ROLE OF IONIC BUFFERING IN THE RELATIONSHIP BETWEEN RECOVERY AND FATIGABILITY

by

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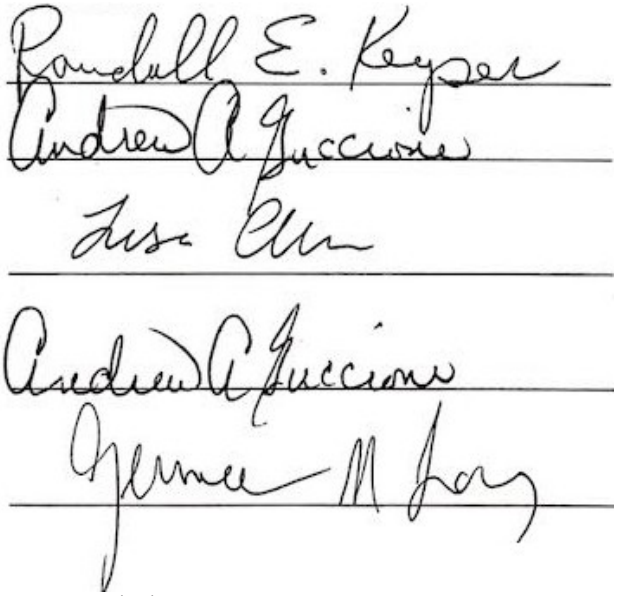

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DEDICATION

This is dedicated my friends and family who have supported me throughout this entire process.

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LIST OF ABBREVIATIONS

ADL – activity of daily living
AET – aerobic exercise training
AT – anaerobic threshold
AT-VO_2 – VO_2 at the anaerobic threshold
AT-VCO_2 – VCO_2 at the anaerobic threshold
AT-W – watts at the anaerobic threshold
ATP – adenosine triphosphate
BMI – body mass index
bpm – beats per minute
CO_2 – carbon dioxide
CPET – cardiopulmonary exercise test
CWRT – continuous work rate test
Electrocardiogram – EKG
End1, End2 – total endurance test time
HR – heart rate
HRR – heart rate reserve
K – rate constant
Kt – transition constant
MRT – mean response time
nmVCO_2 – non-metabolic carbon dioxide
ORI – oxidative response index
pkCPET – peak cardiopulmonary exercise test
pk-HR – peak heart rate
pk-VCO_2 – peak rate of carbon dioxide expiration
pk-VO_2 – peak rate of oxygen consumption
pk-W – peak watts
RER – respiratory exchange ratio
RMR – resting metabolic rate
ROS – reactive oxygen species
RPM – revolutions per minute
SD – standard deviation
td – time delay
τ – tau
Ve – minute ventilation
VCO_2 – rate of carbon dioxide expiration
VO_2 – rate of oxygen consumption

W – watts
WR – work rate
ΔVO_2 – change in VO_2 amplitude
ΔVCO_2 – change in VCO_2 amplitude

ABSTRACT

ROLE OF IONIC BUFFERING IN THE RELATIONSHIP BETWEEN RECOVERY AND FATIGABILITY

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Purpose: This study aimed to characterize the role of ionic buffering capacity, as measured by non-metabolic expired carbon dioxide (nm-$\dot{V}CO_2$), in the relationship between recovery and performance fatigability. **Methods**: Twenty healthy adults (men, n=9, age =44.7±13.9 years; women, n=11, age=50.3±11.1 years) completed peak cardiopulmonary exercise (CPET) and submaximal constant work rate tests (CWRT) on the cycle ergometer on separate days before and after a vigorous, 4-week aerobic exercise training (AET) regimen. Each test was followed by 10-minutes of passive recovery and an endurance test at 70% of the peak watts attained during the CPET. Performance fatigability was measured by the endurance test durations following both the peak CPET (End1) and CWRT (End2), peak CPET time (pk-Time), and peak CPET watts (pk-Watts). Metabolic indices were total $\dot{V}CO_2$ (t-$\dot{V}CO_2$), metabolic $\dot{V}CO_2$ (m-$\dot{V}CO_2$), and non-metabolic $\dot{V}CO_2$ (nm-$\dot{V}CO_2$), and recovery capacity indices were $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic oxidative response index (ORI). Data were analyzed using paired t-tests,

correlations, and regressions and compared before and after AET. **Results**: Significant improvements in recovery ($\dot{V}O_2$ -off ORI 7.48±7.52 ml/s, $p<0.001$ and $\dot{V}CO_2$-off ORI 4.11±5.05 ml/s, $p<0.001$) and performance fatigability measures (End 1 265±337 s ; End2 321±392 s ; pk-Time 63±40 s, $p<0.001$; pk-Watts 24±19, $p<0.001$) were observed after AET, along with significant increases in t-$\dot{V}CO_2$ (1512±2225 ml, $p<0.01$) and nm-$\dot{V}CO_2$ (608 ± 666 ml, $p<0.001$). However, a significant change in m-$\dot{V}CO_2$ (904±2255 ml, $p=0.089$) was not observed. Relationships between measures of recovery and PF measures were observed, although the strengths of the relationships were diminished (pk-Time, pk-Watts) or became non-significant (End1, End2) after controlling the analyses for the effect of nm-$\dot{V}CO_2$. **Conclusion**: The current study suggests that ionic buffering capacity may moderate the relationship between recovery and performance fatigability. These results could have implications regarding AET induced buffering dynamics and its role in fatigue resistance when performing physical activities above moderate intensities.

CHAPER ONE

THE INTRODUCTION

I. Fatigue

Experienced by individuals of all ages and unconfined to those with acute or chronic illnesses[1–4], fatigue has the ability to negatively impact daily, occupational, and social functioning, ultimately disrupting overall quality of life.[3] An estimated 38% of community dwelling individuals and 42% of primary care patients[2] report significant fatigue making it one of the most common complaints voiced in primary care settings.[4] Furthermore, studies have estimated between 5-45% of the population suffer from debilitating fatigue and between 2-11% report fatigue lasting a minimum of 6 months.[5] In older adults, fatigue is one of the most commonly reported symptoms, especially among those with chronic conditions[6] and as persons aged 65 years and older represent the fastest growing age-group of the total population[7], reports of fatigue in this population have the potential to increase concomitantly. Among individuals with chronic medical conditions, such as cancer, cardiovascular disease, autoimmune disease, osteoarthritis, and neurologic disease[5,8–11] severe fatigue is highly prevalent, with rates between 40-74% and consequences such as long-term disability needs.[12] Demographically, prevalence estimates of fatigue have been found to be higher in women and minorities in the middle-aged and older adult populations.[13]

Despite the apparent epidemiologic importance of fatigue, the complex concept has challenged researchers and clinicians alike for decades. The understanding of fatigue in the literature has been hindered by several issues that have collectively contributed to the fragmentation of its body of work and inhibited progress towards improved management in clinical practice.

A. Defining Fatigue

One of the central obstacles hindering the synthesis and collective advancement of fatigue research lies in its lack of a uniform definition and that one definition holds standard across all disciplines. This has created ambiguities and has the potential to silo those researchers and health professionals with the unified goal of improving fatigue management. Due to complex and multidimensional nature of the construct, numerous definitions of fatigue exist and may vary across disciplines, across studies, and between investigators. Despite this variation, fatigue definitions often fit into one of the following models.

i. Energy Utilization Model

Many definitions of fatigue found throughout the literature are centered around the theoretical premise that fatigue is a disorder of energy balance resulting in a subjective report of low mental or physical energy.[6,14] For example, Eldadah[15] in 2010 defined fatigue as "a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual or desired

activities." Similarly, Ricci et al[16] in 2009 utilized "a feeling of weariness, tiredness or lack of energy" as the definition of fatigue. While definitions of fatigue that fall into this category suggest that energy imbalance may lead to the subjective reports of low or decreased energy, the specific physiological processes underlying the energy imbalance may differ. For example, it has been suggested that energy imbalance is rooted in metabolic theory using a model of finite energy availability.[6] Succinctly put, the energy required to maintain life at rest, or the resting metabolic rate (RMR), which consumes 60-70% of all the energy consumed by an healthy individual one day, leaving a specific amount of energy available for cognitive and physical activity.[6] In the presence of pathology or physiologic dysregulation, the RMR is increased along with the increased efforts aimed at maintaining homeostasis, leaving less energy for physical activity, such as those required to perform activities of daily living (ADL).[6] Alternatively, the disruption of function or decline in number of mitochondria could also mechanistically result in energy disorder. As mitochondria are the energetic machineries of all human cells, age-related declines in mitochondrial oxidative and phosphorylation activity[17], mutations, and the susceptibility to reactive oxygen species (ROS)[6] could attenuate energy levels.

ii. Central Fatigue Model

Central fatigue is thought to originate in the central nervous system (CNS) and is associated with alterations within the brain and spinal cord that reduce the neural

input or drive to the skeletal muscle. Thus, muscle function and performance are affected by factors within the CNS and not at the peripheral muscle level. For example, neurophysiologic studies in healthy subjects suggest that changes in motor cortex and spinal excitability are associated with fatigability during motor tasks, as evidenced by transcranial magnetic stimulation (TMS).[5] Additionally, McMorris et al[18] proposed a model of exercise-induced fatigue based on motivation and the interoception control system to explain perceived fatigue.

Central fatigue itself has many sub-definitions that may contribute to the overall lack of clarity in fatigue definitions. For example, central fatigue can be used to refer to the effects of fatigue on the CNS, decrements in performance on a cognitive tasks, change in motivation or drive, CNS causes of fatigability, or subjective reports of fatigue in general.[5] Though, all have the fundamental theoretical basis that fatigue originates in the CNS.

iii. Peripheral Fatigue Model

As opposed to central fatigue, peripheral fatigue is defined as a decline in muscle function that originates from non-central nervous system mechanisms and is a result of over activity.[19] The peripheral fatigue model is rooted in the idea that fatigue is mediated by alteration of cross-bridge function within the muscle cell contractile apparatus.[19] Ionic metabolite accumulation, primarily hydrogen ions (H^+) and inorganic phosphate (Pi), as a result of increased reliance on anaerobic metabolism to supplement aerobic metabolism above the anaerobic threshold,

inhibits cross bridge formation and activation through decreases in intracellular pH.[19–21] Ultimately, it is the direct effects on the myofilaments of the contractile apparatus of the muscle cell and inhibition of oxidative phosphorylation that lead to deleterious functional changes such as decreased performance.[19,21]

iv. Psychosocial Model

Fatigue due to a lack of enthusiasm or motivation is the theoretical basis for the psychosocial model of fatigue.[22] Noakes et al[23] proposed that fatigue occurs due to a decision at the subconscious level to avoid damage or death that may occur during exercise. Thus fatigue is perceived and activity is ceased. Ultimately, the psychosocial model of fatigue is intimately linked to the central fatigue model in that both relate fatigue to a decrease in motor control activation.[22]

These models listed above are ones more commonly found throughout the literature, though are not all-inclusive. In addition to these models, fatigue it is often dichotomized categorically through classifications of normal and pathological (disease models) or as acute and chronic. Ultimately, these models of fatigue and their specific definitions are a result of a reductionist approach taken by researchers to help further our understanding of fatigue. Consequently, the large number of fatigue definitions silos research fatigue making it difficult to create a comprehensive collection of insight into the phenomenon.

B. Fatigue Prevalence Estimates

Determining accurate estimates of fatigue prevalence is another issue impeding fatigue understanding. Inconsistencies in measurement of self-reported fatigue are evidence by the large discrepancy in fatigue estimates. For example, fatigue estimates among adults can vary from approximately 5%-50%. Furthermore, some studies have shown no differences in fatigue reports among older adults and younger adults.[24] This is likely due to the phenomena of self-pacing in which individuals titrate their level of activity in order to maintain perceived fatigue within an acceptable range.[15,24] Thus, they may be more fatigable but not perceive so as they have reduced their overall activity level altering our ability to accurately estimate fatigue prevalence.

C. Operationalizing Fatigue

Closely related to inaccuracies in prevalence estimates is another issue hampering the advancement of fatigue literature, the difficulty that lies in objectively operationalizing fatigue. Self-reports of fatigue fail to capture or illuminate the context in which the fatigue symptom is in. For example, if one individual has a full-time job requiring significant walking or standing and watches her grandchildren after work and another individual is retired and able to participate in recreational hobbies that are a mix of standing and sitting throughout the day, yet they report the same fatigue levels on a visual analog scale, there is no context to which the fatigue can be related. Historically, fatigue research has been hindered by the inability to normalize the symptom to the level of activity until more recently.

D. Mechanisms of Fatigue

Finally, fatigue has no defined etiology and unmasking the complex pathophysiological mechanisms behind the symptom still remains a challenge across all fatigue research. Improving measurements and standardizing definitions may help move the field of research onward toward implication of mechanisms and interventions that may act on those mechanisms.

II. Fatigability

Despite all the challenges facing fatigue researchers over the decades, progress has been made in recent years igniting renewed interest in moving towards a better understanding of the phenomenon. In 2010, the construct of fatigability was introduced by Eldadah et al[15], defined as a phenotype of fatigue in which self-report measures and/or performance measures are normalized to a component of the activity being performed, such as duration, intensity, or frequency. By normalizing the fatigue to the activity level, measures of fatigability not only allow for meaningful comparisons between individuals and groups but also provide insight into whether, and to what extent, fatigue impacts the ability to perform physical activity.[15] Measures of fatigability control analyses for the effects of self-pacing or the titration of activity level in order to manage feelings of fatigue.[15,25,26]

Fatigability can be broken down into two subtypes in which the National Institutes of Health further defines as either a change in the perception of tiredness (perceived

fatigability) or a decline in performance (performance fatigability) as a function of the duration, intensity, or frequency of an activity. (NIH/NIA 34 PA-12-227) Measures of fatigability control analyses for the effects of self-pacing or the titration of activity level in order to manage feelings of fatigue.[15,25,26]

A. Performance Fatigability Severity

Performance fatigability severity measures assess an individual's change in performance in the context of activity, normalizing to either the duration, intensity, or frequency of the activity.

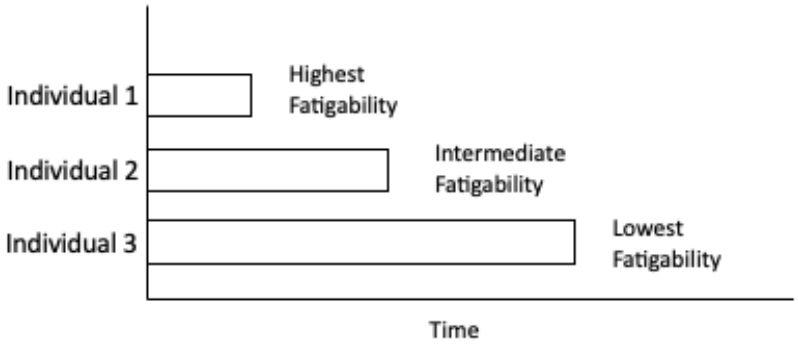

Figure 1. Schematization of performance fatigability severity. If work rate is held constant and individuals are told to walk as long as they are able to, the person with the shortest duration is the most fatigable, while the individual able to walk for the longest duration is least fatigable.

B. Perceived fatigability severity

Perceived fatigability severity is measured as a change in perceived fatigue from the beginning of an activity to completion of the activity while holding a component of this activity constant. The change in perceived tiredness following the activity, in which the duration, intensity, or frequency of the activity is held constant, is measured using the fatigue and fatigability scale below.

