The Relationship Between Blood Potassium, Blood Lactate, And Electromyography Signals
Related to Fatigue in a Progressive Cycling Exercise Test

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Abstract

MATTHEW TENAN: The Relationship Between Blood Potassium, Blood Lactate, And Electromyography Signals Related to Fatigue in a Progressive Cycling Exercise Test
(Under the direction of Robert McMurray)

Local muscle fatigue may be related to potassium efflux from the muscle cell and lactate accumulation within the muscle. However, this has not been verified. The primary purpose of this investigation was to determine if there was a relationship between fatigue related changes in median power frequency (MPF) of EMG signals from the vastus lateralis and blood lactate (LAC) or potassium (K) during a progressive cycling test. This was tested in eight trained cyclists and triathletes under normal and glycogen reduced conditions to modify the LAC environment. No significant relationships were found between LAC ($p>0.27$) or K ($p>0.16$) in either condition during exercise or recovery. Though both lactate and potassium have been implicated as factors relating to neuromuscular fatigue, neither is significantly related to changes in MPF during or after progressive exercise on a cycle ergometer.

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CHAPTER I

Introduction

The study of fatigue is of interest to many exercise scientists. The fields of motor control and exercise physiology have reached a consensus that neuromuscular fatigue is defined as the “failure to maintain the required or expected power output” (Edwards, 1983). Electromyography (EMG) has been extensively studied as a method of identifying neuromuscular fatigue (Jansen, 1997; Kuorinka, 1988; Lepers, 2002; McHugh, 2001; Nyland, 1993; Viitasalo & Komi, 1977). A decrease in the median frequency (MPF) of the EMG’s power spectral density (PSD) has been identified as a valid method of determining neuromuscular fatigue in isometric conditions (Viitasalo & Komi, 1977). Studies involving MPF in dynamic exercises have concluded that MPF may increase, decrease or not change at all (Masuda, 1999; Gerdle, 2000; Bouissou, 1989; Jansen, 1997; Ament, 1996). The conflicting results may be related to changes in neural drive related to the increases in force output dictated by the protocol when the EMG is collected (Dupont, 2000; Gamet, 1990). The inconsistent results may also be due to issues of signal non-stationarity related to data collection during dynamic activities.

The MPF is related to motor unit (MU) recruitment strategies. The slower MUs are generally comprised of Type 1 fibers which twitch at a lower frequency. Larger, faster MUs are generally comprised of Type 2a and Type 2b fibers which have a higher twitch frequency (Goswami, 2001). Type 2 fibers are also the largest contributor to increases in blood lactate in exercise, due to their incomplete metabolism of glucose (Ivy, 1980). Thus, the prevailing

belief is that the decreasing MPF occurs because of a slower conduction velocity along the sarcolemma due to accumulation of metabolites such as lactate within the muscle (Bouissou, 1989). The increase of lactate in type 2 fibers causes an increase in recruitment of type 1 fibers to maintain force production, causing a decrease in MPF. The lactate hypothesis has been partially verified by the finding that McArdle's patients, who are unable to metabolize glycogen and thus produce lactate, do not exhibit a decrease in sarcolemma conduction velocity in exercise until there is an additional change in force output (Linssen, 1996). However, this hypothesis has also been refuted by findings that MPF changes and restitution post-exercise are unrelated to blood lactate levels (Jansen, 1997).

It has been suggested that local muscle fatigue may be related to potassium efflux from the muscle cell (caused by contraction) as well as lactate accumulation within the muscle. The MPF alterations may be related to a combination of lactate as well as the efflux of potassium from contracting muscles, but this has not been tested in a well controlled study. It is known that in a glycogen normal state, lactate and potassium concentrations in the bloodstream both increase with exercise (Lindinger, 1995); however, a glycogen reduced state causes a decrease in blood lactate accumulation during a cycling progressive exercise test (Osborne, 2006). There are no documented studies indicating what the effect of exercise induced glycogen reduction has upon blood potassium in aerobic exercise. Muscle afferents from group III-IV detect both potassium and lactate concentrations in muscle fibers (Hug, 2003; Garland, 1991; Kaufman & Rybicki, 1987; Rotto & Kaufman, 1988). The group III-IV afferents may then exert an inhibitory response on alpha motoneurons, creating altered motor unit recruitment patterns (Woods, 1987). Despite the suggestion that both lactate and

potassium may alter motor unit recruitment patterns in fatigue, they have never been examined in a well-controlled study.

The purpose of this study was to examine the relationship between blood lactate and potassium accumulations, and the MPF of the EMG spectrum using a maximal voluntary isometric contraction (MVIC) while seated on a cycle ergometer. These parameters were measured at 5-minute intervals during a graded exercise test under two conditions: 1) a glycogen normal state and 2) a glycogen reduced state. The glycogen reduced condition created an environment whereby lactate production is severely decreased but blood potassium is the same as in the glycogen normal condition. According to the available literature, this was the first time a discontinuous $\text{VO}_{2\text{max}}$ protocol with MVIC's has been utilized. MVIC's were utilized to limit changes in the total spectrum power and MPF due to changes in length-tension and force-velocity over the time of data collection.

Research Questions

1. Does glycogen reduction modify the relationship between plasma potassium concentration changes from baseline ($\Delta\%K$) and MPF ($\Delta\%MPF$) during isometric contractions collected during and after an exercise test?
2. Does glycogen reduction modify the relationship between blood lactate concentration changes from baseline ($\Delta\%LAC$) and $\Delta\%MPF$ during isometric contractions collected during and after an exercise test?

Research Hypothesis

1. $\Delta\%MPF$ will decrease with increasing exercise intensity in both glycogen normal and glycogen reduced conditions.

2. $\Delta\%$ MPF will increase during the recovery period in both glycogen normal and glycogen reduced conditions.
3. $\Delta\%$ K will increase with exercise intensity in both conditions and decrease to resting levels in recovery.
 - a. During exercise, increases in $\Delta\%$ K will be related to decreases in $\Delta\%$ MPF in both conditions.
 - b. Post-exercise, decreases in $\Delta\%$ K will be related to increases in $\Delta\%$ MPF in both conditions.
4. $\Delta\%$ LAC will increase with exercise intensity in both conditions and decrease during recovery.
 - a. During exercise in the glycogen normal condition, increases in $\Delta\%$ LAC will be related to decreases in $\Delta\%$ MPF.
 - b. During exercise in the reduced condition, increases in $\Delta\%$ LAC will not be related to decreases in $\Delta\%$ MPF.
 - c. Post-exercise, $\Delta\%$ LAC will not be related to changes in $\Delta\%$ MPF in either condition.
5. If a relationship exists between $\Delta\%$ LAC or $\Delta\%$ K and $\Delta\%$ MPF, the relationship will be stronger for $\Delta\%$ K than $\Delta\%$ LAC.

Definition of Terms

1. Blood Lactate Concentration [La⁻] – The concentration of circulating lactate or dissociated lactic acid in the blood stream.
2. Peak Oxygen Uptake (VO_{2Peak}) – The highest amount of oxygen used on a per minute basis during a graded exercise test.

3. Surface Electromyography (sEMG) – Ag/AgCl electrodes which reside on the surface of the skin. They record the myoelectric signals produced during the activation of a motor unit.
4. Motor Unit (MU) – The smallest functional unit to describe neural control of a muscular contraction. A MU encompasses the motor neuron, the multiple branches of its axon, and all the muscle fibers it innervates.
5. Power Spectrum Density (PSD) – This is an estimate of the frequency contents of the EMG signal. It is derived by running the EMG signal through a Fast Fourier Transform (FFT).
6. Median Power Frequency (MPF) – The parameter that divides the area under the PSD curve into two equal parts. A decrease in the MPF of an EMG signal is considered an indicator of fatigue.
7. Maximal Voluntary Isometric Contraction (MVIC) – A voluntary maximal muscle contraction in which the length of the muscle does not change.
8. Graded Exercise Test (GXT) – An incremental test in which work rates are increased until the subject reaches a predetermined end point.
9. Fast Fourier Transform (FFT) - The mathematical algorithm used to decompose a signal into its power spectrum density.
10. Stationarity - A prerequisite for using the fast Fourier transform on an electrical signal. Stationarity can be affected by changes in neural drive related to force output throughout the range of motion measured, changes in muscle and fiber length, and movement of the neuromuscular junction in relation to the detection electrodes. The

effects of non-stationarity in EMG can be minimized by choosing isometric contractions over dynamic.

11. $\Delta\%$ LAC - The percent change in lactate from a baseline measurement.
12. $\Delta\%$ K - The percent change in potassium from a baseline measurement.
13. $\Delta\%$ MPF - The percent change in median power frequency from a baseline measurement.

Delimitations

1. All subjects used their own bike saddle, clipless pedals and shoes to increase familiarity and facilitate normal motor patterns.
2. The glycogen reduction protocol was standardized for all subjects.
3. Standard racing handlebars on the cycle ergometer, including anatomical drops provided the subjects with a familiar position to normalize motor patterns.
4. All subjects must have been recreationally or competitively involved in cycling/triathlons for at least 60 days and were thus familiar with maximal exertion and cycling motor patterns using clipless pedals.
5. The electrodes were placed according to anatomical landmarks for that subject. Their skin was marked with a permanent black marker and digital photos were taken in order to ensure similar EMG electrode placement between the two conditions.
6. No more than 14 days elapsed between conditions to limit the effects of training/detraining on MU recruitment patterns.
7. EMG electrodes were placed in parallel with the muscle fibers to limit the effects of cross-talk. This was verified by manual muscle tests to confirm no EMG activity is detected with hip flexion.

