6.78	6.60	5.89
	1	6.57	6.25	6.66	6.25	6.34	6.40	6.60	6.78	6.60	6.03
	2	6.60	6.25	6.62	6.34	6.34	6.36	6.60	6.85	6.46	6.07
	3	6.60	6.34	6.69	6.34	6.30	6.40	6.52	7.43	6.45	6.14
	6	6.71	6.64	6.73	6.46	6.68	7.15	6.77	6.77	6.84	7.00
	10	6.86	7.55	7.12	6.95	7.14	7.04	7.10	7.07	7.04	6.49
	15	6.99	6.99	7.21	6.88	6.95	7.00	6.99	6.99	7.10	6.77
ACC	0	6.32	6.36	7.17	6.43	6.33	6.54	6.64	6.86	6.54	6.72
	1	6.40	6.36	6.56	6.47	6.32	6.43	6.61	6.75	6.54	6.69
	2	6.36	6.40	6.59	6.50	6.32	6.50	6.61	6.79	6.54	6.90
	3	6.40	6.35	6.63	6.54	6.70	6.63	6.68	6.86	6.54	6.96
	6	6.55	6.56	6.63	6.85	6.71	6.95	6.71	6.71	6.58	6.83
	10	6.85	6.94	7.06	7.03	7.06	6.86	7.11	7.30	7.20	7.23
	15	6.91	7.08	7.08	7.07	6.97	7.12	6.97	7.46	7.08	7.08
POST	0	6.64	6.31	6.78	6.38	6.41	6.45	6.92	6.63	6.44	6.51
	1	6.57	6.27	6.75	6.34	6.33	6.48	6.68	6.56	6.48	6.58
	2	6.54	6.17	6.75	6.34	6.32	6.45	6.68	6.56	6.40	6.51
	3	6.50	6.75	6.68	6.34	6.32	6.56	7.03	6.60	6.48	6.54
	6	7.29	6.20	6.61	6.46	6.82	7.02	6.96	7.16	6.59	6.51
	10	7.07	7.06	7.23	6.99	6.98	7.04	6.79	7.26	7.08	7.11
	15	7.16	7.27	7.11	7.06	6.97	7.21	7.03	7.17	7.30	7.07

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.

APPENDIX 13: *Exercise performance during exercise performed at 90% pre-acclimatization $\dot{V}_{O_2\ max}$ at sea level before (PRE) and following (POST) altitude acclimatization, and at 3,800m upon arrival (AH) and after 3 weeks of acclimatization (ACC).*

	test period	individual data								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**
time to fatigue (min)	PRE	3.57	3.97	4.58	5.43	5.85	3.15	4.08	4.62	4.68
	AH	3.23	2.67	2.55	2.67	3.85	1.25	2.37	3.33	2.78
	ACC	3.45	4.23	3.45	4.17	4.58	1.92	2.97	4.77	2.68
	POST	4.85	8.58	7.47	10.03	10.00	4.65	5.00	6.55	4.03

The untrained subject group consists of individual subjects 1 through 5; the trained subjects are numbered 6 through 10.