Table 2. Perceived fatigability scale. Used with permission from Dr. John F. Schnelle.[24]

Fatigue and Fatigability Scales

(Before Walking Test)		(After Walking Test)
Fatigue ScaleItems	**Score**	**Fatigability Scale Items**
Extremely tired	7	Extremely more tired
Somewhat tired	6	Somewhat more tired
Alittle tired	5	A little more tired
Neither tired nor energetic	4	Neither more tired or energetic
Alittle energetic	3	A little more energetic
Somewhat energetic	2	Somewhat more energetic
Extremely energetic	1	Extremely more energetic

However, as a relatively new construct, the underlying mechanisms and functional limitations associated with fatigability are not completely understood. Clinical manifestations of fatigability most often culminate in reduced exercise tolerance and

altered indices of cardiorespiratory function.[27] Most commonly, outcome variables reported by previous studies have been delimited to traditional indices of aerobic capacity such as peak oxygen consumption[28] (peak-VO_2) or VO_2 at the anaerobic threshold[27], and performance measures such as timed-walk test results and CPET duration. While these measures provide valuable insight into aerobic capacity, they fail to localize or isolate more intricate mechanisms, which could improve the sensitivity of fatigability measurements and further our understanding of how fatigue is propagated and how it can be further mitigated. Identifying and utilizing indices that target the pathomechanisms specific to fatigability may help to improve our understanding and thus management of fatigue.

III. Clinical Significance

Experienced by all individuals of all ages[1–4], fatigue disrupts daily living, social, and occupational activities negatively affecting overall quality of life.[2,3,16] Furthermore, in older adults fatigue has been shown to create significant health implications as it is associated with poorer mobility, functional limitations, disability, and mortality.[25,29,30] Fatigue also presents an important public health concern costing an estimated $136 billion per year for United States employers.[16] Although it is thought to be more commonly associated with diagnosed medical conditions, only one-third of all fatigue complaints can be attributed to disease.[1]

Previous literature suggests that fatigability increases with advanced aging and is a leading reason for restricted activity in older adults.[1,28] Furthermore, it has been

hypothesized that one aspect of the etiology of fatigability in older adults may be age-related alterations of mitochondrial energetics.[28] With increasing life expectancies, fatigability in the older adult population will continue to present significant challenges to healthcare professionals and researchers alike.

Fatigue can be overwhelming and distressing to the degree that it limits physical functioning and social participation capacity.[31] Furthermore, it is a key component of frailty syndrome in older adults and can worsen morbidity and mortality outcomes.[31] In rehabilitation settings, fatigue can act as a barrier to exercise and limit adherence to exercise protocols, ultimately moderating the outcomes achieved in these settings. It is vital that awareness of the challenges facing fatigue research and clinical settings are enhanced and adaptations are considered when planning research studies, clinical assessments, and health interventions to improve fatigue management on a global scale.

CHAPTER TWO

THE THEORECTICAL FRAMEWORK

I. Exercise Perturbation Model

Exercise perturbation provides a model for understanding the homeostatic processes of multiple body systems under conditions of controlled metabolic stress and their disruption. Physiological regulatory and support system integration allows for muscle contraction to occur and be sustained by defending against the homeostatic challenges presented. Failure of any of these systems to function adequately results in the inability to initiate or sustain muscle contraction.

Cardiopulmonary exercise testing (CPET) allows for simultaneous evaluation of the intimate interaction between the cardiovascular and respiratory systems to provide the link between internal and external respiration, or gas exchange between the cells and the atmosphere.[32] The cardiovascular and ventilatory systems in particular are efficiently coupled with skeletal muscle metabolic activity in order to support the transformation of chemical to mechanical energy. Inefficiencies in this coupling can be incurred by increasing the stress on the systems involved ultimately resulting in performance limitations. Thus, cardiopulmonary exercise testing (CPET) provides a classic model to study fatigability, its clinical manifestations, and the potential mechanisms.

II. Exercise Physiology

All forms of biological activity require the common chemical intermediate adenosine triphosphate (ATP) to fuel cellular work, including skeletal muscle contraction and relaxation that allow for physical activity participation.[19,32–34] In skeletal muscle, ATP is necessary for optimal cross-bridge function, thus force generation. More specifically, its hydrolysis allows for optimal myosin positioning prior to the powerstroke and its attachment to myosin allows myosin to release from actin resulting in relaxation.[19,32,33] Furthermore, ATP is required FOR cellular processes supporting muscular contraction including maintenance of sarcolemmal excitability and reuptake of calcium into the sarcoplasmic reticulum (SR).[34] Substrates from ingested food must be converted and stored in the terminal phosphate bond of ATP as the free energy of these substrates is not used directly for skeletal muscle contraction.(cite) The terminal phosphate bond of ATP has a high free energy of hydrolysis (ΔG), thus is considered a high-energy phosphate (~P). Per ~P, as much as 12 to 14 Kcal/mole of ΔG is estimated in contracting skeletal muscle. Skeletal muscle is thought to use one ~P per myosin cross-bridge linkage and release from actin, thus the chemical energy of ATP is converted to mechanical energy, externally manifesting as muscle contraction. (Equation 1)

Equation 1 $$ATP + Actin + Myosin \longrightarrow Actomyosin + Pi + ADP + Energy$$

Given that skeletal muscle ATP stores are low, only capable of providing enough energy for the first 1-2 seconds of work, ATP replenishment is critical for sustained muscle contraction beyond this time frame. Maintenance of skeletal muscle ATP homeostasis

during exercise perturbation is one of the primary principles of physiology and while the turnover can increase 100-fold above rest, intramuscular [ATP] is remarkably well maintained over a wide range of exercise intensities and durations as regulatory processes help to buffer large [ATP] reductions .[34] In skeletal muscle, three main energy sources function with the primary purpose of supporting ATP homeostasis in response to exercise perturbation: immediate energy sources, nonoxidative (fast glycolytic) energy sources, and oxidative (slow glycolytic) energy sources.

Immediate Energy Sources

At the onset of exercise, three immediate sources of energy are available in muscle. 1) The hydrolysis of ATP mediated by the enzyme ATPase (Equation 2); 2) The splitting of the ~P phosphocreatine (PCr) mediated by creatine kinase (Equation 3); and 3) The reaction of two ADP mediated by adenylate kinase, or myokinase (Equation 4).

Equation 2	$ATP + H_2O \rightarrow ADP + Pi$	(enzyme: ATPase)
Equation 3	$PCr + ADP \rightarrow ATP + C$	(enzyme: Creatine kinase)
Equation 4	$ADP + ADP \rightarrow ATP + AMP$	(enzyme: Adenylate kinase)

While these energy sources are available immediately, they only have the ability to provide energy for the initial seconds of exercise. PCr, another ~P, plays an important role in maintaining ATP homeostasis in addition to the rephosphorylation of ADP, by providing a larger reserve (5-6 times greater muscle stores than ATP) of phosphate energy to regenerate ATP.[33]

Nonoxidative (Fast Glycolytic) Energy Sources

The breakdown of glucose, or glycolysis, provides a nonoxidative energy source for muscle contraction. Glycolysis (Equation 5) is the catabolic reaction of glucose that yields energy as well as other byproducts.

Equation 5 $$Glucose \rightarrow 2ATP + 2Lactate^{1} + 2H^{+}$$

Glucose is initially broken down into two pyruvates, though in with no oxygen present, this pyruvate is converted to lactic acid. Skeletal muscle is densely packed with glycolytic enzymes allowing for rapid breakdown of glucose and the ability to supply energy for a longer duration than immediate energy sources (4-50 seconds). Though combined, the immediate and nonoxidative glycolytic energy sources can only provide energy for short durations and must be supplemented in order for sustained muscle contraction beyond these short time frames.

Oxidative (Slow Glycolytic) Energy Source

Unlike the immediate and nonoxidative glycolytic energy sources which do not require oxygen, the oxidative energy source requires the presence of oxygen in order to produce ATP. Oxidative metabolism, similar to nonoxidative, is also a catabolic reaction of glucose though in the presence of oxygen yields far more ATP than nonoxidative glycolysis. (Equation 6)

Equation 6 $$Glucose + O_2 \rightarrow 36\ ATP + CO_2 + H_2O$$

This overarching representation depicts the process as the breakdown of metabolic substrate in the presence of oxygen to yield energy, water (H_2O), and carbon dioxide (CO_2). Though, the entire process is much more complex and can be broken down into three stages.

The first stage of aerobic metabolism is glycolysis (Equation 7) though in the presence of oxygen yielding different products than nonoxidative glycolysis.

Equation 7 $$Glucose \rightarrow 2Pyruvate + 2\ ATP + 2NADH$$

In the presence of oxygen, this form of glycolysis yields pyruvate that does not get converted into lactate. The two pyruvates produced will enter the mitochondrial matrix and are oxidized into acetyl coenzyme A (acetyl-CoA) in preparation for the next stage of aerobic respiration, the citric acid cycle (CAC), also known as the tricarboxylic acid (TCA) or Kreb's cycle. The acetyl-CoA produced enters the Kreb's cycle where it reacts with oxaloacetate to form citrate, initiating a series of redox reactions that directly produce energy in the form of two ATP. In addition to the energy produced in the form of ATP molecules, the Kreb's cycle indirectly produces energy as it yields ten NAHD and two flavin adenine dinucleotide ($FADH_2$) molecules which will deposit their electrons into the electron transport chain (ETC) to drive the synthesis of ATP through oxidative phosphorylation (OXPHOS). OXPHOS is the primary source of ATP within the body, thus the energetic basis for all biological activity,[19,32] and can be broken down into two closely connected components: the ETC and chemiosmosis. Electrons are delivered to the ETC by NADH and $FADH_2$ where they then move from areas of higher energy to lower

energy, or pass down the chain, releasing energy along the way. Some of the energy released during the electron transport drives protons [H^+] out of the mitochondrial matrix into the intermembrane space creating an electrochemical gradient called the proton-motive force. Though, as the increased [H^+] in the intermembrane space are unable to pass directly through the phospholipid bilayer of the membrane and down their concentration gradient back into the mitochondrial matrix, specific channels are required.

Chemiosmosis refers to this movement of H^+ down their electrochemical gradient across the semipermeable membrane with the help of ATP synthase. During this process, ATP synthase provides the channel for H^+ to move back into the mitochondrial matrix and concomitantly catalyzes the addition of phosphate to adenosine diphosphate (ADP), creating energy in the form of ATP. While glycolysis and the Kreb's cycle each yield 2 ATP, OXPHOS yields approximately 30 ATP producing a net 34 ATP per glucose molecule. Oxygen is critical to OXPHOS as it is the terminal electron acceptor at the end of the ECC.

Each of the three main energy sources described above function with the ultimate goal of maintaining ATP homeostasis. During periods of high ATP turnover, byproducts such as AMP, Pi, and ADP stimulate ATP replenishment to setpoint levels limiting the decline of ATP during exercise. Furthermore, PCr breakdown is one of the primary ATP buffering mechanisms and is fundamental in promoting rapid ATP resysnthesis.[35]

III. Physiologic Basis of Fatigue

At the onset of exercise, metabolic activity increases in effort to meet the energy production demands of the activity. At submaximal intensities, energy production via oxidative metabolism is sufficient to meet the energy demand and both VO_2 and VCO_2 will eventually plateau, at approximately 3 and 4 minutes, respectively. When exercise intensity advances beyond submaximal levels, a greater reliance upon non-oxidative metabolism must occur, increasing its contribution towards the total ATP production.[33,36,37] The increased reliance on fast glycolysis above the anerobic threshold (AT) results in increased production of lactic acid in muscle and blood. As a strong acid under physiologic conditions, lactic acid readily dissociates a hydrogen ion.[33] Along with the increase in $[H^+]$ from glycolysis, ATP catabolism also yields H^+ further contributing to the increased $[H^+]$. When ionic production exceeds the capacity of the bicarbonate (HCO_3) – carbonic anhydrase buffering mechanism, the increase in sarcoplasmic $[H^+]$, decrease in tissue pH [38]and the imbalance between the release of protons and the rate at which they can be buffered and removed leads to metabolic acidosis.[39] Thus, above the AT, fatigue is eminent.

Intracellularly, the increased $[H^+]$ and decreased pH has negative effects including inhibition of phosphofructokinase (PFK), slow glycolysis,[40] and glycogenolysis[41] as well as displacement of calcium (Ca^{2+}) from troponin during the crossbridge cycle.[19,22,42] The increase in H^+ liberated into the blood can also have negative effects such as inhibition of

oxyhemoglobin formation in the lungs[43] as well as limitations on free fatty acid release into the circulation.[22,44]

In order to maintain homeostasis and minimize the potential negative effects of pH reductions and maintain pH homeostasis, the H^+ must be buffered. Mitochondrial respiration and the buffering molecules within the cell can act to counteract the intracellular decreases in pH.[39] The intracellular buffering system includes bicarbonate (HCO_3^-) along with amino acids, proteins, phosphate, and PCr hydrolysis which either bind or consume the intracellular protons in protection against further accumulation.[39,45] Bicarbonate buffers the lactate and associated hydrogen ions, although the increase in intracellular lactate production along with the decrease in bicarbonate (due to buffering) creates concentration gradients that drive lactate out of the cell and bicarbonate into the cell.[36] In the blood, the bicarbonate-carbonic acid buffering system is the primary H^+ buffering mechanism. (Equation 8)

Equation 8 $$CO_2 + H_2O \overset{\text{Carbonic anhydrase}}{\leftrightarrow} H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

As evident in Equation 8, CO_2 is a byproduct of H^+ buffering. This CO_2 production is in excess of the CO_2 produced by the Kreb's cycle of aerobic metabolism.

IV. Recovery Following Exercise

In addition to fatigability and its severity, limitations physical activity intolerance on bouts that are subsequent to previous bout are in part a function of recovery following activity. Previous reports have primarily focused on indices of cardiorespiratory capacity

measured during the active portion of exercise tests[27,28] whereas the construct of recovery and its relationship to fatigability has yet to be fully conceptualized. Measures of recovery, operationally defined as restoration of pre-perturbation homeostasis, have been shown to be associated with survival rate, related to severity of disease, and predictive of functional capacity.[46,47,48] Moreover, these measures also provide valuable insight into one's level of cardiovascular fitness[48–51] and into the ability of skeletal muscle metabolism to return to a state of homeostasis[52,53] following an imposed stress, such as fatiguing exercise. As functional activities are interspersed with rest periods throughout the day, the ability to recover sufficiently during the rest period may be a vital component of the ability to sustain function.

Fatigability and recovery are both profoundly influenced by mitochondrial energetics[28] but the relationship between the two constructs remains unclear. As a measure of ionic buffering, which is required by both the capacity to sustain intense activity and to recover from it, estimates of $NMVCO_2$ may provide information regarding the relationship between fatigability and recovery and a potential mechanism for their association. As the incurrence of fatigue is inevitable in all individuals, understanding the fatigue-recovery relationship may inform novel approaches to fatigue management in various population subsets.

V. Measures of Total VCO_2 and Components

Measures of VO_2 have been used almost exclusively to assess potential physiologic processes underlying fatigability and associated performance limitations.[25] While

measures of VO_2 provide valuable insight into one's aerobic capacity, muscle fatigue during exercise above moderate intensities is believed to be mediated by ion inhibition of cross-bridge cycling resulting from competitive binding with the actin-myosin binding sites and the myosin head binding sites for ATP. The ability to maintain exercise above moderate intensities thus depends on the ability to efficiently buffer these ions by, among other mechanisms, the lactic acid and bicarbonate buffering systems, which diminishes plasma ion concentration by increasing expired non-metabolic CO_2 (VCO_2).[19,33,54,55] Therefore, total non-metabolic VCO_2 (nm-VCO_2), measured during CPET, may serve as a novel and more specific laboratory measurement of fatigability.

A small number of previous studies have attempted to characterize and calculate excess CO_2 kinetics[56–58] during exercise. One of these studies by Hirakoba[58] et al went on to assess the impact of endurance training on excess CO_2 and the relationship between this excess CO_2 and distance running performance. While these studies have provided a foundational basis for measuring and assessing excess CO_2 during exercise, variations in methodology, calculations, and sample population have left many unanswered questions. Furthermore, measurements of total expired CO_2 (VCO_2) obtained during CPET and its components, energy repletion or metabolic (m-VCO_2) and buffering or non-metabolic VCO_2 (nm-VCO_2), have yet to be studied in relation to performance fatigability in response to chronic high intensity exercise training.

The proposed study attempted to calculate estimations of total VCO_2 (t-VCO_2), metabolic VCO_2 (m-VCO_2), non-metabolic VCO_2 (nm-VCO_2), and total VO_2 (t-VO_2) using the following calculations.

