THE EFFECTS OF HYPOXIA ON MOTOR OUTPUT DURING A 60 MINUTE LOWER LIMB CYCLING PROTOCOL

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THE EFFECTS OF HYPOXIA ON MOTOR OUTPUT DURING A 60 MINUTE LOWER LIMB CYCLING PROTOCOL

By

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ABSTRACT

The muscles working together to produce motion around a joint is called muscle coordination, and there are specific recruitment patterns for every movement. Exposure to a lowered oxygen environment can cause an acceleration of locomotor muscle fatigue (Romer et al., 2007), and when the muscle becomes fatigued, a change in the pattern of activation may be induced (Gandevia, 2001). The objective of this study was to investigate changes in muscle activation patterns (a cause of central fatigue) during cycling between hypoxic (15% O2) and normoxic (20.93% O2) conditions, and whether they take place before, during, or after the development of peripheral fatigue. Ten endurance trained males participated in three laboratory sessions. The first session was an incremental ramp cycling test to determine VO2max and peak power output (PPO). In the second and third sessions, the participants randomly underwent an hour long cycling test in hypoxia or normoxia consisting of eight 3-minute work intervals (70% of PPO) and eight 4.5-minute active rest intervals (35% of PPO). Electromyography (EMG) was collected continuously throughout the test from the vastus lateralis (VL), biceps femoris (BF), tibialis anterior (TA) and lateral gastrocnemius (LG) muscles. Maximal Voluntary Isometric Contractions (MVICs) were performed after every work interval. Heart rate (HR) was significantly lower between conditions in the active rest intervals, while rate of perceived exertion (RPE) and arterial oxygen saturation (SpO2) were significantly higher throughout the full test in hypoxia. MVIC force values decreased throughout the test in both conditions. Muscle activation changes included a main effect for time in RMS amplitude measures of the VL, BF and LG. There was a main effect for condition for VL:BF coactivation and VL delta time (length of activity over one second). Due

to technical difficulties with the experimental setup, peripheral indicators of fatigue could not be identified; however, indicators of central fatigue were present. No conclusive remarks can be made on whether the limiting factor in the cessation of exercise was related to central or peripheral fatigue.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

BDC – Bottom Dead Center

BF – Biceps Femoris

CAR – Central Activation Ratio

CNS – Central Nervous System

CO_2 – Carbon Dioxide Output

EMG – Electromyography

HR – Heart Rate

ITT – Interpolated Twitch Technique

LG – Lateral Gastrocnemius

m – Meters

mm – Millimetre

MVC – Maximal Voluntary Contraction

MVIC – Maximal Voluntary Isometric Contraction

O_2 – Oxygen

PNS – Peripheral Nervous System

PPO – Peak Power Output

RPE – Rate of Perceived Exertion

rpm – Revolutions per Minute

s – Second

SD – Standard Deviation

SE – Standard Error

SpO_2 – Arterial Oxygen Saturation

TA – Tibialis Anterior

TDC – Top Dead Center

TMS – Transcranial Magnetic Stimulation

TTF – Time to Fatigue

VCO_2 – Carbon Dioxide Output

VL – Vastus Lateralis

VO_2 – Oxygen Output

VO_2max – Maximal Oxygen Consumption

W – Watts

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1.0 REVIEW OF LITERATURE

1.1 Introduction

Motor coordination is described as the combination and interaction of body segments in order to efficiently execute a desired movement (Prilutsky, 2000). This is accomplished by muscle coordination which is described as the muscles working at different proportions of muscle activation and motor output among individual muscles to provide movement at a given joint (Prilutsky, 2000). The pattern the activation of these muscles follow is a muscle synergy. In rhythmic movements such as walking and cycling, all of the above factors play a part in having a smooth continuum of movement.

During cycling, the muscles often follow a certain pattern of activation [measured through electromyography (EMG)] until fatigue is induced in the muscles. Neuromuscular fatigue is described as a decrease in the ability of a muscle to produce a desired force over time during physical activity, which may induce a change in the activation pattern (Gandevia, 2001). At this time however, the muscle activity during fatiguing cycling is still not completely understood (Macdonald, Farina, & Marcora, 2008). Muscle (peripheral) fatigue has been reported to be one of the major limitations of performance during prolonged cycling due to alteration of cycling motion and activation patterns in the lower limb muscles (Castronovo, De Marchis, Bibbo, Conforto, Schmidt, & D'Alessio,2012; So, Ng, & Ng, 2005). Timing of muscle activation during cycling has also been widely reviewed and a distinctive view of muscle recruitment during cycling has been adopted (Castronovo et al., 2012; So et al., 2005).

High intensity interval training is a fatiguing series of repeated exercise sessions (usually at 80% of VO_2max or above) interspersed with rest periods. The exercise sessions can be any length (usually up to 5 minutes) and the rest periods are of equal length or longer. This type of exercise may induce fatigue in a non-experienced population; however, if completed regularly (over an extended period of time), it may actually increase the fatigue threshold (Smith, Moon, Kendall, Graef, Lockwood, Walter, Beck, Cramer, & Stout, 2009; Faria, 1978; Keul et al., 1966; Reindall et al., 1962; Knuttgen et al., 1973; Faria & Cavanagh, 1978), meaning it would take longer for an individual's muscle to fatigue.

Oxygenation of the muscle is known to be a factor in fatigue. In decreased oxygen concentration settings (such as high altitude) there is less oxygen delivery to the muscles (Dempsey & Wagner, 1999), which can lead to reduced maximum voluntary contraction (MVC) force, muscle activation and in turn muscle coordination (Rasmussen, Nielsen, Overgaard, Krogh-Madsen, Gjedde, Secher & Petersen, 2010; Romer, Haverkamp, Amann, Loevering, Pegelow & Dempsey, 2007). Research has also shown that during hypoxia, fatigue is expedited. The common understanding is that this may be due to limitations of the central nervous system and its ability to relay signals to the peripheral locomotor muscles. This would in turn affect the muscle coordination patterns seen during such a test.

This review of literature will discuss factors that affect muscle coordination as well as how muscle coordination is changed by fatigue. In addition, our current understanding regarding muscle coordination during hypoxia as well as interval training will be discussed. To the author's knowledge, all of the above factors have not been studied together as a whole. From the literature to be discussed comes the theory that exercising under the influence of hypoxia causes a greater rate of perceived exertion as well as muscle coordination changes

most likely due to central fatigue factors; while one session of interval training brings about peripheral changes in the muscle. Thus, this research is needed to correctly identify which type of fatigue is present as well as determining if it is the limiting factor to exercising under these conditions.

1.2 Muscle Coordination

Evidence regarding the typical timing of muscle activation during cycling has been previously reviewed, and thus an accepted view of muscle recruitment has been developed (Castronovo et al., 2012, So et al., 2005). During one revolution of the crank on a cycle ergometer, there are four overlapping phases; the propulsive phase (from top dead center (TDC) - 0° - to bottom dead center (BDC) - 180°), the pulling phase (from BDC to TDC), and two transitional phases (±10° on either side of TDC and BDC) as seen in Figure 1 (Fonda & Sarabon, 2010). It is generalized that single joint muscles are force producers during cycling, and that force is then transferred to the bi-articular muscles which then translate that energy onto the pedals (Ericson et al., 1985; Fonda & Sarabon, 2010).

Among the single joint muscles that are active during cycling are the vastus lateralis (VL) and the tibialis anterior (TA) , while the bi-articular muscles that are active include the biceps femoris (BF) and the lateral head of the gastrocnemius (LG), among others (Fonda & Sarabon, 2010). The muscle activity of the muscles will range for different people. Below, approximate values for muscle activation are reported from Ryan & Gregor, 1992 who studied muscle activation during cycling in experienced cyclists from fine wire electrodes. The VL is responsible for extending the knee and is active from approximately 300° to 130° in the cycle, and has peak electrical activity at 30° (Ryan & Gregor, 1992). The LG becomes active at 350° in the cycle and remains active until 270° with a peak in activation at 110°. The

LG is responsible mainly for stabilizing the tarocrural joint and plays a part in knee flexion as well (Ryan & Gregor, 1992). The BF - which flexes the knee and extends the hip - also peaks in activation at 110°, but is only active between 350° and 230° of the cycle (Ryan & Gregor, 1992). Finally, the TA flexes and stabilizes the tarocrural joint, and is normally active throughout the entire cycle, with peak electrical activity at 280° (Ryan & Gregor, 1992). The difference in activation timing in the aforementioned muscles illustrates the different roles of each. VL is a power producer during cycling, while BF and LG improve the transfer of energy between joints at the end of the propulsive phase into the pulling phase (Raasch & Zajac, 1999; So et al., 2005). The TA is a specialized muscle in that it produces power at the end of the propulsive phase and helps the cyclist transition into a new cycle. It also helps with the energy transfer between the limb and the pedals (Raasch & Zajac, 1999). While cycling is simply the movement of the legs in a predefined circular fashion (Hug & Dorel, 2009) the smallest changes in geography or cycling experience may have a large impact on the biomechanical pattern of cycling (Fonda & Sarabon, 2010).

1.2.1 Experienced vs. Inexperienced Cyclists

Cycling experience has an influence on the cycling pattern whether it be through: joint mechanics (Hoshikawa, Takahashi, Ohashi & Tamaki, 2007), muscle recruitment patterns (Chapman, Vicenzino, Blanch & Hodges, 2007), or the pedalling cadence (Faria, 1978; Marsh & Martin, 1995).

Many authors have stated that trained cyclists usually prefer a higher cadence when cycling compared to untrained participants (Faria, 1978; Cavanagh & Sanderson, 1986; Kroon, 1983; Drake 1993). Marsh & Martin (1995) has shown contradictory evidence indicating that there is no significant difference in preferred pedalling cadence between

cyclists and non-cyclists (85rpm vs. 91rpm). This was supported by Chapman, Vicenzino, Blanch & Hodges in 2005 - 77rpm vs. 79rpm for cyclists and non-cyclists respectively.

Although there was a change in peak muscle activity in various cadences in novice and experienced cyclists, novice cyclists have been found to have an increase in duration of muscle activity in the LG and TA at higher cadences compared to experienced cyclists (Chapman, Vicenzino, Blanch & Hodges, 2005). Coactivation was also seen to increase with cadence in non-experienced cyclists. Further research by Chapman et al., (2007), showed that the pattern of muscle recruitment was highly similar between trained triathletes and novice cyclists. This was explained by the interruption in motor learning of cycling in triathletes due to the combination of training regimes, or the adaption of muscle recruitment to maximize training potential for multiple disciplines. In comparison to trained cyclists, the novice cyclists showed greater and more variable coactivation between the muscles of the lower leg, in addition to less muscle activity with higher cadence in only three minutes of cycling (Chapman, Vicenzino, Blanch & Hodges, 2007). There was no change in muscle activity in trained cyclists with increased cadence. Candotti, Loss, Bagatini, Soares, da Rocha, de Oliveira & Guimaraes (2008) compared the EMG activity of the upper leg muscles (BF & VL) of triathletes and cyclists, and found that the triathletes had significantly more coactivation than the cyclists at four different cadences (Candotti, Loss, Bagatini, Soares, da Rocha, de Oliveira & Guimaraes, 2008).

Finally, Theurel et al., (2012) performed a study to examine muscle fatigue and mechanical efficiency throughout two trials of different pedalling techniques (a preferred technique with no feedback, and a "pulling" technique with feedback). The "pulling technique" was representative of the technique that novice cyclists used when fatigued. They

found no time difference in activation for any of the muscles in any of the conditions. They found that there were decreased activation levels in rectus femoris (RF) and VL, in both conditions, but the pulling technique showed a greater decrease. MG, TA and soleus muscles showed no change in activation in either condition (Theurel, Crepin, Foissac & Temprado, 2012). In addition, Theurel et al., had their participants complete three MVCs (one every 15 minutes) throughout the test. There was a significant decrease in every MVC when compared to the pre-test measure in both conditions. By the end of the test, the reduction in force was -15 ± 9% for the feedback condition and 7 ± 12% for the preferred technique.

1.2.2 Changes in Workload & Cadence

As cycling experience has an effect on the tiring of the muscles involved in cycling, maintaining a certain workload or cadence would naturally have the same effect. When the muscles are fatigued, there are changes in the muscle coordination. Most of the mechanical work that happens in cycling is produced in the propulsive phase (Broker & Gregor, 1994). Therefore, we can assume that changes in muscle coordination that occur with changes in workload would take place in the muscles that are the most active during this time. Schmidt (1994) supported this theory in his work. He stated that the quadriceps are the most important muscles for producing power when cycling and the lower leg muscles are responsible solely for maintaining the cycling motion. MacIntosh, Neptune & Horton (2000) showed that there existed a relationship between revolutions per minute (rpm) and resistance that was not unlike that of the force velocity relationship for muscles (Hermansen & Saltin, 1969). The relationship showed that as the optimal cadence (least amount of EMG per given workload) increased, the power output increased (MacIntosh et al., 2000). A low pedalling frequency during maximal effort may cause great tension in the quadriceps muscle with each

turn of the crank (Faria, 1978). This tension can cause muscle fatigue and may limit performance before there is a maximal demand on any other system (ie. cardiorespiratory). The muscle force needed to maintain equivalent power outputs increases as pedalling speed becomes slower (Bannister & Jackson, 1967; Dickinson, 1929; Hoes et al., 1967). However; this may only be true in trained cyclists.