8. An EMG ground electrode was affixed to the antero-medial side of the proximal tibia to limit artifact signals.
9. Glycogen reduction in the glycogen reduced condition created an environment with decreased lactate production but normal potassium changes during exercise and recovery. This will allow for comparison between the glycogen normal and glycogen reduced conditions of lactate's relationship with MPF.

Limitations

1. A decrease in the MPF due to fatigue is thought to be related to increase in intramuscular lactate accumulation. This study collected venous blood lactate accumulation, which is an indirect measure of intramuscular lactate in the working muscle.
2. While the nature of a GXT requires a constant cycling speed, this may not be fully generalizable to real-world conditions.
3. Due to the sample population of our study, the results may only apply to trained cyclists and triathletes.

Significance of the Study

The significance of this study resides in the use of a new methodology for investigating two established research questions, the relationship between MPF, blood lactate, and blood potassium during progressive exercise. Numerous previous studies have found conflicting results regarding the alterations of MPF in dynamic activities (Petrofsky, 1979; Viitasalo, 1985; Kuorinka, 1988; Bouissou, 1989; Gamet, 1990; Ament, 1996; Jansen, 1997; Masuda, 1999; Gerdle, 2000; Singh, 2007).

By collecting EMG data at each stage of a progressive cycling test using an MVIC on the ergometer, we attempted to circumvent the potential confounding factor of length-tension relationship artifact in the PSD. The MVIC also limits signal non-stationarities affecting the PSD. If this method proves effective, it may allow for more novel approaches to examining neuromuscular fatigue in other dynamic activities. It may also explain some of the discrepancies in the presently conflicting literature regarding occurrence of neuromuscular fatigue in dynamic exercise.

The methodology also has the potential of clarifying the existing disagreement between intramuscular lactate or potassium as the driving factor behind the decrement in MPF observed during local muscle fatigue. Because this study did not measure intramuscular concentrations, we cannot definitively answer this research question. However, because both elements escape into the bloodstream as a consequence of muscular work, we are able to gauge general changes in lactate and potassium efflux from the muscle. The alterations in lactate production associated with glycogen reduction, and a presumably normal efflux of potassium, may give indication as to which mediates the change in MPF.

CHAPTER II

Review of Literature

Introduction and Basics of Local Muscle Fatigue

This chapter reviews literature concerning the physiology of local muscle fatigue and the proposed methodologies of determining local muscle fatigue via EMG. The introduction provides a brief overview of the basics of local muscle fatigue. The following section discusses some of the proposed mechanisms of local muscle fatigue. Subsequent sections will discuss electromyography, the various factors affecting the EMG signal, and possible underlying physiologic mechanisms causing fatigue-related changes in EMG.

Local muscle fatigue (used interchangeably with the term neuromuscular fatigue) has been described as a “failure to maintain the required or expected power output” (Edwards, 1983). Lepers (2002) more recently described neuromuscular fatigue as a decrease in the muscles’ ability to exert a maximal voluntary force; however, this definition does not relate the functional aspect of work capacity which requires that muscle maintain both force and velocity.

The origins of local muscle fatigue are complex. Bigland-Ritchie (1984) described eight potential sites of fatigue. Four of these sites are classified as central and neuromuscular transmission: (1) input to higher motor centers, (2) excitatory drive to lower motor neurons, (3) excitability of the motor neuron itself, (4) and transmission at the neuromuscular junction. The other four sites are peripheral in nature at the muscle itself: (1) sarcolemma excitability, (2) excitation-contraction coupling, (3) the mechanical contractile mechanism, and (4) local

energy supply and resulting metabolites. Evidence suggests that the mode, intensity and duration of the physical activity as well as the subject's experience with the activity and muscle fiber types influence fatigue (Fitts, 1981). The central and peripheral sites of fatigue are further complicated by Group III/IV afferents. Group III/IV afferents sense many of the mechanical, electrolytic and metabolic factors released or created during muscular contraction (Kaufman & Rybicki, 1987; Rotto & Kaufman, 1987). The sensory input from Group III/IV may sense peripheral causes of local muscle fatigue and reflexively cause changes centrally (Woods, 1987). The impact of peripheral factors on central sites, combined with the difficulty of measuring central factors in exercise has caused the majority of research to focus on the periphery.

Physiology of Local Muscle Fatigue

Multiple factors are likely involved in local muscle fatigue. There are currently two major models proposed to explain the concept of fatigue in exercise. Most recently, Lambert, St. Clair Gibson and Noakes (2005) have proposed an integrative model of fatigue. This model integrates a subject's teleoanticipation regarding the duration and intensity of the exercise, peripheral feedback from the local muscles mechanoreceptors and chemoreceptors, prior exercise experience (reference signals), actual and perceived metabolic reserves and redundancy systems of command. The integrative model is contrary to a reductionist model which examines muscle fatigue as a phenomenon originating and manifesting at the local muscle tissue. The reductionist model is extensively reviewed by Fitts (1994). Updates to Fitts' model regarding the physiology of local muscle fatigue in relation to potassium and lactate follow in this section.

Fitts (1994) states that “the force-depressing agent is H^+ and not lactate”. However, more recent studies do not support this statement. When experiments are conducted at near-physiologic temperatures, the lactate molecule is highly related to characteristics of fatigue and pH has little effect on force suppression. On the contrary, decreasing pH has been demonstrated to protect against the force suppression caused by potassium efflux from the muscle cell during contraction.

Hogan (1995) examined the effects of increased arterial lactate concentrations in relation to gastrocnemius force output in canines at a constant pH. They stimulated canine muscle via the sciatic nerve for 60 minutes with normal blood perfusion and with a high lactate/normal pH blood infusion. Their results indicated that high lactate conditions had a significantly lower VO_2 , oxygen extraction percentage, and ability to develop tension. The high lactate levels were physiologic (12-15 mmol). The investigators concluded that increases in lactate, independent of changes in pH, cause fatigue-like changes in skeletal muscle at physiologic temperatures.

Pate (1995) examined the effect of reduced pH on rabbit muscle mechanics at high and low temperatures. They examined the muscle at pH's of 7.0 and 6.2 as well as at temperatures of 10 degrees Celsius and 30 degrees Celsius. They replicated earlier studies which demonstrated that lowered pH at 10 degrees Celsius cause an inhibition of maximum shortening velocity and total isometric tension. They then demonstrated that the lowered pH had no effect on maximal shortening velocity and a very minimal effect on total isometric tension at 30 degrees Celsius. Pate concluded that, at physiologic temperatures, it is highly unlikely that decreases in pH act directly on the muscle to inhibit maximum shortening velocity and total isometric tension.

Westerblad (1997) reviewed the effect of intracellular pH on the contractile function of single fibers of mouse muscles at three temperatures. They showed that acidified fibers had a reduced tetanic force (28%) at 12 degrees Celsius, compared to a non-significant reduction in tetanic force (10%) at 32 degrees. The authors concluded that the effect of pH decreases with increasing temperature. The direct inhibition of force production by decreases in pH are small at 32 degrees Celsius and may not be a major factor in fatigue at physiologic temperatures.

An efflux of potassium from the muscle fiber has also been proposed as a mechanism of local muscle fatigue. Cairns (1995) investigated the relationship between extracellular potassium, membrane potential and contraction in rat soleus muscle. They reported that elevating the extracellular potassium concentration from 4 mM to 8-9 mM resulted in minimal depression of tetanic force. However, a further small increase to 11-14 mM created a large reduction in force. Their experiment suggests that changes in the concentration gradient across the muscle membrane may be an important factor in restoring excitability and force production.

Previous studies had indicated that high levels of extracellular potassium, lactate, and decreases in pH all decrease force production in skeletal muscle. Nielsen (2001) examined the effect of lactic acid in combination with potassium in relation to force production. Rat soleus was incubated in 11 mM extracellular potassium. This reduced tetanic force production by 75%. They then incubated the muscle in 20 mM of lactic acid at 37 degrees Celsius. The lactic acid incubation resulted in an increase in force production from baseline (pre-potassium incubation levels). They reproduced this finding with propionic acid instead of lactic acid and had similar results. The researchers suggest that acidification of the muscle

counteracts the force depressing effects of elevated extracellular potassium. There findings actually suggest that, contrary to popular opinion, muscle acidosis may protect against fatigue.

Surface Electromyography and Factors Affecting Power Frequency Spectrum

Surface electromyography (sEMG) equipment allows the researcher to record and quantify the sum of the electrical contributions made by active motor units (MUs). Every muscle fiber is surrounded by a sarcolemma. A resting electrical gradient is created along the sarcolemma by concentrations of potassium (K^+), sodium (Na^+), and chloride (Cl^-). This gradient is established by the Na^+/K^+ ATPase pump and the cell membrane's greater permeability for K^+ ions (Lamb & Hobart, 1992). sEMG is traditionally a two electrode set up which is able to extract the global measure of MU activity in the muscle underlying the skin on which the two electrodes are placed. Two common characteristics of the sEMG signal, amplitude and frequency spectrum, are dependant on both sarcolemma membrane properties and the timing (frequency) of MU action potentials. As such, the sEMG signal is a result of both central and peripheral properties of the neuromuscular system (Farina, 2004).

