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Differences in achievement of V0 2 max during continuous and intermittent graded exercise test in collegiate soccer players

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DIFFERENCES IN ACHIEVEMENT OF VO_{2max} DURING CONTINUOUS AND INTERMITTENT GRADED EXERCISE TEST IN COLLEGIATE SOCCER PLAYERS

BY

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To the Dean of the School of Human Performance and Leisure Sciences:

I am submitting here with a thesis written by Ryan Alexander entitled "Differences in achievement of VO_{2max} during continuous and intermittent graded exercise test in collegiate soccer players". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Movement Science with a specialization in Exercise Science.

Constance

Constance Mier, Thesis Committee Chair

We, members of the thesis committee,
have examined this thesis
and recommend its acceptance.

Accepted:

Chair of Department of Sport and Exercise Sciences

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Dean of School of Human Performance and Leisure Sciences

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Barry University
Abstract

Differences in Achievement of VO_2max during Continuous and Intermittent Graded Exercise Test in Collegiate Soccer Players

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The purpose of the present study was to determine intermittent graded exercise results in a higher stage intensity and higher maximal heart rate, minute ventilation, respiratory exchange ratio, and oxygen uptake compared to continuous graded exercise. The participants (n=11) were collegiate soccer players from the local university. They completed two separate protocols on different days separated by at least 48 hours. The continuous protocol was a graded treadmill test (GXT) consisting of running while grade increased 2.5% every 2 minutes. The intermittent protocol followed the same intensity progression, but with a 1-min active recovery between each stage. During both protocols there was a breath-by-breath analysis of oxygen uptake, minute ventilation, respiratory exchange ratio, and heart rate. VO_2max during the intermittent protocol was higher ($p < 0.05$) compared to the continuous protocol (57.7 ± 5.8 $ml \cdot kg^{-1} \cdot min^{-1}$ vs. 55.7 ± 5.7 $ml \cdot kg^{-1} \cdot min^{-1}$). There was also a significant increase ($p < 0.05$) in the maximum heart rate achieve during the intermittent protocol compared to the continuous protocol (190 ± 6 bpm vs. 186 ± 6 bpm). Minute ventilation and respiratory exchange ratio did not differ between the intermittent and continuous protocols. All 11 participants satisfied the plateau criteria (increase < 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ with stage increase, Taylor et al., 1955). We concluded that the classic continuous graded exercise test does not effectively measure

true maximum oxygen uptake in collegiate soccer players. The intermittent nature of soccer player's training produces adaptations such as increased sodium-potassium pump subunits, increased efficiency of lactate clearance, and re-synthesis of PCr and other energy substrates that contribute to increased performance during the intermittent protocol, which allows for the achievement of the player's maximum oxygen uptake.

CHAPTER I

INTRODUCTION

Soccer is characterized as a high-intensity sport that combines intermittent and random bouts of anaerobic and aerobic activities such as jogging, shuffling, short sprints, rapid acceleration and deceleration, turning, jumping, kicking, and tackling (Al Hazza et al., 2006; Wisloff, Helgerud, & Hoff 1998; Ekblom, 1996; Kirkendall, 1985; Bloomfield, 2006). Fitness coaches of elite soccer clubs profile individual players to determine the best training needed for success in competition. By the nature of the sport, profiling soccer players can include a myriad of performance factors from technical/biomechanical, tactical, and physiological (Stolen, Chamari, Castagna, & Wisloff, 2005).

Fitness testing is important for a number of reasons, including assessment of an athlete's current level of fitness, evaluation of a training program's effectiveness, and the development of optimal training sessions and programs to address an individual athlete's strengths and weaknesses (Sayers, Sayers, & Brinkley, 2008). The majority of studies concerning soccer fitness testing have concentrated on the relationship between match-play success of individuals or teams, and specific performance variables that can either be tested in a lab or field setting (Arnason et al, 2004). Maximal oxygen uptake (VO_2max) quantifies the aerobic capacity of an individual and is an important performance indicator in soccer (Da Silva, Bloomfield, & Marins, 2008). It is often tested to evaluate the success of an individual player or a team. For instance, higher pre-season VO_2max values among an Icelandic soccer league were associated with finishing the regular season higher in the standing (Arnason et al. 2004).

There are a variety of different protocols used to measure athletes' VO_2max (Arnason et al., 2004; Bangsbo, Mohr, & Krustrup, 2006; Grant et al., 1995; Krustrup & Bangsbo, 2001). The most common method of measuring VO_2max is the graded treadmill exercise test (GXT). GXT protocols can vary in magnitude of increments and stage durations depending upon the individual or population being tested and the purpose of the test. Most GXT protocols begin with a designated warm-up period of three to five minutes. Depending on the purpose of the test, the first stage of the GXT is usually the same as or slightly higher than the warm-up intensity which estimates the intensity for the participant at approximately 60-70% VO_2max. Duration of each stage is typically one to three minutes. Each successfully completed stage is followed by an increase in speed or grade until the participant self-terminates the test due to volitional exhaustion.

The testing protocol is a main determinant of the validity of the aerobic capacity test. The increase in intensity can be a progression of speed and/or grade. Other aspects of the graded exercise test that may affect the success of reaching VO_2max are magnitude of intensity progression, duration of the stages, whether or not the protocol is continuous or discontinuous, and overall length of the test. There has been the implementation of a verification phase at the end of the continuous or discontinuous protocols to determine if VO_2max was attained during the protocol (Midgley and Carroll, 2009). The verification phase involves a single square wave bout of exercise performed shortly after the incremental phase. The intensity is 'supramaximal' or greater than the last successfully completed intensity in the protocol (Thoden et al., 1982).

The test administrator decides the magnitude of the increase. At the completion of each stage in a graded exercise test there is an increase in elevation, speed, or both.

With too great of an increase in the magnitude the participant may reach volitional exhaustion too early in a test or before reaching VO_2max. Although this protocol would be considered a maximal test, the concern of the researcher would be on whether or not the physiological restrictions of the protocol were based on limitations of the oxygen delivery and utilization by the muscle, neuromuscular factors that may have restricted the anatomical ranges of motion due to the increase in speed or grade, or other factors that may have caused muscle fatigue (Midgley and Carroll, 2009).

The duration of the stages will affect the total duration of the test. One-minute stages are commonly utilized in aerobic capacity protocols because of the researcher's desire to get the participant to VO_2max as quickly as possible. Three-minute stages are the longest stage durations commonly seen in literature for VO_2max testing and are used when steady state measures are desired. However, longer stages result in longer test duration, which can result in volitional exhaustion before the limits of the oxygen delivery capabilities of the cardiorespiratory system are reached. Two-minute stages are also utilized in some studies, and researchers seem to have the same concern for the two-minute protocols as with the three-minute protocols.

The majority of testing protocols found in recent literature can be classified as discontinuous or continuous. The continuous protocol is more commonly used because of its shorter duration and physiological relation to obtaining an individual's VO_2max. A continuous protocol is a single bout of incremental increases in intensity and is not interrupted or delayed by a decrease in the intensity or rest period. A continuous protocol allows maximum effort to be achieved in a relatively short period of time, thus reducing the possibility that the termination of the test will be from muscle fatigue. On the other hand, discontinuous protocols have traditionally included 3 to 5 minutes stages separated by one to three days (Taylor et al., 1955; Duncan et al., 1997). More recently, an intermittent protocol that incorporated 30-second rest periods was used to assess the effects of test duration on VO_2max in healthy men (Midgley, McNaughton, & Carroll, 2006). Others have implemented five to ten second rests in between working stages for field tests (Krustup et al., 2003).

A common VO_2 max criterion is the attainment of a VO_2max plateau, or a lack of increase in VO_2 with an increase in intensity (Midgely and Carroll, 2009; Howley, 1995). The continuous protocol has traditionally been the protocol used to measure VO_2max because of its reliability and reported high incidence of plateau achievement. However, low frequency of plateau achievement has been reported among athletes (Niemela et al., 1980; Lucia et al., 2006; Doherty et al., 2003; St. Clair Gibson et al., 1999). This is an interesting phenomenon among highly motivated individuals suggesting that true VO_2max may not have been achieved among those not reaching a plateau. If this is the case then it appears that these athletes' ability to reach true VO_2max was limited physiologically in a non oxygen-dependent manner during a continuous protocol.

The continuous protocol increases the intensity to volitional exhaustion in a linear manner that does not replicate the intermittent nature of a competitive soccer match. By utilizing a continuous VO_2max protocol, the researcher is eliminating the importance of an athlete's ability to recover from short, high-intensity exercise. It is possible that a continuous protocol, because of the absence of recovery periods; limits the soccer athlete's ability to achieve a high enough intensity that elicits a VO_2max.

During physical activity, three energy systems, the phosphagen (ATP-PCr) system, glycolytic, and the oxidative system contribute to the energy demands of the contracting muscles (Poortmans, 1984). The coordination of these systems provides the necessary energy for an athlete to complete the tasks demanded during competition. At the beginning of a continuous GXT protocol, there is an initial decline of the phosphocreatine and ATP stores in the muscle. As the test progresses the increase in intensity prohibits any significant re-synthesis of ATP and phosphocreatine in the muscle. This places further demand on the glycogenolytic and glycolytic energy pathways. In contrast, a discontinuous GXT protocol that includes short rest periods allows re-synthesis of ATP and PCr stores in the muscle during recovery with the amount dependent on the duration of the recovery period. Furthermore, recovery periods will allow some buffering and the removal of hydrogen ions from the muscle. Thus, with the addition of recovery periods during the discontinuous GXT there is the potential for delayed muscular fatigue that could otherwise limit the athlete's ability to reach VO_2max during a continuous GXT protocol.

An athlete must have the conditioning necessary to alternate between the high-intensity and recovery bouts that make up the game of soccer for the duration of the match. Although soccer players spend the majority of their time below 70% of the maximum aerobic capacity it has been documented that an average elite soccer player performs 150 to 250 brief intense actions during a game (Bangsbo, Mohr, and Krustrup, 2006). Thus the ability of a soccer player to sustain intensities at or above VO_2max is likely the result of training adaptations associated with intermittent high intensity bouts of exercise. Therefore, it seems much more logical to test soccer athletes' VO_2max in a more applicable manner, where the ability to repeatedly perform increasingly intense exercise bouts and re-synthesize energy substrate stores during brief recovery periods is applied (Krustrup & Bagnsbo, 2001).

The purpose of the present study was to determine intermittent graded exercise results in a higher stage intensity and higher maximal heart rate, minute ventilation, respiratory exchange ratio, and oxygen uptake compared to continuous graded exercise.

Significance of the Study

The gold standard of testing an individual's VO_2 max has been for many years, the graded exercise test on a treadmill in a laboratory (that is excluding cyclists, rowers, and swimmers). The classic continuous graded exercise test is short in duration (i.e. 8 to 12 minutes) and can assure the researcher with a high degree of confidence that the participant was not restricted due to muscle fatigue and that a VO_2max plateau can be achieved. However, the fact that a low prevalence of a plateau during continuous GXTs among athletes has been reported, it is possible that the continuous protocol due to the high levels of work rates achieved by the athletes, is limited by anaerobic capacities rather than the cardiorespiratory capacity. The intermittent nature of a soccer match does not emphasize the same energy demands as a continuous protocol. Therefore, a intermittent GXT protocol maybe a more effective means of measuring true VO_2max in soccer players.

Limitations

1. Recruitment for the study was confined to one geographical area.
2. Some participant's may not have been able to complete all testing protocols due to injury sustained from competition.

3. Testing took place during the pre-season of the semi-professional schedule; therefore, not all athletes involved may have attained their maximum fitness level during the testing period.

Delimitations

1. All measurements were performed in the HPL.
2. All participants were Division II collegiate athletes.
3. All participants were soccer players.

Assumptions

The following assumptions were inherent in the design of the study:

1. All participants provided accurate information about their past medical and health information, as well as, their current health status.
2. All participants put forth a maximal effort during the testing protocols.
3. All participants followed pre test instructions regarding preparation for examination in the laboratory.