The influence of cadence on muscle coordination is highly conflicting. Hansen & Ohnstad, (2008) stated that cadence is set by robust neural networks and therefore it remains unchanged when the mechanical or physiological workload changes. Sarre and Lepers (2005) had participants cycle at 65% of their maximum power output at 50 rpm, 100 rpm, and a freely chosen cadence – mean 87.9 rpm. They found that the VL, LG and BF all had significant increases in EMG activity at the 110 rpm cadence. Another study reported that with a higher cadence (90-120 rpm), muscle activation increases sooner in the cycle for all active muscles (Neptune, Kautz & Hull, 1997). Faria (1978) found that there were no activation changes in cadences of 40, 60 and 80 rpm.

Many of the above studies used standardized workloads as opposed to customized workloads. As the workload plays an important part in the biomechanics of cycling it should always be modified to the capabilities of the individual being tested (Fonda & Sarabon, 2010).

Since there are no clear significant conclusions between muscle activation and different cadences, it seems that a central component of fatigue might be the limiting factor in cycling performance. Lepers et al., (2000) found that at high pedalling rates, the neural input to the VM and VL muscles remains unchanged and central drive is less altered when a "high"

(69-103 rpm) pedalling rate is used. This means that at higher cadences central input is not altered, and that freely chosen cadences do not minimize the effects of fatigue on the leg extensors subsequent strength capacity.

1.2.3 Changes in Muscle Activity

There have been two types of studies performed in order to further understand the coactivation of muscles during cycling: repeated sprint studies and prolonged steady state cycling. There have been a variety of results when examining repeated sprint exercise and muscle coordination. One study showed that 15, 5s sprints lead to no change in EMG for VL, and LG, and a significant decrease in BF EMG (Hautier, Arsac, Deghdegh, Souquet, Belli & Lacour, 2000). The large decrease in antagonist EMG led the researchers to look at VL:BF coactivation and found that there was a decrease. They then came to the conclusion that fatigue of the power producers (VL) may have forced the subjects to adapt to a muscle coordination pattern where the antagonist muscle (BF) were used to transfer the power and force to the pedals more efficiently (Hautier et al., 2000).

Billaut, Basset & Falgairette (2005), showed that after 10, 6 s sprints there was no significant change in amplitude of VL and BF. However, they did find that there was an increased silent period between the VL and the BF, due to earlier antagonist (BF) activation (phase change), meaning an increase in coactivation between the two muscles. They attributed this increase in coactivation to the increase in pedalling rate, which has a tendency to shift the EMG activity to an earlier time in the cycle (Marsh & Martin, 1995). The overall results from the sprinting studies are very similar, while the results of steady state cycling are a bit more variable.

Most studies that have examined coactivation in steady state cycling have shown a decrease in EMG of the power producer (VL) and an increase in EMG of the antagonist muscle (BF) (Dorel, Drouet, Couturier, Champoux & Hug, 2005; Theurel, Crepin, Foissac & Temprado, 2011). A study performed by St. Clair-Gibson, Schabort & Noakes (2001) also found a decrease in VL EMG; however that was the only muscle that was monitored.

Bini, Diefenthaeler & Carpes (2011) had subjects perform a 40km time trial as fast as possible. They hypothesized that because it was a time trial, participants would show decreased coactivation, and if the participants showed increased coactivation that it would lead to premature fatigue. They found that there was an increase in EMG for the VL; however there was no change in BF, TA, or medial gastrocnemius (MG). There was also no change in coactivation between the muscles (Bini et al., 2011).

In 2009 Dorel et al., showed that during steady state cycling, the RMS amplitude of the TA and gastrocnemius muscles decreased significantly, while the BF increased significantly. When the timing of activation (coordination) between the muscles was taken into account, they found that LG, TA, and VL were activated later in the cycle towards the end of the test (Dorel, Drouet, Couturier, Champoux & Hug, 2009). This test was performed at 80% of peak power and went to exhaustion (13.8 ± 6 minutes).

During cycling it is often the power producing monoarticular muscles (such as VL and gluteus maximus) that fatigue the fastest. When these muscles produce less force and power, there would be an expected decrease in the efficiency of the EMG pattern (the amount of muscle activation that is actually contributing to the movement of the limb through the cycle). However, as these muscles fatigue, lower activation of the antagonist muscles

(usually BF) mediates and effectively transfers the force and power to the pedal (Faria, Parker & Faria, 2005).

1.3 Intervals

Interval training is a system of environments in which metabolic systems of the body are exposed to brief but regularly recurring periods of work interspersed with designated rest periods. With interval training, both aerobic and anaerobic power are improved, meaning an improvement in cycling capacity in both circumstances (Faria, 1978). With repeated training, muscle strength and endurance becomes the limiting factor for exercise (Faria, 1978). As the circulatory and respiratory systems adapt to the work, the oxygen supply to the tissues is improved and the anaerobic system is used less (Keul et al., 1966; Reindall et al., 1962). Therefore the use of interval training can bring about a large increase in the transport and utilization of oxygen in a short period of time (Knuttgen et al., 1973).

It is known that with interval training, less fatigue is experienced over time; however, what remains to be seen is the amount and type of fatigue present after a single training session. In a study performed by Villerius, Duc, & Grappe (2008) where subjects performed a 10 minute cycling time trial with 15 minutes of active rest, only a difference in power output was achieved. The power output increased significantly in every time trial by the second minute and remained constant throughout the rest of the trial. There were decreases seen in the last minute of every trial, however, they were not significant. They noted that there were no significant differences for heart rate (HR) between or within any trial, but found the rate of perceived exertion (RPE) did increase significantly by trial 3. EMG activity of the VL, VM, BF, and medial hamstring muscles were monitored, however there was no significant change in amplitude or timing of any of the muscles. Although there were no

muscle coordination changes in this study the increase in RPE suggests a central influence on the participant.

Skof & Strojnik (2005) expanded on the above in order to determine whether peripheral or central fatigue was the limiting factor in a single interval training session. From previous work they understood that peripheral fatigue was present after a steady state bout of exercise and were interested to discover if the same was true after a bout of intervals. They found that after five running sprints, maximal twitch force decreased, while MVC and muscle activation remained the same. As there was no change in MVC or muscle activation, but still a decrease in twitch force, they concluded that one bout of intervals caused fatigue in the peripheral system.

1.4 Hypoxia

Hypoxia is an environment with a reduced oxygen concentration, the intensity of which depends on the length of duration and the level of altitude (Levine, 2002). Durations may include acute (minutes to hours – such as used in this study) up to permanent (live at altitude) or native (adapted through generations). Levels are classified as low (<1600 m), moderate (1600 – 3000m – as seen in this study) or high (>3000 m). Moderate altitude is representative of an environment of 15% oxygen. As oxygen uptake can be limited in a hypoxic environment, it may induce whole body fatigue (Dempsey & Wagner, 1999) as well as locomotor fatigue (Romer, Haverkamp, Amann, Loevering, Pegelow & Dempsey 2007). In a study performed by Rasmussen, Nielsen, Overgaard, Krogh-Madsen, Gjedde, Secher & Petersen (2010), 16 males performed a 20 minutes arm cycling exercises in which the oxygen concentration was reduced by 25% (approximately 15.7% O_2) and participants experienced a decrease in muscle activation and MVC force.

In severe hypoxia (13% O_2) it was found that when participants cycled at 90% of their VO_2 max to exhaustion they experienced a decrease in time to fatigue (33%), decreased muscle activation and a decrease in MVC force in the quadriceps (Romer, Haverkamp, Amann, Loevering, Pegelow & Dempsey, 2007). There was also a 15% decrease in potentiated twitch force in the quadriceps performed by transcranial magnetic stimulation (TMS).Goodall, Gonzalez-Alonso, Ali, Ross & Romer (2012) showed that in a cycling task at 80% peak power output (PPO) in 13% hypoxia, exercise time is greatly reduced (54%). They used near-infrared spectroscopy to assess cerebral oxygen delivery and found that it was reduced significantly in hypoxia. In both hypoxia and normoxia they found decreases in MVC force, however, the decrease was significantly greater in hypoxia compared to normoxia (25% vs. 17%). Finally, they found that EMG was reduced by 16% in normoxia, but the difference was not significant.

A unique study performed by Fulco, Lewis, Frykman, Boushel, Smith, Harman, Cymerman & Pandolf (1996) examined muscle fatigue during a one leg knee extension in normoxic and extreme (4300m) hypoxic conditions. They used a specially designed knee extension apparatus, and had participants perform submaximal knee extensions at a constant rate (moving from 90° to 150° at a rate of 1 hertz) until exhaustion. MVCs were taken periodically throughout the exercise. They found that both conditions showed a similar decrease in MVC force; however the time to exhaustion was 56% shorter in hypoxia than in normoxia. They also found that throughout the exercise in both conditions there was a continual rise in quadriceps (RF, VL, VM) muscle activity, and by minute 10 in each test the difference was statistically significant. In addition they found that there was no change in the BF muscle activity.

Not only are the muscle themselves affected by hypoxia, but the nerve fibres may also be affected. It is known that peripheral fatigue can cause metabolite accumulation and tension in the muscle (Amann, 2011). These are transmitted to the central nervous system (CNS) by chemoreceptors, mechanoreceptors, and group III and IV pain afferents (Amann, 2011). Group III and IV nerve fibres are located within the muscle and project feedback about the cardiovascular and ventilatory reflex responses from the muscles to the central nervous system (CNS), thus making them very important for exercise in hypoxia, as often times muscle O_2 delivery will be reduced. When this reduction is detected, in moderate hypoxia, it can potentially lead to a decrease in alpha motorneuron activation and reduce the central motor drive (Bigland-Ritchie et al 1986; Duchateau & Hainaut, 1993; Martin et al, 2006; Amann, 2011). This could decrease reciprocal inhibition and lead to greater agonist/antagonist coactivation, subsequently altering muscle coordination.

1.5 Fatigue

Fatigue is inherent in any type of exercise, and causes reduced power and velocity (Gandevia, 2001). Fatigue can take place at the muscle (peripheral) or somewhere between the brain and the muscle (central). Both types of fatigue cause decrements in performance, but the type that is the limiting factor for this type of exercise is yet to be determined.

With fatigue, there may be changes in force (motor unit twitch force and contraction velocity) without changes in EMG amplitude - indicative of peripheral fatigue (Carpenier et al 2001; Fuglevand et al 1999; Thomas et al 1991). Peripheral fatigue may also be shown through a consistent increase in muscle activity throughout the task (Behm, Button, Barbour, Butt & Young, 2004). In contrast there may also be changes in EMG (changes in the shape and propagation velocity of the motor unit action potentials) without parallel changes in force

– indicative of central fatigue (Dimitrova and Dimitrov 2003; Keenan et al 2005). Changes in EMG may include a decrease in muscle activity throughout the task (Behm et al., 2004).

Afferents from the peripheral muscles inform the CNS about changes in the muscles (Enoka & Stuart, 1992). These signals require modulation of spinal cord activity by supraspinal centers to match the conditions in the periphery. The supraspinal center then uses this information to alter the activation signal sent back to the periphery to assist with continuing the activity. The effort associated with sustaining physical activity does have consequences and can produce central fatigue. Lepers, Millet & Maffiuletti (2001) noted that sustained cycling impairs force generating capacity of the muscle and is associated with changes in contractile and nervous properties of the leg extensors. Therefore the net motor unit activity is related to the magnitude of the signal discharged by the spinal cord and may be monitored through the use of EMG (Enoka & Stuart, 1992).

Voluntary activation can cause fatigue; however voluntary activation may be impaired by intermittent high force contractions (Enoka, Baudry, Rudroff, Farina, Klass, Duchateau, 2011). This is known to add to fatigue in prolonged low level force tasks (Enoka et al., 2011). By using electrical stimulation of the nerve, muscle activation can be measured if administered during an MVC. Usually stimulation protocols such as the interpolated twitch technique (ITT) or the central activation ratio (CAR) method are used in this case. The amount of activation (or inactivation) present can help to determine if central fatigue is present in the muscle.