The seminal work by Basmajian and De Luca, *Muscles Alive* (1985), eloquently describes and graphically depicts the summation of MU action potentials contributing to the sEMG signal. Basmajian and De Luca (1985) also describe the filter functions of the tissue whereby fat tissue and skin act as a low-pass filter to the sEMG signal. Additionally, they suggest that the sEMG electrode be aligned in parallel with the muscle fibers as action potentials travel 'down' the length of the muscle fiber. Weir (1999) examined the effect of electrode placement in parallel and perpendicular to the muscle fibers of the vastus lateralis on sEMG indicators of fatigue, amplitude and power spectrum. They found amplitude

changes between both conditions to have a strong relationship once amplitude was normalized to a maximal isometric contraction in each electrode orientation. The frequency spectrum changes between both orientations resulted in a non-significant correlation ($r=.05$, $p>.05$).

The sEMG changes in frequency spectrum have been the most frequently used indicator of local muscle fatigue. The use of changes in the frequency power spectrum as an indicator of fatigue was first accepted through the work of Carlo De Luca (1984). It has been demonstrated that changes in the median power frequency of the sEMG spectrum indicate changes in the conduction velocity of muscle fibers in vitro (Solomonow, 1990; Brody, 1991). Even after controlling for skin impedance and electrode orientation, there are a number of anatomical and physiological factors which affect muscle fiber conduction velocity and thus the frequency spectrum of sEMG. The following paragraphs will discuss inconsistencies in types of muscles examined, types of contractions performed and differing force outputs, making direct comparisons difficult.

sEMG only measures the electrical activity of the muscle under the electrodes at the moment of data collection. This makes valid sEMG data collection difficult during dynamic tasks. The electrode may not move in relation to the skin, but the electrode detection area does move in relation to muscle as it shortens and lengthens under the skin. This movement creates inconsistencies in signal characteristics (Ebenbichler, 2002).

Muscle fiber type influences sEMG frequency spectrum. The rationale for this influence is that larger type 2b muscle fibers have a larger diameter than type 1 and thus more surface area on which to conduct the action potentials at a higher rate (De Luca, 1997).

To verify the hypothesis that muscle fiber distribution affects the sEMG frequency spectrum, Viitasalo and Komi (1978) examined the muscle morphology of the vastus lateralis in relation to the percent change in the median power frequency in isometric contractions sustained at 30, 50, and 70% of maximal force to exhaustion. They found that during the 70% of maximal intensity, those subjects with less than 49% slow twitch fibers exhibited a faster decrease in median power frequency compared to subjects with greater than 59% slow twitch fibers. This is logical because subjects with greater proportions of fast fatiguing fibers will likely see a de-recruitment of these fibers as they fatigue.

Gerdle (2000) examined the mean frequency of the power spectrum of the vastus lateralis throughout 100 isokinetic knee extensions of 20 subjects in relation to muscle morphology. They found that mean frequency was positively correlated with the proportion of type 1 and type 2a fibers and negatively correlated with the proportion of type 2b fibers. These findings contradict the rationale that type 2b fibers should be positively correlated with increases in frequency power. The study recorded sEMG data during the dynamic contractions, introducing extra variability into their data. This study may indicate that muscle morphology only affects sEMG during isometric contraction.

Both the fiber content of the muscle as well as increasing neural drive related to increased force output can affect the sEMG frequency spectrum. Bilodeau (1994) examined the median frequency of soleus, lateral gastrocnemius, and medial gastrocnemius throughout a ramp-force plantarflexion contraction from 10-80% of maximal force. The soleus, a muscle with expected higher type 1 fiber content than gastrocnemius, exhibited a lower median frequency throughout the ramp-force contraction (80-100 Hz) compared to the gastrocnemius muscles (100-150 Hz). Both the soleus and the medial gastrocnemius

exhibited a progressive increase in median power frequency (MPF) with increasing force.

The investigators concluded that MPF increases with increasing neural drive, irrespective of muscle morphology.

A number of studies have confirmed the findings of Bilodeau (1994) that increases in force output cause changes in muscle fiber conduction velocity and MPF. Zwarts & Arendt-Nielson (1988) examined both the vastus lateralis and biceps brachii to determine how force output affected muscle fiber conduction velocity. They determined that conduction velocity and force to have a positive and linear relationship when examining discrete collection points at force levels 10-100%. When a maximal contraction is held, the relationship is still positive, but the relationship is curvilinear as force output decreases due to fatigue. Dupont (2000) were concerned with biceps brachii muscles in bifunctional activation tasks; however, they qualitatively reported that MPF and force output have a positive linear relationship until a plateau between 60-90% of force output. Increases in neural drive, associated with increases in force output, cause a significant rise in MPF.

The sEMG signal, especially the sEMG frequency spectrum, is strongly affected by factors unrelated to changes in physiology. It is important when attempting to derive physiologic changes from the sEMG signal to account for electrode orientation, muscle morphology, contractions utilized for data collection, and changes in neural drive related to force output.

Conflicting Results For Dynamic Fatigue Analysis in sEMG

Many studies have found conflicting results when investigating sEMG power spectrum changes, including MPF, during dynamic conditions. These results may be due to inconsistent methodologies which do not take into account all of the variables indicated in

the previous section detailing the factors affecting sEMG power frequency spectrum. This section details the conflicting results in the literature.

Potvin (1997) examined the effects of elbow joint angle and fatigue on MPF in dynamic flexion/extension exercises. He found MPF values to be similar at rest for both concentric and eccentric contractions. He reported a decrease in MPF due to fatigue, but noted that the greatest decreases in MPF were shown with the elbow in flexion. At the end of the trial, the MPF was similar at all joint angles. Potvin hypothesized that this may reflect a biological minimum conduction velocity before contraction failure.

Potvin & Bent (1997) compared MPF in isometric tests with MPF in isotonic biceps brachii flexion exercises to fatigue. They reported a high correlation coefficient ($r=0.76$) at rest between isometric flexion at 90 degrees and an isotonic contraction collected between 80-100 degrees of flexion. This coefficient was lower when compared to isotonic data collected though the entire range of motion ($r=0.45$). When they examined the change in MPF due to fatigue, correlation coefficients faired similarly (isometric vs. isotonic at 80-100 degrees, $r=0.48$; isometric vs. entire isotonic, $r=0.28$).

Masuda (1999) examined the changes in muscle fiber conduction velocity and MPF in isometric and isotonic knee extensions of 50% maximal isometric contraction to exhaustion. They found that both muscle fiber conduction velocity and MPF decreased significantly during the isometric trial. Interestingly, MPF was found to decrease significantly during isotonic exercise, but muscle fiber conduction velocity did not significantly change. Both MPF and muscle fiber conduction velocity were significantly higher in isotonic exercise compared to isometric. The authors suggest that because blood flow is maintained in dynamic contractions by venous return, muscle fiber conduction

velocity is affected by the metabolic state, including lactic acid and potassium concentrations of the muscles, and the changes in MPF cannot be explained by changes in muscle fiber conduction velocity.

Ament (1996) investigated the MPF changes of the soleus and gastrocnemius muscles during exhaustive uphill treadmill running. They found no systematic decrease in MPF during the exhaustive run. However, when they compared an isometric contraction before the run to an isometric contraction after the run, a significant decrease in MPF was observed. They also recorded EMG 1, 2, 3, 4, 5, 7, 10, 15, and 20 minutes after exercise. The subsequent MPF values demonstrated a restitution of the EMG signals approximating pre-exercise levels.

Petrofsky (1979) examined MPF during exercise at different intensities on a cycle ergometer. At loads of 20 and 40% $\text{VO}_{2\text{max}}$ held for 80 minutes, MPF increases in the initial 20 minutes of work and progressively decreased as work continued. At workloads of 60, 80 and 100% $\text{VO}_{2\text{max}}$, MPF progressively decreased from onset of exercise. The findings indicate that decreases in MPF are related to fatigue during cycling; however, Petrofsky did not report any hypothesis testing so determining the significance of this study is difficult.

Hug (2003) investigated the various EMG signs of neuromuscular fatigue from the vastus lateralis and their relation to ventilatory threshold during exhaustive cycling exercise. EMG data was collected dynamically throughout the exercise test. Of the 40 subjects tested in this study, only 8 subjects exhibited a decrease in MPF. Those subjects who did exhibit a decrease in MPF during exercise also demonstrated a significant relationship ($r=0.932$; $p<0.001$) with ventilatory threshold. Their findings reinforce the variability of sEMG data collected during dynamic contractions, but also indicate that MPF decreases may be related

to similar metabolic and neurologic factors commonly associated with the ventilatory threshold in exercise.

Despite the high variability of sEMG collected during dynamic contractions, it does appear that decreases in MPF can be utilized to determine levels of local muscle fatigue.

Possible Underlying Physiology of Local Muscle Fatigue in sEMG

Anatomical and methodological changes in the sEMG MPF aside, there are number of physiologic changes in the local muscle tissue which cause alterations in the MPF.

Muscles Alive (Basmajian & De Luca, 1985) describes many of the aforementioned anatomical considerations as “widely accepted” when examining changes in MPF. However, they then proceed to caution their reader that the relationship between intramuscular pH and conduction velocity (and thus MPF) is “less well documented and not as uniformly familiar”. In the 20+ years since the publication of *Muscles Alive*, the relationship between pH and changes in MPF has not been generally supported by the literature in vivo. The metabolic state of the muscle and other non-pH factors relating to decreases in muscle fiber conduction velocity may cause the decreasing MPF related to fatigue.