Total VO_2 (t-VO_2) (Equation 9)

$$[(\text{peak time} - \text{AT time}) * (\text{peak } \dot{V}O_2 - \text{AT } \dot{V}O_2)] / 2$$

Total VCO_2 (t-VCO_2)

$$[(\text{peak time} - \text{AT time}) * (\text{peak } \dot{V}CO_2 - \text{AT } \dot{V}CO_2)] / 2$$

Metabolic VCO_2 (m-VCO_2)

$$[(\text{peak time} - \text{AT time}) * (\text{estimated peak metabolic } \dot{V}CO_2 - \text{AT } \dot{V}CO_2)] / 2$$

Non-metabolic VCO_2 (nm-VCO_2)

$$\text{t-}\dot{V}CO_2 \; - \; \text{m-}\dot{V}CO_2$$

Figure 3.

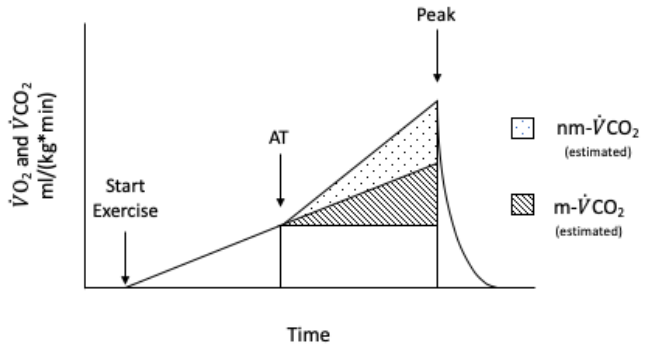

Figure 3. Schematization of total VCO_2 and its energy repletion (m-VCO_2) and buffering (nm-VCO_2) components.

CHAPTER THREE

Short Title: Non-metabolic CO_2 Expiration

Title: CO_2 Expiration and its Relationship with Performance Fatigability

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Abstract

Purpose: This study examined components of carbon dioxide expiration (VCO_2) and their relationship with performance fatigability in adaptation to chronic exercise perturbation. **Methods**: Twenty healthy adults (men, n=9, age=44.7±13.9; women, n=11, age=50.3±11.1) completed peak cardiopulmonary exercise (CPET) and constant work rate tests (CWRT) on separate days before and after a vigorous, 4-week aerobic exercise training (AET) regimen. Each test was followed by 10-minutes of passive recovery and an endurance test at 70% of the peak watts attained during the CPET. Performance fatigability was indexed by the endurance test durations following both the peak CPET (End1) and CWRT (End2), peak CPET time (pk-Time), and peak CPET watts (pk-Watts). Metabolic indices were calculated as total VO_2 (t-VO_2), total VCO_2 (t-VCO_2), metabolic VCO_2 (m-VCO_2), and non-metabolic VCO_2 (nm-VCO_2). Data were analyzed for significant ($p<0.05$) changes and relationships amongst variables were assessed using paired t-tests and regression analyses. **Results**: Significant reductions in performance fatigability along with significant increases in tVO_2, tVCO_2, and nm-VCO_2 were observed after AET. A change in m-VCO_2 was not observed. nm-VCO_2 was strongly predictive of performance fatigability (pk-Time $R^2=0.735$, pk-Watts $R^2=0.692$, end1 $R^2=0.284$, end2 $R^2=0.465$, $p<0.001$). **Conclusion**: The current study demonstrated significant increases in the buffering component of total carbon dioxide expiration but not in the component associated with energy repletion, suggesting the two components may adapt independently to aerobic exercise intervention. The buffering component may be a

predictor of performance fatigability as it was directly associated with measures of sustained performance.

Keywords: Performance Fatigability, Non-metabolic VCO_2, Recovery, Buffering, Cardiopulmonary Exercise Testing (CPET)

Introduction

It is widely accepted that at exercise above the anaerobic threshold (AT), excess carbon dioxide (CO_2) is produced in addition to that produced by oxidative metabolism[1,2] (Figure 1). Expiration of the excess CO_2 is understood as an endpoint of bicarbonate buffering of fatigue-inducing hydrogen ions (H^+), dissociated from metabolic acids in both skeletal muscle and blood.[1,2] Exercise continued above the AT most often leads to fatigue, resulting from encroachment on the buffering capacity of this system, which occurs in accordance with both the intensity and duration of the activity. Ionic accumulation follows, which impairs cross-bridge cycling, and mitochondrial and sarcolemma functioning.[3,4]

Cardiopulmonary exercise tests (CPET) have been used as a method of gaining further insight into performance-limiting fatigue, as well as the performance fatigability phenotype (operationally defined as the decline in performance normalized to the intensity, duration or frequency of the activity level).[5] Previous research has utilized VO_2 or time at the AT and peak, as well as VO_2 cost of an activity as physiological measures when assessing relationships between cardiorespiratory function and performance fatigability.[6–9] While these measures provide a practical method of acquiring valuable insight into oxidative metabolism and the onset of exercise induced fatigue, they lack the ability to account for the mechanisms that limit high energy phosphate production and utilization.[2] Thereby, the approach of the current study to exercise performance limitations was focused on less commonly utilized measures that may infer to buffering capacity and its impact on aerobic capacity.

A small number of studies have attempted to characterize excess CO_2 production (VCO_2) kinetics[10–12] during exercise. For instance, Hirakoba et al[12] assessed the impact of endurance training on excess VCO_2 by examining the relationship between excess VCO_2 and running distance performance. While these studies have provided a foundational basis for measuring and assessing excess CO_2 production during exercise, there remains informational gaps regarding the adaptation of total VCO_2 obtained during CPET, as well as its components related to the Kreb's cycle, metabolic activity in the energy repletion process, and ionic buffering to a chronic exercise challenge.

This study examined the dynamics of the metabolic (m-VCO_2) and non-metabolic (nm-VCO_2) components of VCO_2, respectively indexing Krebs cycle function and ionic buffering, their association with performance fatigability, and the overall VCO_2 adaptation to chronic exercise perturbation.

Methods

Study Design

This longitudinal study used a pre-post experimental design with a single cohort of healthy adults. The regimen included two pre-exercise training testing sessions, followed by four weeks of continuous, high-intensity aerobic exercise training, and two post-exercise training testing sessions.

Study Population

Subjects were recruited from the greater Washington D.C. metropolitan area by word of mouth, newspapers and community social network advertisements, and flyers. The study was registered with ClinicalTrials.gov (identifier: NCT03800342) and the protocol was

reviewed and approved by the George Mason University Institutional Review Board. Informed consent was obtained from participants prior to participation in accordance with U.S. federal regulations and the Declaration of Helsinki.[13]

Inclusion/Exclusion Criteria

Subjects consisted of adults whom reported no history of medical conditions that would affect their ability to respond or adapt to aerobic exercise, or make participation unsafe. Specifically, subjects were eligible for the study if they were between the ages of 18-60, had a body mass index (BMI) greater than 19 but less than 35 kg/m^2, were able to pedal a leg cycle ergometer, and were able to speak fluent English. Subjects were deemed ineligible if they had a history or present symptoms of uncontrolled diabetes (fasting plasma glucose >125mg/dL), significant obstructive or restrictive pulmonary dysfunction, pulmonary vascular disease, coronary artery disease, all forms of chronic or congestive heart failure, uncontrolled hypertension (resting blood pressure >160/100 mm/Hg on or off medications), anemia (hemoglobin <10 g/dL), stroke, cancer (other than melanoma), thyroid disease, autoimmune disease, severe muscular disease, neurological disease, metabolic or mitochondrial disease, bone disease, mitochondrial myopathies or insufficiencies, hepatic disease, chronic renal insufficiency (eGFR < 60 ml/min/1.73 m^2), psychiatric disease that could be worsened by exercise or influence exercise capacity, cognitive impairment, chronic infection requiring antiviral or antibiotic treatment, anticoagulant therapy, or hormone replacement/supplementation therapy (other than birth control). Those who were currently pregnant, smoking, taking any medication/s that may have limited exercise capacity or the ability to adapt to an aerobic exercise training, or

who were involved in active substance abuse were also ineligible to participate in the study. Subjects completed a medical history form and PARQ+ prior to consent to ensure they met all inclusion and exclusion criteria.

Pre and Post-Training Exercise Testing

Pre- and post-training testing consisted of subjects reporting to the lab on two separate occasions (conducted at least 48 hours and 7 days of the previous visit). During visit 1, subjects completed a peak cardiopulmonary exercise test (pk-CPET) on an electronically braked Monark cycle ergometer followed by a 10-minute recovery period and then an endurance bout immediately following the recovery period. The pk-CPET and endurance bouts ended at volitional exhaustion, defined as the subject indicating he/she must stop exercising despite significant encouragement to proceed by the investigational team. The pk-CPET ramping protocol consisted of a progressive increase in workload, advancing by 25 Watts each minute, while the subject maintained 60 revolutions per minute (RPM). Measures of cardiorespiratory function were made during the pk-CPET, which included total VCO_2 ($tVCO_2$), metabolic VCO_2 ($m\text{-}VCO_2$), non-metabolic VCO_2 ($nm\text{-}VCO_2$), and total VO_2 (tVO_2) as described in detail below. The endurance bout was performed at 70% of the peak wattage attained during the pk-CPET and was used to obtain a measure of performance fatigability in total time the subject was able to maintain 60 RPM, or time to fatigue.

During visit 2, subjects completed a submaximal continuous work-rate test (CWRT), followed by a 10-minute recovery period and a subsequent endurance ride at 70% of their peak work rate as done during visit 1. The CWRT consisted of a constant square wave

test in which subjects cycled for 6-minutes at a work-rate corresponding to 80% of their anaerobic threshold (AT) determined from their pk-CPET. Following this 6-minute bout, subjects rested passively for an 8-minute period. Subjects completed 3-cycles of the work-rest combination with a 10-minute passive recovery following the third 6-minute active bout.

Cardiorespiratory function during and following each CPET and endurance bout was assessed using a pulmonary gas exchange analysis system (MedGraphics CardiO2 CPET system; Medical Graphics Corp, St Paul, MN), which was calibrated according to manufacturer's specifications prior to each exercise test. Heart rate (HR) was measured continuously by 12-lead electrocardiogram (ECG).

Continuous High-Intensity Aerobic Exercise Training (AET)

Following pre-training testing, subjects reported to the lab 4 times a week for 4 weeks to complete the aerobic exercise training portion of the study. Training consisted of leg-cycling using the choice of a Monark, spin, or upright bike for a duration of 45 minutes at a target intensity of 65 to 75% of the subject's heart rate reserve (HRR) determined from the pk-CPET according to the following equation:

65 and 75% HRR = [0.65 and 0.75 x (peak HR – resting HR)] + resting HR

Subjects warmed up for approximately 5 minutes prior to the start of the 45-minute training session. Subjects were encouraged to cool-down for 5-10 minutes following each training session.

Determination of variables:

Cardiorespiratory Function: Peak VO_2 and VCO_2 were determined from an 8-breath average at the end of the pk-CPET or by the VO_2 and VCO_2 at the end of the last completed stage, whichever was higher. The AT, a marker denoting the onset of exercise-induced fatigue[14], was determined using the V-slope method of Beaver and Whipp[15] applied to breath-by-breath iterations of VO_2 and VCO_2 and reported as AT-VO_2 and AT-VCO_2.

Performance Fatigability: The primary measures of performance fatigability were total time in seconds (s) observed during the CPETs (pk-Time) and pre-training (END1) and post-training (END2) endurance tests and the peak watts attained on the CPETs (pk-Watts).

VO_2 and VCO_2 Volumes (Figure 1):

- Total VCO_2 was calculated using the following formula (area of a triangle): [(peak time – AT time) * (peak VCO_2 – AT VCO_2)] / 2
- Metabolic VCO_2 was estimated using the same formula but first calculating the estimated peak metabolic VCO_2 (using the slope of VO_2 line from time zero to the anaerobic threshold and extending to peak test duration time in seconds) and substituting this value in for peak VCO_2.
- nm-VCO_2 was estimated by calculating the difference between total VCO_2 and metabolic VCO_2 and converting to liters (ml).

Statistics. The main variables of interest were volumes of VO_2 and VCO_2 (t-VCO_2, t-VCO_2, nm-VCO_2, m-VO_2) and measures of performance fatigability (End1, End2, pk-Time, pk-Watts). Data were assessed for normalcy using histograms and Shapiro-Wilk

tests and analyzed for significant associations using Pearson's product-moment correlation and regression analyses. Differences between pre and post-exercise training variables were analyzed using paired t-tests. Statistical significance was accepted at $p < 0.05$.

Results:

Subjects were 11 women and nine men who reported no acute or chronic health conditions on the PARQ+ (Table 1). Mean resting HR for this sample was 80±13 bpm pre-AET and 78±13 post-AET. All subjects approached peak physiologic effort at volitional exhaustion[16] based on attainment of a respiratory exchange ratio (RER) of at least 1.10[16] with 16 of the 20 subjects reaching more rigorous criteria of 1.15. All but one subject attained 85% of their age-predicted peak HR with the one remaining subject attaining 83% of age-predicted peak HR during the post intervention CPET. With respect to age-predicted maximal HR, subjects achieved a mean of 96±0.10% at pre and 95±0.06% at post training CPETs.

RestingVO_2 (312 ± 90.9 ml/min), VCO_2 (278 ± 80.5 ml/min), and RER (0.90 ± 0.07) pre-AET were not significantly different from post-AET (VO_2 318 ± 91.0 ml/min, p=0.56; VCO_2 291 ± 97.9 ml/min, p=0.223; and RER 0.91 ± 0.07, p=0.64). No significant changes were found in BMI post intervention (-0.12 ± 0.37, p=0.153).

Significant changes in AT-VO_2, pk-VO_2, pk-VCO_2, and RER were observed following AET (Table 2). AT-Time (8±56 s, p=0.523), AT-VCO_2 (-6.85±153 ml/min, p=0.843), VeVCO_2 (-0.15±3.0, p=0.825), and PetCO_2 (-0.9±4.6 mmHg, p=0.397) between pre and post testing. Significant increases were observed in t-VO_2, tVCO_2, and nm-VCO_2 after

exercise training (Figure 2, Panel A), however no changes were observed for m-VCO_2 (Figure 2, Panel A). Significant reductions in performance fatigability measures can be seen in Figure 2, Panel B.

VCO_2 and VO_2 volumes were observed to have significant and predictive relationships with measures of performance fatigability (Table 3, Figure 3). Furthermore, nm-VCO_2 was a stronger predictor of performance fatigability compared to traditional measures of aerobic capacity, including peak-VO_2 and AT-VO_2 (Table 3, Figure 3). Significant associations were also found between nm-VCO_2 and both minute ventilation (Ve) and tidal volume (Vt) at peak exercise (Figure 4) and trended toward significance in its association with peak respiratory rate (R^2=0.93, p=0.056). Additionally, nm-VCO_2 was significantly associated with Total VO_2 (R^2=0.667, p<0.001), VO_2 (R^2=0.764, p<0.001, and AT-VO_2 (R^2=0.454, p<0.001).

Discussion:

This study investigated relationships between the components of VCO_2 and performance fatigability following a standardized AET intervention in healthy individuals. Previous studies[7,9] have highlighted indirect relationships among measures of performance fatigability and cardiorespiratory capacity, suggesting that cardiorespiratory impairment may increase performance fatigability to levels limiting the ability to engage in or sustain activities of daily living. Yet, commonly reported measures fail to take into account the increased reliance on glycolysis for ATP resynthesis above the AT and the associated impact on aerobic metabolism and performance during sustained exercise. Above the AT

there is increased reliance on glycolysis to support oxidative pathways for ATP replenishment in order to meet the energy demand of the activity. This is concomitant to an increase in the production of both intracellular and arterial lactic acid that dissociates into a lactate ion and hydrogen ion (H^+). Ultimately, it is this increase in [H^+] that causes both intracellular and arterial pH to decrease and the imbalance between the release of protons and the rate at which they can be buffered and removed leads to metabolic acidosis. Intracellularly, the increased [H^+] and decreased pH have negative effects including inhibition of phosphofructokinase (PFK), slow or oxidative glycolysis, and glycogenolysis, as well as displacement of calcium (Ca^{2+}) from troponin during the crossbridge cycle. Thus, while cardiorespiratory capacity may be associated with measures of performance fatigability, the current suggests that this relationship may be mediated by H^+ accumulation induced inhibition of oxidative metabolism.