Hypotheses

The following hypotheses have been created as a way to evaluate the effectiveness of this study:

Hypothesis 1a: A higher intensity will be achieved during the intermittent protocol compared to the continuous protocol with respect to the grade of the treadmill at the final completed stage.

Hypothesis 1b: A higher maximum heart rate will be achieved during the intermittent protocol compared to the continuous protocol

Hypothesis 1c: A higher respiratory exchange ratio will be achieved during the intermittent protocol compared to the continuous protocol.

Hypothesis 1d: A higher maximum minute ventilation will be achieved during the intermittent protocol compared to the continuous protocol.

Rationale: The ability of a soccer player to sustain intensities at or above VO_2max is likely the result of training adaptations associated with intermittent high intensity bouts of exercise. Therefore, an intermittent protocol that more adequately simulates a soccer player's training method should provide the athlete the means to achieve a higher work intensity and thus higher heart rate, RER and ventilation.

Hypothesis 2: The intermittent protocol will elicit larger absolute and relative VO_2 max values compared to the continuous protocol.

Rationale: The continuous protocol increases the intensity to volitional exhaustion in a linear manner that does not replicate the intermittent nature of a competitive soccer match. By utilizing a continuous VO_2max protocol, the researcher is eliminating the importance of an athlete's ability to recover from short, high-intensity exercise. It is possible that a continuous protocol, because of the absence of recovery periods; limits the soccer athlete's ability to achieve a high enough intensity that elicits a VO_2max.

Definition of Terms

Aerobic capacity: The maximum rate at which an athlete can produce ATP through oxidation of energy resources and is usually expressed as a volume of oxygen consumed per kilogram of body weight per minute (Baumgartner, 1987).

Maximal Oxygen Uptake (VO_2 max): maximum rate of oxygen utilization during aerobic exercise (Howley, 1995).

VO_2 max plateau: "In running the oxygen requirement increases continuously as the speed increases attaining enormous values at the highest speeds: the actual oxygen intake, however, reaches a maximum beyond which no effort can drive it… The oxygen intake may attain its maximum and remain constant merely because it cannot go any higher owing to the limitations of the circulatory and respiratory system." (Hill and Lupton, 1923)

Discontinuous Protocol: Protocols designed to measure maximum oxygen uptake where the stages are increased incrementally, but are typically separated by 6 to 24 hours and last at least 3 minutes for steady state measurements. Tests usually lasted up to one week in time.

Continuous Protocol: Protocol which varies intensity by increasing the speed or grade incrementally with no decrease in intensity until the subject terminates the tests due to volitional exhaustion.

Intermittent Protocol: Protocol which increases intensity from work stage to stage either by increasing speed or grade and implements short (i.e. 1 minutes) active or passive recovery stage in between work stages.

CHAPTER II

LITERATURE REVIEW

The purpose of this chapter is to examine past and recent literature that pertains to the current study. The literature is presented in the following categories: physiology of soccer, importance of VO_2max in soccer performance, physiology of recovery, and testing VO_2 max in soccer players.

Physiology of Soccer

In a competitive soccer match there are a variety of different actions carried out by each player that are dependent upon their position, tactics, playing style, playing surface, and even geographical location of the game (Bloomfield, Polman, & O'Donoghue, 2007). In a recent study, Bangsbo et al. (2006) concluded with the aid of video analysis that the majority of field players during a match cover a distance of 10 to 13 kilometers. The activity level of these players regardless of their position is considered intermittent, and very few players spend more than 45 minutes above their lactate threshold (Grant et al., 1995).

Activities executed during a soccer match are 10-20 sprints, high-intensity running approximately every 70 seconds, approximately 15 tackles, 10 headings, 50 involvements with the ball, 30 passes, and changing pace and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure (Eklbom, 1986; Withers et al., 1982; Bangsbo, Norregaard, and Thorsoe, 1991; Reilly and Thomas, 1976; Helgerud et al., 2001; Mayhew and Wenger, 1985). Reilly and Thomas (1976) performed a motion analysis on the different positional field players in professional soccer. They found that a player changes activity every 5 to 9 seconds and on average sprints 15 meters every 90 seconds for a total distance of 8 to 11 kilometers during a match (Bangsbo, Norregaard, and Thorsoe, 1991; Reilly and Thomas, 1976). A quarter of the distance covered during that match was while walking, 37% while jogging, 21% running below top speed, 11% sprinting and 6% running backwards. Withers et al. (1982) completed a very similar movement analysis study that showed 27% of the distance covered in a match was walking distance. They found 46% of the distance was covered while jogging, 13% of the distance while running at a moderate pace, 6% during maximal sprints, and 8% completed while in a backwards-running movement. Motion analysis of the physiological demands of soccer has also been expressed in units of time. Apor (1988) and Ohashi et al. (1988) depicted the duration of single sprint time to be between 3 to 5 seconds. Apor and Ohashi measured the time between sprints to be 30 to 90 seconds.

Dependent upon play position, different tasks are performed during each match. Mohr et al. (2003) reported that fullbacks and attackers sprint significantly more than midfielders and central defenders. Bloomfield, Polman, Donoghue (2006) studied the 2003-2004 season of the English Premier League. A total of 55 players (18 defenders, 18 midfielders, and 19 strikers) were analyzed throughout the course of the season. Multi-camera systems were set up around the field and the players were analyzed in the match for an analysis of the types of movements, the repetitions of each movement, and the distance covered during each movement. Bloomfield created a movement classification system that defined all purposeful movements completed during the soccer game. According to this movement system, midfielders spent over a quarter of the game jogging spent a greater percentage of the game sprinting, running, and jogging-compared to strikers and defenders. Each position covered between 8 and 13 kilometers per game. There was a vast decrease in distance covered in the second half compared to the first half for all playing positions.

The physiological demands of a soccer match involve a combination of anaerobic and aerobic energy systems being taxed throughout the entire ninety-minutes (Wisloff, Helgerud, & Hoff, 1998). The intermittent bouts of increased intensity activity are separated with short low-intensity active recovery periods. By measuring heart rate in elite soccer players from Denmark, Bangsbo (1994) estimated that soccer players stay at approximately 70% of maximal oxygen uptake during a match. From these heart rate values, as well as muscle biopsy samples extracted periodically throughout the match, he concluded that more than 90% of the total energy consumption was accounted for by the aerobic energy system. Reilly (1990) studied English League soccer and found that, with respect to the traditional English formation of four defenders, three midfielders, and three forwards, the average heart rate was 157 beats per minute. This work rate is equated to approximately 75% of the participant's VO_2max.

The aerobic system will contribute mostly during recovery while the players are walking, jogging and running below maximum. Conversely, the ATP-PCr and anaerobic glycolytic system contribute to ATP production during high-intensity periods, and upon initiation of exercise or an increase in intensity. Over the course of a ninety-minute match the aerobic energy system is heavily relied upon to provide energy substrates during the lower intensity bouts, but also to re-synthesize ATP-PCr stores immediately following high-intensity bouts. The ability to replenish energy stores contributes to the success of the player during an entire match.

The capacity to perform high-intensity intermittent exercise may be influenced by factors such as phosphate levels, muscle glycogen levels, pH, and blood and muscle lactate concentration. To determine whether anaerobic glycolysis is significant during soccer, researchers have analyzed blood lactate of soccer players during different intermittent activities. Denadai et al. (2005) studied the lactate threshold and maximal lactate steady state in 12 male soccer players. The participants completed 8 different testing sessions. The first was an incremental treadmill test where they ran 3-min stages at an increasing speed until exhaustion. At the completion of each stage lactate samples were taken until lactate threshold was reached. Lactate threshold is defined as the VO_2 corresponding to the starting point of an accelerated lactate accumulation of around 4 $mmol^{-1}$ and was expressed as % VO_2 max (Aunola & Rusko, 1984). The remaining 7 sessions determined the subjects lactate threshold by performing constant velocity runs on a treadmill for 30 minutes. The different sessions would vary in running speed by 0.5 km/hr. The lactate threshold was defined as the highest velocity at which the blood lactate concentration did not increase by more than 1mM between minutes 10 and 30 of the constant velocity run (Jones and Doust, 1998). As predicted this study concluded that soccer players with higher OBLA (onset of blood lactate accumulation) were able to attain higher speeds before reaching lactate threshold. This in turn could represent the significance of the anaerobic capacity of soccer players and their ability to transfer lactate from the muscle to the blood where it can be transferred to another muscle or the liver for metabolism.

Brooks (1986) concluded that most (75 %) of the lactate formed during sustained, steady state exercise is oxidized in the mitochondria during exercise, and only a minor fraction (~20%) is converted to glucose via the liver. The effectiveness of this oxidization is dependent on the activation of the oxidative energy system at the onset of exercise. The transfer of pyruvate from areas of high glycogenolytic rate to areas of high cellular respiration through the mitochondrial wall represents an important means by which substrate is distributed, metabolic "waste" is removed, and the functions of various tissues are coordinated during exercise (Brooks 1986). With the intermittent training of soccer players this important transfer of lactate occurs at a much more efficient rate because of the trained efficiency between the aerobic and anaerobic systems.

Lactate serves as a gluconeogenic precursor in the liver and most of the glucose will be released into the circulation during high-intensity exercise (Brooks, 1986). Glucose generated from lactate can help restore liver and muscle glycogen during low-intensity recovery bouts. It is the restoration of these substrates that helps enable soccer player to continue intermittent high intensity play. Furthermore, when the lactate shuttle system effectively removes lactate from the muscle the potential oxygen delivering capacity of the blood increases via the Bohr Effect. The Bohr Effect is the increased dissociation of oxygen from hemoglobin in the blood as a result of the increased carbon dioxide and decrease in pH of the blood. With the increase in the acidosis of the blood as a result of the higher concentration of blood lactate, there appears to be an increase in the oxygen utilization. As a result, what was once believed to be a limitation to intermittent exercise is now suggested to accelerate the oxygen utilization capabilities of the muscle, which will promote further oxidative processes within the muscle.

Match performance characteristics such as position and team role of soccer players can be determined by the distribution of fiber types in the muscle. Muscle fiber types are categorized as fast twitch or slow twitch based on the speed of response when stimulated. This classification distinguishes between slow oxidative (type I), fast glycolytic (Type IIb), and fast oxidative glycolytic (Type IIa). Analysis of work rate of soccer players during match-play suggests that an ability to sustain physical effort over 90 min, albeit discontinuously at different exercise intensities varying from low to high intensity is critical to soccer performance (Drust et al., 1998). Studies suggest that there is a relative balance between fiber types in soccer players. Jacobs et al. (1982) studied Swedish professional soccer players and found balanced results between fast twitch (FT 59.8 %) and slow twitch (ST 40.2%) muscle fibers in the vastus lateralis. The percentage FT area was 65.6% depicting a FT/ST mean fiber area of 1.28 (Jacobs et al., 1982). These values suggest that although there is a more equal distribution fiber type in the leg muscle of soccer players, there is still a closer relationship between soccer players and sprinters than soccer players and endurance trained athletes with respect to fiber type composition.

Essen (1978) attained muscle biopsies of the vastus lateralis after the completion of continuous and intermittent exercise to study glycogen depletion within the different fiber types. After the 60 minutes of continuous exercise at a moderate intensity there was a significant depletion of the type I muscle fibers compared to the type IIa or IIb (277 mmol/kg dry weight vs. 113 mmol/kg dry weight). With 60 min intense intermittent exercise a significant and similar depletion occurred in both type I (213 mmol/kg dry weight) and type II (A + B) fibers (203 mmol/kg dry weight) (Essen, 1978). These data indicate that soccer players recruit heavily both type I and II fibers and that glycogen depletion is likely an inevitable outcome and source of muscle fatigue during a match.

The intermittent and diverse nature of a competitive soccer match promotes the importance of the aerobic and anaerobic energy systems. The multitude of movements and sprints executed during the game at both high and low intensities warrants an elite level of fitness that competitive soccer demands. Without a significant contribution from robust anaerobic and aerobic energy systems, the soccer player would not be able to perform high intensity sprints and jumps continually throughout an entire match.