Viitasalo (1985) investigated anaerobic and aerobic thresholds in ergometer cycling in relation to EMG activity of knee extensor muscles. They found significant increases in blood lactate in a progressive cycling test, but no significant change in MPF during the five data collection points. The investigators noted extreme variability in the shape of the MPF curve among subjects. This is likely caused by the EMG data being collected under dynamic conditions. The EMG was averaged from three descending phases of pedaling at different time epochs of each workload. This accounts for the high levels of variability both within

subjects and between subjects, leaving their lack of findings between blood lactate and MPF in question.

Bouissou (1989) examined sEMG power spectrum changes in relation to intramuscular lactate and pH during cycling exercise at 125% of $\text{VO}_{2\text{max}}$. Subjects performed two trials, one under normal conditions and one after NaHCO_3 was administered to produce an alkaline environment. They observed significant increases in total spectrum power of the EMG signal during both trials. They also observed steady decreases in MPF throughout the trials, though greater decreases were more evident in the alkalosis trial. Interestingly, the study reported no relationship between percent change in MPF and pH, but did report a significant correlation ($R=0.64$, $P<0.01$) between intramuscular lactate and percent change of MPF. The authors suggest that the relationship is not causative, but the EMG changes are related to the metabolic state of the muscle. It should again be noted that the authors collected the EMG data under dynamic conditions which may have introduced variability into the results.

Jansen (1997) examined the relationship between blood lactate and systemic changes in MPF in cycle ergometry. They collected dynamic EMG data from the vastus lateralis and isometric data from the flexor digitorum superficialis along with blood lactate concentrations during and after a progressive cycling test. Their findings resulted in no significant changes in MPF for either the vastus lateralis or flexor digitorum superficialis. They did note a non-significant decline in MPF of the vastus lateralis collected one minute post-exercise. They assumed that the dynamic vastus lateralis EMG data collected could be considered “a wide-sense stationary EMG signal”. This assumption may not have been correct and introduced variability into their dynamic EMG data. The isometric flexor digitorum superficialis MPF

results indicated that systemic blood lactate changes do not affect nerve conduction velocity in non-working muscles.

Singh (2007) attempted to determine muscular fatigue in three quadriceps muscles and two heads of the gastrocnemius in a wingate-style cycling test. They reported significant median frequency decreases pre and post fatigue (characterized by a decrease in power output greater than 33%). Their EMG was collected dynamically during the test and analyzed by averaging sectioned parts of the EMG burst from 10 second collection intervals. They reported significant intramuscular changes before and after the test for muscle creatine, muscle PCr, muscle ATP and muscle lactate. Their findings and EMG reduction may indicate that MPF is related to the metabolic state of the muscle, but their use of 15 non-adjusted t-tests bring their results into question.

We know that local muscle fatigue during exercise is a complex phenomenon. Recent in vitro research suggests lactate accumulation and potassium efflux from the muscle are strong mediators suppressing muscle force output. Contrary to previous studies, pH appears to have a protective effect against local muscle fatigue. This protective effect may have credence based upon findings that decreases in MPF, commonly associated with fatigue, are unrelated to increases in pH. In vivo sEMG fatigue studies on dynamic activity have been muddled by inconsistent and possibly invalid data collection techniques. This study attempts to clarify the literature on the physiologic changes in working muscles in relation to changes in MPF using a stable data collection protocol.

CHAPTER III

Methodology

Subjects

Eight male and female subjects ages 18-50 were recruited for this study. The subjects were moderate to highly experienced triathletes or cyclists who had been training for their respective sport a minimum of 60 days. The subjects were familiar with clipless cycling pedals and able to bring in their own shoes, pedals and bicycle saddle for the test. All subjects read and signed the consent form to act as a human subject (Appendix I), filled out the department medical history form, and completed a training history form (Appendix II).

Instrumentation

Oxygen Uptake

All oxygen uptake measures were collected using the Parvo TrueMax 2400 Metabolic System. The system was calibrated before each subject's cycling trial. All standard operating procedures were followed according the manufacturer's instructions.

Blood Lactate & Blood Potassium

Blood potassium was measured using the Vitros DT60 II Chemistry System. The whole blood was cold centrifuged, and the plasma was extracted. The plasma was then pipetted onto dry chemistry slides read by the system. The dry chemistry slide contains two ion-selective electrodes. Each electrode produces an electrical potential in response to the amount of potassium that is applied to the slide. The electrical potential difference is proportional to the potassium concentration. Blood lactate was measured using the

Accutrend Lactate analyzer. Whole blood placed on the test strip reacts with existing reagents and the analyzer measures the light refraction of the color change.

Electromyography

A Delsys Bagnoli desktop system was utilized for EMG data collection. This system configuration is: interelectrode distance = 10 mm; amplification factor = 10,000 (20-450 Hz); CMMR @ 60 Hz > 80 dB; input impedance > $10^{15} \text{ } \Omega/\text{pF}$. This study used only one channel, one active preamplified surface electrode. The sampling frequency was 4096 Hz.

Procedure

Overview

All subjects attended the lab for a total of four sessions. The first session was a screening trial intended to collect demographic data, fill out paperwork, and perform a submaximal exercise test. The second session was a glycogen normal progressive cycling trial. The third session was a two hour timed exercise session performed at night which was designed to reduce the glycogen stores of the subject. The fourth session was a glycogen reduced progressive cycling trial performed the morning after session three.

Screening Session

Subjects reported to the Applied Physiology Laboratory on the day of their test preparation. The subjects underwent a cardiovascular screening to determine if they were fit to act as a subject in the study. They also completed an informed consent agreement to be a human subject, a health history form, and a training history form. The subjects were required to bring in the saddle, pedals, and cycling shoes from their normal road bicycle. The saddle and pedals were installed on the mechanically braked cycle ergometer in the lab. They were requested to wear either a cycling or triathlon jersey for the trials.

The subject then performed a submaximal exercise test (Appendix III) on the cycle ergometer. This was done to familiarize the subject with the cycle ergometer and also to obtain a $\text{VO}_{2\text{peak}}$ estimation for the glycogen reduction protocol.

Glycogen Reduction Timed Cycle Exercise

The glycogen reduction condition consisted of two parts: an exercise session to reduce muscle glycogen and the glycogen reduced trial designed to examine the effects of decreased lactate production on EMG. For the glycogen reduction protocol, the participant utilized the same cycle ergometer set-up on which they performed the submaximal exercise test in the screening trial. The participant was fitted with an appropriate mouthpiece which was connected to an oxygen analyzer. This cart was used to ensure that the participant was exercising at 65-70% of his/her predicted maximal exercise capacity. The maximal exercise capacity was predicted from the submaximal exercise test performed during session one. The participant was also outfitted with a heart rate monitor which was used to keep the investigators continually appraised of the participant's exercise workload. The participant was allowed to warm up and stretch as he/she desired. Once he/she was warmed up, the participant inserted the mouthpiece and started cycling on the ergometer. The workload was continually increased until the metabolic cart indicated that the participant was working at 65-70% of his/her maximal capacity. The duration of the test was 120 minutes. The participant had their metabolic work rate assessed every 20 minutes to ensure that he/she was working consistently at 65-70% of the maximal capacity. The participant was allowed and encouraged to consume water throughout the protocol. Heart rate and the participants' perceived exertion were continually assessed to limit the possibility of any complications. The absolute workload on the bike was continually adjusted so that the participant's effort

was maintained within 65-70% of maximal aerobic capacity. This protocol was first described by Gollnick, et. al. in 1974, and variations of their protocol have been used for many years without any reported complications (Glass, 1997; Sabapathy, 2006). The day following the glycogen reduction session the participant completed the glycogen reduced exercise test to examine the effect of decreased lactate production on EMG.

Glycogen-Reduced Condition and Glycogen Normal Condition Rides

Prior to arriving to the Applied Physiology Laboratory, participants were asked to refrain from vigorous exercise for at least 24 hours for the glycogen normal condition and about 8 hours for the glycogen-reduction condition. Participants performing the glycogen-reduced condition were asked to verify that they had not eaten anything since the reduction protocol by having their urine tested for ketone bodies. The participants were allowed to drink as much water as they wished, but were asked to avoid any caffeinated or alcoholic beverages for at least eight hours prior to exercise.

Upon arrival, the participant was fitted with the electromyography (EMG) lead and electrode. The EMG electrode and wire were run through the cycling shorts on the participant's dominant side. The electrode was then attached to the skin over the vastus lateralis at the midpoint between the greater trochanter and patella. Care was taken to align the electrode in parallel with the muscle fibers. Manual muscle tests were administered for knee extension and hip flexion to verify proper electrode placement and limit cross-talk. The electrode was outlined with a black permanent marker to ensure proper placement for the second test. A digital picture of the electrode placement was also taken to verify similar placement between conditions.

The participant then had a small 20 gauge catheter inserted into his/her antecubital vein by experienced personnel. The catheter was cleared with 2 mL of saline upon insertion to ensure future blood draws were clear. This allowed for quick and clean blood draws throughout the exercise trial. All blood draws via catheter followed sterile and standard procedures: 1) draw .5 mL blood to clear catheter and discard blood 2) draw 3 mL blood and 3) inject sterile saline to keep catheter clean.