In order to maintain homeostasis and minimize the potential negative effects of pH reductions and maintain pH homeostasis, the H^+ must be buffered. Mitochondrial respiration and the buffering molecules within the cell can act to counteract the intracellular decreases in pH. The intracellular buffering system includes bicarbonate (HCO_3^-) along with amino acids, proteins, phosphate, and PCr hydrolysis which either bind or consume the intracellular protons in protection against further accumulation. Synthesis of carbonic acid from ion released from the metabolic acid and bicarbonate buffers the lactate by encumbering the ions and further dissociating the carbonic acid to CO_2 and water. The CO_2 is ultimately stored again as bicarbonate or released into the ambience via alveolar expiration.

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Where H_2CO_3 is carbonic acid and HCO_3^- is bicarbonate.

In the current study, the buffering component of total carbon dioxide expiration, nm-VCO_2, was significantly associated with all measures of performance fatigability, including timed endurance tests, pk-Time, and pk-Watts obtained during exercise testing. This finding and the finding that the energy repletion component of VCO_2 was not significantly associated with all performance fatigability measures, underscored the importance of adequate respiratory buffering during sustained activity and particularly activity above the anaerobic threshold.[3] Furthermore, significant associations were observed between nm-VCO_2 and measures of cardiorespiratory capacity, including total VO_2, peak-VO_2, and AT-VO_2, implicating nm-VCO_2 as an important consideration when evaluating aerobic capacity beyond the oxygen delivery and extraction components. That the buffering component was directly associated with measures of both cardiorespiratory capacity and performance provides plausibility that it may be a strong predictor of performance fatigability and thus a biomarker unaffected by motivation.

An additional novel finding of this study was the significant increase in nm-VCO_2 demonstrated following the 4-week AET protocol. These findings are partial in contrast to previous work by Hirakoba[12] et al in which no change was found in excess VCO_2 following exercise training, though significant relationships were demonstrated between excess VCO_2 in relation to body weight and lactate concentration, and distance running performance similar to the current study. The contrast in findings between changes in

excess VCO_2 post exercise training may be due to the differences in training protocol as in the previous study, subjects performed individual endurance training consisting of interval and paced running and the training period ranged from 25-102 days between subjects. Thus, training was not standardized but varied by subject making comparisons more difficult. Furthermore, when assessing the relationship between distance running performance and excess VCO_2, excess VCO_2 was reported as a ratio between excess VCO_2 relative to body mass and blood lactate accumulation. The current study calculation is based entirely on non-invasive measures of VCO_2 that may be more applicable to clinical settings.

The plausibility presented in this study is conceptually supported by findings of concomitant increases in the buffering component of VCO_2 and t-VCO_2, cardiorespiratory capacity measures, and performance fatigability following only a short period, 4-weeks, of high-intensity aerobic exercise training. The increase in nm-VCO_2 may be due to increases in bicarbonate buffering system capacity, increased capacity for encroachment on a bicarbonate buffering reserve or increased reliance on intracellular non-bicarbonate buffering mechanisms, which would facilitate lactate secretion from the cell and into the plasma to eventually be buffered.[12,17] It has also been suggested that potential increases in excess VCO_2 post-AET could be due to improved peripheral circulation and increases in muscle capillarity, resulting in greater CO_2 efflux from the skeletal muscle to blood.[12] While it has been suggested that Ve is not entirely determined by VCO_2 during exercise[18], the current findings support the idea that the ventilatory response to peak exercise, specifically at the respiratory compensation threshold and above, is a compensatory

response to exercise induced metabolic acidosis. In this study, nm-VCO_2 was significantly associated with peak V_t and Ve, suggesting that the ventilatory response at peak exercise is likely altered from its original trajectory as a compensatory mechanism in response to metabolic acidosis and ionic buffering capacity that approaches a peak capacity.[19]

It should be noted that the calculations used in this study are estimations based on common theoretical assumptions. The respiratory compensation threshold, the onset of hyperventilation and disproportionate rise of Ve to VCO_2, was not incorporated into the calculation of nm-VCO_2 in this study. This exclusion is likely a conservative one, as breathing efficiency can be expected to decrease above this point. Including this threshold may potentially increase the absolute trends demonstrated here. Furthermore, the sample size, comprised of a non-randomized convenience sample, may limit generalizability of the results of this study to a larger population. Subjects in this study were determined to be healthy based on a self-report method and medical evaluations were not conducted as a diagnostic or differential diagnostic component of the methodology to rule out unrealized conditions. Thus, trends toward Type 2 error and sample bias may circumvent accurate generalization of the data to the public at large. Conversely, the findings of the study may elucidate potential predictors of performance fatigability and cardiorespiratory function and capacity.

Conclusion

The current study demonstrated significant increases in the buffering component of total carbon dioxide expiration but not in the component associated with energy repletion. The

buffering component was directly associated with measures of performance, providing plausibility that the buffering component may be a predictor of performance fatigability.

Table 1. Demographic Characteristics and Baseline

Characteristic	(n = 20)
Age, years (median, IQR)	52 (46 – 55)
Female, n (%)	11 (55)
Male, n (%)	9 (45)
BMI, kg/m^2 (median, IQR)	26.5 (23.1 – 29.7)
Activity Level, Subjective Report	
Meet ACSM Guidelines per week, n (%)	11 (55)
Minutes of Moderate Intensity Activity per week, median (IQR)	120 (40 – 24)
Minutes of High Intensity Activity per week, median (IQR)	25 (0 – 120)

Table 2. Cardiorespiratory Variable Change Pre and Post AET

Variable	pre-AET	post-AET	change	p-value
AT-VO_2 (ml/kg/min)	16.3 ± 4.5	17.5 ± 4.7	1.25	p<0.01*
AT-VO_2 (ml/min)	1270 ± 401	1360 ± 423	89.4	p<0.01*
Peak VO_2 (ml/kg/min)	28.0 ± 7.6	31.9 ± 8.6	3.9	p<0.001*
Peak VO_2 (ml/min)	2181 ± 705	2484 ± 832	303.8	p<0.001*
Peak VCO_2 (ml/min)	2817 ± 868	3038 ± 995	220.9	p<0.05*
Peak RER	1.32 ± 0.10	1.22 ± 0.09	-0.10	p<0.001*

Table 2. Pre-AET, post-AET, and change scores for AT-VO_2, Peak-VO_2, Peak-VCO_2, and RER. Data are reported as mean ± SD. Significance levels are reported as p-values.

Table 3. Associations between measures of Performance Fatigability and Cardiorespiratory Indices

	pk-Time	pk-Watts	End1	End2
t-VCO_2 (ml)	$R^2 = 0.662$ $p < 0.001$*	$R^2 = 0.669$ $p < 0.001$*	$R^2 = 0.183$ $p < 0.01$*	$R^2 = 0.296$ $p < 0.001$*
t-VO_2 (ml)	$R^2 = 0.602$ $p < 0.001$*	$R^2 = 0.601$ $p<0.001$*	$R^2 = 0.223$ $p < 0.01$*	$R^2 = 0.309$ $p < 0.001$*
m-VCO_2 (ml)	$R^2 = 0.351$ $p < 0.001$*	$R^2 = 0.384$ $p < 0.001$*	$R^2 = 0.060$ $p = 0.129$	$R^2 = 0.095$ $p = 0.056$
pk-VO_2 (ml/(kg*min)	$R^2 = 0.668$ $p < 0.001$*	$R^2 = 0.670$ $p < 0.001$*	$R^2 = 0.252$ $p < 0.001$*	$R^2 = 0.392$ $p < 0.001$*
AT-VO_2 (ml/(kg*min)	$R^2 = 0.478$ $p < 0.001$*	$R^2 = 0.499$ $p < 0.001$*	$R^2 = 0.166$ $p < 0.01$*	$R^2 = 0.252$ $p < 0.01$*

Table 3. Associations between measures of performance fatigability (Peak Time, Peak Watts, Endurance 1, and Endurance 2) and both volumes of total VCO_2, total VO_2, and metabolic VCO_2, and traditional measures of aerobic capacity (peak-VO_2 and AT-VO_2). R^2 and p-values are reported. Associations between non-metabolic VCO_2 and measures of performance fatigability can be seen in Figure 3.

Figure 1

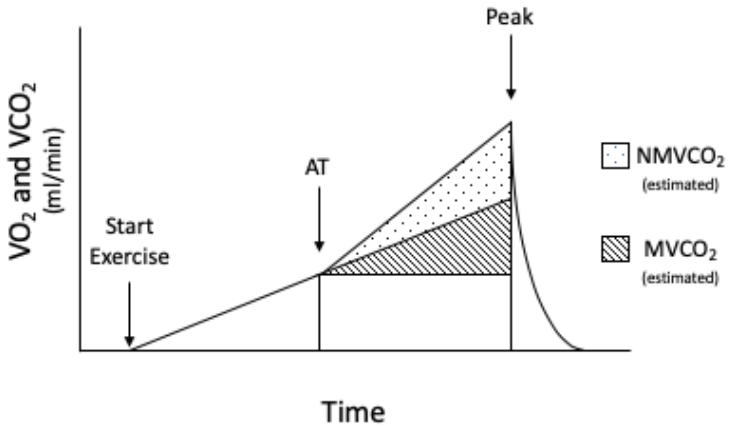
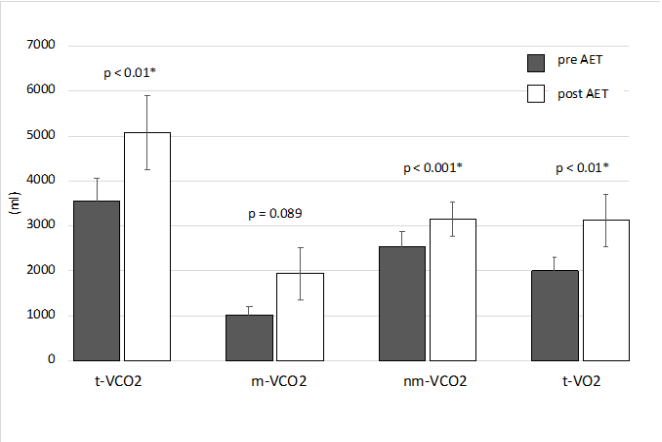


Figure 1. Schematization of VCO_2 and its energy repletion (m-VCO_2) and buffering (nm-VCO_2) components.

Figure 2

Panel A



Panel B

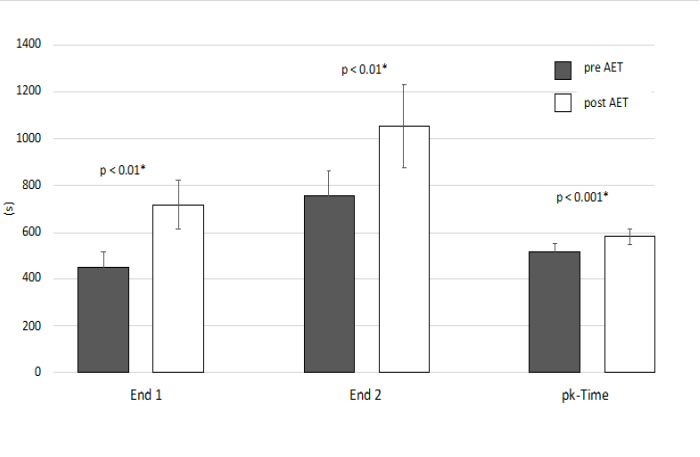


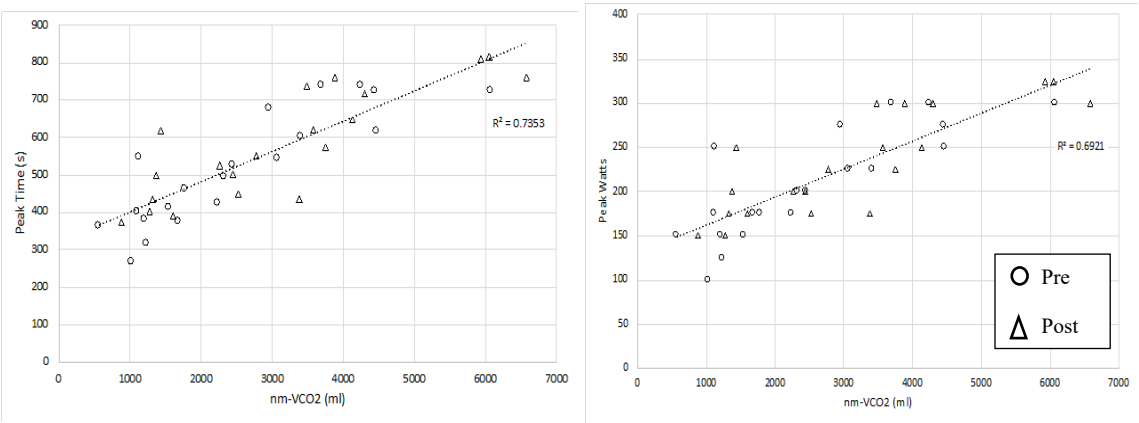
Figure 2

Panel A. Change in volumes (ml) of total VCO_2, metabolic VCO_2, non-metabolic VCO_2, and total VO_2 pre and post continuous high-intensity exercise training protocol. Data are reported in means with standard error bars and significance levels reported as p-values.

Panel B. Change in performance fatigability measures (Endurance 1, Endurance 2, and Peak Time) pre and post continuous high-intensity exercise training protocol. Data are reported in means with standard error bars and significance levels reported as p-values.

Figure 3.

Panel A. Panel B.



Panel C. Panel D.

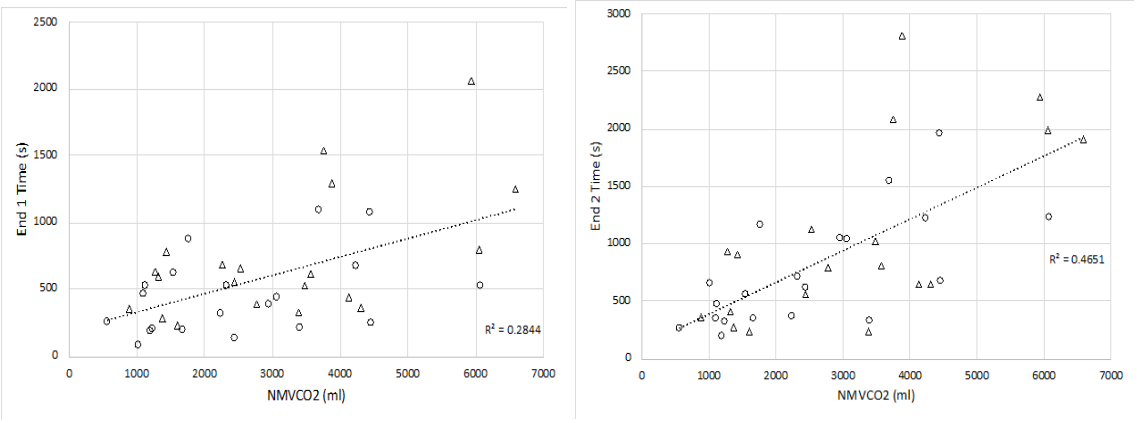


Figure 3. Associations between nm-VCO_2 represented on the x-axis and performance fatigability as measured by Peak Time (Panel A), Peak Watts (Panel B), End 1 (Panel C), End 2 (Panel D) represented on the y-axes. R^2 values are reported. nm-VCO_2 and Peak Time (Panel A) $p<0.001$*, Peak Watts (Panel B) $p<0.001$*, End 1 (Panel C) $p<0.001$*, End 2 (Panel D) $p<0.001$*.