The Importance of VO_2 max in Soccer Performance

No doubt, the emphasis of the high-intensity sprints during a soccer match is on the-capacity of the anaerobic energy system which plays an essential role of supplying creatine phosphate and glucose at high rates-during intensive exercise bouts. However, the majority of energy is supplied by the aerobic system. There is evidence that the aerobic system is extremely important when aiding in the shuttling of lactate from the muscle and providing the energy substrates necessary during recovery so the re-synthesis of PCr and ATP stores is possible. The importance of the aerobic energy system is evident from the relatively high maximal oxygen uptake values reported in elite soccer players, ranging from 55 to 65 ml/kg/min (Al Hazza, et al., 2001; Ekblom, 1986; Stolen, Chamari, Castagna, Wisloff, 2005).

Reilly and Thomas (1976) observed a positive correlation between VO_2max and the distance players were able to cover in a game. These data are supported by Smaros (1980) who studied twelve male English professional soccer players over the duration of one full off-season to examine the energy demands of a soccer match. Match analysis and recording of off-season training showed significant correlation between players with a greater aerobic power and the distance covered during a match. These two studies support the evidence that aerobic capacity is important to soccer performance possibly through the ability to recover from high-intensity bursts quickly. It is during the rest periods that a large flow of blood is required to shuttle the inorganic phosphate and oxygen to the muscle, as well as remove the lactic acid and hydrogen ions produced by the anaerobic bouts. The quicker this is achieved, the sooner a player can repeat the high-intensity sprints, and thus cover more distance and attempt more sprints.

VO_2max in soccer players may be dependent on player position. In support of this idea, Stroyer et al. (2004) observed significantly different values for VO_2max measurements among soccer players with respect to their positions. They studied 27 male soccer players ranging in age from 12 to 14 years. The participants executed a submaximal and maximal treadmill test in the laboratory. Comparing midfielders and attackers, Stroyer measured a 12% higher VO_2max in midfielders (65 ml/kg/min vs. 58 ml/kg/min).

Although the sport of soccer is categorized by its intermittent activity there seems to be some justification for the importance of the aerobic system. Although the aerobic system is not as fast as the anaerobic system in providing ATP, it is the aerobic system that aids in the success of athletes, especially late in the match. Relative to the general population, soccer players do have elite maximum oxygen uptake levels, but there is evidence that the players with higher VO_2max values are more capable of completing bouts of sprints late in the game.

Physiology of Recovery

Aerobic fitness enhances recovery from high intensity intermittent exercise through increased aerobic response, improved lactate removal and enhanced PCr regeneration and is linked to power recovery during repeated efforts of high-intensity exercise (Tomlin and Wenger 2002). With respect to a soccer match, the replenishment of PCr stores in the muscle aids in the glycogen sparing factor to a minor extent, but also contributes to a decreased lactate build-up by the continued anaerobic metabolism of glycogen in the cytoplasm of the muscle. Further, studies appear to support a relationship between endurance fitness and PCr recovery following both submaximal work and repeated bouts of maximal intensity exercise (Bogdanis, 1995).

Bogdanis et al (1995) examined the effects of repeated sprints on a cycle ergometer in 14 male cyclists. Each participant performed two 30-sec maximal sprints on three separate occasions. The passive rest between the sprints was either 1.5 minutes, 3 minutes, or 6 minutes. Muscle biopsies were taken from the vastus lateralis before the first sprint, immediately after the first sprint, near the end of the passive recovery, and at the end of the second sprint. Results from the muscle biopsies at the end of the first sprint showed that PCr and ATP contents were 19.7 ± 1.2% and 70.5 ± 6.5% of the resting values, respectively. Muscle lactate was 119.0 ± 4.6 mmol (kg dry weight) and muscle pH was decreased 6.72 + 0.06. During recovery, PCr increased rapidly to 65.0 ± 2.8% of rest after 1.5 min, but reached only 85.5 ± 3.5% of rest after 6 min of recovery. At the same time ATP and muscle pH remained low (19.5 ± 0.9 mmol (kg dry weight) and 6.79 ± 0.02, respectively).

A second investigation by Bogdanis et al. (1996) attempted to study the contribution of phosphocreatine and aerobic metabolism during multiple repeated bouts of sprint exercise. Eight male subjects performed two cycle ergometer sprints separated by 4 minutes of recovery. The protocol was repeated twice. The first sprint lasted 30 seconds and the second sprint was either 10 or 30 seconds in duration. Muscle biopsies were obtained before the sprints, immediately after the first sprint, 15 seconds before the second sprint, and after the second sprint. At the end of the first sprint the PCr level was 16.9 ± 1.4%, and muscle pH decreased to 6.69 ± 0.02. Fifteen seconds before the second sprint muscle pH remained unchanged 6.80 ± 0.03, but PCr resynthesis had raised stores to 78.7 ± 3.3% of resting values. Most of the PCr during the second sprint was utilized during the first 10 seconds and remained unchanged until its completion. The anaerobic ATP turnover, as calculated from changes in ATP, PCr, and lactate, was 235 ± 9 $mmol\cdot kg^{-1}$ during the first sprint but was decreased to 139 ± 7 $mmol\cdot kg^{-1}$ during the second 30-s sprint, mainly as a result of an approximately 45% decrease in glycolysis (Bogdanis, 1996). During the second 30-second sprint there was a decrease in total work done by approximately 18%. This decrease in power output during the second sprint is correlated with the increased contribution of aerobic metabolism, indicative of the increase in oxygen uptake during sprint two (2.68 ± 0.10 vs. 3.17 ± 0.13 $l\cdot min^{-1}$; sprint 1 vs. sprint 2; $p < 0.01$).

In another study by Bogdanis et al, (2006) the effects of active recovery on metabolic responses, cardiorespiratory responses, and power output during repeated sprints was examined (Bogdanis, 2006). Thirteen male subjects performed two maximal 30-second cycle ergometer sprints, 4 min apart, on two separate occasions with either an active (cycling at 40% of maximal oxygen uptake) or passive recovery. Active recovery resulted in a significantly higher mean power output during sprint 2, compared with passive recovery (603 W and 589 W, $p < 0.05$). The improvement in power output was attributed to a 3.1% higher power generation during the initial 10 seconds of the second sprint following the active recovery ($p < 0.05$), since power output during the last 20 seconds of the second sprint were the same. Heart rate between the two 30-second sprints and oxygen uptake during the second sprint were higher for the active compared with passive recovery (148 ± 3 vs 130 ± 4 beats min^{-1}, $p < 0.01$ and 3.3 ± 0.1 versus 2.8 ± 0.1 min^{-1}, $p < 0.01$; respectively). These data suggest that recovery of power output during repeated sprint exercise is enhanced when low-intensity exercise is performed between sprints. The beneficial effects of an active recovery are possibly mediated by an increased blood flow to the previously exercised muscle.

The altering of high-intensity bouts and active recovery is the nature of competitive soccer. Recovery is enhanced with increased circulation to exercised muscle. During passive recovery, the lingering sympathetic response imposes vasoconstriction while the vasodilation to skeletal muscle has been reduced because muscle contraction has stopped. As a result, when exercise is initiated again the muscle has not been supplied with the oxygen to restore its lost energy substrate stores from the last bout of exercise. With active recovery between high-intensity sprint bouts in soccer matches, the blood flow to the exercising muscle is sustained at a higher rate, therefore oxygen delivery and lactate shuttling occurs and the muscle is more prepared for the next high-intensity bout of exercise.

Testing VO_2 max in Soccer Players

One of the most common measurements in exercise physiology is maximal oxygen uptake (VO_2 max). Obtaining accurate and valid VO_2max values is of physiological importance when comparing groups or individuals, and when tracking longitudinal changes. Laboratory tests such as treadmill running for determination of

VO_2max and shuttle run tests performed in the field to estimate VO_2max have been developed to evaluate aerobic performance in soccer (Krustrup et al., 2003; Leger & Lambert, 1982). Studies have commonly utilized continuous protocols for the measurement of maximal oxygen uptake (Al Hazza et al., 2001; Bangsbo & Lindquist, 1992; Davis, Brewer, & Atkin, 1992). A continuous protocol has been traditionally used because of its effectiveness in getting an athlete to VO_2max within a short amount of time. The decreased time to maximum assures that the termination of the test is due to oxygen uptake capacity and not muscle fatigue.

VO_2max has long been regarded as the gold standard measure of cardiorespiratory fitness. The work of Hill and Lupton (1923) introduced the VO_2 max concept; however issues relating to the appropriate protocols and test procedures for its true attainment are unresolved. In 1975 the American College of Sports Medicine published the first edition of the guidelines for Graded Exercise Testing and Prescription (ACSM, 1975). McConnell (1988) adopted many of these guidelines when submitting a standardized protocol for continuous maximal testing on a treadmill with the objective of implementing test uniformity. The guidelines stated that the warm-up should be completed at 60-70% of VO_2max for 5 minutes followed by a brief rest. The test begins with an initial intensity of 60-70% VO_2max with a rate increase of approximately 5% VO_2max per minute. This workload increase would result in total test duration of 8-10 minutes.

The test duration of a VO_2max protocol has been widely disputed since the 1983 study of Buchfuhrer et al. Buchfurer et al (1983) compared VO_2max measured during five treadmill tests of varying durations from 7 to 26 minutes. The conclusion from the findings was that the test administrator should select a work rate increment that will progress to maximum tolerance between 8-12 minutes (Buchfurer et al., 1983). The subjects in this study were five moderately fit men; therefore, it cannot be concluded with great power the practical application of this conclusion to a greater population. Even if these results are consistent within this population, there is still question about the application of the 8-12 minute protocol for sedentary individuals or well-trained athletes (Midgley et al., 2008). Midgley et al. (2008) concludes that current evidence suggests that valid VO_2 max values have been found for treadmill tests with protocols lasting between 5 to 26 minutes, but that after the conclusion of the continuous incremental test there should be a rest period followed by a supramaximal bout of exercise to exhaustion to verify that maximum oxygen uptake had been reached during the protocol.

Taylor et al. (1955) observed that in response to multiple discrete bouts of exercise, each with a higher workload than the previous, an upper limit of oxygen uptake per unit of time was reached. As a result, a termination criterion for determination of a true maximal effort was established. They found an increase in oxygen uptake with a 2.5% grade increase at 7 mph to be 4.2 ± 1.1 (X ± SD) $ml \cdot kg^{-1} \cdot min^{-1}$. Thus an increase of less than 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ suggests that oxygen uptake had achieved a maximum level and could be considered a plateau.

There have been multiple studies that have reported the incidence of VO_2 plateau, results ranging from 0% to 100% of participating subjects (Froelicher et al., 1974; Duncan et al., 1997; Astorino et al., 2005; Rossiter et al., 2006). Thus, the issue with the VO_2 plateau as a criterion method for determining VO2max achievement is that many participants including athletes do not attain the plateau. For instance, Day et al. (2003)

reported that 19 of 71 (17%) subjects demonstrated an accelerated VO_2 response as they approached fatigue during the end of an incremental test.

The lack of robustness of the VO_2 plateau highlights the need for secondary criteria to establish whether a maximal effort has been given (Midgely & Carroll, 2009). Secondary termination criterion was standardized in support of the argument against a maximal effort not being given if there was no incidence of a plateau. The secondary criteria in the absence of a plateau have been designated as an increase in heart rate to maximum values estimated by age (Martiz et al., 1961), a respiratory exchange ratio (RER) of 1.15 or greater (Issekutz, Birkhead, and Rodahl, 1962), and post exercise blood lactate levels greater than 8 $mmol \cdot l^{-1}$ (Astrand, 1952). Studies have found no difference between the maximal heart rates, respiratory exchange ratios, or post-exercise blood lactate concentrations among subjects who demonstrated a VO_2 plateau compared with those participants without a plateau (Astrand, 1952; Rowland and Cunningham, 1992; Rivera-Brown & Frontera, 1998).