At this time, the participant was asked to mount the cycle ergometer and rest quietly 5 minutes prior to the exercise testing. During the early portion of the rest period the participant was fitted for the breathing mouthpiece. After the end of the 4th minute in the 5 minute rest period, the participant's oxygen consumption was recorded. Once the rest period was completed, a 3 mL blood sample was drawn via the installed catheter. The blood sample immediately was tested for lactate concentration. The remaining blood was separated into two parts, one for cell measurements and the second for the blood potassium determination. The blood collection was immediately followed by a maximal voluntary isometric contraction (MVIC) on the cycle ergometer. The MVIC was conducted by placing a block under the pedal of the dominant foot when the crank arms were parallel to the floor and instructing the participant to place as much force as possible on the dominant-foot pedal. The 90-degree angle of the cycle crank arm was chosen because Jorge & Hull (1986) indicated that this angle was an area of high vastus lateralis activity during normal cycling. A belt was placed around the waist of the participant restraining them to the saddle of the bike.

Once these resting procedures were completed, the participant began an active warm-up on the cycle ergometer at a low self-selected intensity for 3.5 minutes. After the warm-

up, the progressively increasing exercise test (GXT) commenced. The exercise test consisted of 5 minute segments of cycling at progressively higher workloads. The exercise segments were alternated with 5 minute active recovery periods where the tension was released from the cycle and the participant was able to ‘spin’ the pedals at a comfortable speed. At the end of each exercise stage, an MVIC and blood draw were performed according to previously mentioned parameters. The participant’s heart rate and perceived exertion were also assessed at the end of each exercise stage. The active recovery periods allowed the participant to remain comfortable on the bike throughout an otherwise strenuous exercise test. The participant was allowed and encouraged to consume water during the active recovery periods. The GXT was completed once the participant’s pedal frequency dropped below 70 rpm’s for a 30 second period. The GXT is graphically depicted in Appendix IV (also see Figure 1).

Figure 1. Exercise GXT

Once the exercise was completed, the participant remained seated on the ergometer so the blood and MVIC data could be collected 3, 6, 9 minutes post exercise. Once these measures were completed, the participant got off the ergometer and sat in a comfortable chair for the remaining 51 minutes of recovery. During this recovery period quiet activities were allowed but all physical exertion was minimal. Also during this period, additional blood and EMG samples occurred at minutes 30 and 60. The participant was allowed to consume water as he/she wished during this recovery period, but was asked to remain seated throughout the time period. Once all EMG data was collected, the EMG equipment and catheter were carefully removed. At this point the participant was allowed to ask any questions and scheduled an appointment the following trial.

The glycogen normal condition followed an identical protocol as the glycogen reduced condition. However, in the glycogen reduced condition, participants performed the glycogen reduction protocol and fasted a minimum of 8 hours before testing. A counter-balanced randomized assignment of glycogen reduced vs. glycogen normal was used to determine the order in which the conditions were performed.

Analysis

EMG Data Reduction

The raw EMG data were first bandpass filtered with a fourth order Butterworth filter 20-350 Hz and notch filtered 59.5-60.5 Hz. The EMG waveform was next reduced to its frequency power spectrum, from which the median power frequency (MPF) was derived. The MPF was calculated for each contraction using the fast Fourier transform. An averaged power spectrum was obtained from 2048 data points by taking four 512 point increments and

Hanning windowing the data with a 50% overlap. The median of this spectrum was then calculated as the frequency which divides the spectrum into two regions of equal power.

Statistical Analysis

All data collected were first normalized to the percent change in relation to the baseline value. This normalization for each subject's percent of baseline was done because each person typically falls within a wide range of normal values. Normalizing as percent of baseline values removes the range of this variability.

Because not all subjects performed the same number of stages during the exercise tests, all data had to be normalized as a percent of $\text{VO}_{2\text{peak}}$ during that test. The test was terminated when the subject was no longer able to maintain 70 rpms. All subjects had varying levels of fitness and muscle mass, leading to termination at different stages between subjects and conditions. With lactate, this was done by applying an exponential line of regression based on the raw data (R^2 range: 0.88-0.99; R^2 mean: 0.95). Data normalized for percent of $\text{VO}_{2\text{peak}}$ during exercise for potassium (R^2 range: 0.44-0.99; R^2 mean: 0.86) and MPF (R^2 range: 0.16-0.97; R^2 mean: 0.65) were extrapolated by applying a cubic or 4th order polynomial spline to the raw data. All normalization and interpolation of data was performed in Matlab. The exercise lactate, potassium and MPF data for 20, 40, 60, 80 and 100 percent of $\text{VO}_{2\text{peak}}$ were extrapolated via the applied spline for each subject. It should be noted that the first exercise stage typically was above 20% of $\text{VO}_{2\text{peak}}$. This may cause the extrapolated values for 20% of $\text{VO}_{2\text{peak}}$ for all measures to have questionable validity. However, this measure was included in analyses because visual inspection of the data appeared to provide reasonable results.

The lactate and potassium concentrations and EMG data were analyzed using a series of one-way repeated measures ANOVAs with Bonferroni adjusted repeated measures t-tests to determine statistical significance. Repeated measures t-tests were utilized to determine significance between glycogen normal and glycogen reduced conditions. Level of significance was set at $p \leq 0.05$ with standard Bonferroni adjustments.

The relationships between the values for percent change in lactate, potassium, and MPF were assessed by simple correlation analysis. Separate correlation analyses were conducted for data collected during exercise and post-exercise to answer the separate research hypotheses. P-values were Bonferroni adjusted when the same averaged MPF values are used to examine correlations with potassium and lactate. The R was used to determine strength of correlation between the variables.

CHAPTER IV

Results

The primary purpose of this study was to investigate the relationship between the changes in median power frequency ($\Delta\%$ MPF) of the EMG signal and changes in blood lactate ($\Delta\%$ LAC) and blood potassium ($\Delta\%$ K) during and after progressive graded exercise. This chapter will first discuss the subjects' characteristics and verification that one of the conditions was performed in a glycogen reduced state. Thereafter, the results will be discussed as they occurred during exercise and then recovery from exercise in both glycogen normal and glycogen reduced conditions.

Subject Characteristics

Table 1. Means ($\pm$ standard deviations), minimums and maximums physical characteristics of participants (n=8) in the study.

Characteristic	Mean $\pm$ std	Minimum	Maximum
Body Mass (kg)	68.4 $\pm$ 8.1	57.3	80.2
Height (cm)	172.8 $\pm$ 10.0	159.0	186.0
Age (years)	28.0 $\pm$ 6.4	21	38
Predicted VO _{2max} (mL/kg/min)	62.3 $\pm$ 7.4	47.0	71.7
Resting HR (bpm)	55.0 $\pm$ 6.8	46	67

This study involved 8 subjects, all of whom completed the two condition study in its entirety. The maximum, minimum, and means with standard deviations of physical characteristics for the subjects are shown in Table 1. The subject population was divided evenly between males (4) and females (4). All had been training for triathlons for more than

60 days. Based on predicted $\text{VO}_{2\text{max}}$ and resting HR, our population was highly trained considering their gender and age.

Verification of Glycogen Reduced Status

The study had two conditions, one under normal glycogen conditions and one in a glycogen reduced state. The results of repeated measures t-tests indicate that the subjects were glycogen reduced for one condition; significant differences were found in respiratory exchange ratio (RER) and lactate between conditions (Table 2). However, there was no significant difference between the trials in number of stages completed ($p=0.454$), maximum heart rate ($p=0.239$) or peak VO_2 ($p=0.627$).

Table 2. Means ($\pm$ standard deviations) for maximum RER, lactate, and heart rate, number of stages completed, and peak VO_2 for glycogen normal and reduced conditions.

	Condition	Mean $\pm$ std
Max RER*	Glycogen Normal	1.02 $\pm$.04
	Glycogen Reduced	.91 $\pm$.07
Maximum Lactate (mmol/L)*	Glycogen Normal	11.1 $\pm$ 2.4
	Glycogen Reduced	6.2 $\pm$ 1.8
Number of Stages Completed	Glycogen Normal	7.5 $\pm$ 1.6
	Glycogen Reduced	6.9 $\pm$ 1.5
Maximum HR (bpm)	Glycogen Normal	181.3 $\pm$ 10.8
	Glycogen Reduced	174.8 $\pm$ 10.2
$\text{VO}_{2\text{peak}}$ (mL/kg/min)	Glycogen Normal	53.2 $\pm$ 8.6
	Glycogen Reduced	50.988 $\pm$ 9.4

* $p<0.05$ Normal vs. Reduced Trials

Exercise

The lactate, potassium and EMG results as related to percent $\text{VO}_{2\text{peak}}$ are presented in Table 3. In the normal condition, the one-way ANOVA for $\Delta\%$ LAC resulted in omnibus significance ($p<0.001$), indicating that in general, the $\Delta\%$ LAC increased as intensity

increased. A similar trend was observed for the reduced condition ($p<0.001$). When comparing $\Delta\%LAC$ between the two conditions, significant differences were found at 40% ($p=0.027$), 60% ($p=0.004$), 80% ($p=0.002$), and 100% ($p=0.012$) of VO_{2peak} . The one-way ANOVA for $\Delta\%K$ also resulted in omnibus significance in the normal condition ($p<0.001$). In general, the $\Delta\%K$ increased as intensity increased. A similar trend was observed for the reduced condition ($p<0.001$). $\Delta\%K$ was not significantly different between glycogen conditions. $\Delta\%MPF$ did not have significant changes for intensity during either glycogen conditions (normal, $p=0.229$; reduced, $p=0.315$). Figure 2 depicts the individual subjects $\Delta\%MPF$ at each intensity for the normal glycogen condition. Five out of the eight subjects exhibited expected $\Delta\%MPF$ changes (noted by solid lines). There was no significant difference between glycogen conditions for $\Delta\%MPF$.