1.6 Fibre Type

Another factor involved with the fatigue of the muscle is the fibre type. All muscles have fast and slow twitch fibres, however, the amount of each type will play a role in how fast a muscle fatigues. The surface fibres of the VL seem to have about a 40:60 split of slow twitch fibre to fast twitch fibre composition; however with endurance training the size of slow twitch fibres will increase – as measured in cadavers (Johnson, Polgar, Weightman & Appleton, 1972). This is refuted by Scholz et al., (1959), and Barnard et al., (1970) who claim that with aerobic training the number of slow twitch oxidative fibres will actually increase. Research has shown that subjects with high percentage of fast twitch fibres are more sensitive to fatigue than subjects with a higher percentage of slow twitch fibres (Colliander, Dudley & Tesch, 1988). However, according to Lepers et al., (2001) there appears to be no relationship between fibre type recruitment pattern and neuromuscular fatigue and subsequent reduction in strength during cycling. The BF has a much higher percentage of slow twitch fibres (65:35 - slow to fast) (Johnson et al., 1972) which may explain why in previous work there is an increase in BF EMG with time. The BF is still recruiting motor units, while the motor units of the VL become fatigued. No conclusive remarks have been made for the LG or TA in the literature for changes in EMG while cycling, perhaps due to their minor role in cycling. The LG is fairly homogeneous in its fibre composition, while the TA has a high percentage of slow twitch fibres (approximately 70%) (Johnson et al., 1972).

1.7 Conclusion

The muscles working together to produce motion around a joint is called muscle coordination, and there are specific recruitment patterns for every movement. Exposure to a

lowered oxygen environment (hypoxia) can cause an acceleration of locomotor muscle fatigue (Romer et al., 2007), and when the muscle becomes fatigued, a change in the pattern of activation may be induced (Gandevia, 2001). This change in muscle coordination is indicative of central fatigue, while it has been stated that in one session of interval training, fatigue is usually expedited by peripheral factors (Skof & Stronjnik, 2005), although it seems that participants still reported higher RPE values in this type of exercise (Villerius et al., 2008). The combination of all of these factors will provide us with a novel study, in which results will show how muscle coordination is affected by hypoxia and interval training combined, as well as determining whether the limiting factor in this type of exercise is related to central or peripheral mechanisms.

1.8 Objectives & Hypothesis

The objectives of this research are as follows:

1) To gain insight into lower limb muscle coordination and force output during a fatiguing cycling protocol with and without moderate hypoxia.
2) To determine if central fatigue takes place before, during or after the development of peripheral fatigue

The following hypotheses are addressed in this study:

1) There will be an increase in the amplitude of the RMS EMG signals from all muscles, except the VL, which will decrease. This is expected to be true especially in the hypoxic condition. In fatiguing cycling it has been noted that the VL amplitude normally decreases, while the BF amplitude increases. It is

expected that the effect of hypoxia and the intervals will have a combined effect and cause the amplitude of the TA and LG to increase as well.

2) It is hypothesized that muscle activation will shift to an earlier spot in the cycle and the muscle will be active for longer near the end of the test in hypoxia, when the participant is the most fatigued, and that cycling cadence will most likely decrease over time.
3) Signs of central and peripheral fatigue will develop simultaneously; however, central fatigue will be more prevalent and have a greater effect on the how the participant feels during exercise (RPE), as well as the time to fatigue.

1.9 Significance of the Study

The results of this study will provide the research community with further comprehension on the role that changes in neuromuscular function (muscle coordination) and central motor drive play in coping with hypoxic stress. This research will be valuable in specific communities (ie. cycling and mountaineering) to ensure proper training and preparation for expeditions.

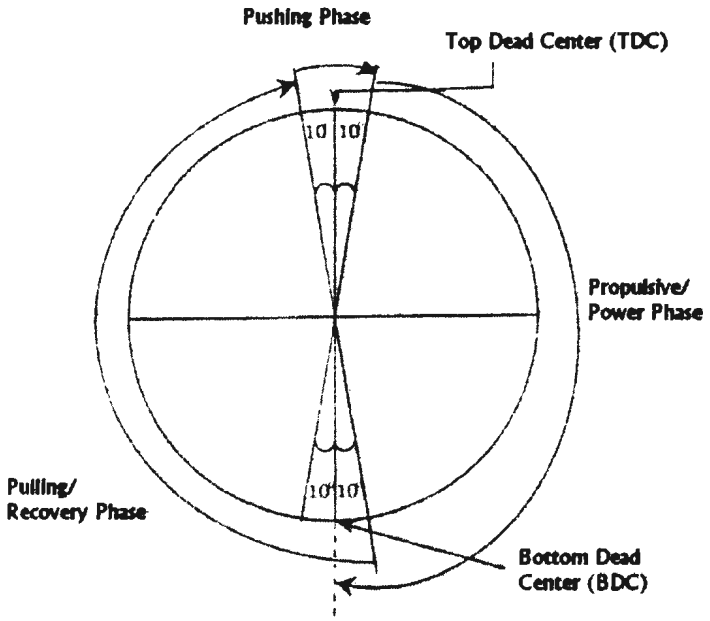


Figure 1: The phases of the crank cycle during the action of cycling (So, Ng, Ng, 2005)

2.0 METHODOLOGY

2.1 Subjects

Ten endurance trained males (height: 178.4 ± 7.3 cm, mass: 75.4 ± 6.1 kg, age: 27.6 ± 4.8 yrs) participated in this study. All participants were endurance athletes (running, cycling or swimming) and trained an average of ten hours per week (57.39 ± 6.89 $mL \cdot kg^{-1} \cdot min^{-1}$). All participants were verbally informed of all procedures and provided written and informed consent. Participants also completed a Physical Activity Readiness Questionnaire (PAR-Q) as well as a training inventory questionnaire prepared by the researchers. Memorial University's Human Research Ethics Authority (HREA) approved this study.

2.2 Experimental Approach

A single blinded randomized cross-over study design was used. Participants attended the lab for three different sessions. In the first session, participants underwent an incremental ramp cycling protocol (Storer, Davis, Caiozza, 1990) to determine their peak power output (PPO). They were familiarized with the knee extensor MVC protocol as well as stimulation of the femoral nerve, the hypoxic condition (15% O_2) that they would receive in one of the two subsequent sessions. As a part of the familiarization session all participants were introduced to MVIC force completed on a knee extension table (Technical Services, Memorial University of Newfoundland). Resting heart rate and blood pressure as well as anthropometric (height and weight) and cycle ergometer measurements (saddle and handlebar height) were taken at this visit. The cycle ergometer measurements were recorded when the knee angle was 90° and the hip angle was 70° as measured by a goniometer, and were used in all subsequent sessions. This session lasted approximately one hour. The second and third sessions (hypoxic or normoxic condition) were randomized, separated by one week, and

lasted approximately 90 minutes (see Figure 2). Participants were blinded as to which condition they received during the 60-minute cycling protocol in sessions two and three. Furthermore, participants were asked to record all physical activity (frequency, intensity, time, type) between sessions to ensure consistency in training. Prior to participation in this research study none of the participants had any experience exercising in artificial or natural hypoxic conditions. Muscle coordination was measured and compared within and between normoxic and hypoxic conditions during the 60-minute cycling protocol.

2.3 Experimental Conditions

2.3.1 $VO_{2\ max}$ and Peak Power Output Determination

On their first visit to the lab, participants performed a $VO_{2\ max}$ test using an incremental ramp cycling protocol in the normoxic condition to determine maximum oxygen uptake (VO_2). VO_2 and carbon dioxide output (VCO_2) were continuously collected through a two valve mouthpiece connected to a gas analyzer (Sable Systems International, Las Vegas, NV). The VO_2max protocol consisted of cycling starting at a power output of 50W, increasing by 1W every three seconds (Storer, Davis, Caiozza, 1990). A self-selected pace was allowed, as long as it was kept above 60 revolutions per minute (rpm). Participants continued the test until volitional fatigue, or until their rpm dropped below 60. The last completed power output performed by the participant was determined to be their peak power output (PPO). After the ramp protocol was completed, a five minute rest was given and a verification test was performed to ensure that the VO_2max that was reached was accurate (Workman & Basset, 2012). The verification test consisted of cycling at 105% of the PPO received in the ramp protocol, as hard and as fast as possible, until unable to maintain a cadence of at least 60 rpm.

2.3.2 Cycling Test

A 50 second ramp protocol (wattage increased by 100 watts (W) every 5 seconds) designed by the research team was utilized as a dynamic MVC to determine maximal EMG in the lower limb muscles while cycling. EMG measurements during the hypoxic and normoxic conditions were then normalized to the dynamic MVC EMG values, thus giving an indication of the intensity of muscle activation. Participants completed this protocol before starting the warm-up in sessions two and three.

The warm-up consisted of cycling for five minutes at a self-selected pace and was completed at 35% PPO as determined in the ramp protocol. Following the warm up, participants completed a 60-minute cycling task composed of 8 - three minute work intervals at 70% PPO interspersed with 8 - four and a half minute active rest intervals at 35% PPO. Participants cycled at a self-selected pace, but were required to maintain a cadence of at least 60 rpm for all intervals. After the test was complete, participants were required to stay on the cycle ergometer until their heart rate returned below 100 bpm. Heart rate (PolarElectro, Kempele, Finland), rate of perceived exertion (RPE – Borg Scale, 1998) and levels of arterial oxygen saturation (SpO_2) were taken at the end of every interval (work and active rest). Participants performed the cycling task in either a hypoxic or normoxic condition.

2.3.3 Hypoxic Condition

The hypoxic condition was created using the GO_2 Altitude System (Biomedtech, Melbourne, Australia). This system uses a generator equipped with a semi permeable nitrogen filtration membrane, and continuously pumps air at a flow rate of 20 l/min into an oro-nasal mask on the participant's face. An oxygen sensor (Cambridge Sensotec, Cambus, UK) monitored the gas concentrations and a pulse oximeter (Radical 7 SET, Massimo, Irvine,

CA) placed on the subject's forehead monitored the SpO_2 to ensure it did not drop below 80% (Workman & Basset, 2012). This study replicated a hypoxic environment in which the oxygen concentration is 15% as opposed to 20.93% according to standard ambient temperature and pressure (SATP). This is equivalent to moderate altitude, approximately 2200-2500m (Levine, 2000).

Each experiment ended if participants: 1) recorded an SpO_2 level below 80%, 2) did not feel well enough to continue, 3) recorded an RPE of 20, or 4) could not maintain a cadence of 60 rpm. Following each session, participants' completed the Lake Louise Hypoxic Scale (Appendix B) to determine if there were any hypoxia induced symptoms such as dizziness or nausea and the intensity of these symptoms.

2.4 Dependent Variables

2.4.1. Knee Extension Force

Before the dynamic MVC was performed, participants were asked to perform at least two Maximum Voluntary Isometric Contractions (MVICs) with a two minute rest period between each to determine their maximum isometric force output on the cycle ergometer. The MVICs were performed as part of the CAR method, to normalize EMG during all subsequent MVICs. In order to ensure a consistent maximal effort, the participants proceeded with the test if there was less than 5% difference between the two MVICs (Behm et al., 2004).

Participants removed their feet from the pedals, and placed their right thigh on a padded extension on an aluminum pole (Technical Services, Memorial University) for support. They remained seated on the cycle ergometer and braced themselves on the handlebars. Their right ankle was inserted into a padded strap attached by a high-tension

wire that measured force using a Wheatstone bridge configuration strain gauge (Omega Engineering Inc. LCCA 250, Don Mills, Ontario). A wooden box was placed under the left foot for support (Figure 3). All forces were amplified, sampled at 200 Hertz (Hz) (Biopac Systems Inc. DA 100 and analog to digital (A/D) converter MP100WSW; Holliston, MA) and monitored on computer. Raw EMG data were sampled at 2000 Hz – A/D converted and stored on a computer for further analysis using a commercially designed software program (AcqKnowledge 4.1, Biopac Systems Inc., Holliston, MA). The peak-to-peak (P-P) amplitude of the MVC was obtained after every workload and used for analysis.

2.4.2 Muscle Activation

EMG was used to assess muscle activation. EMG was measured from the right lower limb muscles continuously throughout sessions 2 and 3. Surface EMG electrodes [Meditrace Ag/AgCl, disc shape, and 10 mm in diameter (Graphic Controls Ltd., Buffalo, NY)] were placed with an interelectrode distance of 2 cm centre to centre, over the muscle belly of the VL, BF, TA and LG according to guidelines published by Kamen, 2010. A ground electrode was secured on the fibular head. Thorough skin preparation included shaving the area, removal of dead epithelial cells with an abrasive (sand) paper followed by cleansing with an isopropyl alcohol swab.

There were two sensors (one positioned on the crank, the other on the cycle ergometer) that were used as a measure of one full revolution of the crank. When the two sensors came in contact with each other (90° into the 360° cycle), an analog signal was sent to the Acqknowledge software program along with EMG to indicate a new cycle.