Aitken and Thompson (1988) explored the relationship between oxygen uptake (% VO_2 max) and RER (VCO_2/VO_2) during a maximum progressive treadmill exercise test in endurance athletes, weight-trained athletes and team athletes and untrained men. Overall, they found the mean RER to range from 1.01 to 1.11 among the groups when VO_2 values had leveled off. Forty-five endurance trained men had a lower $\%VO_2max/RER$ relationship and higher RER at VO_2max (1.11 ± 0.02, ($p < 0.01$)), 98 team athletes had a lower $\%VO_2max/RER$ relationship, but lower RER at VO_2max (1.06 ± 0.03, ($p < 0.01$)), and the weight trained ($n = 19$) had a higher $\%VO_2max/RER$ relationship and lower RER at VO_2max (1.01 ± 0.02, ($p < 0.01$)), all compared to controls

(Aitken and Thompson, 1988). In another study, RER values less than 1.00 despite leveling values for oxygen uptake were measured in young male ice hockey athletes (Cunningham et al, 1977). Clearly, it is evident that RER $\geq$ 1.15 is not a universal finding that is reliable to determine maximal oxygen uptake tests. It is evident that this criterion cannot be used solely as determination of a maximal test due to the variance among population. However, used in conjunction with other termination criteria, RER values can aid in the determination of a maximal effort.

Doherty, Nobbs, and Noakes (2003) studied 51 elite athletes (male n=35 and female n=16) for the prevalence of specific termination criteria (i.e. the VO2max plateau) during a continuous incremental treadmill protocol. The three termination criteria were a plateau in the VO_2 defined as an increase of less than 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$, a final RER of 1.1 or above, and/or a final heart rate above 95% of the age predicted maximum (220 – age). The criteria for RER and heart rate were satisfied by 72% of male athletes and 56% females, and 55% males and 69% females, respectively. In contrast only 39% of males and 25% of females showed a plateau in VO_2. Conclusion from this study shows that the absence VO_2 plateau is evidence of prior muscular fatigue before the athletes are reaching their maximum values or there is a lack of motivation from the participant to exercise to their maximum. It is possible that the continuous incremental protocol is restricting these athletes in obtaining their VO_2max.

Duncan, Howley, and Johnson (1997) studied the VO_2 max termination criteria in continuous and discontinuous treadmill protocols. During the continuous protocol, subjects ran at 7.0 miles per hour and zero percent grade for 3 minutes. The elevation was then increased 2.5% after every completed minute of the protocol until the subject could no longer continue the test. For the discontinuous protocol, subjects ran at 7.0 miles per hour for a minimum of three minutes and a maximum of five minutes. Stages for this study were separated by 2-3 days. VO_{2max} did not differ between the discontinuous and continuous protocols (56.8 ± 4.7 versus 55.8 ± 4.2 $ml \cdot kg^{-1} \cdot min^{-1}$). Maximal ventilation (150.7 ± 16 and 149.5 ± 17.5 $l \cdot min^{-1}$ BTPS) and heart rates (186.3 ± 7.7 $beats \cdot min^{-1}$ and 191.7 ± 6.7 $beats \cdot min^{-1}$) were also similar between the discontinuous and continuous protocols. However, RER (1.28 ± 0.05 versus 1.22 ± 0.05) and lactate (14.3 ± 2.7 versus 11.9 ± 2.7 $mmol \cdot l^{-1}$) were greater ($P < 0.05$) during the discontinuous protocol.

There has also been the implementation of a verification phase to aid in the justification of true VO_2 max attainment, especially when a plateau is not evident. Thoden et al. (1982) recommended that after 15 minutes of recovery from the last incremental phase of the testing protocol, a constant bout of exercise with an equivalent workload to the last completed stage in the incremental test protocol be performed until volitional exhaustion. It was recommended that if the verification phase lasted more than 6 minutes than retesting was necessary. An update by Thoden et al. (1991) suggested a recovery time between 5 minutes and 15 minutes to obtain a heart rate of 100 beats per minute before performing the verification phase. They also suggested increasing the workload one stage higher than the last completed stage of the incremental testing protocol.

Midgely et al. (2006) used the verification phase at a speed equivalent to one stage higher than that attained during the last completed stage of the incremental protocol by 16 male distance runners. After the final incremental phase the subjects performed a

10-minute recovery walk and a verification phase at 0.5 km•h^{-1} higher than the last completed stage of the incremental protocol. The verification phase had no definite duration. The participant terminated the stage when they stopped from exhaustion or the researcher noticed a plateau in VO_2 values. Verification criterion for this study was a peak oxygen uptake $\leq 2\%$ higher than the incremental phase value and peak heart rate values within 2 beats/min^{-1} of each other. From the 32 tests performed, 26 satisfied the oxygen uptake verification criterion and 23 satisfied the heart rate verification criterion. Maximum heart rate was lower ($p = 0.001$) during the verification phase than during the incremental phase, and 7 tests exhibited peak oxygen uptake values over 100 mL/min−1 ($\geq 2\%$) lower than the peak values attained in the incremental phase (Midgley et al., 2006).

The verification phase is utilized as support for the achievement of a maximal oxygen uptake values even without a measureable plateau. With the lower heart rate and VO_2 values during the 'supramaximal' stage it is concluded that the oxygen uptake values had plateau during the last stage of the incremental protocols and the oxygen delivery capabilities were now being hindered by other variables such as cardiac output or central fatigue. In coordination with the incremental protocol the verification phase is utilized to assure the researcher of a maximal effort.

The continuous protocol of the traditional VO_2 max test is not representative of the activity within a soccer match because of the absence of any active recovery. Thus, the trained soccer player that improved recovery from high intensity exercise may not be able to sustain continuous high intensity exercise longs enough to achieve VO_2max. It seems much more logical to test the soccer athletes' VO_2max with a protocol that incorporates active rest periods, thus capitalizing on their ability to repeatedly perform intense exercise bouts and recover quickly (Krustrup & Bagnsbo, 2001). This is why more recent studies have adopted the intermittent recovery field test, the Yo-Yo Intermittent Recovery Test Level 1 (YYIRTL1) developed by Bangsbo. Krustrup et al. (2003) were able to demonstrate the reliability of the YYIRTL1 among thirty-seven elite soccer players. Compared with previous results analyzing elite players ability to cover distances in a match, the Yo-Yo test results showed proportional results with respect to forwards, midfielders, and defenders (Bloomfield, Polman, & O'Donoghue, 2006; Krustrup et al., 2003).

The Yo-Yo Intermittent Recovery Test Level 1 is repetitive bouts of sprints performed over twenty meters at increasing speeds. Unlike continuous exercise protocols, the YYIRTL1 protocol implements an aspect of active recovery that adapts to a more game-like testing environment. This adaptation makes the YYIRTL1 protocol more likely to produce a more accurate depiction of match fitness among soccer players. Match analysis has provided evidence that during competitive soccer; players perform a great deal of activity involving turning and changes of directions over a variety of intensities (Withers, Maricic, Wasilewski, & Kelly, 1982). This analysis decreases the theoretical effectiveness of the laboratory treadmill protocols because of the lack of match-simulated movements during such tests. Efforts by Krustrup et al. (2003) have confirmed that the physiological demands involved during these soccer specific endurance tests are similar to those required during a soccer match. For these reasons, these field tests are commonly chosen by coaches to represent players conditioning throughout a season.

In order to assess the contribution of aerobic capacity to a soccer player's success, the demands of match play have been examined by making observations during a match, obtaining physiological measures during real and simulated games and determining the physical capacity of elite players from field tests of performance (Bangsbo, 1994b). The problem with the validity of these measures is that they are estimates, or the means of obtaining these values have in themselves hindered the performance of the participant. Therefore, there is a need to find an accurate, standardized simulation of match play to directly measure aerobic capacity. Regarding VO_2max measurements, a discontinuous protocol that incorporates short active rest periods between stages will better replicate the match play of soccer competition and therefore be a more valid laboratory test for soccer athletes.

The gold standard of testing an individual's VO_2 max has been for many years, the graded exercise test on a treadmill in a laboratory (that is excluding cyclists, rowers, and swimmers). The classic continuous graded exercise test is short in duration (i.e. 8 to 12 minutes) and can assure the researcher with a high degree of confidence that the participant was not restricted due to muscle fatigue and that a VO_2max plateau can be achieved. However, the fact that a low prevalence of a plateau during continuous GXTs among athletes has been reported, it is possible that the continuous protocol due to the high levels of work rates achieved by the athletes, is limited by anaerobic capacities rather than the cardiorespiratory capacity. The intermittent nature of a soccer match does not emphasize the same energy demands as a continuous protocol. Therefore, a discontinuous GXT protocol maybe a more effective means of measuring true VO_2max in soccer players.

CHAPTER III

METHODS

Experimental Design

In the present investigation, an experimental design was used to test for significant differences between continuous and intermittent protocols in soccer players. Each participant executed two graded treadmill protocols to test VO_2max, one protocol per day, one continuous and one intermittent protocol with a verification phases following each. All testing was performed in the Human Performance Laboratory at Barry University. Prior to the treadmill tests, participants visited the laboratory for a familiarization trial. Each protocol included two-minute stages performed at a running pace that approximates 60-70% VO_2max. Intensity was increased by 2.5% grade after the completion of each stage. During the intermittent protocol, each stage, except for the final one was followed by a 1-min active recovery period. A 10-minute active recovery followed the completion of the final stage. At the end of the active recovery a constant exhaustive bout of exercise at one stage intensity higher than the last completed stage was executed until volitional exhaustion.

Eleven collegiate soccer players from the local university participated in the two testing protocols. Each individual testing session was separated by forty-eight hours that included light to moderate intensity exercise with no physical contact. The orders of the tests were randomized.

Participants

The eleven collegiate soccer players were all participating in championship season training selected during the study. All subjects in the study reported at least twelve years of competitive soccer experience at the amateur level. Prior to the testing all soccer players trained for three consecutive months without interruption. Current training patterns include three to five skill/team sessions per week and two or three resistance training sessions. All participants participate in approximately 20 hours per week of intermittent soccer related activities. Training focused on intermittent, match-like simulations, skill sessions, resistance training, and conditioning. The skill and team training averaged eight to ten hours per week during the season period. Any additional aerobic training or sprint work was considered voluntary and was not able to be documented for the purpose of this study. Conditioning protocols included, but were not limited to, high-intensity repetitive bouts of sprinting with active recovery or interval training. Continuous aerobic running sessions were reserved for post-match recovery training days, and were limited to less than one time per week. All participants were active in resistance training programs approximately 2-3 times per week, each session lasting 1-2 hours.

At the onset of the study the 11 soccer players convened for an informational meeting to learn about the exercise protocols. Each participant read and signed a written informed consent approved by Barry University's Internal Review Board prior to participation. At this meeting, participants were instructed to not consume a large meal within three hours before any of the test sessions, as well as to not consume any beverage with measurable amounts of caffeine for at least eight hours before any of the testing protocols (Bangsbo, Mohr, & Krustrup, 2006). Other guidelines for the participants was to maintain a daily diet that is high in carbohydrates to make sure that all subjects have maximized their glycogen stores at test time, and all participants were directed to abstain from any drug or alcohol consumption the week prior to testing. At the conclusion of the informational portion of the meeting each participant completed a sub-maximal treadmill protocol to familiarize them with the equipment.

The sub-max protocol consisted of the participant be outfitted with a Hans Rudolph two-way breathing valve apparatus. The subject completed two to three stages at increasing intensities perceived to be a 14-15 on the RPE (Rate of Perceived Exertion) Borg Scale. VO_2max and heart rate measures were not taken during this protocol; this was utilized to familiarize the subjects with the headgear, mask, and changes of intensity during the protocol.

Maximal Oxygen Uptake (VO_2 max) Testing Procedures

All subjects active in this experiment were familiar with the testing protocols to insure stable and reliable results. All assessments were executed at a similar time of day to maintain consistency for all participants during protocols. The laboratory graded exercise tests were executed on an electronic treadmill (Quinton Med-Track SR60). Upon arrival to the laboratory body weight and height were measured. Body weight was measured with a calibrated SECA electronic scale (± 0.1 kg), and height was measured with the SECA wall-mounted measurement ruler (± 0.1 cm). Each participant then executed a five-minute warm-up run on the treadmill at seventy-five percent of the participant's maximal perceived intensity (approximately 13 or 14 on the Perceived Exertion (RPE) Borg Scale).