Figure 4

Panel A. Panel B.

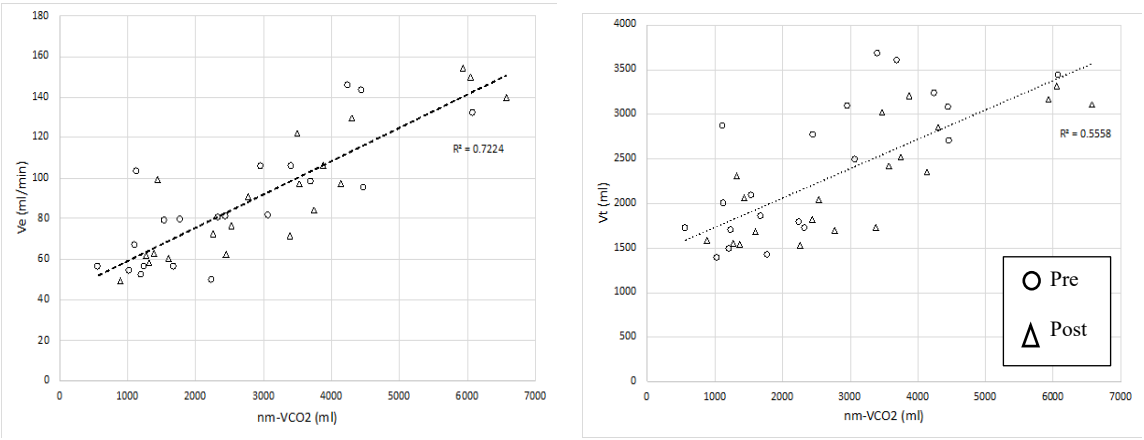


Figure 4. Associations between nm-VCO_2 represented on the x-axis and ventilatory response measures of minute ventilation (Ve; Panel A) and Tidal Volume (Vt; Panel B) represented on the y-axes. R^2 values are reported. Panel A $p<0.001$* and Panel B $p<0.001$*.

CHAPTER FOUR

Short Title: Respiratory Buffering , Recovery, and Performance Fatigability Adaptation to Exercise Training

Title: The Role of Respiratory Buffering in the Relationship Between Recovery and Performance Fatigability Following Aerobic Exercise Training

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Purpose: This study aimed to characterize the role of ionic buffering capacity, as measured by non-metabolic expired carbon dioxide (nm-$\dot{V}CO_2$), in the relationship between recovery and performance fatigability. **Methods**: Twenty healthy adults (men, n=9, age =44.7±13.9 years; women, n=11, age=50.3±11.1 years) completed peak cardiopulmonary exercise (CPET) and submaximal constant work rate tests (CWRT) on the cycle ergometer on separate days before and after a vigorous, 4-week aerobic exercise training (AET) regimen. Each test was followed by 10-minutes of passive recovery and an endurance test at 70% of the peak watts attained during the CPET. Performance fatigability was measured by the endurance test durations following both the peak CPET (End1) and CWRT (End2), peak CPET time (pk-Time), and peak CPET watts (pk-Watts). Metabolic indices were total $\dot{V}CO_2$ (t-$\dot{V}CO_2$), metabolic $\dot{V}CO_2$ (m-$\dot{V}CO_2$), and non-metabolic $\dot{V}CO_2$ (nm-$\dot{V}CO_2$), and recovery capacity indices were $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic oxidative response index (ORI). Data were analyzed using paired t-tests, correlations, and regressions and compared before and after AET. **Results**: Significant improvements in recovery ($\dot{V}O_2$ -off ORI 7.48±7.52 ml/s, $p<0.001$ and $\dot{V}CO_2$-off ORI 4.11±5.05 ml/s, $p<0.001$) and performance fatigability measures (End 1 265±337 s ; End2 321±392 s ; pk-Time 63±40 s, $p<0.001$; pk-Watts 24±19, $p<0.001$) were observed after AET, along with significant increases in t-$\dot{V}CO_2$ (1512±2225 ml, $p<0.01$) and nm-$\dot{V}CO_2$ (608 ± 666 ml, $p<0.001$). However, a significant change in m-$\dot{V}CO_2$ (904±2255 ml, $p=0.089$) was not observed. Relationships between measures of recovery and PF measures were observed, although the strengths of the relationships were diminished (pk-Time, pk-Watts) or became non-significant (End1, End2) after controlling the analyses

for the effect of nm-$\dot{V}CO_2$. **Conclusion**: The current study suggests that ionic buffering capacity may moderate the relationship between recovery and performance fatigability. These results could have implications regarding AET induced buffering dynamics and its role in fatigue resistance when performing physical activities above moderate intensities.

Keywords: Performance Fatigability, Non-metabolic $\dot{V}CO_2$, Recovery, Buffering, Cardiopulmonary Exercise Testing (CPET)

Introduction

Fatigue is a universal symptom of over activity reported by healthy individuals with normal functional capacity, which occurs during everyday life.[1] This symptom is also one of the most commonly reported complaints in primary care medical settings.[1] Conversely, even though fatigue is exacerbated by many acute and chronic illnesses, only about one-third of all cases of fatigue reported in the general population can be explained by a diagnosed medical condition.[2] Minorities and women report the highest prevalence of fatigue,[3] and fatigue is commonly reported in elderly adults of all population subsets.[2,4–6] The impact of fatigue is widespread as evidenced by associations between fatigue severity and increases in mortality[7], morbidity[8,9], risk of disability onset[2,10], functional limitations[11–13] and decreases in mobility[2,11], and participation in physical activity.[2,7,14] The impact of fatigue on human performance is less well understood, in part due to measurement challenges derived from discrepancies in the operational definitions of fatigue. Consequently, Eldadah[6] introduced the construct of fatigability in 2010, which included a performance fatigability phenotype that was operationally defined as a decline in performance normalized to the intensity, duration, or frequency of the activity being performed.[6] For example, if one individual can cycle at a given work rate for thirty minutes before noticing a measurable decline in performance, while another can ride only twenty minutes before noticing a similar decline, the former would be less fatigable than the latter. By normalizing fatigue to a specific function of the activity, in this case work

intensity, more objective evaluations and comparisons can be made with respect to functional capacity and an individuals' ability to sustain activity.

The construct of fatigability has gained considerable interest over the last decade. however, a 2017 integrative literature review that focused on measurements of fatigability reported only six studies that included measures of performance fatigability.[11] Only four of these six studies assessed relationships between fatigability and indices of cardiorespiratory function obtained during the active portion of a cardiopulmonary exercise test (CPET) such as oxygen cost, peak oxygen consumption ($\dot{V}O_2$), or $\dot{V}O_2$ at the anaerobic threshold (AT).[11,15–18] Published information on performance fatigability and the capacity to recover from strenuous physical activity (another aspect of cardiorespiratory fitness), remains to the best of our knowledge unavailable. In the authors' unpublished observations, what appear to be significant associations between measures of performance fatigability and recovery oxygen and carbon dioxide off-kinetics following cardiopulmonary exercise testing (CPET) have been identified. Additionally, the observations have included apparent relationships between measures of performance fatigability and measures of non-metabolic expired carbon dioxide (nm-$\dot{V}CO_2$) during exercise at intensities above the AT during CPET.

Briefly, nm-$\dot{V}CO_2$ is the portion of total $\dot{V}CO_2$ expired in excess of the CO_2 produced by the Kreb's cycle and is a crucial end product of the bicarbonate buffering mechanism. Thus, the buffering processes that produce nm-$\dot{V}CO_2$ as a byproduct may play a significant role in modulating performance fatigability.[19] We hypothesize that buffering capacity, as indexed by nm-$\dot{V}CO_2$, may modulate a relationship between performance

fatigability and recovery from strenuous physical activity. Therefore, the purpose of this study was to assess the role of nm-$\dot{V}CO_2$ in the relationship between recovery and performance fatigability.

Methods

Study Design

The study used a pre-experimental, longitudinal design comprised of a single arm of apparently healthy subjects. All subjects underwent identical exercise test batteries in a pre-training, control condition and following a 4-week, exercise training challenge, which was operationally defined as the trained (experimental) condition. The exercise tests included both peak and submaximal exercise testing methods. The training regimen included four weeks of continuous, vigorous aerobic, cycle-ergometer exercise training, with supervised training sessions, conducted in an exercise laboratory.

Study Population

Subjects were recruited from the greater Washington D.C. metropolitan area by word of mouth, newspaper and community social network advertisement, and flyers. The study was registered with ClinicalTrials.gov (identifier: NCT03800342) and the protocol was reviewed and approved by the George Mason University institutional review board. Informed consent was obtained from participants prior to participation in accordance with U.S. federal regulations and the Declaration of Helsinki.[20]

Inclusion/Exclusion Criteria

Subjects were eligible for the study if they were between the ages of 18-60, had a body mass index (BMI) greater than 19 but less than 35 kg/m^2, able to pedal a leg cycle

ergometer, and able to speak English fluently. Subjects were determined to be ineligible if they reported having a history or present symptoms of uncontrolled diabetes, significant obstructive or restrictive pulmonary dysfunction, pulmonary vascular disease, coronary artery disease, all forms of chronic or congestive heart failure, uncontrolled hypertension, anemia, stroke, cancer (other than melanoma), thyroid disease, autoimmune disease, severe muscular disease, neurological disease, metabolic or mitochondrial disease, bone disease, mitochondrial myopathies or insufficiencies, hepatic disease, chronic renal insufficiency, psychiatric disease that could be worsened by exercise or influence exercise capacity, cognitive impairment, chronic infection requiring antiviral or antibiotic treatment, anticoagulant therapy, or hormone replacement/supplementation therapy (other than birth control). Those who were currently pregnant, smoking, taking any medication/s that may have limited exercise capacity or the ability to adapt to an aerobic exercise training, or who were involved in active substance abuse were also ineligible to participate in the study. Subjects were assessed for eligibility using PARQ+[21], and a medical history questionnaire.

Pre and Post-Training Exercise Testing

Pre and post-training testing consisted of subjects reporting to the lab on two separate occasions with a minimum of 48 hours and maximum of 7 days in between each visit. During visit 1, subjects completed a peak cardiopulmonary exercise test (pk-CPET) on an electronically controlled cycle ergometer (Lode Corival, Medical Graphics Corp, St Paul, MN) followed by a 10-minute recovery period in the sitting position. An endurance bout (End1) was then performed immediately following the recovery period. Both the pk-

CPET and End1 ended at volitional exhaustion defined as, when despite significant encouragement to continue by the investigational team, the subject could no longer maintain the a priori determined pedaling cadence. The pk-CPET protocol consisted of a progressive increase in workload, advancing by 25 Watts each minute, while the subject maintained 60 revolutions per minute (RPM). The pk-CPET was performed to obtain measures of subjects' cardiorespiratory fitness, total $\dot{V}CO_2$ (t-$\dot{V}CO_2$), metabolic $\dot{V}CO_2$ (m-$\dot{V}CO_2$), non-metabolic $\dot{V}CO_2$ (nm-$\dot{V}CO_2$), and total $\dot{V}O_2$ (t-$\dot{V}O_2$). The intensity for End1 was set at 70% of the peak wattage attained during the pk-CPET and the measure of performance fatigability was the total time the subject was able to maintain the 60 RPM pedaling cadence.

During visit 2, subjects completed a submaximal, continuous work-rate test (CWRT), using a square-wave procedure, followed by a 10-minute recovery period and an endurance ride procedure identical to End1 (End2). For the CWRT, subjects completed 3-cycles of a work-rest combination consisting of 6-minutes at a work-rate corresponding to 80% of their anaerobic threshold (AT) determined from the pk-CPET and an 8-minute period of passive seated rest.

During each CPET and CWRT, cardiorespiratory function was assessed by measuring pulmonary gas exchange (MedGraphics CardiO2 CPET system; Medical Graphics Corp, St Paul, MN). The system was calibrated according to manufacturer's specifications prior to each CPET and CWRT. Heartrate (HR) was measured continuously by electrocardiogram (EKG).

Continuous High-Intensity Aerobic Exercise Training (AET)

Following the pre-training tests, subjects reported to the lab 4 times a week for 4 weeks to complete the aerobic exercise-training (AET) regimen. Training consisted of cycling using the choice of a mechanically braked (Monark 868, Healthcare International Inc, Seattle, Washington), upright (Nautilus U614, Nautilus Inc, Portland, Oregon; NordicTrack GX 4.6 Pro, NordicTrack, Logan, UT), or spin (Keiser M3, Keiser Corp, Fresno, Ca) stationary cycle for a duration of 45 minutes and at a target intensity of 70% of the subject's heart rate reserve (HRR) determined from the pk-CPET. A target HR range of 70% ± 5 bpm was individualized for each subject using the following equation[22]:

Target HR range= 65 and 75% HRR = [0.65 and 0.75 x (peak HR – resting HR)] + resting HR

Subjects warmed up for at least 5 minutes prior to the start of the 45-minute training session.

Determination of variables:

Cardiorespiratory Function: Peak $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by an 8-breath average, at the end of the pk-CPET or at the end of the last completed stage, whichever was higher. The AT, a marker denoting the onset of exercise-induced fatigue[23], was determined using the V-slope method of Beaver and Whipp[24] applied to breath-by-breath measurements and reported as the VO_2 and VCO_2 value at the AT (AT-$\dot{V}O_2$ and AT-$\dot{V}CO_2$, respectively).

Recovery off-kinetics following Peak CPET: Pulmonary gas exchange was analyzed breath-by-breath throughout the test. $\dot{V}O_2$ and $\dot{V}CO_2$ off-transient response was modeled

using nonlinear, least squares regression fitting techniques (Origin, OriginLab Corp., Northhampton, MA, USA) with a mono-exponential function of the form:

$$\dot{V}O_2(t) = (\Delta\dot{V}O_2 endexercise) + Amplitude(1 - e^{-(t-TD)/\tau})$$

Where $\dot{V}O_2(t)$ represents $\dot{V}O_2$ as a function of time (t) throughout the exercise transient; $\dot{V}O_2$endexercise is the $\dot{V}O_2$ data collected immediately upon exercise cessation; Amplitude is the amplitude increase in $\dot{V}O_2$ above the baseline value; tau (τ) is the time constant, or the time taken to reach 63% of the steady-state response; and TD is the time delay.[25] This formula was adapted from the model for off-kinetics and that used by Myers et al.[26] $\dot{V}CO_2$ off-kinetics were determined using the same model substituting $\dot{V}CO_2$ values and iterations in place of $\dot{V}O_2$. From these models, a mean response time (MRT) was estimated as the sum of tau and the time delay. The oxidative response index (ORI) was calculated as the $\Delta\dot{V}O_2$ /tau or $\Delta\dot{V}CO_2$ /tau, as specific to the analyses, and was utilized to normalize the response time to the amplitude.[27] The fit for both $\dot{V}O_2$ and $\dot{V}CO_2$ recovery kinetics began at the cessation of exercise and terminated at the end of the 10-minute recovery period.

Performance Fatigability: The primary measures of performance fatigability were total time in seconds (s) observed during End1 and End2 and peak time (pk-Time), and peak watts (pk-Watts) obtained during pk-CPET. End1 denotes the total time of the endurance bout following the pk-CPET and End2 denotes the total time of the endurance bout following the CWRT.

Total $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}CO_2$ Component Calculations:

- Total $\dot{V}CO_2$ (t-$\dot{V}CO_2$) and total $\dot{V}O_2$ (t-$\dot{V}O_2$) were calculated using the following formula:

[(peak time – AT time) * (peak ($\dot{V}O_2$ or $\dot{V}CO_2$) – (AT $\dot{V}O_2$ or $\dot{V}CO_2$))] / 2

- Metabolic $\dot{V}CO_2$ (m-$\dot{V}CO_2$) was estimated using the same formula but first calculating the estimated peak metabolic $\dot{V}CO_2$ (using the slope of $\dot{V}O_2$ line from time zero to the anaerobic threshold and extending to peak test duration time in seconds) and substituting this value for peak $\dot{V}CO_2$.
- Non-metabolic $\dot{V}CO_2$ (nm-$\dot{V}CO_2$) was estimated by calculating the difference between total $\dot{V}CO_2$ and metabolic $\dot{V}CO_2$ and converted to milliliters (ml).