2.4.3 Muscle Inactivation

To evoke a maximal twitch force of the knee extensors, electrical stimulation was applied to the femoral nerve during rest. The femoral nerve was electrically stimulated via adhesive Ag-AgCl electrodes (diameter 10 mm) fixed to the skin over the inguinal triangle (cathode) and the greater trochanter (anode). Current pulses (200 μs duration, 400-800 mA of a 400 V square-wave pulse) were delivered via a constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The electrical stimulation was progressively increased until the knee extensor resting twitch force no longer increased. This stimulation intensity was used in all subsequent MVCs to determine inactivation.

2.5 Data Analysis

EMG analysis consisted of amplitude, duration, and onset and offset times of two flexors [(biceps femoris (BF) and tibialis anterior (TA)] and two extensors [(vastus lateralis (VL) and lateral gastrocnemius (LG)] of the lower limb. Quadriceps muscle force, activation, percent inactivation and evoked contractile properties [half relaxation time, evoked twitch force and electromechanical delay (EMD)] were assessed at predetermined times during the 60-minute cycling protocol.

While EMG was collected for the full duration of the cycling task, only the last 30 seconds [to ensure a minimum of 20 cycles for the average (Hug et al., 2005)] from the first, middle and last work interval of each cycling test were analyzed. Software analysis consisted of finding the root-mean-square (RMS) of the EMG for all muscles using a 30ms moving average window. The 30s sample was averaged in 1s intervals (triggered by the crank position) and the averaged output was graphed (see Figure 4). The maximum amplitude from baseline and the mean amplitude was taken from this data and normalized to the dynamic

MVC (for each muscle). Timing properties of the muscle activation including onset and offset of activation (threshold of 25% of maximum – onset was defined as the first time the amplitude of muscle activation crossed the threshold and continued to increase, offset was the last time that muscle activation crossed the threshold and continued to decrease) (Hug & Dorel, 2009), the delta time (how long the muscle was active over a 1s period, and the cycle period (the actual length of the cycle in seconds) were obtained. After the cycle period was obtained all measurements were made relative to the averaged output for that time point.

Coactivation levels were calculated at the same time points as muscle activation by using a ratio created from the normalized (to the dynamic MVC) duration values calculated for muscle activation. Coactivation was measured between the VL:BF and TA:LG as well as VL:LG and BF:TA.

The central activation ratio (CAR) method was used to describe the amount of inactivation (an indicator of central fatigue) at the quadriceps muscle. Two seconds prior to performing a MVIC, subjects were administered an initial evoked stimulation, relaxed, and then told to maximally contract their knee extensors for four seconds. During the MVIC, subjects received an additional evoked stimulation, which lead to a superimposed twitch and then were instructed to relax. A third evoked stimulation (potentiated twitch) was administered 2 seconds following the completion of the MVIC. The CAR was calculated comparing the amplitude of the superimposed stimulation with the pre-stimulation force value of the MVIC to estimate the extent of inactivation during a voluntary contraction (value of superimposed twitch / value of MVIC prior to stimulation x 100 = % of muscle inactivation) (Behm, Power & Drinkwater, 2001). After every work interval, the participant stopped cycling, their leg was strapped into a strain gauge in the same fashion as the

isometric MVC, and the twitch protocol was performed. Then the participant was immediately removed from the apparatus and continued to cycle. The CAR method was used to calculate inactivation of all the muscles after every work interval.

2.6 Statistical Analysis

SPSS for Windows (SPSS, Version 17.0, Polar Engineering and Consulting) was used for all statistical analysis. For muscle activation during the cycling protocol, a 2-way (condition – hypoxia, normoxia vs. time – beginning, middle, end) analysis of variance (ANOVA) with repeated measures was performed for each separate parameter (peak amplitude, mean, delta time, duration and coactivation). For all other parameters (force, inactivation, HR, RPE, SpO_2) 2-way (condition vs. time – every interval) ANOVA with repeated measures for time were performed. Descriptive statistics for all parameters includes means ± standard deviations. A value of $p < 0.05$ was considered statistically significant. Post hoc analysis was performed where interactions were deemed significant using the Least Significant Difference (LSD) test.

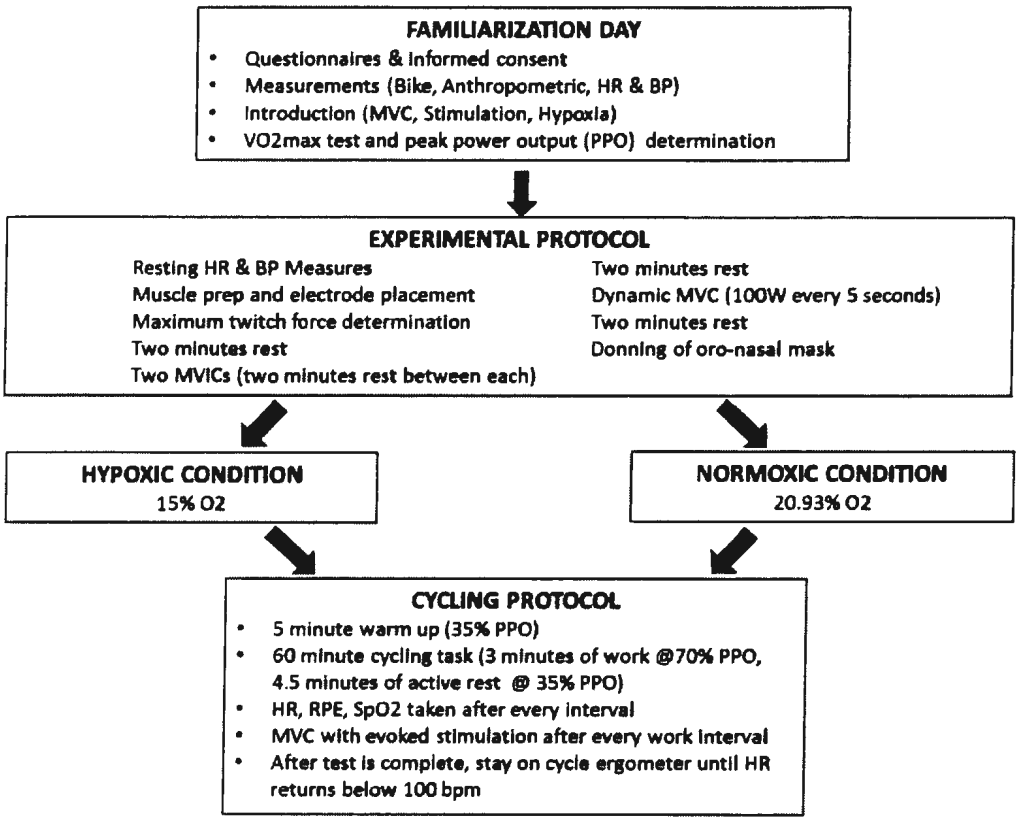


Figure 2: Flow chart of experimental sessions

Figure 3: A) Stimulation Apparatus. B) The participant releasing their foot from the clips. When finished the black padded part (circled) is swung under the thigh and locked into place. Foot is strapped into the ankle strap and MVIC with twitch protocol is performed.

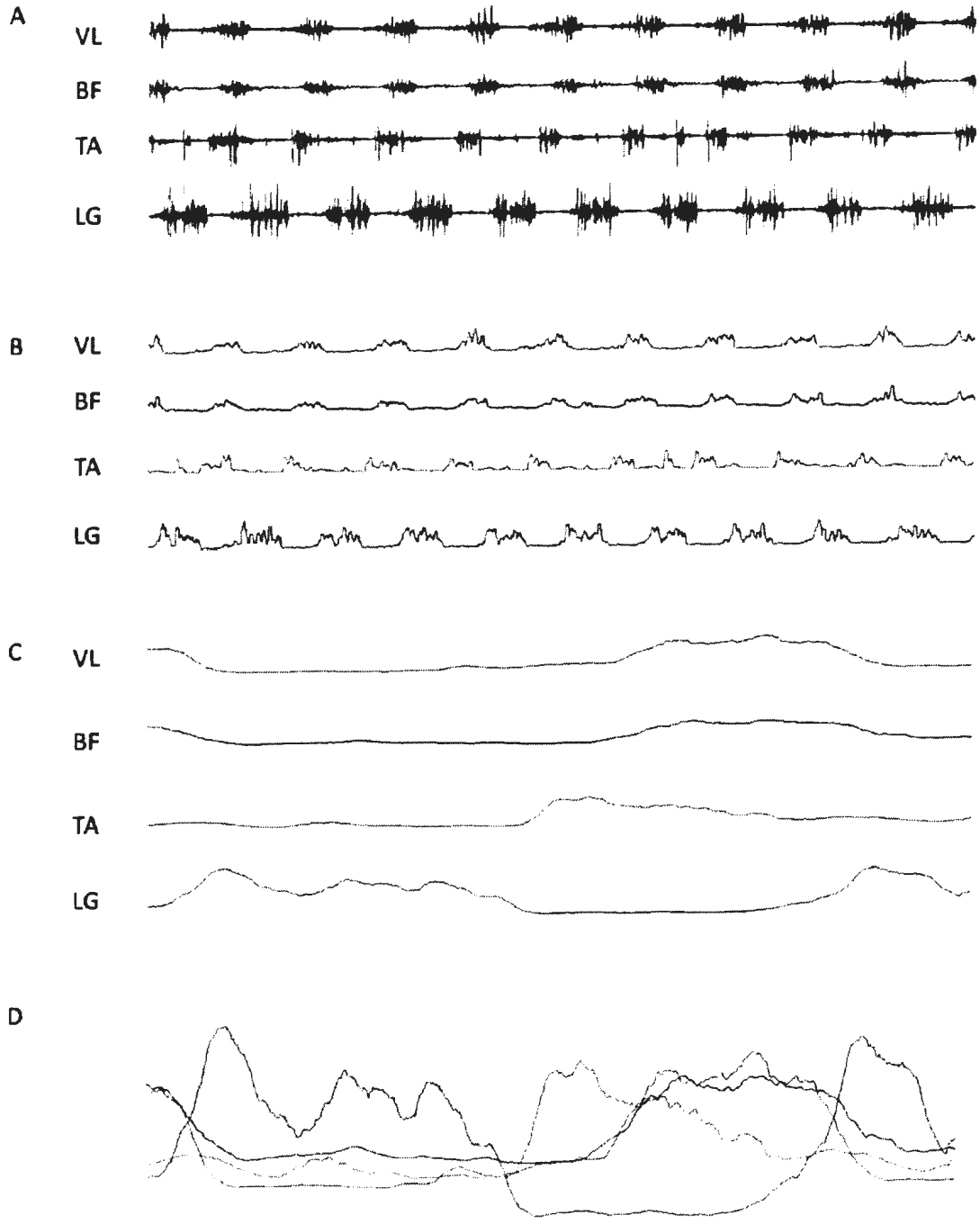


Figure 4: A) Raw data, B) RMS of EMG, C) Average Cycle over 30 seconds, D) Overlapped Averages

3.0 RESULTS

3.1 Time to Finish

Twelve participants started the testing; however two participants dropped out due to time commitment issues. In the normoxic condition all ten participants completed all intervals, so the time to finish was 60 minutes. In the hypoxic condition, four participants completed all intervals; three completed seven intervals, two completed four intervals and one completed three intervals. This made the average time to finish for the hypoxic condition 44.4 minutes, approximately 74% of the time to finish in the normoxic condition (See Figure 5). RPE, HR and SpO_2 were analyzed according to whether the participant was performing a work interval or a rest interval for a more accurate illustration of what was happening between conditions. Since most (7/10) participants made it to the seventh work interval, the results are presented for only these seven participants. The data was collapsed over time for each participant, so the data presented represents the first, middle and last work interval specifically for each of the seven participants.

3.2 Rate of Perceived Exertion

There was a significant ($p= 0.001$) main effect for condition on RPE recorded during the seven work intervals. RPE was 18.7% higher in the hypoxic condition (16.55 ± 0.43) compared to the normoxic condition (13.94 ± 0.72) (Figure 6A). There was also a significant main effect ($p = 0.01$) for condition on RPE recorded over the six active rest intervals. RPE for the active rest intervals was 18.4% higher in the hypoxic condition (11.93 ± 0.33) compared to the normoxic condition (10.07 ± 0.45).

3.3 Heart Rate

There was a significant ($p = 0.004$) interaction between condition and intervalfor HR recorded during the active rest intervals. Heart rate was 6.7% lower in the normoxic (135.03 ± 6.28 bpm) compared to the hypoxic condition (144.13 ± 7.70 bpm) (Figure 6B).