Table 3. Means $\pm$ standard deviations for percent changes in lactate ($\Delta\%LAC$), potassium ($\Delta\%K$), MPF ($\Delta\%MPF$) and absolute values for hematocrit (Hct) with increasing intensity in all conditions.

		Percent VO_{2peak}				
	Glycogen Status	20%	40%	60%	80%	100%
$\Delta\%LAC$	Normal*	37 $\pm$ 47	33 $\pm$ 57 $\dagger$	101 $\pm$ 85 $\dagger$	285 $\pm$ 127 $\dagger$	714 $\pm$ 372 $\dagger$
	Reduced*	66 $\pm$ 64	15 $\pm$ 25 $\dagger$	-6 $\pm$ 38 $\dagger$	92 $\pm$ 84 $\dagger$	279 $\pm$ 173 $\dagger$
$\Delta\%K$	Normal*	7 $\pm$ 10	15 $\pm$ 7	17 $\pm$ 8	19 $\pm$ 12	30 $\pm$ 9
	Reduced*	-1 $\pm$ 10	12 $\pm$ 9	13 $\pm$ 6	23 $\pm$ 11	23 $\pm$ 16
$\Delta\%MPF$	Normal	-12 $\pm$ 18	5 $\pm$ 19	-1 $\pm$ 15	-8 $\pm$ 21	-9 $\pm$ 25
	Reduced	11 $\pm$ 34	-0 $\pm$ 19	-8 $\pm$ 10	-2 $\pm$ 22	-11 $\pm$ 27
Hct (%)	Normal	42 $\pm$ 3	43 $\pm$ 2	43 $\pm$ 3	44 $\pm$ 2	46 $\pm$ 2
	Reduced	42 $\pm$ 3	42 $\pm$ 3	43 $\pm$ 3	43 $\pm$ 3	44 $\pm$ 3

a. Hematocrits are displayed for visual assessment only.

* $p<0.001$ between intensities; $\dagger$ $p<0.05$ normal vs. reduced conditions

Figure 2. $\Delta\%$ MPF for each subject at 20, 40, 60, 80, 100% $\text{VO}_{2\text{peak}}$ in normal glycogen condition. Subjects approximating the expected mean values trend line have solid lines and black markers. Subjects not approximating expected trends dotted lines and white markers.

Simple correlations were computed to examine the relationship between $\Delta\%$ MPF and $\Delta\%$ LAC during exercise in each of the two glycogen conditions. The correlations were low in magnitude and not significant for either condition (normal, $r = -0.177$, $p = 0.275$; reduced, $r = 0.041$, $p = 0.802$). Correlations were also computed to examine the relationship between $\Delta\%$ MPF and $\Delta\%$ K during exercise in each of the two glycogen conditions. These correlations were also low and not significant for either condition (normal, $r = 0.229$, $p = 0.155$; reduced, $r = 0.137$, $p = 0.398$).

Recovery

The lactate, potassium and EMG results as related to time of recovery are presented in Table 4. In the normal condition, the one-way ANOVA for $\Delta\%$ LAC resulted in omnibus significance ($p<0.001$), indicating that there were changes over time in recovery. In general, the $\Delta\%$ LAC decreased with progression of time post exercise. A similar trend was observed for the reduced condition ($p=0.002$). When comparing $\Delta\%$ LAC between the two conditions, significant differences were found at 3 minutes post exercise ($p=0.005$) and 6 minutes post exercise ($p=0.002$). The one-way ANOVA for $\Delta\%$ K also resulted in omnibus significance in the normal condition ($p=0.002$). In general, the $\Delta\%$ K increased with time in recovery. A similar trend was observed for the reduced condition ($p=0.008$). $\Delta\%$ K was not significantly different between glycogen conditions. $\Delta\%$ MPF did not have significant changes over time during either glycogen conditions (normal, $p=0.125$; reduced, $p=0.516$). There was no significant difference between glycogen conditions for $\Delta\%$ MPF.

Table 4. Means $\pm$ standard deviations for percent changes in lactate ($\Delta\%$ LAC), potassium ($\Delta\%$ K), MPF ($\Delta\%$ MPF) and absolute values for hematocrit (Hct) by time after exercise in all conditions.

	Glycogen Status	Post Exercise				
		3 minutes	6 min. Post Exercise	9 min. Post Exercise	30 min. Post Exercise	60 min. Post Exercise
$\Delta\%$ LAC	Normal*	780 $\pm$ 208†	830 $\pm$ 292†	715 $\pm$ 283	357 $\pm$ 150	143 $\pm$ 87
	Reduced*	323 $\pm$ 183†	285 $\pm$ 152†	286 $\pm$ 186	151 $\pm$ 117	64 $\pm$ 42
$\Delta\%$ K	Normal*	2 $\pm$ 10	-8 $\pm$ 7	-5 $\pm$ 8	1 $\pm$ 8	17 $\pm$ 16
	Reduced*	2 $\pm$ 8	-1 $\pm$ 7	2 $\pm$ 8	7 $\pm$ 11	17 $\pm$ 10
$\Delta\%$ MPF	Normal	-3 $\pm$ 22	4 $\pm$ 32	10 $\pm$ 40	7 $\pm$ 37	8 $\pm$ 39
	Reduced	-3 $\pm$ 18	6 $\pm$ 27	9 $\pm$ 29	1 $\pm$ 27	1 $\pm$ 26
Hct (%)	Normal	46 $\pm$ 3	46 $\pm$ 2	45 $\pm$ 3	42 $\pm$ 3	42 $\pm$ 2
	Reduced	45 $\pm$ 3	44 $\pm$ 3	43 $\pm$ 3	41 $\pm$ 3	41 $\pm$ 4

a. Hematocrits are displayed for visual assessment only.

* $p<0.05$ between intensities; † $p<0.05$ normal vs. reduced conditions

Correlations were computed to determine the relationship between $\Delta\%$ MPF and $\Delta\%$ LAC during recovery in each of the two glycogen conditions. The correlations were low and not significant for either condition (normal, $r = -0.093$, $p = 0.566$; reduced, $r = 0.303$, $p = 0.060$). Similar correlations also examined the relationship between $\Delta\%$ MPF and $\Delta\%$ K during recovery in each of the two glycogen conditions. These correlation were low and not significant for either condition (normal, $r = 0.078$, $p = 0.634$; reduced, $r = -0.121$, $p = 0.464$).

CHAPTER V

Discussion

Previous research has indicated that decreases in the median power frequency of the EMG signal can be utilized to assess muscular fatigue during exercise (Jansen, 1997; Kuorinka, 1988; Lepers, 2002; McHugh, 2001; Nyland, 1993; Viitasalo & Komi, 1977). While the use of MPF has been well validated during fatigue in purely isometric exercise, its use has not been consistently valid in studies of dynamic exercise (Petrofsky, 1979; Viitasalo, 1985; Kuorinka, 1988; Bouissou, 1989; Gamet, 1990; Ament, 1996; Jansen, 1997; Masuda, 1999; Gerdle, 2000; Singh, 2007). Additionally, it has long been hypothesized that the decrease in MPF is an indicator of decreased conduction velocity along the sarcolemma as a result of ionic or pH changes resulting from exercise (Bouissou, 1989). The purpose of this study was to examine MPF during dynamic progressive aerobic exercise and determine if systemic changes in blood lactate and blood potassium are related to alterations in MPF using maximal voluntary isometric contractions (MVIC) interspersed within the exercise.

Blood potassium and blood lactate responded as expected with significant increases during exercise and decreases during recovery in both conditions. Also as expected, blood potassium did not change between normal and reduced conditions, but lactate accumulation was suppressed in the glycogen reduced condition. However, MPF did not respond with significant systemic changes during exercise or recovery.

Effects of Glycogen Reduction

The timeline of lactate production during progressive aerobic exercise is well documented, and our subjects exhibited expected changes during exercise and recovery. Osborne (2006) previously reported lactate data for subjects cycling above ventilatory threshold for 8 minutes under glycogen normal and glycogen reduced conditions. They reported significant decreases in lactate accumulation during the glycogen reduced exercise conditions. The data from the present study agree that glycogen reduction does cause significantly lower accumulation of blood lactate during exercise. Accordingly, our study also recorded a significantly lower accumulation of blood lactate between the two conditions in recovery. All of these results are logical because an incomplete oxidation of glycogen in muscles is the major contributor to changes in blood lactate during exercise, and the glycogen reduced condition had less substrate from which to produce lactate.

The timeline of potassium accumulation during progressive aerobic exercise has not been established, nor has a timeline of recovery from such exercise. Our study demonstrated that during progressive aerobic exercise, plasma potassium concentration increases in a generally linear fashion with increases in exercise intensity. This increase was significant between 20% of $\text{VO}_{2\text{peak}}$ and 40%, 60%, 80% and 100% of $\text{VO}_{2\text{peak}}$ in both normal and reduced conditions ($p=0.001$); however, 40%, 60%, 80% and 100% of $\text{VO}_{2\text{peak}}$ were not significant from each other in either condition. The changes in potassium are likely related to the increase in muscle fibers recruited with increasing intensity of exercise. The greater number of fibers depolarized, a greater amount of potassium would be expected to accumulate in the blood. Depolarization of the muscle fiber occurs, in part, because of an efflux of potassium ions from the muscle cell into the interstitial space. There was no

significant difference between normal and reduced conditions. The lack of a difference between the two conditions is possibly related to the lack of difference in the number of stages performed between the two conditions. Each stage required a consistent power output to complete. If the same number of stages was completed, then power output would be the same; a similar number of muscle fibers would have been depolarized to cause similar levels of blood potassium. In recovery, there was a significant increase in blood potassium between blood draws at 6 minutes post-exercise and 60 minutes post-exercise ($p=0.046$). Again, there was no significant difference between the normal and reduced conditions.