Variables and Statistics. The main variables of interest were t-$\dot{V}O_2$, t-$\dot{V}CO_2$, m-$\dot{V}CO_2$, nm-$\dot{V}CO_2$, recovery $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic ORI, and indices of performance fatigability including End1, End2, pk-Time, and pk-Watts. Data were assessed for normalcy using histograms and Shapiro-Wilk tests. Pearson's product-moment correlation and regression analyses were used to determine the strength of relationships among the variables of interest and significant pre and post-exercise training differences were determined using paired t-tests. Statistical significance was set at $p \leq 0.05$ for all analyses. Numerical data are means ± one standard deviation.

Results

Demographic characteristics of the subjects are provided in Table 1. Resting HR was 80±13 bpm pre-AET and 78±13 post-AET. All subjects approached peak physiologic effort at volitional exhaustion[22] based on attainment of a respiratory exchange ratio

(RER) of at least 1.10[22] with 16 of the 20 subjects reaching more rigorous criteria of 1.15. All but one subject attained 85% of their age-predicted peak HR with the one remaining subject attaining 83% of age-predicted peak HR during the post intervention CPET. Subjects achieved a 96±0.10% at pre-training and 95±0.06% at post-training of their age-predicted maximum HR on the respective CPETs. Adherence AET to the aerobic training regimen was 99.7% with only one subject missing one training session.

Pre and Post-AET changes in metabolic and performance fatigability variables are reported in Table 2 while pre and post changes in $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic variables are reported in Table 3. There were no significant changes in resting $\dot{V}O_2$ (5.5±90.0 ml/min, p=0.562), resting $\dot{V}CO_2$ (12.35±43.9 ml/min, p=0.223), resting RER (0.010±0.08, p=0.635), AT-Time (8±56 s, p=0.523), AT-$\dot{V}CO_2$ (-6.85±153 ml/min, p=0.843), Ve$\dot{V}CO_2$ slope(-0.15±3.0, p=0.825), and $PetCO_2$ (-0.9±4.6 mmHg, p=0.397) following AET. Significant relationships between measures of recovery and PF measures were observed, although the strengths of the relationships were diminished (pk-Time, pk-Watts) or became non-significant (End1, End2) after controlling the analyses for the effect of nm-$\dot{V}CO_2$. (Table 4) Significant changes following AET in both $\dot{V}O_2$ and $\dot{V}CO_2$ recovery kinetics as measured by the ORI can be seen in Figure 1. Significant relationships between recovery kinetics, both $\dot{V}O_2$ (Panel A) and $\dot{V}CO_2$ (Panel B), and nm-$\dot{V}CO_2$ can be seen in Figure 2. Additionally, relationships between peak minute ventilation (Ve) and nm-$\dot{V}CO_2$ can be seen in Figure 3.

Discussion

The findings of the current study support the hypothesis that ionic buffering may play a modulating role in the relationship between performance fatigability and the capacity to recover following physical activity. As fatigue is eminent at work rates above the AT, these findings may have implications regarding AET mediated buffering dynamics and its role in fatigue resistance when performing physical activities above moderate intensities. In addition, significant improvements in recovery after CPET, as measured by $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetics, were observed following AET. The study may have implications in healthy adults, however, more information is necessary for inferences to be made to highly sedentary and clinical populations[29] in which AT is attenuated at lower levels of intensity that more closely reflect the energy demands associated with activities of daily living.[30]

The findings of the current study present insight regarding cardiorespiratory adaptions following AET using a short duration (4-week) continuous, high-intensity protocol. More commonly utilized are continuous, moderate-intensity, as well as high-intensity, interval training (HIIT)[31–33] protocols. Both methods have been shown to induce cardiorespiratory adaptions, such as increases in both peak $\dot{V}O_2$ [33,34] and the lactate threshold[34], also demonstrated using the protocol in this study. These findings may have implications in populations in which HIIT at peak intensities is contraindicated, as well as in populations that require more time efficient[35] interventions.

Improvements in the capacity to return to a state of homeostasis have previously been demonstrated following submaximal[36] and maximal exercise.[37] The findings of the

current study were in agreement with these previous studies, while further underscoring significant relationships among gas exchange off-kinetics, nm-$\dot{V}CO_2$, and laboratory measures of performance fatigability. Furthermore, the current study underscored associated changes in recovery $\dot{V}CO_2$ off-kinetics. and those previously reported by Takahashi et al [37] that suggest that the flux of CO_2 delivered to the lungs is an important determinant of ventilatory drive during recovery. While the exact mechanisms for improvement in recovery $\dot{V}O_2$ and $\dot{V}CO_2$ kinetics following exercise are not completely understood, the improved capacity to replenish high energy phosphates in the acute phase of recovery likely plays a dominant role [38] and the post-exercise recovery process is thought to be largely mediated by oxidative mechanisms. These processes and others can be inhibited by decreases in intracellular and plasma pH, implicating improvements in buffering capacity of function in the reduction of the time course of $\dot{V}CO_2$ off-kinetics and metabolic acidemia, in turn permitting faster $\dot{V}O_2$ off-kinetics.

The hypotheses that the ability to sustain exercise at intensities above AT can be inhibited and the rate at which post-exercise recovery can occur may be prolonged by metabolic academia has been presented.[39] The current findings support these hypotheses in that improvements in ionic buffering capacity were concurrent with improvements in the recovery-fatigability relationship. Hirakoba et al[40] reported that the ratio of relative excess CO_2 to lactate production was a potential determinant of running performance in distance runners. Moreover, Gaesser and Brooks[38] in their review of recovery following exercise, stated that much of post-exercise gas exchange kinetics may represent recovery metabolism. Because aerobic metabolism appears to play a determinant role in the acute

recovery phase following exercise, it remains susceptible to inhibition by by-products of anaerobic metabolism which were accumulated during the active portion of exercise. Performance of routine daily activities requires both the ability to resist fatigue during the activity and to recover once the activity has been completed. Both performance fatigability and recovery are intensity dependent and thus inextricably linked. Previous work[41] has demonstrated the strength of their relationship using timed walk tests and peak time and watts measured during CPET. In the current study, concurrent measures of performance fatigability were strongly correlated with measures of recovery for both pre and post AET. Furthermore, recent studies[42] have reported associations between measures of both performance fatigability and recovery, and nm-$\dot{V}CO_2$ calculated above the AT and it is well-understood that decreases in intracellular and arterial pH can inhibit oxidative phosphorylation and the cross-bridge cycle of the sarcomere. This would suggest that the ability to buffer byproducts of anaerobic metabolism is vital not only for sustaining activity but for recovery following activity.

Limitations

It should be noted that the calculations used in this study are estimations based on common theoretical assumptions. Variables such as nm-$\dot{V}CO_2$ cannot be measured directly by current gas exchange measurement technology. In addition, plasma lactate and pH were not measured so direct associations regarding acidity and respiratory buffering could not be made. Nonetheless, nm-$\dot{V}CO_2$ is an end product of the bicarbonate buffering mechanism and as such represents an index of buffering capacity given the accuracy of the estimates.

The sample of subjects in the current study, although sufficient in size for observing significant post-training changes and relationships among the variables in these subjects, was comprised of a non-randomized convenience sample. Thus, the nature and size of the sample limits the ability to generalize the findings to a larger population or more specific subsets such as those with acute and chronic illnesses. Also, the ability to generalize the findings of this work are limited to continuous, high-intensity AET interventions.

Conclusion

Clinical implications of these findings may exist as no population is immune to fatigue. Fatigue may indeed act as a barrier or reduce adherence to participation in rehabilitation programs potentially moderating the outcomes.[8,43] Activity limiting fatigue may reduce participation creating a spiraling effect in which fatigue is exacerbated by the activity limitation itself. In the current study, not only was AET successful in decreasing performance fatigability in this healthy population, but the protocol used was of short duration (4-weeks), which could result in higher adherence rates, as seen in this sample. The findings of the current study suggest that ionic buffering capacity, as measured by non-metabolic $\dot{V}CO_2$ estimations during CPET, may moderate the relationship between recovery and performance fatigability. These results could have implications regarding AET induced buffering dynamics and its role in fatigue resistance when performing physical activities above moderate intensities.

Table 1. Subject Demographics

Characteristic	(n = 20)
Age, years (median, IQR)	52 (46 – 55)
Female, n (%)	11 (55)
Male, n (%)	9 (45)
BMI, kg/m^2 (median, IQR)	26.5 (23.1 – 29.7)

Table 1. Demographic characteristics and baseline BMI (kg/m^2) prior to aerobic exercise training intervention. Data are reported in number per sample (n) and percentage of total sample (%), median, and interquartile range (IQR).

Table 2. Pre and post AET Changes in Cardiorespiratory, Metabolic, and Performance Fatigability Measures

Variable	pre	post	change (95% CI)	p value
Commonly Utilized Measures				
Peak $\dot{V}O_2$ (ml/min)	2181 ± 705	2484 ± 832	304 (187 – 421)	< 0.001*
Peak $\dot{V}CO_2$ (ml/min)	2817 ± 868	3038 ± 995	221 (39 – 402)	< 0.05*
Peak RER	1.32 ± 0.10	1.22 ± 0.09	-0.10 (-0.13 – -0.06)	< 0.001*
AT-$\dot{V}O_2$ (ml/min)	1270 ± 401	1360 ± 423	89 (27 – 152)	< 0.01*
Metabolic Measures				
t-$\dot{V}CO_2$ (ml)	3610 ± 217	5069 ± 3717	1460 (428 – 2491)	< 0.01*
t-$\dot{V}O_2$ (ml)	1986 ± 1334	3113 ± 2595	1127 (393 – 1861)	< 0.01*
m-$\dot{V}CO_2$ (ml)	1027 ± 811	1926 ± 2602	899 (-156 – 1954)	0.091
nm-$\dot{V}CO_2$ (ml)	2536 ± 1478	3146 ± 1683	611 (299 – 922)	< 0.001*
Performance Fatigability Measures				
Pk-Time (s)	519 ± 151	582 ± 147	63 (40 – 82)	< 0.001*
Pk-Watts	209 ± 61	233 ± 59	24 (15 – 33)	<0.001*
End1 (s)	453 ± 295	718 ± 470	265 (107 – 423)	<0.01*
End2 (s)	734 ± 484	1054 ± 776	321 (132 – 510)	<0.01*

Table 2. Pre and post AET changes in traditional cardiorespiratory, metabolic, and performance fatigability variables. Data are reported in mean ± SD and change scores (95% confidence interval). RER=respiratory exchange ratio, AT=anaerobic threshold, t-$\dot{V}O_2$ and t-$\dot{V}CO_2$=total volumes of $\dot{V}O_2$ and $\dot{V}CO_2$, m-$\dot{V}CO_2$=metabolic $\dot{V}CO_2$, nm $\dot{V}CO_2$=non-metabolic $\dot{V}CO_2$, pk-Time=peak Time, pk-Watts=peak Watts, End1 and End2 represent the endurance bouts.

Table 3. Pre and post AET $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic changes

Variable	pre	post	change (95% CI)	p value
$\dot{V}O_2$ off				
Δ $\dot{V}O_2$ (ml/min)	-1700 ± 604	-2046 ± 162	-346 (-427 – -220)	< 0.01*
TD (s)	6.6 ± 2.6	7.4 ± 0.9	0.85 (-1.22 – 2.93)	0.399
Tau (s)	55.6 ± 7.2	52.0 ± 6.2	-3.61 (-6.23 – -0.99)	< 0.01*
MRT (s)	62.2 ± 8.4	59.5 ± 8.0	-2.71 (-5.63 – 0.21)	0.067
$\dot{V}CO_2$ off				
Δ $\dot{V}CO_2$ (ml/min)	-2250 ± 783	-2505 ± 873	-253 (-422 – -87)	< 0.01*
TD (s)	8.1 ± 5.3	10.0 ± 5.7	1.91 (-1.21 – 5.04)	0.216
Tau (s)	86.4 ± 9.1	80.3 ± 8.4	-6.11 (-9.95 – -2.27)	<0.01*
MRT (s)	95.7 ± 10.1	90.3 ± 10.2	-4.35 (-9.08 – 0.39)	0.070

Table 3. Pre and post AET $\dot{V}O_2$ and $\dot{V}CO_2$ off-kinetic changes. Data are reported in mean ± SD and change scores (95% confidence interval). $\Delta\dot{V}O_2$ and $\Delta\dot{V}CO_2$ (ml/min) represents the change in amplitude from the start of recovery to the baseline/plateau; TD (s) represents the time delay; tau (s) represents the time constant or 63% of the time taken to reach baseline; MRT (s) represents the mean response time or $\Delta\dot{V}O_2$ or $\Delta\dot{V}CO_2$ divided by tau.

Table 4. Role of nm-VCO_2 in the Relationship between Recovery and Fatigability

	End1	End2	pk-Time	pk-Watts
$\dot{V}O_2$-off ORI	-0.412, p<0.01*	-0.514, p<0.01*	-0.822, p<0.001*	-0.836, p<0.001*
covarying nm-$\dot{V}CO_2$	-0.022, p=0.893	-0.005, p=0.976	-0.527, p<0.001*	-0.579, p<0.001*
$\dot{V}CO_2$-off ORI	-0.563, p<0.001*	-0.650, p<0.001*	-0.911, p<0.001*	-0.912, p<0.001*
covarying nm-$\dot{V}CO_2$	-0.2365, p=0.147	-0.151, p=0.364	-0.647, p<0.001*	-0.698, p<0.001*

Table 4. Relationships between measures of recovery and performance fatigability with and without accounting for the nm-$\dot{V}CO_2$ as a potential moderator between the two phenomena. Measures of performance fatigability are on the x-axis and measures of recovery are on the y-axis.

Figure 1.

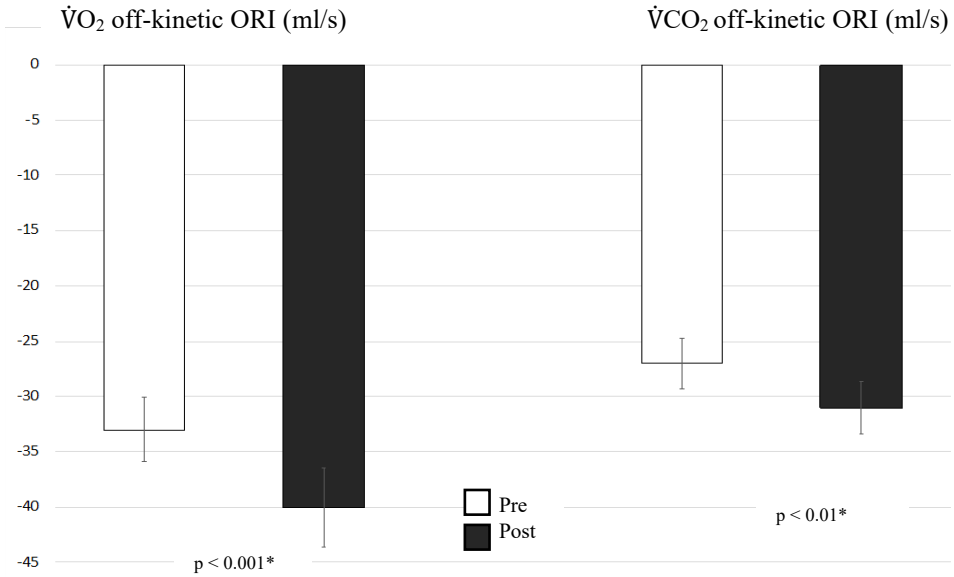
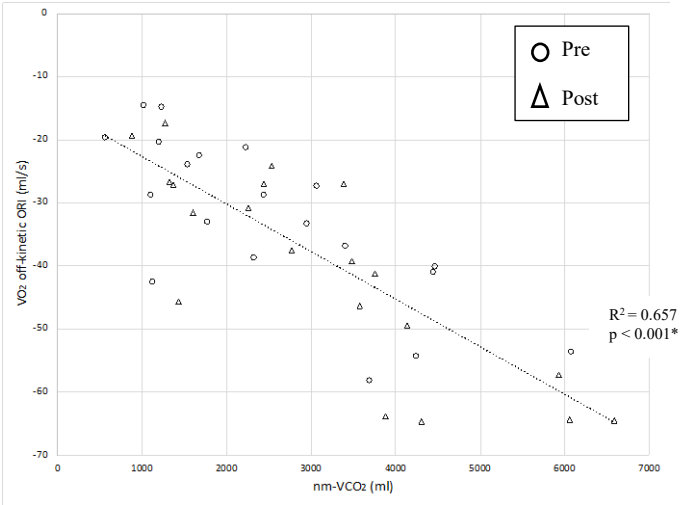


Figure 1. Change in $\dot{V}O_2$ and $\dot{V}CO_2$ recovery off-kinetics following AET. Recovery is measured by the off-kinetic transient oxidative response index (ORI ml/s). A larger negative value suggests a better ability to recover. Pre-AET means are represented by the white bars while post-AET means are represented by the black bars. Standard error bars and significance levels are reported.