After the completion of the warm-up each participant was fitted with a Hans Rudolph two-way valve as described above. A Polar T-31 electrode heart monitor was then fitted to the participant's chest to obtain heart rate readings during the testing protocol.

VO_2max was measured continuously during each stage, using open circuit spirometry (Parvomedics TrueOne 2400 Metabolic Measurement Gas Analyzer, Sandy, Utah). Expired gases were collected and analyzed for volume and fractional oxygen and carbon dioxide. Calibration measurements were performed prior to each test. A two-point calibration of gases was performed with an exact compound mixture (16.01% Oxygen, 4.01% Carbon Dioxide, BAL% Nitrogen) and room air. A 3-L syringe was used for the flow-meter calibration. Data was collected continuously for VO_2, ventilation (VE) and respiratory exchange ratio (RER). All values were retrieved in breath-by-breath measurements.

When the participant was ready to begin testing, the speed was raised to the pre-determined 65% to 75% percent intensity and the protocol began. For the continuous protocol speed was maintained and the percent grade was increased 2.5% every two minutes. This test was terminated when the participant reached volitional exhaustion.

For the intermittent protocol a 1-min active recovery period between each 2-min work stage was included during which the speed was decreased to 4 mph, and grade returned to zero. After the completion of the active recovery stage, the speed was returned to the seventy-five percent intensity speed and the grade was then increased 2.5% from the last stage. This pattern is followed until volitional exhaustion. At the end of the test, the speed is decreased to 2.5 mph without grade. This active recovery intensity was maintained for 10-minutes. With the completion of the 10-minute active recovery the treadmill was raised to one stage intensity higher than the last completed stage of the protocol. The participant ran at this intensity for two-minutes to complete the verification phase. All oxygen uptake values were measured by breath-by-breath analysis through the Parvomedics system. The criteria utilized for the determination of plateau during the verification phase was an increase of less than $2.1 \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with an increase in stage intensity (Taylor et al., 1955).

Statistical Analyses

Analyses of data were completed using the Statistical Package for the Social Sciences (SPSS), version 16 for Windows. Statistical significance was set at $p \leq 0.05$ and data were described as mean ± standard deviation. For the VO_2 max tests paired samples t-test was applied to determine the significant difference between the continuous protocol, intermittent protocol, and verification phase for each protocol. Dependent variables were VO_2max, maximum heart rate, RER, and maximum ventilation values. Maximum values for each dependent variable are determined by taking the average of the last two 15-second values in the final stage of the test protocol, whether the stage was completed or not.

CHAPTER IV

RESULTS

The purpose of the present study is to determine if the implementation of a 1-minute active recover stage in the intermittent graded exercise test protocol better replicates soccer training, and results in a higher stage intensity being reached, as well as, higher heart rate, minute ventilation, respiratory exchange ratio, and maximum oxygen uptake. It was predicted that the intermittent protocol was allow the participants to achieve a higher stage intensity, as well as, achieve higher maximum heart rate, minute ventilation, respiratory exchange ratio, and maximum oxygen uptake.

Descriptive Data

There were eleven participants that completed both protocols. All participants were able to complete both of their protocols at the same time of day, and all protocols were executed between noon and five o'clock in the afternoon to assure consistency in the participants eating habits prior to the protocols.

Table 1. Descriptive characteristics of participants.

	Men	Women	Total
N	6	5	11
Age	21.8 ± 1.7	20.6 ± 2.0	21.3 ± 1.9
Height, cm	180.3 ± 7.3	164.9 ± 6.4	173.3 ± 10.3
Body mass, kg	74.8 ± 10.0	60.5 ± 6.1	68.3 ± 34.9

Continuous VO_2max Protocol and Intermittent VO_2max Protocol

From the eleven participants that were able to complete both protocols, 8 of the 11 achieved higher oxygen uptake values during the intermittent protocol. From those 8 participants, 7 of them achieved values outside of the plateau criteria range. There were similar values measured for ventilation and respiratory exchange ratio, but there was a significant difference in the total maximum heart rate average of the eleven participants. All but three participants achieved a higher heart rate and minute ventilation during the intermittent protocol

Table 2 displays the difference between the maximum oxygen uptake achieved during the continuous and intermittent protocols. 8 out of 11 participants achieved a higher VO_2max during the intermittent protocol compared to the continuous protocol. From those 8 participants, 7 had an increase that was outside of the 2.1 ml·kg^{-1}·min^{-1} range to satisfy the plateau (Taylor et al., 1955). Furthermore, 6 out of the 8 achieved a value that was at least 5% higher than their measured VO_2max during the continuous protocol. Combined, the 11 participants displayed a 3.8% increase from their continuous maximum oxygen uptake ($p < 0.05$).

Table 2. Intermittent and Continuous VO_2max achieved

Participants	Continuous	Intermittent	Difference (%)
	VO_2	**VO_2**	
1	50.5	52.6^{β}	4.2
2	49.3	52.5^{β}	6.5
3	56.7	53.9	-4.9
4	47.9	51.4^{β}	7.3
5	54	56	3.7
6	58.9	61.9^{β}	5.1
7	51.2	54.5^{β}	6.4
8	64.7	64.7	0.0
9	63.9	70.1^{β}	9.7
10	55.7	58.5^{β}	5.0
11	59.4	59	-0.7
Total	55.7 ± 5.7	57.7 ± 5.8**	3.8

Ventilation Comparison Continuous vs. Intermittent

Table 3 displays the ventilation values achieved during the continuous and intermittent protocols. There was no significant difference ($p > 0.05$) between the maximum minute ventilation values of the continuous and intermittent protocol.

Table 3. Max ventilation achieved during continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	100.7	110.4	9.6
2	110.9	119.4	7.7
3	144.8	145.8	0.7
4	105.3	106.1	0.8
5	109.6	109.0	- 0.5
6	193.5	185.5	- 4.1
7	140.3	160.7	14.5
8	127.7	128.8	0.9
9	163.8	162.0	- 1.1
10	128.2	136.1	6.2
11	180.8	176.0	- 2.7
Average	136.9 ± 31.3	140.0 ± 28.0	2.9

Table 4 displays the respiratory exchange ratio (RER) averages of the last 30-seconds of each protocol. There was no significant difference ($p > 0.05$) in the RER values of the continuous and intermittent protocols.

Table 4. Respiratory exchange ratio values for continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	1.17	1.17	0.0
2	1.11	1.13	-1.8
3	1.23	1.24	-0.8
4	1.27	1.12	11.8
5	1.18	1.16	1.7
6	1.15	1.04	9.6
7	1.21	1.14	5.8
8	1.08	1.11	-2.8
9	1.26	1.17	7.1
10	1.11	1.11	0.0
11	1.19	1.17	1.7
Average	1.17 ± 0.06	1.14 ± 0.05	2.9

Table 5 maximum heart rate values of the continuous and intermittent protocols. Although the average increase in heart rate was only 1.6% for the entire population there was an increase of at least 1.5% for 7 of the 11 participants. The 1.6% difference in maximum heart rate during the intermittent protocol equals an increase of 4 beats per minute higher. This was another significant difference ($p < 0.01$).

Table 5. Maximum heart rate values for continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	180	181	0.6
2	192	199	3.6
3	188	192	2.1
4	201	201	0.0
5	188	192	2.1
6	182	186	2.2
7	191	190	-0.5
8	184	188	2.2
9	188	187	0.5
10	182	185	1.6
11	179	184	2.8
Average	186 ± 6	190 ± 6	1.6

Table 6 displays the values calculated from the last completed stage of the intermittent protocol and the last 30-seconds completed of the verification phase following the intermittent protocol. Four out of the five female participants were unable to achieve a higher value on the verification phase following the intermittent protocol. Five of the six male participants achieved a lower VO_{2max} value during the verification phase compared to the last completed stage of the intermittent protocol. The two participants that did achieve a higher value were within the 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ plateau criterion. The two participants that achieved 5.8 and 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ less than the intermittent completed approximately 60 seconds of the verification phase.

Table 6. VO_2max values for participants last completed stage of intermittent protocol and Verification Phase

Participants	Inter VO_2max	Verification VO_2max	Difference
Female			
1	52.6	50.8	-1.8
2	52.5	52.3	-0.1
3	53.9	52.2	-1.7
4	51.4	45.0	-6.3
5	56.0	56.8	0.7
Male			
1	61.9	56.0	-5.8
2	54.5	53.9	-0.3
3	64.7	66.5	1.9
4	70.1	67.4	-2.5
5	58.5	58.3	-0.1
6	59.0	58.8	-0.1
Total	57.7 ± 5.8	56.2 ± 6.6	-1.5 ± 2.6

VO_2max values are expressed as $ml \cdot kg^{-1} \cdot min^{-1}$.

CHAPTER V

DISCUSSION

The purpose of the present study was to determine intermittent graded exercise results in a higher stage intensity and higher maximal heart rate, minute ventilation, respiratory exchange ratio, and oxygen uptake compared to continuous graded exercise. There have been a number of previous studies that have examined the achievement of an individual's maximum oxygen uptake. The necessary criteria to determine a maximum effort and the incidence rate of a plateau at the completion of the classic continuous graded exercise test have been disputed for many decades. This study demonstrates that a continuous GXT protocol may limit some athlete's ability to achieve true VO_2max and that an intermittent protocol maybe more appropriate for intermittently trained athletes.

Hypothesis 1: A higher intensity will be achieved during the intermittent protocol compared to the continuous protocol. As a result, a higher heart rate, RER and minute ventilation will be achieved.

All participants on the intermittent protocol compared to the continuous protocol achieved higher grade intensity on the treadmill. Although there was a slightly higher average for the intermittent ventilatory response, there is no evidence that this was the leading factor in the attainment of a higher VO_2max. There was also no significant difference between the respiratory exchange ratio during the last completed stage of the continuous and intermittent protocol ($p > 0.05$). The RER was higher in the continuous protocol compared to the intermittent protocol. However, this did not result in a significant difference ($p > 0.05$) a higher maximal ventilation or RER for the continuous and the intermittent protocols. The maximum heart rate attained during the last minute of the completed stage of the intermittent protocol was significantly higher ($p < 0.05$) than during the continuous protocol. Higher heart rate is directly correlated with the higher intensity. Increased heart rate during the intermittent protocol is an attempt to maintain sufficient cardiac output at a higher intensity.

It is assumed that because of the intermittent protocol's active recovery stage that there is the opportunity for re-synthesis of PCr, muscle glycogen, and ATP. Therefore, the RER is not expected to be as high as a continuous exhaustive bout of increasing intensity exercise. The ventilation measures were not significantly different either because there is more time during the active recovery to maintain lower levels of CO_2 in the blood. Therefore the ventilation demands are not as high at specific intensities because there has been more time to expel CO_2 during the active recovery.

Of the eight participants that achieved a higher maximum oxygen uptake during the intermittent protocol compared to the continuous, six demonstrated an increase greater than the 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ plateau criterion. Of these participants only four achieved a higher heart rate, and average increase was less than 4 beats per minute. Similarly, 4 participants who achieved higher oxygen uptake values during the intermittent protocol also achieved a higher maximum ventilation value. In general, there were too many inconsistencies between the eleven participant's ventilation and maximum respiratory exchange ratios to conclude a significant difference between the two protocols. The participant's ability to achieve a higher maximum heart rate is hypothesized to aid in maintaining sufficient cardiac output at higher intensity to aid in the delivery of oxygen to the working muscle.