The present study did not find any effect of progressive exercise on MPF or any effect of glycogen depletion on MPF. This is in contrast to a previous study which reported differences between conditions (Osborne, 2006). Osborne found that when cycling above ventilatory threshold, MPF was significantly depressed in the glycogen reduced condition after 5, 6, 7, and 8 minutes of exercise. Our study did not have an operational hypothesis for MPF based on differences between the two glycogen conditions, but we did not find a difference between the glycogen conditions in either exercise ($p=0.399$) or recovery ($p=0.856$). This may indicate that glycogen reduction does not significantly alter motor unit recruitment patterns during progressive exercise, though further research is needed.

Relationships Between Lactate, Potassium and MPF

A number of in-vitro studies have suggested both lactate and potassium have direct effects on force production and VO_2 suppression in the muscle fiber, theoretically acting as MPF suppressing agents (Hogan, 1995; Cairns, 1995; Nielsen, 2001). During exercise, changes in potassium and lactate concentrations were not significantly related to alterations in MPF in either normal or glycogen reduced conditions. Though correlations were not

significant, it is interesting to note that the direction of the weak correlation existing between potassium and MPF was positive, not negative as would be expected. The study did hypothesize that glycogen reduced conditions would produce an environment where lactate was less related to changes in MPF compared to the glycogen normal condition. A decrease in correlation did occur, though the relationships in both conditions were weak and non-significant. In recovery, changes in lactate and potassium were not related to changes in MPF. In the reduced condition, lactate was almost significantly related ($p=0.06$), but this is likely a coincidence because it is very weakly related ($r= -0.093$) and in an opposite direction of the glycogen normal condition. As a whole, the results of this study indicate that there is no relationship between the MPF of the vastus lateralis and blood lactate or blood potassium during or after a progressive cycling test. This lack of relationship is likely due to the lack of any significant change in MPF during both exercise and recovery. While the lack of MPF change during and after aerobic exercise opposes the findings of Potvin (1997) and Osborne (2006), it does agree with numerous other published studies (Jansen, 1997; Cannon, 2007; Petrofsky, 1979; Gamet, 1990; Viitasalo, 1985; Ament, 1996).

While our study found no significant relationship between potassium and MPF in recovery, the time course for potassium to return to near-resting values is very similar to the time course for MPF to return to resting values after exercise in studies conducted by Kuorinka (1988) and Ament (1996). Both of these studies reported a return to near-resting MPF values by 7-10 minutes after exercise. This time course is very similar to our return to resting levels for blood potassium. It is possible that blood potassium is highly related to changes in MPF, but that our study was unable to discern this relationship due to lack of consistency in MPF values between subjects.

Given the knowledge of MPF and potassium's quick return to baseline timeline, the discontinuous nature of our exercise protocol may have contributed to our lack of significant findings regarding MPF changes in exercise and MPF's relationship to potassium. However, we were still able to discern significant changes in potassium. If the two variables are strongly related, MPF should have shown significant changes in conjunction with potassium. Additionally, Osborne (2006) noticed significant differences in MPF between normal and glycogen depleted conditions 5 minutes into a supra-ventilatory threshold time trial test. Based on his findings, our protocol should have elicited a difference between the conditions on the 5 minute stage where $\text{VO}_{2\text{peak}}$ was reached, if such a difference existed. Our study indicated no significant MPF difference between glycogen conditions for any of the exercise workloads.

If, as Singh (2007) suggests, the decrease in MPF is caused by phosphagen cycle metabolic changes, our study would not likely have found a change in MPF. Typically, our MVIC EMG data was collected 1 minute after completion of the exercise stage. This time allowed for setting up of the MVIC as well as the venous blood draw. This minute may have been enough for the phosphagen cycle to replenish sufficient intramuscular ATP stores, causing no change in MPF. This hypothesis may also explain why wingate-style tests typically elicit changes in MPF, but lower intensity aerobic exercise does not. This would also explain the lack of significant differences between glycogen conditions in our study, as glycogen reduction should have limited effect on phosphagen cycle replenishment in 1 minute.

Limitations of the Current Study

The greatest limitation of the current study is the sample size. The high variability of EMG between the subjects and resulting lack of significant change during exercise and in recovery made finding significant correlations difficult. A post-hoc power analysis determined that to find a significant difference between MPF at baseline and 100% $\text{VO}_{2\text{peak}}$ the study would have required at least 25 subjects for a glycogen normal condition and 35 subjects for a glycogen reduced condition. Additionally, while the subjects were told to exert maximal force during the MVIC's interspersed within the condition, we did not quantify the force output of these MVIC's. Because the EMG power spectrum can be greatly impacted by changes in force output, this may have resulted in inconsistent EMG data. One might expect that MVIC force output would decrease as the test progresses, causing the MPF to decrease; however, there is evidence to suggest that MVIC force may be augmented after a maximal aerobic exercise test, resulting in an increase in MPF (Tenan, unpublished data). This lack of documented consistent force output during the tests may have resulted in inconsistent EMG data. It is also possible that, with the expansion of muscle tissue during dynamic exercise, the EMG electrode did not remain over similar muscle fibers for every MVIC. All of these factors may have contributed to the study's inability to discern a significant change in MPF.

Conclusions

Evidence of neuromuscular fatigue of the vastus lateralis as measured by the MPF of the EMG signal does not occur during or after an aerobic progressive exercise test on a cycle ergometer with interspersed MVIC's and active rest for data collection. Though both lactate and potassium have been implicated as factors relating to neuromuscular fatigue, neither is

significantly related to changes in MPF during or after progressive exercise on a cycle ergometer. On the basis of these findings, main hypotheses one, two and five, along with all subsets of hypotheses three and four, were rejected.

It was hypothesized that MPF would decrease with increasing exercise intensity and increase during recovery. The study revealed no change in MPF during either exercise ($p=0.363$) or recovery ($p=0.154$). These findings may indicate that, during an aerobic progressive exercise test of this nature, neuromuscular fatigue of the vastus lateralis, as measured by the MPF of EMG, is not a significant limiting factor in performance. While the vastus lateralis is considered a “major mover” in cycling (Jorge, 1986), the individual muscle may not reach a critical level of fatigue, measurable by EMG, before the subject terminates the test. This finding is interesting in that it suggests that termination of exercise due to fatigue occurs for several reasons: local fatigue in a combination of muscle groups involved in the exercise, termination occurs due to cardiovascular limitations in highly trained populations, or a central nervous system control that occurs before the onset of quantifiable local muscle fatigue. The first theory would suggest that training for aerobic exercise should not involve isolating muscle groups. The muscles should be functionally trained together to facilitate proper MU firing patterns, prolonging time to onset of fatigue. With regard to cardiovascular limitations, maximal exertion that occurs during an aerobic capacity test is limited by the ability to provide oxygen (the cardiovascular system) to the working muscles, which could result in termination before measurable local muscle fatigue occurs. The final theory supports the integrative model proposed by Lambert, St. Clair Gibson and Noakes (2005) that a “Central Governor” controls the termination of exercise. Also interestingly, the two blood markers suggested as major contributors to the integrative model are blood

potassium and blood lactate. The cessation of exercise may not have been due to the inability of the muscles to continue contracting; rather, it may be a complex reflex inhibition from the central governor caused by the accumulation of metabolites or ionic changes in the bloodstream (either duration or total concentration of metabolites/ions in the bloodstream) in conjunction with a variety of other perceived factors such as familiarity with the mode of exercise, mental fatigue or a preconceived notion regarding their own maximal capacity.

Recommendations for Future Research

The scope of this study was large for the sample size used. It would be prudent for studies in the future to examine certain variables in isolation with larger sample sizes.

For example, is the MPF of EMG when collected dynamically similar to that which is collected via isometric contractions interspersed within the test? In a study examining this question, it would also be prudent to collect force data from the isometric contraction as well as examine isometric contractions at various levels of percent MVIC.

Upon visual inspection of line graphs for MPF changes with subjects in this study, it was noted that some subjects directly followed expected patterns of decreasing MPF during progressive exercise. There may be a demographic factor (gender, age, body composition, training status, etc) which predicts if a subject will display expected EMG responses and not others. A larger exploratory study, similar to the one undertaken by Hug (2003) should investigate if there are screening factors which may predispose a subject to not display expected changes in MPF.

While conducting this study, unexpected patterns of potassium concentration changes occurred. These changes may be significant if examined in a greater sample population, but also may be different when not using highly trained subjects as this study did. Future studies

should examine the time courses of potassium through progressive and steady state aerobic exercise along with recovery for differently trained/untrained populations.

Alterations in MPF may be largely related to ATP stores available in isometric conditions, including isometric contractions interspersed within an aerobic exercise test. It would be interesting to replicate our current exercise protocol with blood tests for adenosine or multiple muscle biopsies during exercise. However, the financial cost incurred for a study of that magnitude, along with finding a sample of subjects willing to undergo multiple muscle biopsies during exercise may be prohibitive.