Figure 2

Panel A.



Panel B.

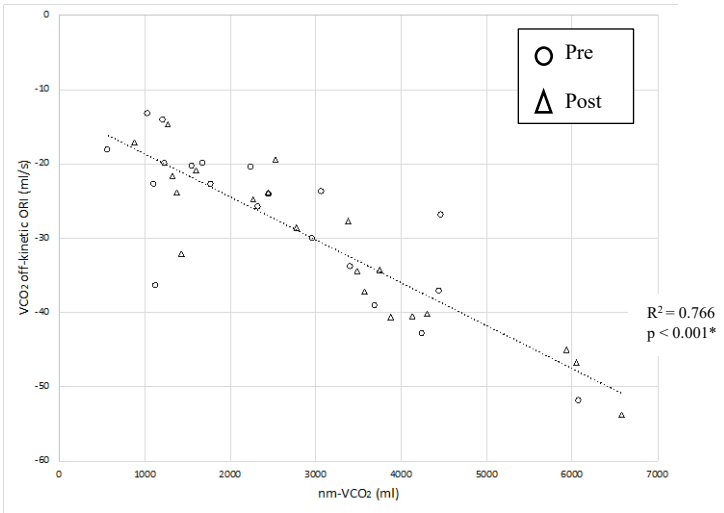


Figure 2.

Panel A. Relationship between recovery measures and non-metabolic $\dot{V}CO_2$. nm-$\dot{V}CO_2$ in ml in represented on the x-axis, while $\dot{V}O_2$ off kinetic ORI (ml/s) is represented on the y-axis. Open circles represent pre AET and triangles represent post-AET. R^2 and significance levels reported.

Panel B. Relationship between recovery measures and non-metabolic $\dot{V}CO_2$. nm-$\dot{V}CO_2$ in ml in represented on the x-axis, while $\dot{V}CO_2$ off kinetic ORI (ml/s) is represented on the y-axis. Open circles represent pre AET and triangles represent post-AET. R^2 and significance levels reported.

Figure 3.

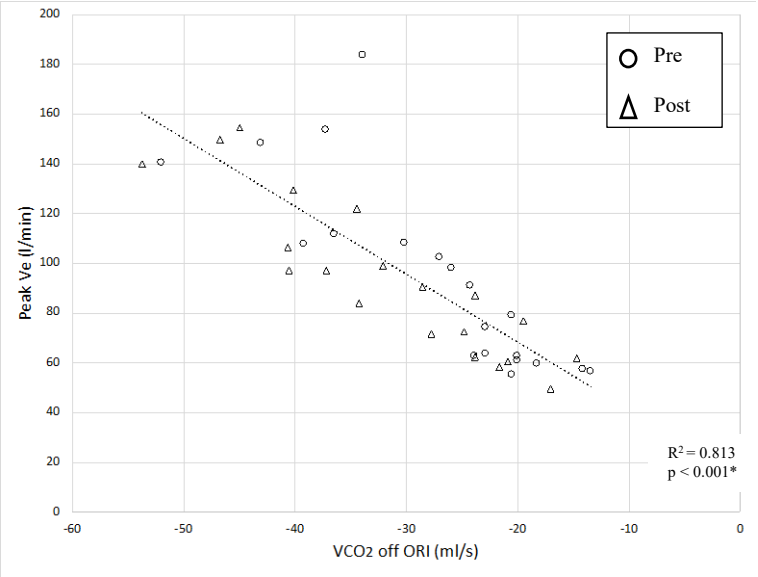


Figure 3. Relationship between $\dot{V}CO_2$ recovery kinetics and minute ventilation (Ve). $\dot{V}CO_2$ off kinetic transient ORI (ml/s) is represented on the x-axis, while Ve (l/min) is represented on the y-axis. Open circles represent pre AET and triangles represent post-AET. R^2 and significance levels reported.

APPENDIX

ORIGINAL DISSERTATION PROPOSAL

Abstract:

Fatigue is experienced by all individuals, not only those with acute or chronic illnesses[1–4], and has the ability to negatively impact daily, occupational, and social functioning, ultimately disrupting overall quality of life.[3] Among primary care patients, 42% report significant fatigue.[2] Yet, as a self-reported measure, quantification of fatigue severity has remained a challenge to researchers, clinicians, and healthcare professionals alike.[15,25,26] Measures of fatigability, determined as changes in fatigue severity normalized specifically to the intensity, duration, or frequency of the activity being performed, control analyses for the effects of self-pacing or the titration of activity level in order to manage feelings of fatigue.[15,25,26] However, the construct of fatigability is relatively new, and thus our understanding of the underlying mechanisms and their associated functional limitations are not completely understood.

The ability to sustain physical activity is dependent on the energy substrate provided by oxidative phosphorylation.[19,33,54] If oxidative capacity is insufficient for meeting this demand entirely or if buffering of the ionic by-products of metabolism is insufficient for maintaining an optimal intracellular environment, decreases in the pH of the sarcoplasm facilitates competitive ionic binding.[19,33] This results in inhibition of crossbridge

formation and metabolic pump function impairing the ability to sustain muscle activity.[19] Mechanisms such as lactic acid formation and the bicarbonate system buffer these fatigue-inducing ions releasing non-metabolic carbon dioxide (CO_2) that can be measured as expired CO_2 (VCO_2) during cardiopulmonary exercise testing (CPET).[33,54] However, the relationship between VCO_2 and fatigability has yet to be investigated.

While the rate at which fatigue ensues and the ability to resist this fatigue during the active portion of an activity are vital components of our understanding of the impact of fatigability on physical activity, the return to homeostasis following the perturbation may also provide valuable insight. While the recovery phase following activity is strictly an aerobic process, this aerobic process can be inhibited by a decrease in pH brought upon by ion accumulation during the activity.[33] Thus, both fatigability and recovery are both profoundly influenced by mitochondrial energetics and ionic metabolite inhibition.[28,33] Still, the relationship between the two has yet to be understood.

Indices of aerobic capacity and maximal exercise performance have been utilized to capture clinical manifestations of fatigability.[27,28,59,60] While these measures have provided valuable insight into cardiorespiratory capacity, they largely omit information about more intricate buffering mechanisms that resist activity-limiting fatigue. My proposed project presents novel research aimed at furthering our understanding of the underlying mechanisms of fatigability and its impact on function. I will examine CPET response differences in the relationships among a) total expired non-metabolic carbon dioxide volume ($NMVCO_2$), b) metabolic recovery kinetics, and c) performance fatigability pre and post aerobic exercise training.

I will measure NMVCO_2 volume and CO_2 on-kinetics, VO_2 and CO_2 off kinetics in apparently healthy individuals. Measurements will be made by cardiopulmonary testing on the cycle ergometer before and after a 4-week aerobic exercise training regimen. The findings of this study will have important implications related to non-metabolic CO_2 a as manifestation of performance fatigability. Furthermore, the results of this study have important clinical implications in that understanding the relationship between recovery and fatigability may inform the management of fatigue and associated debilitation in the clinical setting.

Specific Aims

Fatigue impacts all individuals regardless of health status creating debilitating effects on physical function.[1–4,16] While this self-reported symptom is ubiquitous and imposes serious negative consequences on health-related quality of life, it remains a challenge for healthcare professionals to measure and treat.[4,6,15,26,59] The construct of fatigability was introduced to improve our understanding of the impact fatigue on physical activity by normalizing the change in fatigue (perceived fatigability) or decline in performance (performance fatigability) to the level of activity being performed.[15] While clinical manifestations of fatigability can be observed as limitations in exercise tolerance during cardiopulmonary exercise testing (CPET) our understanding of its mechanisms are not fully understood. Fatigability research has primarily utilized measures of oxygen consumption (VO_2) for primary physiologic variables[27,59,60] as the ability to sustain physical activity is dependent on the energy substrate provided by oxidative phosphorylation.[27,33] However, during activities above moderate intensity the mechanisms underlying increased ion accumulation and resultant carbon dioxide expiration are controversial: one hypothesis is that oxidative capacity is insufficient for meeting the increased work demand and ionic by-products of anaerobic metabolism and another that buffering capacity becomes functionally reduced as it approaches its maximum. In either case, if the buffering rate of these ions is insufficient, muscle contraction is impaired and fatigue ensues, resulting in the inability to further sustain activity. Furthermore, interacting with fatigability is the construct “recovery” or restoration of pre-perturbation homeostasis. Measures of recovery not only provide

valuable insight into one's level of cardiovascular fitness but have also been associated with survival rate, severity of disease, and functional capacity.[48,61–64] Etiologically, measures of recovery provide insight into the ability of skeletal muscle metabolism to return to a state of homeostasis following an imposed stress, such as fatiguing exercise. As recovery is an aerobic process it may also be inhibited and prolonged by insufficient buffering of ionic-byproducts produced during the activity.[33,54] Mechanisms such as lactic acid formation and the bicarbonate system are responsible for buffering the fatigue-inducing ions and result in the production of non-metabolic carbon dioxide (CO_2) that can be measured as expired CO_2 (VCO_2) during cardiopulmonary exercise testing (CPET).[33,54] Thus, the severity of fatigability, and the capacity to recover from activity are each profoundly influenced by skeletal muscle energetics[28], specifically the ability to buffer fatigue-inducing ions produced as a result of anaerobic metabolism. Yet, the role of total expired non-metabolic CO_2 ($NMVCO_2$) on the interdependency between fatigability and recovery has yet to be investigated.

I propose a novel pilot study aimed at assessing the role of $NMVCO_2$ in the relationship between fatigability and recovery and whether $NMVCO_2$ adapts to a brief regimen of aerobic exercise training independently of VO_2. I have completed pilot work in our laboratory that has provided preliminary data to support the proposed specific aims. Furthermore, this pilot study allowed me to acquire the knowledge and expertise necessary for conducting this research. I have assembled a Dissertation Committee of qualified individuals, each with specific expertise to advise me in my work and I will accomplish this work through the following Specific Aims:

1. Determine whether changes in total expired non-metabolic carbon dioxide ($NMVCO_2$) may be independent of changes in oxygen consumption (VO_2) in response to a 4-week aerobic exercise perturbation in healthy individuals. I hypothesize that in adaptation to aerobic exercise training, $NMVCO_2$ will increase independently of increases in peak VO_2.
2. Characterize the association of $NMVCO_2$ with fatigability-recovery relationship in response to a 4-week aerobic exercise perturbation in healthy individuals. I hypothesize that following aerobic exercise training, increases in total $NMVCO_2$ will be observed in concomitance with a) decreases in performance fatigability and b) faster recovery.

Fatigue is a universal symptom impacting not only those with medical conditions, but also those who are healthy, as only one third of all fatigue cases can be attributed to a diagnosed medical condition.[1,3,4,6] Furthermore, in older adults' fatigue has been shown to create significant health implications as it is associated with poorer mobility, functional limitations, disability, and mortality.[1,15,26,29,65] Both fatigability and recovery are determinants of the persistence and severity of fatigue and the effectiveness of rest for resuming normal physical functioning. The underlying mechanisms of and relationships among fatigue and its determinants remains an understudied area and filling the information gaps created by the lack of information regarding this mechanism of physical dysfunction may provide critical insights and novel approaches to fatigue management, having both physical and fiscal implications.

Significance

The National Institutes of Health, National Institute on Aging (NIH/NIA) has defined fatigue as either a perception of tiredness (perceived fatigue) or a decline in performance (performance fatigue) as a result of exertion. (NIH/NIA 34 PA-12-227) Experienced by all individuals of any age[1–4], fatigue disrupts daily living, social, and occupational activities negatively affecting overall quality of life.[2,3,16] Furthermore, in older adults fatigue has been shown to create significant health implications as it is associated with poorer mobility, functional limitations, disability, and mortality.[25,29,30] Fatigue also presents an important public health concern costing an estimated $136 billion per year for United States employers.[16] Although it is thought to be more commonly associated with diagnosed medical conditions, only one-third of all fatigue complaints can be attributed to disease.[1]

Estimates of fatigue prevalence and its impact on functional limitations have varied, and it has become clear that these estimates and thus our understanding of fatigue have been adversely affected by measurement challenges.[6,15,26,59] The phenomenon of 'self-pacing' occurs as individuals titrate their activity level to manage their experience of fatigue, masking the full scope of its impact.[15,26] Consequently, the construct of fatigability was developed to address these limitations by normalizing both perceived and performance fatigue to the level of activity being performed and quantifying it as a function of duration, intensity, or frequency of activity.[15] Measures of fatigability not only allow for

meaningful comparisons between individuals and groups but also provide insight into whether, and to what extent, fatigue impacts the ability to perform physical activity.[15]

As a relatively new construct, the underlying mechanisms and functional limitations associated with fatigability are not completely understood. Clinical manifestations of fatigability most often culminate in reduced exercise tolerance and altered indices of cardiorespiratory function.[27] Most commonly, outcome variables reported by previous studies have been delimited to traditional indices of aerobic capacity such as peak oxygen consumption[28] (peak-VO_2) or VO_2 at the anaerobic threshold[27], and performance measures such as timed-walk test results and CPET duration. While these measures provide valuable insight into aerobic capacity, they fail to localize or isolate more intricate mechanisms, which could improve the sensitivity of fatigability measurements and further our understanding of how fatigue is propagated and how it can be further mitigated. Identifying and utilizing indices that target the pathomechanisms specific to fatigability may help to improve our understanding and thus management of fatigue.

Background

Previous literature suggests that fatigability increases with advanced aging and is a leading reason for restricted activity in older adults.[1,28] Furthermore, it has been hypothesized that one aspect of the etiology of fatigability in older adults may be age-related alterations of mitochondrial energetics.[28] Conversely, recent evidence indicates that it is an age-associated decline in participation of physical activity, and not chronological age, that influences the degradation in mitochondrial energetics of skeletal

muscle[66]; previous reports have not accounted for likely influences of physical activity and cardiorespiratory capacity on the relationship between age and fatigability.[25,66]

Measures of VO_2 have been used almost exclusively to assess potential physiologic processes underlying fatigability and associated performance limitations.[25] While measures of VO_2 provide valuable insight into one's aerobic capacity, muscle fatigue during exercise above moderate intensities is believed to be mediated by ion inhibition of cross-bridge cycling resulting from competitive binding with the actin-myosin binding sites and the myosin head binding sites for ATP.[19] The ability to maintain exercise above moderate intensities thus depends on the ability to efficiently buffer these ions by, among other mechanisms, the lactic acid and bicarbonate buffering systems, which diminishes plasma ion concentration by increasing expired non-metabolic CO_2 (VCO_2).[19,33,54,55] Therefore, total non-metabolic VCO_2 ($NMVCO_2$), measured during CPET, may serve as a novel and more specific laboratory measurement of fatigability.