3.4 Arterial Oxygen Saturation (SpO_2)

There was a significant ($p = 0.007$) main effect for condition on SpO_2 recorded during the seven work intervals. SpO_2 was 12.5% higher in normoxia (98.10 ± 0.40%) compared to hypoxia (85.86 ± 3.07%). There was also a significant ($p = 0.009$) main effect for condition on SpO_2 recorded during the six active rest intervals. SpO_2 was 8.2% higher in the normoxic condition (98.10 ± 0.50%) compared to the hypoxic condition (90.10 ± 1.95%) (Figure 6C). Finally, there was also a significant ($p = 0.025$) main effect for time during active rest. The post-hoc analysis determined that SpO_2 had a tendency ($p = 0.64$) to decrease 2.7% from the first interval (95.64 ± 0.74%) to the last interval (93.07 ± 1.32%).

3.5 Maximal Voluntary Isometric Contraction (MVIC) Force

Participants produced significantly ($p < 0.001$) greater force (60.44 ± 2.14 kg) on the table than on the bike in both hypoxia by 52.5% (39.63 ± 1.72 kg) and normoxia by 45.7% (41.49 ± 0.84 kg).

There was a significant ($p = 0.017$) main effect for condition on MVIC. MVIC force was 7.8% lower in the hypoxic condition (32.32 ± 1.44 kg) as compared to the normoxic condition (34.85 ± 1.12 kg) (Figure 7). Pre-test values were not significant between conditions; however, in both conditions all values throughout the testing were significantly lower ($p < 0.03$) than pre-test values. A post-hoc test indicated a significant ($p = 0.031$)

interaction for condition*time for MVIC force. The post-hoc test showed that MVIC force was significantly ($p = 0.04$) lower after the first and sixth work interval in hypoxia when compared to normoxia.

3.6 Muscle Activation

3.6.1 Vastus Lateralis

There was a significant ($p = 0.02$) main effect for time on VL RMS amplitude. The post-hoc analysis revealed that VL RMS amplitude significantly ($p = 0.004$ and $p = 0.005$) increased by 14.5% and 16.8% from the beginning (0.531 ± 0.03) to the middle (0.608 ± 0.04) and from the beginning (0.531 ± 0.03) to the end (0.620 ± 0.04) of the test, respectively. The post-hoc test revealed a significant ($p = 0.029$) interaction for condition*time for peak amplitude. A comparison of equivalent time points (t-tests) between conditions showed that the peak amplitude was significantly ($p = 0.049$) higher at the mid-point of the test in hypoxia and tended ($p = 0.082$) to be higher at the end of the test in hypoxia as well (Figure 8A).

There was a significant ($p = 0.037$) main effect for time on VL mean RMS amplitude. The post-hoc test showed that mean amplitude significantly ($p = 0.034$ and $p = 0.008$) increased 15.8% and 18.8% from the beginning (0.112 ± 0.01) to the mid-point (0.129 ± 0.01) and from beginning (0.112 ± 0.01) to the end (0.133 ± 0.01) of the test, respectively (Figure 8B).

There was a significant ($p = 0.038$) main effect for condition on VL delta time. VL delta time was 17.1% higher in the normoxic (0.263 ± 0.01s) compared to the hypoxic (0.308 ± 0.03 s) condition (Figure 8C).

3.6.2 Biceps Femoris

There was a significant ($p = 0.02$) main effect for time on BF peak RMS amplitude. The post-hoc analysis showed that BF peak amplitude significantly ($p = 0.004$ and $p = 0.013$) increased 16.9% and 25.3% from the beginning (0.533 ± 0.1) to the mid-point (0.623 ± 0.16) and beginning (0.533 ± 0.14) to the end of the test (0.668 ± 0.18), respectively (Figure 9A).

There was a trend ($p = 0.071$) for time on BF mean amplitude. The post-hoc test revealed that BF mean amplitude increased 24.1% and 29.1% from the beginning (0.141 ± 0.03) to mid-point (0.175 ± 0.04) and beginning (0.141 ± 0.03) to the end (0.182 ± 0.04) of the test, respectively (Figure 9B).

A significant ($p = 0.042$) interaction for condition*time was found for BF delta time. The post-hoc analysis showed that during hypoxia the BF delta time significantly ($p = 0.028$) increased by 10.1% at the end of the test compared to normoxia (Figure 9C).

3.6.3 Lateral Gastrocnemius

There was a significant ($p = 0.004$) main effect for time on LG mean amplitude. The post-hoc test showed that LG mean amplitude significantly ($p = 0.001$) decreased by 14.7% from the beginning (0.143 ± 0.009) to the mid-point (0.122 ± 0.006) of the test. Overall, there was a 9.1% decrease from the beginning to the end of the test. There was also a trend ($p = 0.082$) for the LG mean amplitude to be 10.1% higher in normoxic condition than the hypoxic condition. A significant ($p = 0.048$) interaction for condition*time was found for LG mean amplitude. The post-hoc analysis showed that during hypoxia the LG mean amplitude was significantly ($p = 0.016$) lower by 2.1% at the end of the test compared to normoxia (Figure 10B).

There was a trend for an interaction ($p = 0.07$) effect for delta time. The post-hoc analysis showed that during hypoxia the LG delta time tended ($p = 0.06$) to be 23.6% higher at the end of the test compared to normoxia (Figure 10C).

3.6.4 Tibialis Anterior

There were no significant differences for TA activation during all testing.

3.7 Coactivation

VL:BF coactivation was 10.3% ($p = 0.009$) lower in the normoxic condition (0.833 ± 0.05) compared to the hypoxic condition (0.919 ± 0.06) (Figure 11). There were no other effects for muscle coactivation.

3.8 Muscle Inactivation & Evoked Contractile Properties

There were no significant differences found for muscle inactivation using the CAR method. Unfortunately, due to technical difficulties when recording evoked contractile properties in the experimental set-up, the contractile properties: half relaxation time, rate of force development, evoked twitch force and electromechanical delay (EMD) were unable to be analyzed.

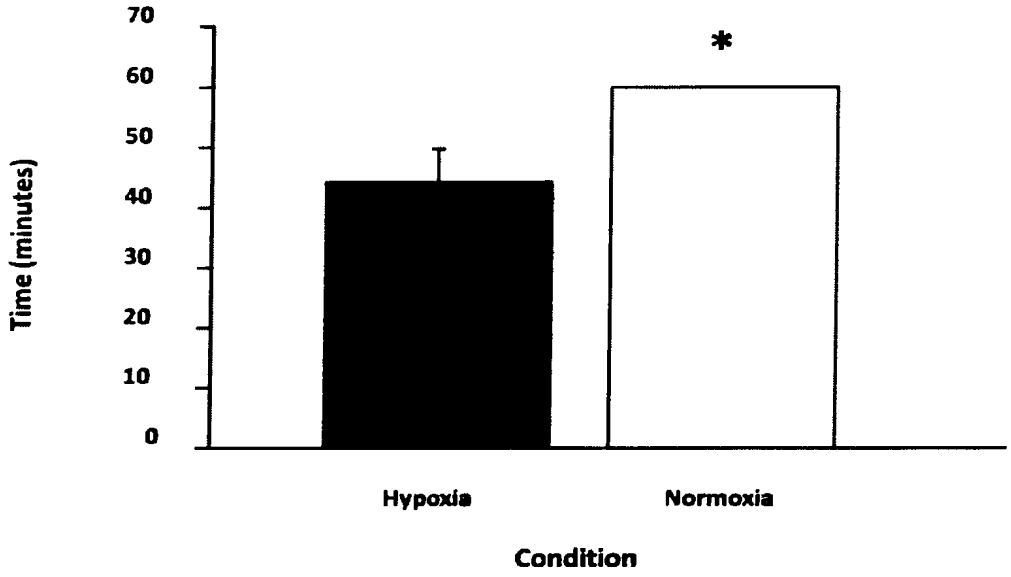


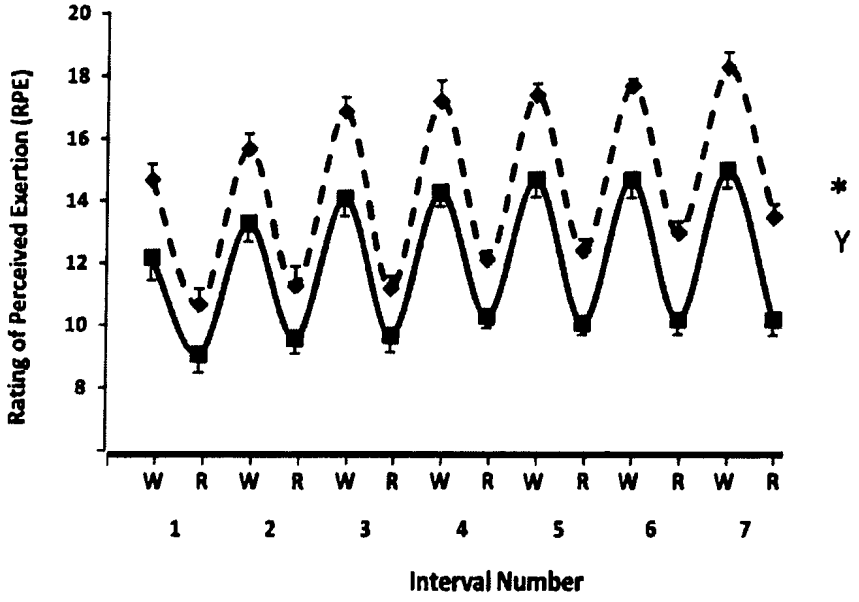
Figure 5: Average Time to Fatigue for hypoxic and normoxic conditions

Note: For all figures:

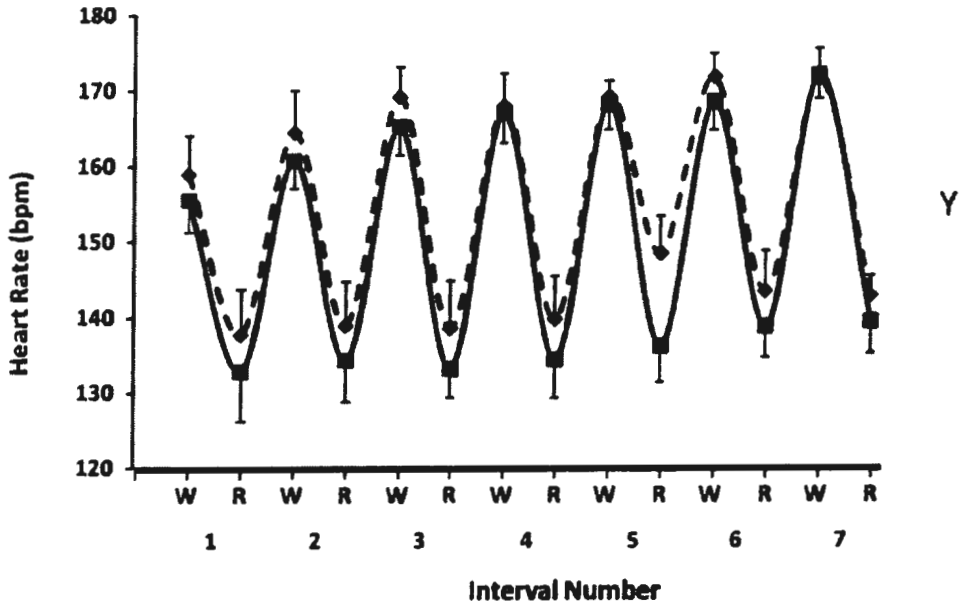
Hypoxia

Normoxia

6 A)



6 B)



6 C)

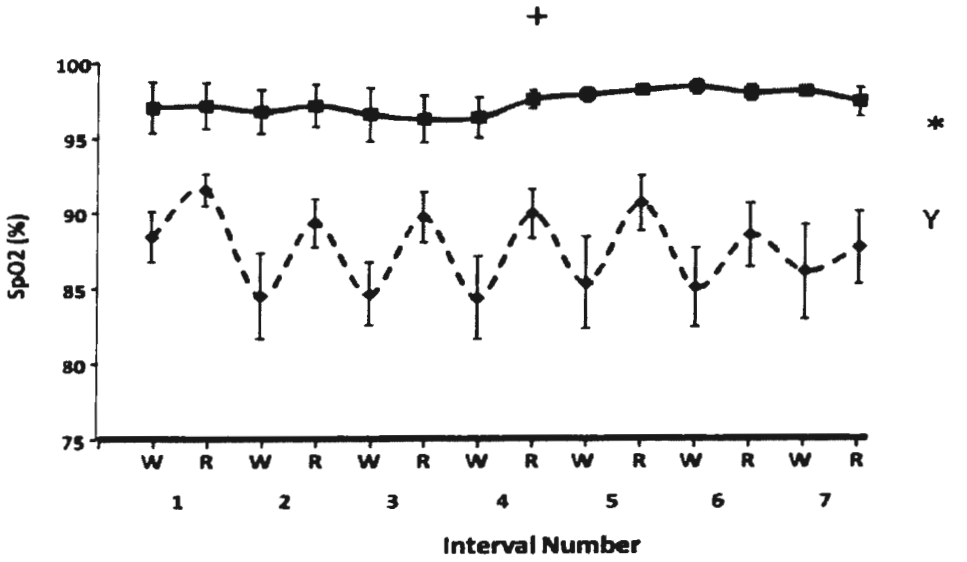


*Figure 6: Average recordings of A) RPE, B) HR and C) SpO2 during the work intervals (W) and active rest (R) intervals during hypoxic and normoxic tests. * represents significant ($p < 0.01$) main effect for condition during W intervals and Y represents significant ($p < 0.01$) main effect during R intervals. + represents a significant ($p < 0.05$) effect for time. All data are reported as mean ± SE.*