Appendix I:

Consent to Participate In a Research Study

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form

IRB Study # 08-1843

Consent Form Version Date: 12/07/08

Title of Study: The Relationship Between Blood Potassium, Blood Lactate, EMG Signals, and Immune Response Related to Local Muscle Fatigue in a Progressive Exercise Test

Principal Investigators: Matthew S. Tenan, BS ATC; Kyle Leppert, BA

UNC-Chapel Hill Department: Exercise and Sport Science

UNC-Chapel Hill Phone number: (919) 962-1371

Faculty Advisor: Robert G. McMurray, PhD

Funding Source and/or Sponsor: N/A

Study Contact telephone number: (919) 962-1371 (work); (717) 315-6763 (mobile)

Study Contact email: tenan@unc.edu

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.

You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is twofold: one purpose is to learn about the causes of fatigue and how they change muscle electrical signals, and the second purpose is to evaluate the immune response to exercise and changes caused by alteration of muscle energy stores.

You are being asked to be in the study because you are a moderate to highly experienced triathlete or cyclist, aged 18-50 years, who has been training for your respective sport a minimum of 60 days and are familiar with clipless pedal systems.

Are there any reasons you should not be in this study?

You should not be in this study if you are uncomfortable with blood draws or have any major medical or orthopedic concerns limiting exercise.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately twelve people to participate in this research study.

How long will your part in this study last?

Your total commitment of 10 hours will take place over a two-week period.

The entire study will be collecting data through a 4-month period.

1. The first session including screening, participant descriptives assessment, and a submaximal exercise test will take 1.5 hours.
2. The control maximal exercise test session will take 3 hours.
3. The glycogen reduction protocol session will take 2.5 hours.
4. The experimental maximal exercise test session will take 3 hours.

What will happen if you take part in the study?

You will be asked to attend four sessions at the Applied Physiology Laboratory in Fetzer gym. The first session will be used to familiarize you with the project and obtain your consent. You will be required to bring in the bicycle saddle, cycling shoes and pedal system from the bike on which you typically train. Once you consent, you will complete a medical history form, training history form. You will then, undergo a physical screening examination, listening to your heart, lungs, obtaining your blood pressure and a resting electrocardiogram. Your height and weight will be measured and body composition will be obtained using the "BodPod". The BodPod uses air displacement to obtain fat and muscle mass. All you have to do is sit in it. For an accurate BodPod measurement, it is essential that you do not eat, exercise or fully immerse yourself in water for 3 hours prior to the assessment. Finally, you will perform a submaximal exercise test to estimate your maximal capacity. This will be accomplished by having you work at three progressively harder workloads on a stationary cycle ergometer. During this test your heart rate will not exceed 165 beats per minute. The results of the submaximal test will be used to determine what workload you will use for the glycogen depletion trial, which will be performed at a later date.

The trials consist of a control trial and a two-part glycogen depletion experimental trial. The control and experimental exercise trials will follow the same procedure, except that the experimental glycogen depletion trial will be preceded by an exercise session designed to reduce your energy stores. In this depletion session, you will cycle at 65-70% (moderate-to-hard rate) of your maximal aerobic capacity for 120 minutes. To ensure you maintain a work capacity in the 65-70% zone, an investigator will continually assess your oxygen uptake. Your well-being will be continually monitored by investigators using a 4 lead electrocardiogram and water will be provided. You will not be allowed to eat in the time between the session intended to deplete your muscle energy and the experimental trial. The

day following the depletion you will complete the experimental exercise trial. Before the control trial, you will be requested to not eat in the 3 hours before the test.

The control and experimental exercise trials will be separated by 5-7 days to insure your muscle energy stores have been replenished or you have fully recovered from the control trial. Before each of these two exercise trials you will have a catheter inserted into your antecubital vein, located on the front side of your elbow. This will allow for blood draws during exercise. You will also be fitted with surface electrodes to measure the electrical activity of your thigh muscles and heart. These surface electrodes only measure the electrical activity and do not present any risk to you. You will also be fitted with a device which measures the angle of your knee as you exercise. The exercise protocol is completed on a cycle ergometer and starts out very light and increases intensity every five minutes. The trial will be complete when you indicate you are no longer able to cycle during that workload. This should take approximately 45 minutes or longer depending upon how in shape you are. The exercise segments are alternated with 5 minute active recovery periods where the tension is released from the cycle and you are able to 'spin' the pedals at a comfortable speed. At the end of each exercise segment, a static (non-moving) thigh muscle contraction and a blood draw of 3 mL will be performed on the bike. The active recovery periods will allow you to remain comfortable on the bike throughout an otherwise strenuous exercise test. You will be allowed and encouraged to consume water during the active recovery periods.

Once the exercise is completed, you will need to remain seated on the ergometer so the blood and muscle signals can be collected 3, 6, 9 minutes post exercise. Once these measures are completed, you can get off the ergometer and sit in a comfortable chair for the remaining 51 minutes of recovery. During this recovery period quiet activities are allowed but any physical exertion will be minimal. Also during this period additional blood and muscle signal samples will be taken at minutes 30 and 60. You will be allowed to consume water as you wish during this recovery period, but you will be asked to remain seated throughout the time period. A final blood sample will be collected at 120 minutes post-exercise; after which the catheter will be removed by a trained investigator. The total amount of blood drawn depends on the length of exercise session, but will never exceed 45 mL through 14 blood draws. The area over the puncture will be cleaned and bandaged using sterile techniques.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. The benefits to you from being in this study may be informational sheets detailing your maximal aerobic capacity, lactate threshold during exercise and body fat percentage. This information is highly valuable when training for competitive aerobic events.

What are the possible risks or discomforts involved with being in this study?

You will be asked to perform strenuous exercise bouts and to provide blood samples during the trials. With strenuous exercise, some degree of stress can be expected and may result in some muscle soreness and/or fatigue. The glycogen depletion trial will leave you tired and possibly irritable and grouchy for the next day. This is normal and similar to what many athletes do as part of their carbohydrate loading procedure a week before competition. Once you complete the experimental trial, you will be given food.

The blood draws will be conducted by a trained individual, Dr. McMurray, from the University of North Carolina-Chapel Hill Applied Physiology Laboratory. Dr. McMurray has been performing blood draws for over 30 years without complications. All standard safety and sterile procedures will be used during all blood draws. If minor bruising does develop as a result of the blood draws, ice and compression can be applied to the area. In the event that there are cardiac complications which occur during this study, all investigators are certified by the Red Cross in CPR and in the use of an Automated External Defibrillator (AED). An AED is located within the Applied Physiology Laboratory at all times.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

A subject identification number will identify each participant. You will be identified by a subject identification number only. Code lists linking the participant identification number and your name and email address will be viewed only by the principal investigators and faculty advisor, and will be stored in a locked filing cabinet in the Applied Physiology Laboratory. Email addresses will be obtained solely for the purpose of contacting you to schedule data collection sessions. Once your participation is finished, the address will be discarded. All data will be stored in electronic format on the principal investigator's personal computer. Computer access will be protected via confidential passwords. Code lists identifying subjects will be retained for one year following the completion of the study. After a period of one year following the completion of the study these lists will be destroyed.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will not receive anything for taking part in this study.

Will it cost you anything to be in this study?

There is no cost to you for participating in this study.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research subject?

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Title of Study: The Relationship Between Blood Potassium, Blood Lactate, EMG Signals, and Immune Response Related to Local Muscle Fatigue in a Progressive Exercise Test

Principal Investigators: Matthew S. Tenan, BS ATC; Kyle Leppert, BA

Subject's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

Signature of Research Subject

Date

Printed Name of Research Subject

Signature of Person Obtaining Consent

Date

Printed Name of Person Obtaining Consent

Appendix II:

Training History Form

University of North Carolina-Chapel Hill
Training History Form
Adult Subjects

IRB Study # _____

Title of Study: The Relationship Between Blood Potassium, Blood Lactate, EMG Signals, and Immune Response Related to Local Muscle Fatigue in a Progressive Exercise Test

What sports have you trained for in the past 12 months (please list all)?

Please indicate any competitive events in which you have been a participant in that time period.

If any of the sports you have participated in/trained for involve cycling, did you utilize a clipless pedal system? If so, what kind/brand?

Please describe in detail your average weekly training regimen over the last 60 days
(e.g. number of days exercising per week, mode of exercise, duration of exercise, intensity of exercise, etc.)

Appendix III:

Submaximal Exercise Test

Each participant will begin by cycling at a rate of 50 revolutions per minute against a resistance producing a workload of 150 kilogram-meters per minute (kgm/min). This workload will be maintained for a first stage of 3 minutes. During the third minute, the participant's heart rate will be checked using the Polar Heart Rate Monitor. If the heart rate is below 86 beats per minute (bpm), the workload will be adjusted at the end of the third minute to 600 kgm/min. If the heart rate is between 86 and 100 bpm, the workload will be adjusted to 450 kgm/min. And if the heart rate is above 100 bpm, the workload will be adjusted to 300 kgm/min. The second workload will be maintained for the next three minutes thereafter (second stage). The workload will be increased again at the start of the third and fourth 3-minute stages by an additional 150 kgm/min each. Heart rate will be measured during the last 30 seconds of minutes 2 and 3 within each stage. If those heart rates differ by more than 6 bpm, the workload will be extended an additional minute until heart rate stabilizes. The test will be terminated if the participant's steady-state heart rate reaches or exceeds 85% of the age-predicted maximal heart rate ($\text{Heart Rate} = 220 - (\text{age in years})$) in the third stage, the test will be terminated (Heyward, 2006). The peak oxygen uptake for each participant will be estimated by using the multistage equations based on the oxygen uptake rates of the last two stages.

Appendix IV:

Progressive Exercise Protocol

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