In addition to fatigability and its severity, limitations physical activity intolerance on bouts that are subsequent to previous bout are in part a function of recovery following activity. Previous reports have primarily focused on indices of cardiorespiratory capacity measured during the active portion of exercise tests[27,28] whereas the construct of recovery and its relationship to fatigability has yet to be fully conceptualized. Measures of recovery, operationally defined as restoration of pre-perturbation homeostasis, have been shown to be associated with survival rate, related to severity of disease, and predictive of functional capacity.[46,47,48] Moreover, these measures also provide valuable insight into

one's level of cardiovascular fitness[48–51] and into the ability of skeletal muscle metabolism to return to a state of homeostasis[52,53] following an imposed stress, such as fatiguing exercise. As functional activities are interspersed with rest periods throughout the day, the ability to recover sufficiently during the rest period may be a vital component of the ability to sustain function.

Fatigability and recovery are both profoundly influenced by mitochondrial energetics[28] but the relationship between the two constructs remains unclear. As a measure of ionic buffering, which is required by both the capacity to sustain intense activity and to recover from it, $NMVCO_2$ may provide information regarding the relationship between fatigability and recovery and a potential mechanism for their association. As the incurrence of fatigue is inevitable in all individuals, understanding the fatigue-recovery relationship may inform novel approaches to fatigue management in various population subsets.

Innovation

The proposed study will address two novel aims with the intent of furthering our understanding of fatigue management through identifying relationships between performance fatigability, $NMVCO_2$, and recovery. First, this research will investigate what may be a novel manifestation of performance fatigability, $NMVCO_2$. VO_2 during CPET has been the most often used physiological outcome measure in studying human performance capacity.[67] Fatigability[27,28,60] and recovery studies have also utilized VO_2 as their primary outcome measure. The current study will take a novel more mechanistic

approach by determining if NMVCO2 may provide a cardiorespiratory index that is more closely predictive of performance fatigability, independently of VO_2.

Secondly, the proposed study will investigate the contribution of $NMVCO_2$ on the relationship between fatigability and recovery in response to exercise perturbation. Fatigability as a performance determinant is a more recent construct and was only recently operationally defined in 2010.[5,6] The ability to perform and sustain sequential activities throughout the day depends not only on the ability to resist fatigue during the activity, but also on the ability to recover from the previous activity in preparation for the next. While measures of recovery following CPET are not new, they are underutilized as indices of cardiorespiratory fitness in comparison to those measured during the active portion of CPET. Consequently, the relationship between recovery and fatigability has yet to be investigated, though is vital to our understanding of fatigue and its clinical and functional implications.

Building on my preliminary work, I will characterize relationships between performance fatigability and both $NMVCO_2$ measured during cycle ergometry and VO_2 and VCO_2 off-kinetics during recovery. Despite the inability to demonstrate statistical significance (underpowered study) the r-values in my preliminary data, previously published in peer reviewed abstract form[68], inferred that performance fatigability during a 10-minute walk test may directly correlate with recovery kinetic indices following both maximal and submaximal exercise. (Table 1) This suggests that the recovery rate of the oxidative metabolic system following cessation of both severe and moderate intensity activity could potentially impact optimal performance on a subsequent bout of activity.

Furthermore, total expired VCO_2 was directly correlated with indices of performance fatigability (Table 2) as evidenced by VO_2 on-kinetics measures. These results suggest that higher $NMVCO_2$ may represent more efficient buffering of fatigue-induced by-products of anaerobic metabolism during exercise above moderate intensity. $NMVCO_2$ was also directly correlated with traditional measures of aerobic capacity including peak-VO_2 and the anaerobic threshold VO_2 (AT-VO_2). (Table 2)

Table 1.

	k_t (post-max)	k_t (post-submax)
Performance Fatigability Score	r = 0.68 p = 0.13	r = 0.52 p = 0.29

Table 2.

	PF- ORI	PF- tau	Peak VO_2	AT-VO_2
$NMVCO_2$	r = 0.7835 p = 0.037*	r = -0.8003 p = 0.03*	r = 0.7926 p = 0.034*	r = 0.6979 p = 0.0812

This current study design will build upon the pilot study in the following ways. First, the current study will target more specifically and inclusively the outcome variables of interest. Secondly, the current study will utilize measures of fatigability that cannot be influenced by motivation. Finally, changes in both fatigability and recovery will be assessed before and after an aerobic exercise training regimen.

Approach

Study Design: A single-arm, longitudinal study design will be employed in which subjects will participate in a 4-week aerobic exercise training protocol. This will entail completion of two pre-training exercise testing visits (visits 1 and 2), a continuous high intensity aerobic exercise training protocol (visits 3-19), and two post-training exercise testing visits (visits 20 and 21). Those individuals electing to participate in the study will be required to meet study inclusion criteria, be absent any of the exclusion criteria, and provide informed consent prior to enrollment. Specific details pertaining to the study population, testing protocol, and training protocol are below.

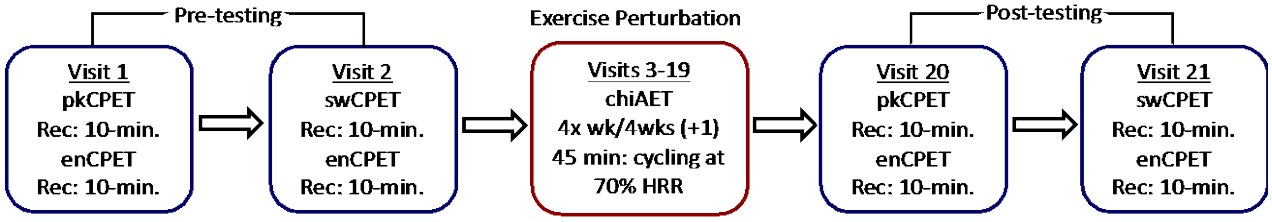


Figure 1. Study Protocol Overview. Abbreviations: 'CPET'- cardiopulmonary exercise test; 'pk'- peak; 'Rec.'- recovery; 'min'- minutes; 'en'- endurance; 'sw'- square wave; 'chiAET'- continuous high intensity aerobic exercise training; 'wk'- week; 'HRR'- heart rate reserve

Study population: Healthy subjects will be recruited from northern Virginia and greater Washington D.C. area. Those meeting the inclusion criteria and not meeting an exclusion

criterion will provide informed consent in accordance with the policies of George Mason University Institutional Review Board and the Declaration of Helsinki prior to enrollment.

Inclusion Criteria:

- age 18-60
- body mass index > 19 to <35 kg/m^2
- able to pedal leg cycle ergometer
- able to speak fluent English.

Exclusion Criteria:

Include a history of the following:

- uncontrolled diabetes mellitus (fasting plasma glucose > 125 mg/dL)
- significant obstructive or restrictive pulmonary dysfunction (eg. COPD, ILD)
- pulmonary vascular disease (all forms of pulmonary hypertension)
- coronary artery disease
- all forms of chronic or congestive heart failure
- uncontrolled hypertension (resting blood pressure > 160/100 on or off medication)
- anemia (hemoglobin < 10 g/dL)
- stroke
- cancer (other than melanoma)
- thyroid disease (e.g. hyperthyroidism, hypothyroidism)
- autoimmune disease (e.g. lupus, sjogren's, rheumatoid arthritis)

- severe muscular diseases (e.g. sarcopenia, McAardle)
- neurological disease (e.g. parkinson's disease, multiple sclerosis)
- metabolic/mitochondrial disease
- bone disease (e.g. osteoporosis, osteomyelitis)
- mitochondrial myopathies and insufficiencies
- hepatic diseases (e.g. hemochromatosis, hepatitis)
- chronic renal insufficiency (eGFR < 60 mL/min/1.73m^2)
- psychiatric disease that could be worsened by exercise or influence exercise capacity
- active substance abuse
- cognitive impairment
- chronic infection requiring antiviral or antibiotic treatment
- taking any medications that may limit exercise capacity or the ability to adapt to aerobic exercise training
- anticoagulant therapy
- therapeutic hormone replacement/supplementation (excluding birth control)
- known current pregnancy
- smoking (no smoking within the past 3 months or during the study;)

Study Procedures:

Cardiopulmonary Exercise Tests (CPET): CPET's will be carried out in the Department of Rehabilitation Science Functional Performance Laboratory, which is housed on the second floor of Peterson Hall on the Fairfax campus of George Mason University. All

three CPET's will be performed on an electronically braked cycle ergometer (Model, Brand and address).

I. Peak CPET (pkCPET): Resistance for the pkCPET will be progressed using a 25 watt per minute RAMP protocol on an electronically braked cycle ergometer. The work rate progression will start with one minute of unloaded pedaling and the work rate will be progressed each minute until volitional exhaustion has been attained. The increase in wattage per time was selected as a best effort attempt to ensure optimal data collection with a test duration of between 8 and 12 minutes. The targeted test endpoint is the attainment of volitional exhaustion defined as the subject's insistence that he or she must stop exercising due to fatigue despite strong encouragement to continue from the testing staff. Cardiopulmonary gas exchange and heart rate (EKG) will be measured throughout the pkCPET.

II. enCPET: The purpose of the enCPET is to obtain a measure of performance fatigability as measured by the total time the subject is able to continue exercising at 70% of their peak wattage attained on the pkCPET. Following the pkCPET and after a 10-minute recovery period, or until the subject's VCO_2 has returned to baseline (whichever happens first) subjects will start the enCPET. During the enCPET, resistance will be increased within 5-10 seconds to 70% of the maximal wattage the subject reached during their pkCPET. The subject will then cycle at 60 rpm until they reach volitional exhaustion or until a target duration of 60 minutes if he or she is able to meet this endpoint. Cardiopulmonary gas exchange and heart rate (EKG) will be measured throughout the enCPET's.

III. swCPET: The purpose of the swCPET is to provide an exercise perturbation for measuring VO2 on-kinetics. swCPET will occur on a separate day (2 – 14) days after the pkCPET. A baseline resting period of three minutes and three 6-minute work intervals separated by 8-minute intervals of passive recovery are required for this test. Subjects will first maintain a consistent 60 rpm while the wattage increases to a predetermined value corresponding to 80% of (20% below) their anaerobic threshold determined from the pkCPET on day 1 of testing. Subjects will exercise at this intensity for 6 minutes followed by a passive recovery period of 8 minutes. A total of three work-recovery cycles will be completed. Cardiopulmonary gas exchange and HR (EKG) will be measured throughout the swCPET.

Cardiopulmonary Gas Exchange Analyses: All gas exchange analyses will be made using a Medgraphics Cardio2 Ultima® (St. Paul, MN), cardiopulmonary exercise testing system. The system will be calibrated before all cardiopulmonary tests. Subjects will interface the system by wearing a form-fitting facemask or a mouthpiece and nose clip, which is connected to the system by small plastic tubing. Subjects will breathe room air through a bidirectional pneumotachometer interface fitted to the mask or mouthpiece. Pulmonary gas exchange will be measured and recorded throughout the pre-test resting, exercise testing, and 10-minute recovery periods for all of the CPETs. Main variables for analysis are total body oxygen consumption (VO_2), total non-metabolic carbon dioxide expiration ($NMVCO_2$), respiratory exchange ratio (RER), which is calculated as the ratio of VCO_2 to VO_2, and the anaerobic threshold (AT), determined by the V-slope method[69].

$NMVCO_2$ Calculation: VCO2 will be plotted on VO_2 during the CPET and $NMVCO_2$ will be estimated as the area above the VO_2 line between the AT and peak VO_2. Calculations of area will be made using the formula: [(peak VCO_2time - AT VCO_2 time) * (peak VCO_2 volume-AT VCO_2 volume)]/2, for both metabolic and total VCO_2 areas. The total area of VCO_2 will be calculated by using the measured peak VCO_2 (volume and time) and the VCO_2 (volume and time) projected at the AT. The estimated peak metabolic VCO_2 will be calculated using the slope of VCO_2 line from the start of exercise to the AT. This value will be used to calculate estimated metabolic VCO_2 volume as an area. The area of metabolic VCO_2 will be subtracted from the total VCO_2 to give a final value for $NMVCO_2$.

Electrocardiographic Measurement of Heart Rate: Heart rate (HR) will be obtained from the on-board electrocardiograph (EKG) interfaced in the cardiopulmonary exercise testing system. 10- electrodes will be placed on the subjects' chests in the standard Mason-Likar exercise-testing configuration. HR will be monitored and recorded continuously throughout all CPETs.

Continuous high intensity aerobic exercise training (chiAET): The chiAET protocol consists of training sessions 4 times a week for a total of 4 weeks. During training sessions, subjects will cycle at 70% of their heart rate reserve (HRR) plus or minus 5%. HRR will determined from the pkCPET as the subjects maximal HR minus their resting HR. This will be multiplied by .70 to obtain 70% which is then added back to the resting HR for a final target HR intensity. Plus/minus 5% of this target HR will be calculated to

provide upper and lower limits for a range of target training intensities surrounding 70% HRR. Subjects will warm up for ~5 minutes until their HR reaches their desired predetermined range. They will then cycle for 45 minutes keeping their HR within target range, followed by a 5-minute active recovery period of progressively decreasing intensity to end each session. HR will be monitored using a polar chest strap, watch, and the HR reading on the cycle ergometer.

Questionnaires:

Medical History Form: The health history form is used to determine whether an individual meets inclusion/exclusion criterion and will provide information about non-exclusionary medications (including vitamins/supplements). This form will also request emergency contact information from subjects in the unanticipated occurrence of an adverse event.

Physical Activity Readiness Questionnaire-plus (PAR-Q+): The PARQ+ is used to determine whether or not an individual is safe to exercise. The form includes 7 general health questions answered with either a 'yes' or 'no'. If the individual marks 'no' to all 7 questions, they are cleared for participation. If they answer 'yes' to any of the questions, they are asked to answer follow up questions to deem eligibility.

International Physical Activity Questionnaire (IPAQ): The IPAQ is composed of a set of 5 sections (total 27 questions) with the purpose of obtaining comparable data on health-

related physical activity. The IPAQ is designed for individuals aged 15-69 years old and asks individuals to recall the type and duration of their physical activity in the last 7-day period.

Perceived Fatigability Scale: The perceived fatigability scale will be given prior to and post both enCPET's. Subjects will rate their perception of fatigue or vigor on a scale of 1-7 using the left side of the Fatigue and Fatigability Scale prior to the CPET and following the test, subjects will be asked "compared to when you started, how would you rate your level of tiredness now" using the right side of the scale. The left side is considered a measure of fatigue because a change in fatigue was not assessed. The right side is considered to be a rating of fatigability because it assesses the change in tiredness. The score for the change in tiredness is then normalized to the total distance covered to calculate the perceived fatigability score: performance fatigability = (change in tiredness / total distance walked) x 100 (multiplied by 100 to facilitate reporting and comparison).

Statistical Analysis Plan

Data will be collected at two time points, pre and post chiAET. Primary variables of interest include measures of fatigability, recovery, and $NMVCO_2$. Fatigability will be measured as time to fatigue during the enCPET and VO_2 on kinetics during the swCPET. Recovery will be measured as VO_2 and VCO_2 off-kinetics following the active portion of CPET's. Non-metabolic VCO_2 will be measured as the $NMVCO_2$ during the peak and enCPET's. Changes in variables compared pre and post-chiAET will be analyzed using

student's T-tests with significance set at $p < 0.05$. For determining the primary aim, changes in $NMVCO_2$ will be compared to changes in VO_2 using student's T-tests. For aim two, the relationship between fatigability and recovery will be determined by Pearson's correlation in which a strong relationship will be defined as an r=0.8 or greater and moderate relationship as an r=0.6 or greater. Analyses of covariance analysis will be used to determine to what extent $NMVCO_2$ plays a role in this relationship. Analyses of covariance will also be run in order to compare the contribution of level of other variables thought to possibly effect results, including physical activity and age of subjects. VCO_2 analyses will be covaried by work rate as the peak work rate elicited during the pkCPET will determine the enCPET and swCPET work rates. Subjects who do not reach volitional exhaustion will be stopped at the 60-minute limit during the enCPET, therefore the total time on the treadmill will be compared using Tobit regression as this model predicts the value given from the regression equation on the independent variables and can predict a value beyond the censoring limit.

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