Unlike the Duncan, Howley, and Johnson (2007) study of 10 males, this study did show a significantly higher heart rate during the intermittent protocol than during the continuous protocol. The RER was, however, higher in the discontinuous protocol when compared to the continuous protocol for Duncan, Howley, and Johnson (2007). Duncan, Howley, and Johnson (2007) also did not show a significant difference in the ventilation measurements of either protocol (150.7 ± 16.0 vs. 149.5 ± 17.5 ·min^{-1} BTPS). The participants in the Duncan, Howley, and Johnson study were recreationally active males who described their training as structured running, bicycling, and weightlifting, but reported nothing "intermittent-like" in their training. Also, the discontinuous protocol was spread across 2-3 days, and therefore was more like continuous protocol with the objective of reaching steady state at each progressive exercise intensity. The progression during the continuous protocol was a speed progression instead of an increase in grade like the present study. This means the reasoning for termination may also have been due to participant's inability to maintain stride length during a particular stage (Duncan, Howley, and Johnson, 2007). Due to the numerous differences in protocol and participant training status it is difficult to accurately compare the results. The specificity of the intermittent protocol in the present study, compared to Duncan, Howley, and Johnson's multiple day discontinuous protocol better emphasizes the training adaptations of soccer players.

Hypothesis 2: The intermittent protocol will elicit significantly larger relative VO_2 max values compared to the continuous protocol

During the intermittent protocol, compared to the continuous protocol, all soccer completed, 1 additional stage at a higher intensity. Although the mean duration of the intermittent protocol (16.26 ± 0.05 minutes) was 6.5 minutes longer than the continuous protocol (9.75 ± 0.05 minutes) soccer players were able to achieve a higher maximum oxygen uptake value, as previously hypothesized. The duration of both test still satisfied the time interval of Buchfurer et al. (1983) that the VO_2max was significantly higher on tests where the increment magnitude was large enough to induce test durations of 8-17 minutes. This is evidence that there are limitations in the progression of oxygen kinetics when performing the continuous protocol for intermittently trained athletes.

Intermittently trained participants (i.e. soccer players) present different physiological adaptations (Iaia et al., 2009; Bangsbo et al., 1992a; Balsom et al., 1999; Roberts et al., 1982; Shepley et al., 1992) to training that may affect their performance in a continuous protocol and result in early termination before the achievement of their maximum oxygen uptake. The model that states the limits of exercise are due to the cardiorespiratory system were first presented by Hill and Lupton (1923). Noakes (2000) has more recently presented four other likely models that explain the termination of exercise at maximal exercise according to one of the following models:

- The energy supply/energy depletion model
- The muscle power/muscle recruitment model
- The biomechanical model
- The psychological model

From the four models presented by Noakes it seems from the present study that the second of the four models is the most likely. The muscle power/muscle recruitment model states that it is the process in skeletal muscle recruitment, excitation and contraction that limits maximal exercise. More specifically, it is local muscle fatigue, including failure of sarcoplasmic reticulum calcium release (Allen et al., 2007), impaired sodium/potassium pump activity (McKenna et al., 2007), and slowed cross-bridge cycling (Fitts, 2008) that may directly limit the rate of muscle contraction and force production in the muscle that would hinder one's ability to exercise maximally.

Na$^+$, K$^+$ pump subunits

For the purpose of this study, soccer players are defined as speed endurance trained athletes. There are many physiological adaptations as a result of speed endurance training that have been documented (Iaia et al., 2009; Bangsbo et al., 1992a; Balsom et al., 1999; Roberts et al., 1982; Shepley et al., 1992). An important adaptation for prolonged performance may be the increase NA$^+$, K$^+$ pump subunits as a result of speed endurance training (Green et al., 2004). The increase in NA$^+$, K$^+$ pump subunits is believed to be the cause for the participant's achieving a higher work intensity, as well as, higher maximum oxygen uptake during the intermittent protocol.

There are many factors that are believed to contribute to fatigue during maximal exercise. Sarcolemmal depolarization due to extracellular K$^+$ accumulation has been suggested to be a primary contributor to fatigue development during maximal intensity exercise (Sejersted & Sjogaard, 2000). Potassium is an essential intracellular ion used to actively convert stored glucose into glycogen in the muscle. Thus, with increased exercise there is a significant potassium flux from the intracellular space of the working muscle (Sejersted & Sjogaard, 2000). This influx has the potential to decrease the membrane potential of the cell to half of its resting value. Sejersted and Sjogaard (2000) describe the flow of potassium as being directed out of the cell, resulting in an outward electro diffusion of K^+. This is normally opposed by K^+ uptake mediated by the Na^+-K^+ pump. Under certain circumstances, the net electro potential driving force for K^+ may be directed into the cell, which means that there may be an inward K^+ flux through the Na^+-K^+ pump. This hypothesis is based on observations that during maximal intensity exercise contracting muscles lose K^+ which progressively accumulates in the extracellular space of the cell (Juel, 1988; Cairns et al., 1995). Speed endurance training has shown to reduce interstitial K^+ accumulation (Nielsen et al., 2004) in response to an increase in the amount of Na^+, K^+ pumps (Clausen, 2003). Increase in Na^+, K^+ subunits allows for increase efficiency in K^+ clearance out of the cell resulting in the continued depolarization of the cell.

Vanders (2008) presents the chronological order in which the muscle is meant to contract from the onset of the action potential in Table 6.

Table 6. Chronological order of cell depolarization from onset of action potential

1. Action potential is initiated and propogates to motor neuron axon terminals
2. Calcium enters axon terminals through voltage-gated calcium channels
3. Calcium entry triggers release of Ach from axon terminals
4. Acetylcholine diffuses from axon terminals to motor end plate in muscle fiber
5. Acetylcholine binds to nicotinic receptors on motor end plate, increasing their permeability to Na^+ and K^+

6. **More Na^+ moves into the fiber at the motor end plate than K^+ moves out, depolarizing the membrane and producing the end plate potential**
7. **Local currents depolarize the adjacent muscle cell plasma membrane to its threshold potential, generating an action potential that propagates over the muscle fiber surface and into the fiber along the T-tubules**
8. Action potential in T-tubules induces DHP receptors to pull open ryanodine receptor channels, allowing release of Ca^{2+} from lateral sacs of sarcoplasmic reticulum
9. Ca^{2+} troponin on the thin filaments, causing tropomyosin to move away from its blocking position, thereby uncovering cross-bridge binding sites into action

***Steps 6 and 7 are the stages of depolarization that will allow for the continuation of the Sliding-Filament Theory.

Without the intracellular build up of potassium the cell is able to continue depolarization and contraction of the working muscle. With respect to the present study, the continuous protocol was highlighted with lower VO_{2max} values possibly because the participants were being limited by the interstitial K^+ accumulation. With the implementation of the one-minute active recovery period between each working stage the soccer players were able to use the increased Na^+, K^+ pump subunits to reduce the interstitial K^+ concentration and allow for the continued depolarization of the working muscle.

There are however other possible training adaptations that also contributed to the success of the participants achieving higher work intensity during the intermittent protocol. There are many physiological benefits to implementing the 1-minute recovery periods between each work stage. During these active recovery stages it is assumed that there is increased lactate clearance, as well as, some energy substrate (i.e. PCr and ATP) re-synthesis that will aid in the achievement of a higher intensity during the end of the protocol.

Effects of Recovery on Performance

The increased concentration of interstitial potassium during the continuous protocol is one assumed variable that causes the early termination of the soccer players VO_2max protocol before reaching their true maximum. The implementation of the 1-minute recovery stage in between each working stage is hypothesized to assist in the regulation of the interstitial potassium concentration, thereby, assisting in the continued depolarization of the muscle for contraction. Other variables hypothesized to play a role in the achievement of a higher intensity during the intermittent protocol are the re-synthesis of ATP and phosphocreatine (PCr) in the muscle and increased lactate clearance to the blood. Results from Bogdanis et al (1995) muscle biopsies at the end stages during repeated sprints tests on a cycle showed the re-synthesis effects of recovery on PCr in the muscle. After the first sprint the PCr and ATP contents were 19.7 ± 1.2% and 70.5 ± 6.5% of the resting values, respectively. During recovery, PCr increased rapidly to 65.0 ± 2.8% of rest after 1.5 min, but reached only 85.5 ± 3.5% of rest after 6 min of recovery. The population for the Bogdanis (1995) study was described as male cyclists and there was no indication of the nature of the participant's previous training.

A second investigation by Bogdanis et al. (1996) attempted to study the contribution of phosphocreatine and aerobic metabolism during multiple repeated bouts of sprint exercise. Eight male had muscle biopsies obtained before the sprints, immediately after the first sprint, 15 seconds before the second sprint, and after the second sprint. At the end of the first sprint the PCr level was 16.9 ± 1.4%. Fifteen seconds before the second sprint PCr re-synthesis had raised stores to 78.7 ± 3.3% of resting values. Most of the PCr during the second sprint was utilized during the first 10 seconds and remained unchanged until its completion. Bogdanis once again highlights the regeneration capabilities of the muscle during repeated bouts of high-intensity exercise.

Similar to the protocol that the current study explored, the cyclists experienced alternating high and low-intensity stages. The low intensity stages offered an opportunity to replenish PCr stores in the muscle, which would aid in the continued performance at the next higher intensity. Evident in both Bogdanis studies (1995 & 1996) the stores were never completely regenerated, but a re-synthesis of 40 – 80% of resting values is going to provide a significant contrast in energy metabolism at the next intensity. With respect to the current study, the active recovery between high-intensity work stages, allowed for increased blood flow to the working muscles. This consistent blood flow provided oxygenated blood to aid in aerobic metabolism during the low-intensity active recovery stages. Increased aerobic metabolism aids in the replenishing of PCr, which is going to aid in anaerobic metabolism at the next subsequent working stage.

Another important aspect of the active recovery during the intermittent protocol is the ability to shuttle the lactate from the muscle into the blood and/or buffer the lactate within the muscle to convert it into usable energy. The main metabolic pathway for lactate elimination is oxidation in the tricarboxylic acid to end products CO_2 and H_2O (Rontoyannis, 1988). It has been reported the approximately 23-27% of lactate is converted to glycogen during recovery (Bangsbo et al., 1991). Coffey et al (2004) studied running performance with respect to blood lactate concentrations. Coffey reported that there are positive effects of active recovery on the clearance of lactate, and also agreed that the increased blood flow, theoretically, should enhance lactate clearance from the muscle, as well as, aid with the delivery of oxygenated blood to the muscle for aerobic metabolism which aids in the conversion of lactate to pyruvate via lactate dehydrogenase enzyme. Bonen and Belcastro (1976) studied 6 individuals recovery, with respect to lactate clearance, between three different recovery methods. Their findings showed an increase clearance of lactate when the 6 individuals performed an active recovery compared to no recovery, or passive recovery.

The 1-minute active recovery at a low-intensity in between each working stage allows for the increased clearance of lactate from the muscle and/or the conversion of lactate to pyruvate in the muscle. The conversion of lactate to pyruvate makes it readily available for aerobic metabolism. The increased blood flow to the working muscle allows the delivery of oxygen to continue aerobic metabolism at the lower intensities, which also promotes the re-synthesis of PCr. These variables coupled with the increased sodium-potassium pump subunits are assumed to be key variables in the achievement of a higher intensity during the intermittent protocol.

Plateau Prevalence during Intermittent Verification vs. Intermittent Protocol

Table 7 displays the attained values from the last 30 seconds of the completed stage from the intermittent protocol and the last completed 30 seconds of the verification phase. During the intermittent protocol plateau criteria was satisfied in 10 of the 11 participants with an increase of less than 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ oxygen uptake with a 2.5% grade increase (Taylor et al., 1955). Two of the participants achieved values much lower than the last completed stage of the intermittent protocol because they were unable to complete more than 1-minute of the verification phase. Of the 9 participants that were able to complete the verification phase, only two achieved values higher than the maximum oxygen uptake measured at the end of the intermittent protocol. One of these participants was on $0.7 \mathrm{ml \cdot kg^{-1} \cdot min^{-1}}$ higher, and the other was $1.9 \mathrm{ml \cdot kg^{-1} \cdot min^{-1}}$. Both of these participants are within the plateau criteria of $2.1 \mathrm{ml \cdot kg^{-1} \cdot min^{-1}}$ (Taylor et al., 1955). Most of the participants were unable to achieve the same value as they had achieved at the last stage of the intermittent protocol.