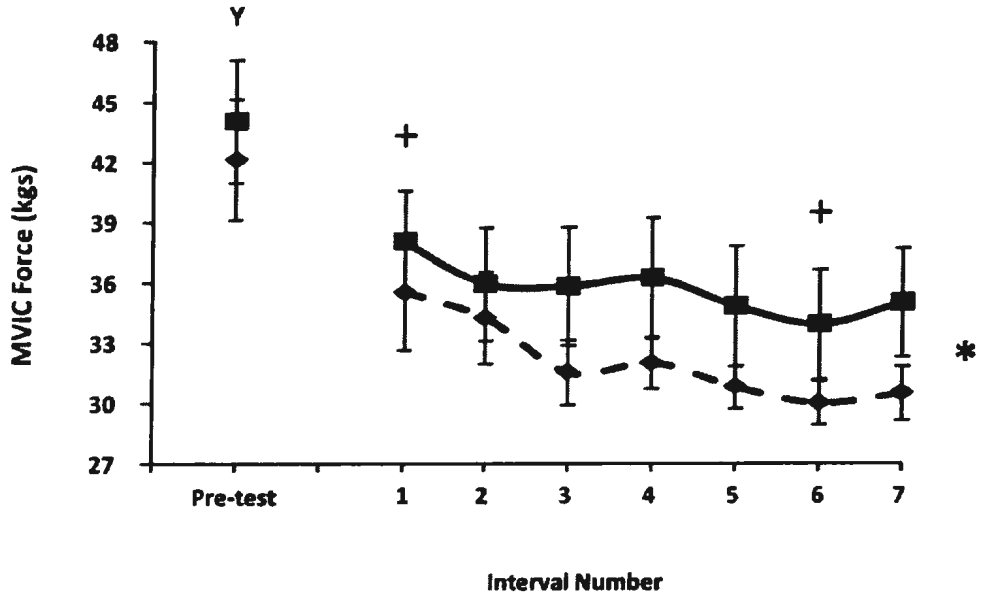
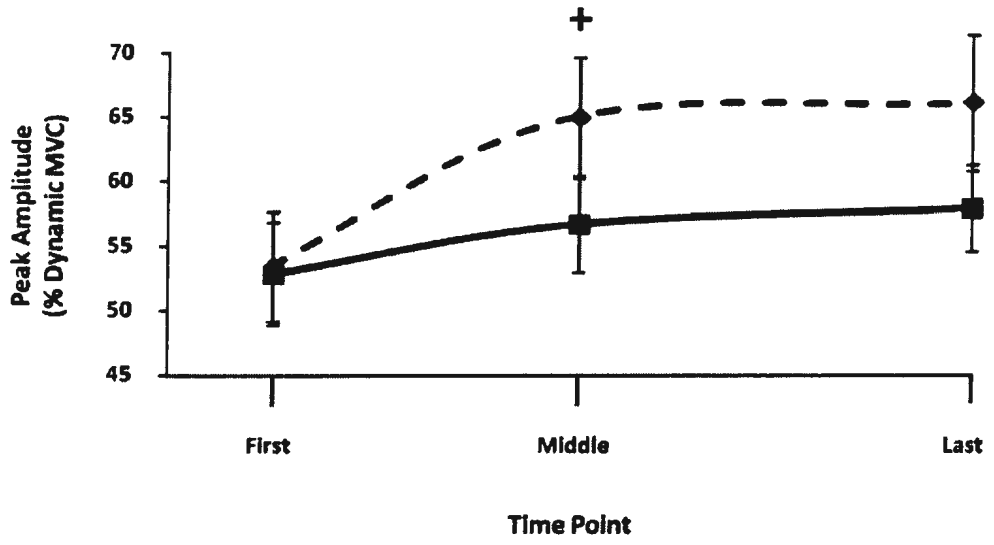
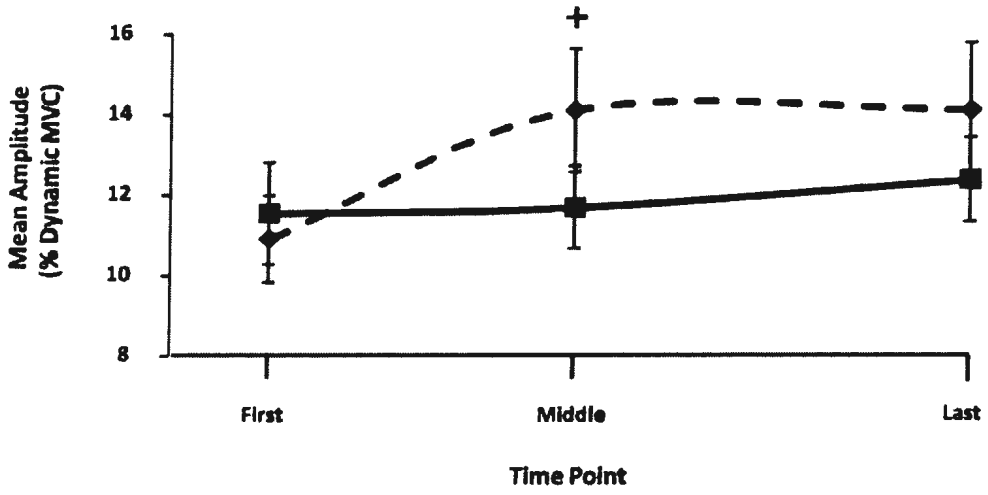


*Figure 7: Average MVC force recorded after each work interval. * represents significant ($p < 0.05$) main effect for condition. Y represents a significant ($p<0.05$) main effect for time (at that point), + represents a significant ($p<0.05$) interaction for condition X time. All data are reported as mean ± SE.*

8 A)



8 B)



8 C)

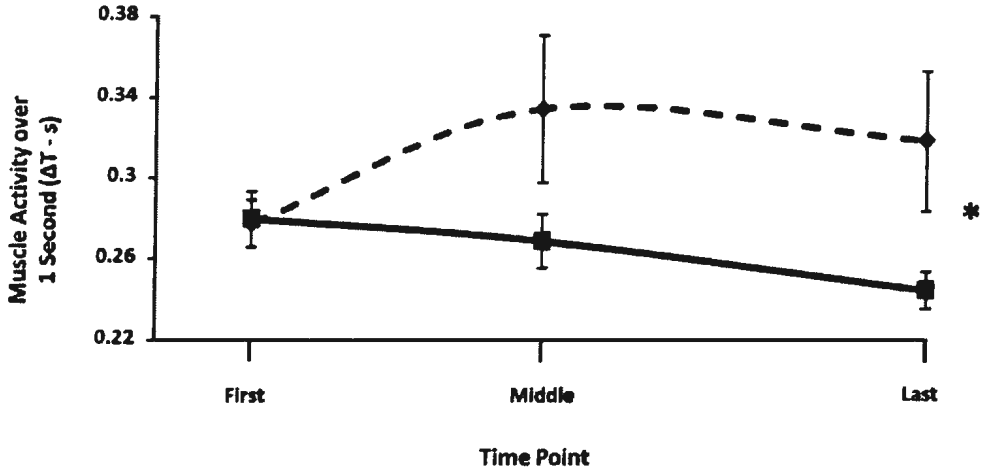
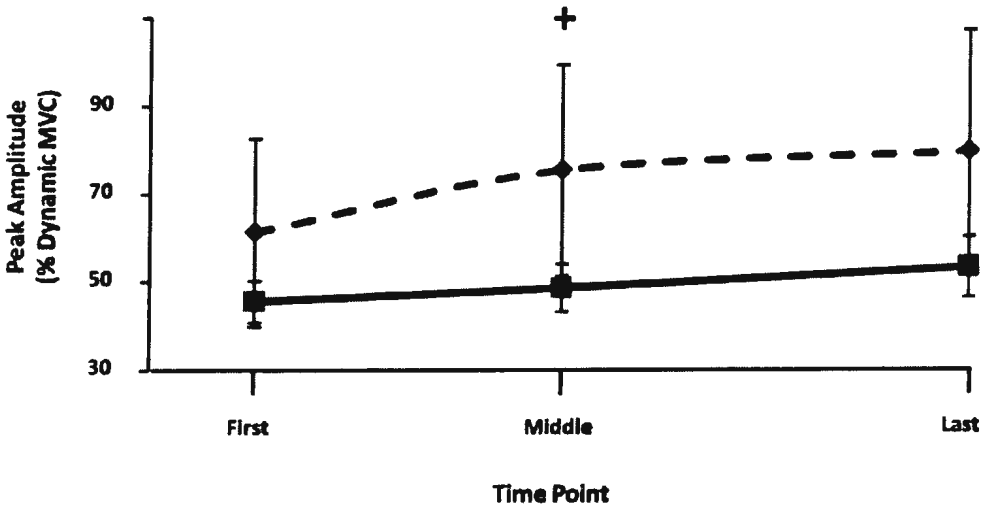
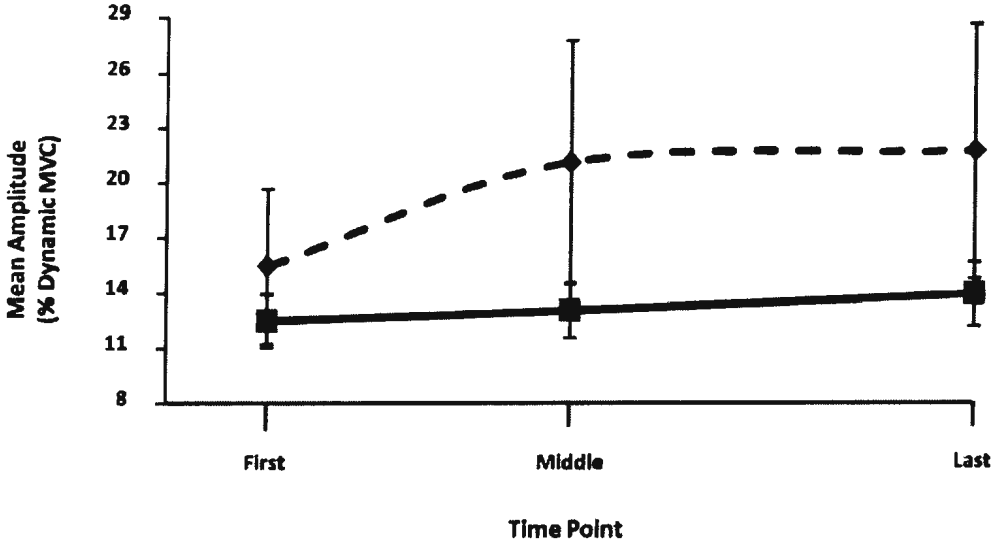


*Figure 8: Vastus Lateralis: A) Peak-to-Peak Amplitude, B) Mean Amplitude, C) Delta Time recorded at the beginning, middle and end of the work intervals during the hypoxic and normoxic test. * represents significant ($p < 0.01$) main effect for condition and + represents significant ($p < 0.05$) interaction for condition X time. All data are reported as mean ± SE.*

9 A)


Peak Amplitude
(% Dynamic MVC)
90
70
50
30
+
First
Middle
Last
Time Point

9 B)


Mean Amplitude
(% Dynamic MVC)
29
26
23
20
17
14
11
8
First
Middle
Last
Time Point