Reasoning for this lies in the oxygen kinetic hypotheses. Xu and Rhodes (1999) stated the two hypotheses as, one suggesting that the rate of the increase in oxygen uptake at the onset of exercise (i.e. verification phase after ten minutes of walking) is limited by the capacity of oxygen delivery to the active muscle. The other hypothesis suggests that the ability of the oxygen utilization in exercising muscle (i.e. during the last stage of the intermittent protocol) acts as the rate-limiting step. Therefore, by the verification phase only being two minutes in duration, and the participants returning to near resting values after 10 minutes of slow walking, the oxygen kinetics during the verification phase were not fast enough to display the same values as the end of the intermittent protocol. The active recovery serves as an ideal time for lactate clearance from the muscle into the bloodstream, but with the low-intensity walking it is hypothesized that there was not total clearance of the lactate from the blood. As a result, increases in blood lactate levels, accompanied with elevation in body temperature, and increased ventilatory work have been shown to increase the magnitude of the slow component (Xu and Rhodes, 1999).

Summary

The hypothesis that an intermittent graded exercise test compared to a continuous graded exercise test can more accurately measure maximal heart rate and VO_2 among intermittently trained athletes is supported in the present study. The increased ability to clear the interstitial potassium by means of the increased Na^+, K^+ pump subunits is believed to play a key role in the achievement of a higher intensity during the intermittent protocol. Performance increases are also due to increased efficiency in lactate clearance, re-synthesis of energy substrates, as well as, regulation of energy metabolism byproducts (i.e. hydronium ions, lactic acid, etc.) Further exploration of interstitial potassium accumulation, blood lactate levels, and electromyography muscle readings during maximal intensities for both protocols is necessary to defend the present study.

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APPENDIX A

Research with Human Subjects Protocol Form

Barry University

Research with Human Participants Protocol Form

PROJECT INFORMATION

1. **Title of Project**

Continuous vs intermittent VO_2max protocol in soccer players

2. **Principal Investigator** (please type or print)

Ryan Alexander
Master's Degree student in Movement Science
423-202-4096
Ryan.alexander@mymail.barry.edu

NOTE: You ***WILL NOT*** *receive any notification regarding the status of your proposal unless accurate and complete contact information is provided at the time the proposal is submitted.*

3. **Faculty Sponsor** (If Applicable)

Constance M. Mier, PhD
Department: HPLS, Dept SES
305-899-3573
cmier@mail.barry.edu

Faculty Sponsor Signature:______________________ Date: ____________

4. **Is an IRB Member on your Dissertation Committee?** **Yes _X No: ________**

5. **Funding Agency or Research Sponsor**
(Name, Address)

6. **Proposed Project Dates**
Start ___September 1, 2010__
End ___January 1, 2011__

Note: It is appropriate to begin your research project (i.e., the data collection process) only *after* you have been granted approval by this board. Proposals that list starting dates occurring before the date of submission will be returned without review. Please allow time for approval when determining your start date.

Please Provide the Information Requested Below

A. Project activity STATUS is: (Check one of the following three as appropriate.)

X **NEW PROJECT**
___ **PERIODIC REVIEW ON CONTINUING PROJECT**
___ **PROCEDURAL REVISION TO PREVIOUSLY APPROVED PROJECT**
(Please indicate in the **PROTOCOL** section the way in which the project has been revised.

B. This project involves the use of an **INVESTIGATIONAL NEW DRUG (IND) OR AN APPROVED DRUG FOR AN UNAPPROVED USE** in or on human participants.

___ YES X NO

Drug name, IND number and company:

____

C. This project involves the use of an **INVESTIGATIONAL MEDICAL DEVICE (IMD)** or an **APPROVED MEDICAL DEVICE FOR AN UNAPPROVED USE.**

___ YES X NO

D. This project involves the use of **RADIATION** or **RADIOISOTOPES** in or on human participants.

___ YES X NO

E. This project involves the use of Barry University students as participants. (If any students are minors, please indicate this as well.)

_X_ YES Barry Students will be participants (Will minors be included? _ YES X NO)
___ NO Barry Students will participate

F. **HUMAN PARTICIPANTS** from the following population(s) would be involved in this study:

___ Minors (under age 18)
___ Abortuses
___ Prisoners
___ Mentally Disabled
___ Other institutionalized persons (specify)
___ Other (specify)
___ Fetuses
___ Pregnant Women
___ Mentally Retarded

____

G. Total Number of Participants to be Studied: _30_

Description of Project

1. **Abstract** (200 words or less)

The gold standard of testing an individual's aerobic capacity (VO_2 max) has been for many years, the graded exercise test on a treadmill in a laboratory (that is excluding cyclists, rowers, and swimmers). The classic continuous graded exercise test is short in duration (i.e. 8 to 12 minutes) and can assure the researcher with a high degree of confidence that the participant was not restricted due to muscle fatigue and that a VO_2max plateau can be achieved. However, the fact that a low prevalence of a plateau during continuous graded exercise protocols among athletes has been reported, it is possible that the continuous protocol due to the high levels of work rates achieved by the athletes, is limited by anaerobic capacities rather than the cardiorespiratory capacity. The intermittent nature of a soccer match does not emphasize the same energy demands as a continuous protocol. Therefore, a discontinuous graded exercise protocol maybe a more effective means of measuring true VO_2max in soccer players.

2. Recruitment Procedures

Describe the selection of participants and methods of recruitment, including recruitment letter if applicable. (**NOTE**: If the investigator has access to participants by virtue of his or her position within the study setting, please provide a brief description of such access.)

All participants in this study will be recruited from the Barry University collegiate men's and women's soccer teams. The primary investigator will arrange a time with each coach to meet with each team. During the meeting, the primary investigator will describe the study protocol and hand out consent forms. Athletes who are younger than 18 years at the time of the meeting will be told that they are not eligible to participant and will be asked to not sign the consent form. However, it is possible that a few athletes will turn 18 during the study. The primary investigator will let athletes know that they may contact him once they are 18 yrs of age if they wish to participate in the study.

The team coaches will not be involved with recruitment for this study and will be asked to not be present when the primary investigator meets with the team. Athletes will be asked to read the consent form during the meeting. After reading the consent forms, athletes will be asked to sign the form if interested in participating. The primary investigator will ask those that signed the consent form to write their name legibly next to the signature so that the participant can be identified by the primary investigator. If an athlete prefers to not participate, he or she will hand back the consent form without a signature, but will be asked to write "no" where the signature is normally placed. This way, all athletes will write on the consent form to avoid revealing who is signing up and who is not.

Via email, the primary investigator will contact each of the athletes that provided a signed consent form to schedule the first visit to the lab.

3. Methods

Describe procedures to which humans will be subjected. Include a description of deceptive techniques, if used, and debriefing procedures to be used on completion of the study. Use additional pages, if necessary.

Participants will be asked to visit the human performance laboratory on three separate occasions, preferably within a 1-2 week period. Visits will be separated by at least one full day and days two and three will be randomly ordered.

Day 1: familiarization and practice test; approximate time 60 minutes
Day 2: VO_2max test #1 (discontinuous or continuous), approximate time 60 minutes
Day 3: VO_2max test #2(discontinuous or continuous), approximate time 60 minutes

On day one, a practice test will be performed during which the participant will become acquainted with the treadmill and the mouthpiece attachments that are used during the actual tests. The participant will run on the treadmill with full gear (see photo below) for about 5 to 10 minutes.

Measuring oxygen uptake during a treadmill test

On days two and three, tests for VO_2max will be performed. VO_2max is a measure of maximal oxygen uptake or aerobic power. To measure it, the participant runs on a treadmill until exhaustion during a graded exercise test (GXT). The test begins at a relatively moderate running pace and intensity is increased every 2 minutes by adjusting the grade (incline) by 2.5% until the participant can no longer run due to fatigue. Approximately 4 to 6 stages are performed during a typical maximal GXT. For these athletes, typical running pace is 5-7 mph and maximal grades achieved can be 4-12%. These values are estimated from the several hundred VO_2max tests performed by the PI and faculty sponsor over the years. A 5-min warm up period at a level of walking and light jogging is provided before beginning the test.

During the test, continuous measurement of heart rate (the participant will be wearing a heart rate monitor consisting of a chest strap as seen in the photo above) and oxygen uptake will be measured (oxygen uptake is measured by the metabolic analyzer system). For oxygen uptake measures the participant breaths room air through a two-way breathing valve that is connected to an expired gas hose. Expired gases are continuously collected and volume and percent of oxygen and carbon dioxide are measured. The test is terminated when the participant signals that he or she is finished or if he or she removes their feet from the treadmill while grabbing hold of the handle bars. At this point, the treadmill grade is decreased to 0% and the speed is reduced to a slow walking pace for recovery.

Two protocols for VO_2max will be performed on separate days, in random order. The continuous protocol will be nonstop, with 2-min stages increasing in intensity as described above. The total time of the test should be between 8 to 12 minutes. The discontinuous protocol will be identical to the continuous protocol regarding running speed and grade increases; however, a 1-min active recovery will take place between the 2-min stages. During this active recovery, the participant will be jogging at a light pace. The discontinuous protocol will last approximately 3 to 5 minutes longer than the continuous because of the 1-min rest periods.

Following each protocol, the participant will be asked to recover for 10 minutes on the treadmill by continuing to walk. Following the recovery period, the treadmill speed and grade will be increased to the same intensity as the last completed stage of the protocol. This intensity will be run for 60 seconds. After 60 seconds the intensity will be increased to the next stage and the participant will again run until volitional exhaustion to complete this verification stage. The verification stage allows the investigator to determine whether or not a "true" VO_2max has been obtained.

Participants will be asked to schedule their tests on a day that follows a relatively light to moderate training day. Further, testing will be performed at a time of day that the participant will not have engaged in training for at least 12-24 hr. For instance, if a participant engaged in a training session during the afternoon hours of Monday, testing will be performed no earlier than late morning hours on Tuesday. This provides the athlete sufficient recovery and avoids undue fatigue during the tests and during subsequent training that might follow later in the day. These tests are much like the intensity an athlete engages in on a regular basis during a normal training session and therefore, will not create abnormal stress and fatigue on the athlete and will not interfere with his or her performance during the training sessions.

Participants will also be asked to eat a light meal 2-3 hr prior to each test in order to avoid a full stomach and a fasted state. If an athlete is experiencing cold or flu symptoms or a musculoskeletal injury or pain, tests will be delayed until the athlete is in good health and can perform the tests without undue stress.

4. Alternative Procedures

Describe alternatives available to participants. One alternative may be for the individual to withhold participation.

Other than not choosing to not participate entirely, there are no alternatives

5. Benefits

Describe benefits to the individual and/or society.

We hope to gain insight into aerobic performance among soccer players and determine an appropriate measure for aerobic capacity in these athletes. Possible direct benefits to the participant may be the personal knowledge gained concerning one's performance.

6. Risks

Describe risks to the participant and precautions that will be taken to minimize them. Include physical, psychological, and social risks.

The risks involved with the VO_2max tests are relatively small for this particular population. The participant may experience temporary fatigue and mild muscle soreness immediately following the test, common for maximal exercise. To minimize any soreness that may result, we ask the participant to not engage in vigorous physical activity at least 12 hrs prior to the test. We have performed over 700 VO_2max tests in the 12 years that Dr. Mier has worked in the human performance laboratory and prior to that, she performed several hundred tests in other locations. The PI has performed over 200 of these tests. Among these tests, there have been no incidences of injury, or any cardiac or pulmonary distress. All tests will be supervised by Dr. Mier.

Vigorous exercise such as the VO_2max tests acutely and transiently increases the risk of sudden cardiac death and acute myocardial infarction. However, exercise only provokes cardiovascular events in individuals with preexisting heart disease. Among individuals younger than 35 yr, the risk of sudden death during exercise is low because the prevalence of occult disease is low. The absolute incidence of death during or immediately following sports participation among United States high school and college athletes has been estimated as one death per year for every 769,000 women. This is an overestimation of the incidence of cardiac events because only 74% of these deaths were the result of cardiac disease.

In observance of this risk, although very minimal, there is an automated external defibrillator accessible in the laboratory. Both the primary investigator and faculty sponsor are certified AED/CPR first responders and emergency medical services can be contacted readily if needed.

7. Anonymity/Confidentiality

Describe methods to be used to ensure the confidentiality of data obtained.