9 C)

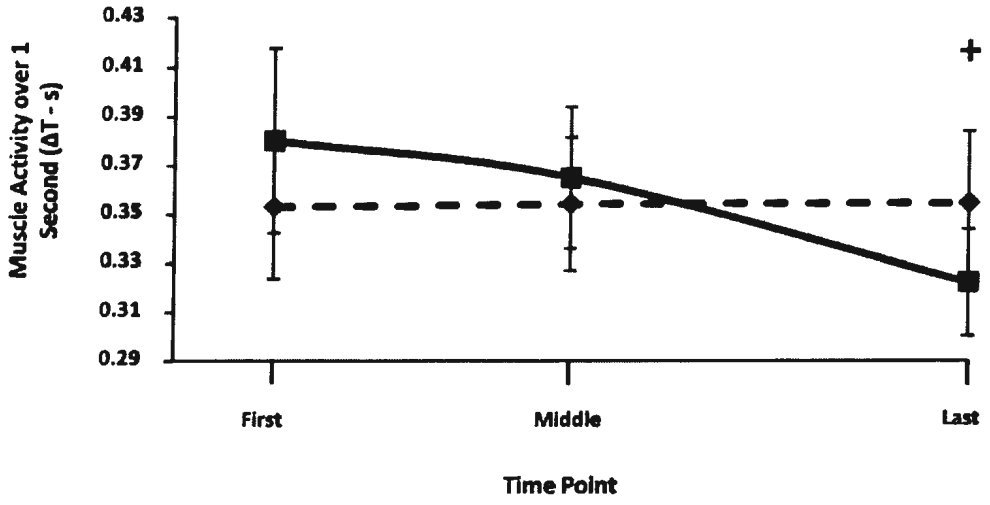
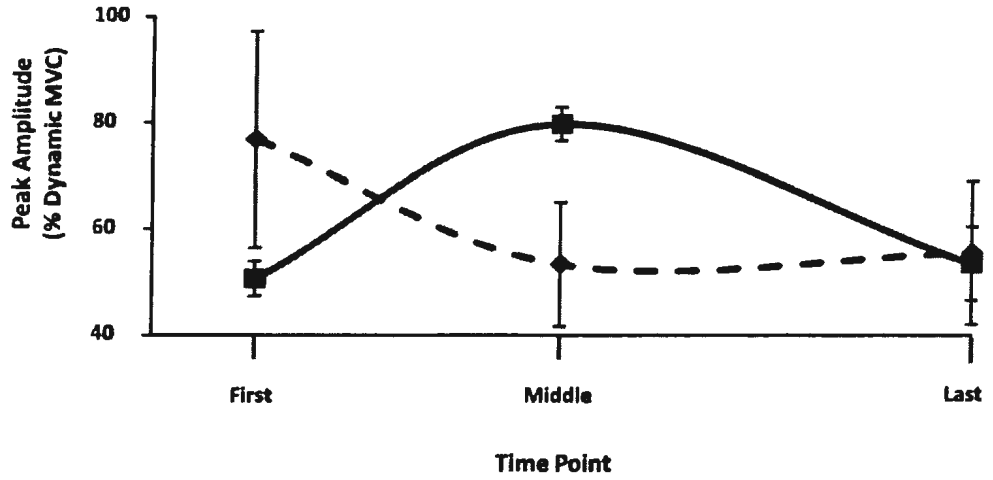
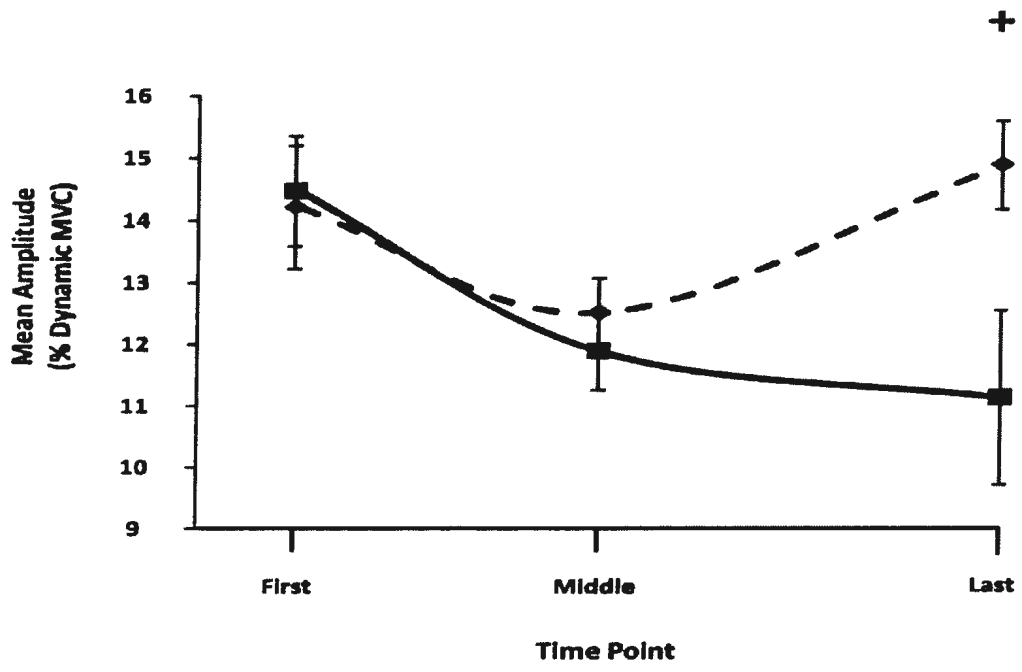


*Figure 9: Biceps Femoris: A) Peak to Peak Amplitude, B) Mean Amplitude, C) Delta Time recorded at the beginning, middle and end of the work intervals during the hypoxic and normoxic test. * represents significant ($p < 0.01$) main effect for condition and + represents significant ($p < 0.05$) interaction for condition X time. All data are reported as mean ± SE.*

10 A)



10 B)



10 C)

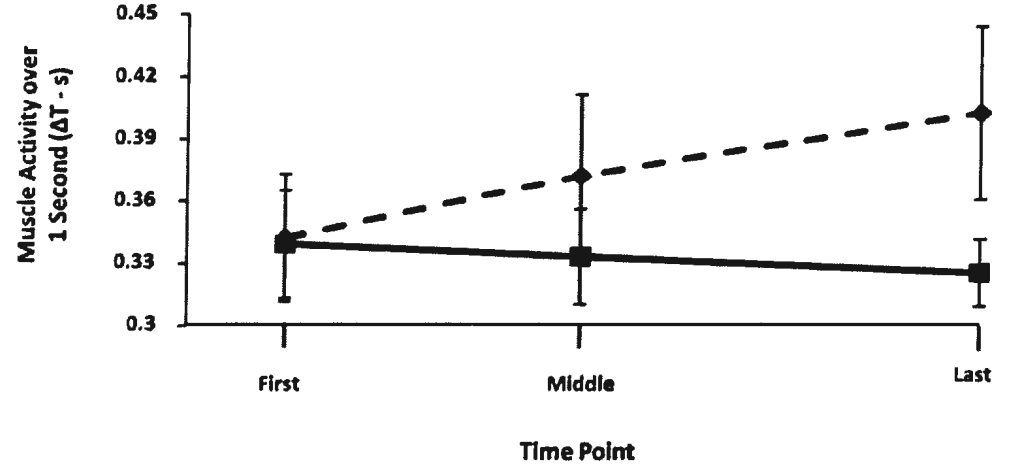


*Figure 10: Lateral Gastrocnemius A) Peak to Peak Amplitude, B) Mean Amplitude, C) Delta Time recorded at the beginning, middle and end of the work intervals during the hypoxic and normoxic test. * represents significant ($p < 0.01$) main effect for condition and + represents significant ($p < 0.05$) interaction for condition X time. All data are reported as mean ± SE.*

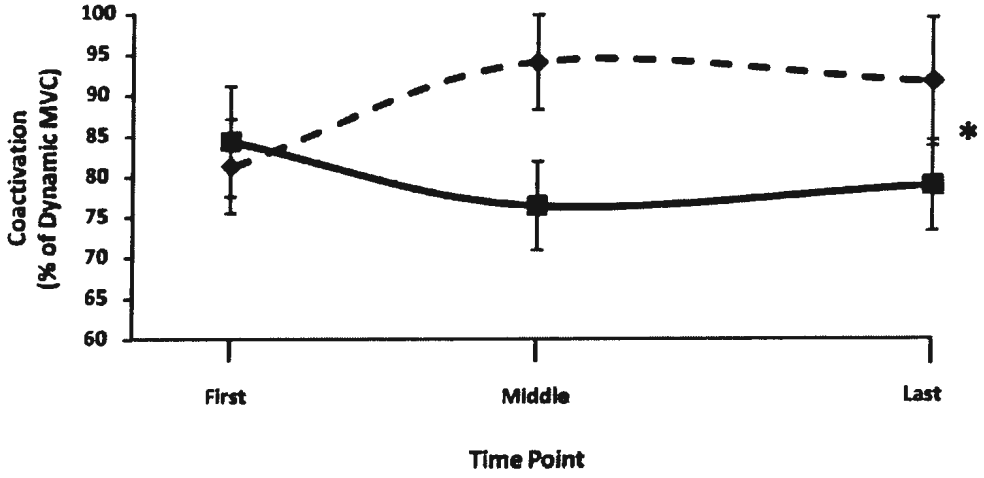


*Figure 11: Coactivation between Vastus Lateralis and Biceps Femoris recorded at the beginning, middle and end of the work intervals during the hypoxic and normoxic tests. * represents significant ($p < 0.01$) main effect for condition. All data are reported as mean ± SE.*

4.0 DISCUSSION

The primary focus of this study was to examine the effects of an intermittent lower limb cycling task in a simulated hypoxic environment has on neuromuscular performance. Compared to normoxia, hypoxia induced greater fatigue in participants at the same relative work intensity. This was evident by participants having decreased TTF and MVIC and increased RPE, SpO_2, and HR (during active rest) during the hypoxic condition. Independent of condition, peak RMS amplitude and mean amplitude was increased over time in the VL and BF muscles, but mean amplitude decreased over time in the LG. There was also a increase in coactivation for VL:BF in the hypoxic condition. The timing of muscle activation was only statistically different between conditions for the delta time of the VL and a trend ($p = 0.082$) for the mean amplitude to be higher in the LG.

4.1 Changes in psychophysiological parameters during intermittent hypoxic cycling

Participants' TTF was reduced by 26% during hypoxia compared to normoxia. This is in agreement with previous studies that have also demonstrated decreases in TTF during cycling in hypoxia (Romer et al., 2007; Goodall et al., 2012). It is noted, however, that the decreases in TTF in the aforementioned studies was either 33% or 56%, respectively. When compared to the 26% reduction in TTF in the current study, the difference may be due to differences in the cycling task. For example, Romer et al. (2007) had participants perform a cycling task (92% of VO_2max in 13% O_2) and found a similar percentage (33%) reduction in TTF. They also used endurance trained participants, who had an average VO_2max of 56.5% $mL \cdot kg^{-1} \cdot min^{-1}$ (similar to the 57.3 $mL \cdot kg^{-1} \cdot min^{-1}$ in our study). Goodall et al. (2012) used a cycling task at 80% PPO in 13% O_2 and found a 54% decrease in TTF. They had endurance trained cyclists (VO_2max: 61.1 $mL \cdot kg^{-1} \cdot min^{-1}$) complete their cycling task. Both of these

studies had participants exercise at a higher work rate, a lesser oxygen concentration than in the present study (i.e. 2% higher O_2 concentration in present study) and the cycling tests were performed to exhaustion using steady state exercise whereas participants in the present study performed interval cycling. Cycling time in hypoxia in those studies were only 4.2 and 3.6 minutes (Romer et al., 2007; Goodall et al., 2012, respectively). However, the relationship between decrease in oxygen concentration levels and TTF during cycling is unknown. In the present study, cycling time during hypoxic work intervals (3 minutes at 70% PPO) ranged from 6 to 24 minutes, with an average duration of 18.3 minutes. These work intervals however were interspersed with active rest intervals that were 4.5 minutes at a reduced PPO (35%). The active rests that participants experienced in this study may have provided them with enough 'recovery' to delay the onset of fatigue, and thus increase their TTF. Thus, TTF differences in hypoxic conditions are not only due to hypoxia itself but also the type of cycling task performed.

Participants' average HR was not different between the hypoxic and normoxic conditions during the work intervals. However, during the active rest intervals participants' average HR was 7% higher in the hypoxic condition. This further supports the idea of a greater recovery in the active rest intervals in normoxia, subsequently prolonging TTF in this condition. A study performed by Goodall et al. (2012) showed that in steady state exercise (80% PPO in 13% hypoxia), HR was significantly higher in normoxia at the end of the test. This may be due to a significantly lower work time in hypoxia compared to normoxia (3.6 minutes vs. 8.1 minutes, respectively). Romer et al. (2007) showed that in a steady state test to exhaustion, (92% VO_2max, 13% O_2), there was no difference in HR between hypoxia and normoxia. Unfortunately, the relationships between HR and PPO during hypoxia remain

unknown, so the question then arises, why there a difference in HR in the active rest intervals and not the work intervals between conditions. Basic physiology suggests that lower O_2 concentrations can activate chemoreceptors which subsequently activate cardioacceleratory centres and inhibit cardioinhibitory centres (Martini, 2004). This dual effect activates the sympathetic nervous system thus increasing HR. In the hypoxic condition the sympathetic nervous system remains stimulated due to low O_2 levels, leading to increased HR during the active rest intervals. The activation of the sympathetic nervous system is reduced in normoxia so the standard relationship of HR and exercise intensity will apply. This theory is further supported by the SpO_2 results found in this study. During normoxia SpO_2 values were consistent whether they were in a work or an active rest interval. In hypoxia, SpO_2 values fluctuated between work and active rest intervals, but were always significantly lower than the values in normoxia.

Although the HR indicated that the participants were working at equal intensities during the work intervals in both conditions, the RPE indicated that their perception of how hard they worked during the hypoxic condition was higher. Unlike HR, participants' RPE values were higher during hypoxia in both active rest and work intervals. Thus, participants reported that they felt more fatigued throughout the whole cycling task during hypoxia compared to normoxia. In fact, the cycling task during hypoxia was so demanding that only seven out of ten participants made it to the seventh interval in hypoxia (whereas they completed all 8 work intervals in normoxia), and the average recorded RPE was 18.5 following the last work interval completed in hypoxia compared to 15.4 in normoxia. Romer et al. (2007) reported no significant change between hypoxic and normoxic RPE values (9.2 vs. 9.6, respectively as recorded on Borg's modified CR10 scale). A direct comparison may

not be appropriate in this case due to the large difference in cycling time between their study and the current one. However, a study with similar cycling time (20 minutes), albeit upper body cycling, reported significantly higher RPE values in the hypoxic condition (Rasmussen et al., 2010).

Increased HR and RPE and decreased SpO_2 while cycling in hypoxia contributed to the decrease in TTF. Other indicators that participants fatigue was enhanced during hypoxia compared to normoxia were further illustrated via changes in various measures of motor output, muscle activation and coactivation.

4.2 Changes in Motor Output

MVIC force decreased by 27.6% in the hypoxic condition and 20.5% in the normoxic condition. This represents a 7.1% difference between conditions. All forces recorded following work intervals were significantly less than the pre-test values in both conditions. Similar results were found by Goodall et al. (2012), who showed a 25% decrease in force in the hypoxic condition and a 17% decrease in force in the normoxic condition when cycling at 80% PPO in 13% O_2 to exhaustion. Another cycling study by Romer et al. (2007) that studied cycling while at 90% of VO_2max in 13% O_2 showed a 24% decrease in MVC force in hypoxia.

The greater decrease in force output in the hypoxic condition could be due to central (O_2 availability) and (or) peripheral factors (metabolic disturbances). Goodall et al. (2012) determined that both peripheral and central fatigue were evident in their study as shown by a decline in: MVC force, force evoked from femoral nerve stimulation, and voluntary activation determined by motor cortex stimulation in the normoxic condition with even

greater reductions in the same parameters in the hypoxic condition. Although there were similar levels of peripheral fatigue between the two conditions, there was a greater reduction in cortical voluntary activation in the hypoxic condition, to which they attributed the drop in voluntary force (Goodall et al., 2012). However, there were no measures of spinal activation measured, so the reduction may only be attributed to the CNS, not specifically supraspinal mechanisms. Although it is evident that the CNS was a mechanism here, the actions of the peripheral nervous system (PNS) must not be ruled out, the decrease in MVIC shown in this study could be a combination of both central and peripheral mechanisms. For instance, the firing of group III and IV muscle afferents is increased in hypoxia (Hill et al., 1992; Arbogast et al., 2000; Amann, 2011). These afferents return information to the brain (CNS) which may inhibit the central motor drive to the muscle (Gandevia, 1998; Martin et al., 2006). Reduced central motor drive may be caused by decreased alpha motor neuron activation (Bigland-Ritchie et al 1986; Duchateau & Hainaut, 1993; Martin et al, 2006; Amann, 2011).

The increases over time in peak and mean amplitude were expected for the BF. Previous literature has stated that the BF often will compensate for reduction in force of the VL (Faria et al., 2005). All of the studies examining muscle activation that were reviewed prior to this thesis showed an increase - although some not significant (Billaut et al., 2005) in BF activity during cycling (Hautier et al., 2000; Dorel et al., 2005; Theurel et al., 2011; Bini et al., 2011). Although there was no decrease in VL amplitude in this study, there was an increase in the BF amplitude, perhaps demonstrating that the BF was preparing for the eventual loss of force in the VL, or perhaps due to the fact that the fatigue experienced was submaximal, indicating that muscle activation would increase regardless.

In the present study there was an increase in peak and mean amplitude over time for the VL. As the VL is the main power producer for cycling (Raasch & Zajac. 1999; So et al., 2005; Faria, Parker & Faria, 2005), and has a large number of fast twitch muscle fibres (Johnson et al., 1972), it is expected that it will fatigue faster than other muscles. In this study, muscle activity amplitude of the VL steadily increased; an indication of submaximal fatigue. There have been a variety of studies examining VL activity during cycling and all have showed a variety of results. It seems that there were no significant results in sprinting studies (Hautier et al., 2000, Billaut et al., 2005) or interval studies (Villerius et al., 2008; Skof & Strojnik, 2005), while most steady state cycling studies have shown a decrease in RMS EMG of the VL (Dorel, Drouet, Couturier, Champoux & Hug, 2005; Theurel, Crepin, Foissac & Temprado, 2011). When hypoxia is introduced into the equation, thigh muscle activation decreases (Rasmussen et al., 2010; Romer et al., 2007). All of the aforementioned studies had a workload above 80% of the maximum Vo_2max or PPO. It is known that EMG amplitude decreases during maximal exercise, explaining why VL EMG was less at the end of the previous studies. In the current study where participants worked at a submaximal intensity (70% PPO), EMG continued to increase, a classical phenomenon that occurs during submaximal fatigue (Behm et al., 2004). Submaximal fatigue may be induced by peripheral factors (ie. changes in the excitation-contraction coupling, or the neuromuscular junction) in the quadriceps muscles (Bigland-Ritchie, Furbush & Woods, 1986) which may be measured by changes in the stimulated femoral nerve force.

Goodall et al. (2012) found that in both hypoxia and normoxia, VL EMG increased, but was not statistically different between conditions. The similar increases in EMG suggest that near the end of the exercise, motor units were still being recruited. The fast twitch motor

units being recruited is known to be associated with the accumulation of metabolic by-products (i.e. lactate, H+ ions). In addition the firing of group III and IV muscle afferents is increased in hypoxia (Hill et al., 1992; Arbogast et al., 2000). These afferents return information to the brain (CNS) which may inhibit the central motor drive to the muscle (Gandevia, 1998; Martin et al., 2006). Reduced central motor drive may be caused by decreased alpha motor neuron activation (Bigland-Ritchie et al 1986; Duchateau & Hainaut, 1993; Martin et al, 2006; Amann, 2012).

In addition to the above results, a decrease in LG peak activation and no change in TA activity were also shown. As these muscles are responsible for stabilization and energy transfer throughout the cycle, not producing the power to execute the motion, they may not have tired as quickly (Ryan & Gregor, 1992; Raasch & Zajac, 1999). It seems that there are usually very small (not statistically different) amplitude changes in the TA and that the timing of muscle activation is more likely to change (Dorel et al., 2009; Chapman et al., 2005). In addition the TA is composed of a higher percentage of slow twitch fibres than fast twitch (Johnson et al., 1973) meaning that it may be more resistant to fatigue.

The change in delta time for the VL between conditions could be a result of the fatiguing muscle. The VL was active longer in hypoxia. This change in VL delta time, probably had an influence on the change seen in the VL:BF relationship, which showed that there was more coactivation in hypoxia. This is similar to literature (Goodall et al., 2012) which stated that in hypoxia there is greater coactivation of the thigh musculature due to a reduction in central motor drive leading to a decrease in reciprocal inhibition. In contrast, Neptune et al. (1997) examined the timing of muscle activation during cycling in normoxia, and showed a change in the timing of the VL as well. This was credited to an increase in

pedalling rate; which has a tendency to shift the EMG activity to an earlier time in the cycle (Marsh & Martin, 1995). This theory was further supported by Pyndt, Laursen & Nielsen (2003) who showed that in normoxia, there were increases in coactivation between the TA and soleus. Their data revealed a decrease in the inhibition of the soleus, which they also attributed to an increase in pedalling rate as well as an increase in workload. Through observation of the participants in the present study, pedalling rate was higher in the normoxic condition, contrary to what the results state. This may indicate that perhaps the hypoxic effect had a greater influence on central motor drive (and therefore reciprocal inhibition) than fatigue alone as Goodall and colleagues stated.

4.3 Conclusion

The results of the present study show that when participants performed an intermittent cycling task at a submaximal intensity, they were fatigued during normoxia which was further increased during hypoxia. This was illustrated by increases in HR and RPE, and a decrease in SPO_2 levels, and subsequently a decreased TTF. Other measures that demonstrated fatigue were a decrease in MVIC throughout the test in both conditions and a continual increase in RMS EMG activity over time. All of the changes and the power output at which participants were cycling would indicate that fatigue was submaximal. Our data suggests that the submaximal fatigue shown in normoxia which was further increased in hypoxia was centrally mediated. However, other research has demonstrated that peripheral contributions to fatigue cannot be ruled out as mechanisms for the fatigue induced changes in the present study. Due to technical difficulties and availability of equipment during data collection, common measures of peripheral fatigue could not be monitored.

4.4 Limitations

Both types of fatigue (central and peripheral) are represented in this study, meaning that there might be a combination of the two; however, one may have been the predominant cause for the cessation of exercise. Unfortunately, we cannot conclude as to which type of fatigue, central or peripheral, contributed more to the changes in TTF, motor output and muscle activation and coactivation while cycling during hypoxia and normoxia.

Two additional parameters that would have been insightful in the current study are lactate accumulation, and muscle and (or) nerve stimulation. Lactate was not measured due to the unreliability of the available equipment at the time of data collection. Femoral nerve stimulation and inactivation of the knee extensors was measured throughout the cycling protocol via the CAR method, however, due to technical difficulties with the experimental setup unforeseen at the time of data collection, this data was unable to be used. With the addition of these two parameters, inferences could have been made as to the presence of peripheral fatigue throughout the study. This would have further helped uncover an objective of the study, whether peripheral fatigue developed before, during or after the onset of central fatigue.

5.0 CONCLUSION

Fatiguing cycling has been widely studied in the literature with varied protocols and participant groups. Numerous parameters have been examined; such as HR, RPE, muscle activation and muscle inactivation. Recently, many factors such as hypoxia and interval training have been introduced into these protocols in order to further understand their impact on fatigue. Although all of the above have been studied in relation to cycling, it seems that to the present day there have not been any conclusive results to what type of fatigue is present during many cycling tasks and how and when it develops.

The objectives of the current study were to examine motor output during a submaximal interval cycling test during hypoxia and compare the results to a normoxic condition. In addition this study aimed to discover if both central and peripheral factors of fatigue were present as well as when they developed in relation to each other.

Results from the study did not support the first hypothesis, in that peak amplitude of the VL and the BF increased while the peak amplitude of the LG decreased. This was most likely due to the fact that the protocol was not maximally fatiguing. The second hypothesis was somewhat supported in that there were changes in the delta time for the VL in hypoxia, but none of the other muscles. This was most likely due to the fact that the VL has a large amount of fast twitch fibres (which fatigue faster) and is a power producer muscle during cycling. The cycling cadence did decrease in hypoxia noticeably for 50% of participants. This was most likely due to the effect of fatigue, and the experience of the subjects. The third hypothesis was also somewhat supported, in that there was a greater RPE recorded during the hypoxic condition as well as a decreased TTF, indicating central fatigue, however it was not evident whether this was the limiting type of fatigue in this exercise.

Results from the present study showed that subjects were fatigued in both conditions, but more so in the hypoxic condition. Results from the measured variables showed that the fatigue was submaximal and centrally mediated; however, due to limitations of the current research as well as previous research peripheral fatigue may also be a contributor to the fatigue experienced. This study supported much of the research already performed in this area. It added insight into the area of intermittent hypoxic cycling and muscle activation during submaximal hypoxic cycling. It seems that results from many recent studies including the current one, are suggesting that central and peripheral fatigue are present in exercise to volitional fatigue, however, it still remains unclear which is the limiting factor.

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APPENDIX A: Training Profile Questionnaire

1. Age

2. Specialization:

3. PB (specify distance/time):

4. Number of years of training (in a structured training program)

5. Numbers of training sessions per week (including morning and garbage – very easy run - runs but excluding weight training)

6. Numbers of training sessions at or greater than 70% maximal aerobic speed

7. How many interval-training sessions per week (excluding tempo run; so, just session with intensity interspaced with short rest period)?

8. Average running/cycling distance per week (how many kilometers in average do you run per week?)

9. Longest running/cycling distance in a week (how many kilometers have you run in your highest running/cycling week?)

10. Longest single distance run/cycle in one week (how many kilometers have you run/cycle in the highest single long run/cycle session?)

11. How many weight-training sessions per week?

12. How many cross-training sessions per week? (Cycling, swimming, elliptical, others activities)

13. In which period of your annual training plan are you? (general preparatory phase, specific preparatory phase, competition phase, taper or transition phase).

14. At which level are you competing, provincial, national, international?

APPENDIX B: Lake Louise Scale for the Diagnosis of Acute Mountain Sickness (AMS)

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

Total Score of:

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