Signed informed consent forms will be kept separate from the data in the faculty sponsor's office. Data sheets for each participant will be coded and will not contain the participant's name or any other identifying characteristic (such as phone number). Only the PI and faculty sponsor will have access to these data. Data will be kept in the PI's personal files at home and also in the faculty sponsor's office. All data and informed consents will be kept indefinitely. These procedures will assure full confidentiality.

8. Consent

Attach a copy of the consent form(s) to be signed by the participant and/or any statements to be read to the participant or informational letter to be directed to the participant. **(A copy of the consent form should be offered to each participant.)** If this is an anonymous study, attach a cover letter in place of a consent form.

9. Certification

I certify that the protocol and method of obtaining informed consent as approved by the Institutional Review Board (IRB) will be followed during the period covered by this research project. Any future changes will be submitted to IRB review and approval prior to implementation. I will prepare a summary of the project results annually, to include identification of adverse effects occurring to human participants in this study. I have consulted with the department or program faculty/administrators and the Dean of the school which is to be the subject of research and have received prior approval to conduct the research and/or to disseminate the results of the study. A copy of that approval has been included with this protocol.

________________________________ ____________________

Principal Investigator *Date*

***Reminder*: Be sure to submit fifteen (15) individually collated and bound (i.e. stapled or paper clipped) copies of this form with your application.**

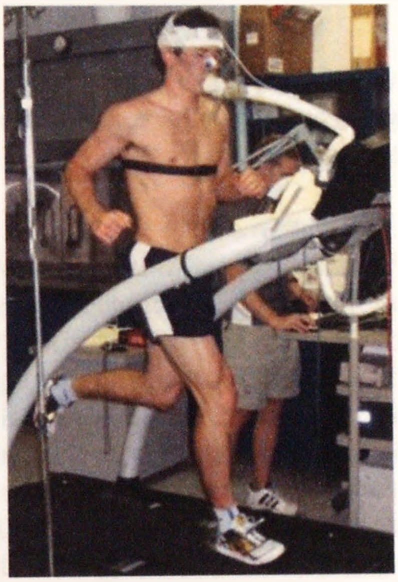
APPENDIX B

Informed Consent Form

Barry University
Informed Consent Form

Your participation in a research project is requested. The title of the study is "Continuous vs Intermittent VO_2max protocol in soccer players". The research is being conducted by Ryan Alexander, a graduate student in the Movement Science with a specialization in Exercise Science Master's degree program in the Department of Sport and Exercise Sciences, Barry University. The aim of the research is to determine the effectiveness of a discontinuous treadmill running protocol on measuring soccer player's aerobic capacity (VO_2max). We anticipate the number of participants to be 30.

For this study, you will be asked to visit the human performance laboratory on the campus of Barry University in Miami Shores, Florida on three separate days. During the first visit, you will be asked to perform a practice test on the treadmill to become acquainted with the VO_2max test protocol. While running for about 5 to 10 minutes, you will breathe through an apparatus, as shown in the photo. Using a mouthpiece, you breathe room air through a two-way breathing valve that is connected to an expired gas hose. In no way does this hinder your ability to breath. This visit should last no more than 60 minutes.



During the next two visits, you will be asked to run on a treadmill until exhaustion. The test protocol begins at a relatively moderate running pace and the intensity is increased every 2 minutes by adjusting the incline until you can no longer run due to fatigue. Approximately 4 to 6 stages are performed with a 5-min warm up period (walking and light jog) prior to the test. During the test, continuous measurements of heart rate (you will be wearing a heart rate monitor consisting of a chest strap as shown in photo above) and oxygen uptake will be performed. For oxygen uptake measures you breathe room air through the apparatus described above. A nose clip is placed on your nose so that you will be breathing only through your mouth during the entire test. This is normal during exercise and will not hinder your ability to exercise. The test is terminated when you signal that you are finished or you remove your feet from the treadmill while grabbing hold of the handle bars. Prepping, warm-up and the actual test requires approximately 60 minutes during each of the two visits.

During one visit you will perform the test as a continuous protocol that is nonstop, with 2-min stages increasing in intensity as described above. The total time of the test should be between 8 to 12 minutes. During the other visit, the protocol will be discontinuous. This protocol is identical to the continuous protocol regarding running speed and incline; however, a 1-min active recovery will take place between the 2-min stages. During this active recovery, you will be jogging at a light pace. The discontinuous protocol lasts about 3 to 5 minutes longer than the continuous protocol. You may perform the continuous protocol first or last, but you will be told ahead of time the order of the tests.

Following each protocol, you will be asked to recover for 10 minutes on the treadmill by continuing to walk. Following the recovery period, the treadmill speed and grade will be increased to the same intensity as the last completed stage of the protocol. This intensity will be run for 60 seconds. After 60 seconds the intensity will be increased to the next stage and you will again run until volitional exhaustion to complete this verification stage.

Your consent to be a research participant is strictly voluntary and should you decline to participate, should you choose to perform only some of the tests or drop out at any time during the study, there will be no adverse effects on your status as a Barry University student or athlete.

The risks of involvement in this study are minimized as much as possible and all exercise tests are performed by experienced technicians. You will feel temporary fatigue and mild muscle soreness, common for maximal exercise. To minimize any soreness that may result, we ask that you not engage in physical activity at least 12 hrs prior to the test. The intensity of exercise required for these tests are not unlike what you are use to performing on a regular basis for your sport. However, there is an increased risk of sudden cardiac death and acute myocardial infarction when performing such exercise. But, these events occur only in individuals with heart disease. Among individuals younger than 35 yr such as you, the risk of sudden death during exercise is low because the prevalence of disease is low. Further, because you engage in regular exercise as an athlete, the risk is even lower than in a non-athlete population. Incidence of death during or immediately following sports participation among high school and college athletes has been estimated as one death per year for every 769,000 women. This is an overestimation of the incidence of cardiac events because only 74% of these deaths were the result of cardiac disease. Thus, these exercise tests present very little risk to you. Both the primary investigator and faculty sponsor are certified AED/CPR first responders and emergency medical services can be contacted readily if needed.

Your participation in this study will help provide useful information for developing effective methods for testing aerobic capacity in soccer athletes. Possible direct benefits to you may be the personal knowledge gained concerning your performance.

As a research participant, information you provide will be held in confidence to the extent permitted by law. Only the investigator and faculty sponsor will have access to your information. Any published results of the research will refer to group averages only and no names will be used in the study. Data will be kept in a locked file in the faculty sponsor's office and the investigator's personal home files. Your signed consent form will be kept separate from the data in the faculty sponsor's office.

If you have any questions or concerns regarding the study or your participation in the study, you may contact me, Ryan Alexander at 423-202-4096 or the faculty sponsor, Dr. Constance Mier, at (305) 899-3573 or the Institutional Review Board point of contact, Barbara Cook, at (305)899-3020. If you are satisfied with the information provided and are willing to participate in this research, please signify your consent by signing this consent form.

Voluntary Consent
I acknowledge that I have been informed of the nature and purposes of this experiment by Dr. Mier and that I have read and understand the information presented above, and that I have received a copy of this form for my records. I give my voluntary consent to participate in this experiment.

______________________ __________

Signature of Participant *Date*

______________________ __________ ______________________

Researcher *Date* *Witness* *Date*

(Witness signature is required only if research involves pregnant women, children, other vulnerable populations, or if more than minimal risk is present.)

Appendix C

Manuscript

ABSTRACT

The purpose of the present study was to determine intermittent graded exercise results in a higher stage intensity and higher maximal heart rate, minute ventilation, respiratory exchange ratio, and oxygen uptake compared to continuous graded exercise. The hypotheses were that all participants would achieve higher stage intensity, with respect to grade reach during the intermittent protocol; all participants would achieve a higher maximum heart rate, respiratory exchange ratio, and minute ventilation; as well as, all participants would achieve a higher relative VO_2max during the intermittent protocol. The participants completed two separate protocols on different days separated by at least 48 hours. The continuous protocol was a GXT a ramp progression of 2.5% with every stage intensity increase. The intermittent protocol followed the same intensity progression, with the implementation of a 1-minute active recovery stage between each working stage. Eight VO_2max achieved during the intermittent protocol was significantly higher ($p < 0.05$) compared to the continuous protocol (57.7 ± 5.8 ml·kg^{-1}·min^{-1} vs. 55.7 ± 5.7 ml·kg^{-1}·min^{-1}). There was also a significant increase ($p < 0.05$) in the maximum heart rate achieve during the intermittent protocol compared to the continuous protocol (190 ± 6 bpm vs. 186 ± 6 bpm). There was no significant difference ($p > 0.05$) between the minute ventilation or respiratory exchange ratio values of the intermittent and continuous protocols. We concluded that the classic continuous graded exercise test does not effectively allow for the achievement of soccer player's true maximum oxygen uptake. The intermittent nature of soccer player's training produces adaptations such as increased sodium-potassium pump subunits, increased efficiency of lactate clearance, and re-synthesis of PCr and other energy substrates that contribute to increased performance during the intermittent protocol, which allows for the achievement of the player's maximum oxygen uptake.

INTRODUCTION

Soccer is characterized as a high-intensity sport that combines intermittent and random bouts of anaerobic and aerobic activities such as jogging, shuffling, short sprints, rapid acceleration and deceleration, turning, jumping, kicking, and tackling (Al Hazza et al., 2006; Wisloff, Helgerud, & Hoff 1998; Ekblom, 1996; Kirkendall, 1985; Bloomfield, 2006). Fitness coaches of elite soccer clubs profile individual players to determine the best training needed for success in competition. By the nature of the sport, profiling soccer players can include a myriad of performance factors from technical/biomechanical, tactical, and physiological (Stolen, Chamari, Castagna, & Wisloff, 2005).

Fitness testing is important for a number of reasons, including assessment of an athlete's current level of fitness, evaluation of a training program's effectiveness, and the development of optimal training sessions and programs to address an individual athlete's strengths and weaknesses (Sayers, Sayers, & Brinkley, 2008). The majority of studies concerning soccer fitness testing have concentrated on the relationship between match-play success of individuals or teams, and specific performance variables that can either be tested in a lab or field setting (Arnason et al, 2004). Maximal oxygen uptake (VO_2max) quantifies the aerobic capacity of an individual and is an important performance indicator in soccer (Da Silva, Bloomfield, & Marins, 2008). It is often tested to evaluate the success of an individual player or a team. For instance, higher pre-season VO_2max values among an Icelandic soccer league were associated with finishing the regular season higher in the standing (Arnason et al. 2004).

There are a variety of different protocols used to measure athletes' VO_2max (Arnason et al., 2004; Bangsbo, Mohr, & Krustrup, 2006; Grant et al., 1995; Krustrup & Bangsbo, 2001). The most common method of measuring VO_2max is the graded treadmill exercise test (GXT). GXT protocols can vary in magnitude of increments and stage durations depending upon the individual or population being tested and the purpose of the test. Most GXT protocols begin with a designated warm-up period of three to five minutes. Depending on the purpose of the test, the first stage of the GXT is usually the same as or slightly higher than the warm-up intensity which estimates the intensity for the participant at approximately 60-70% VO_2max. Duration of each stage is typically one to three minutes. Each successfully completed stage is followed by an increase in speed or grade until the participant self-terminates the test due to volitional exhaustion.

A common VO_2 max criterion is the attainment of a VO_2max plateau, or a lack of increase in VO_2 with an increase in intensity (Midgely and Carroll, 2009; Howley, 1995). The continuous protocol has traditionally been the protocol used to measure VO_2max because of its reliability and reported high incidence of plateau achievement. However, low frequency of plateau achievement has been reported among athletes (Niemela et al., 1980; Lucia et al., 2006; Doherty et al., 2003; St. Clair Gibson et al., 1999). This is an interesting phenomenon among highly motivated individuals suggesting that true VO_2max may not have been achieved among those not reaching a plateau. If this is the case then it appears that these athletes' ability to reach true VO_2max was limited physiologically in a non oxygen-dependent manner during a continuous protocol.

The continuous protocol increases the intensity to volitional exhaustion in a linear manner that does not replicate the intermittent nature of a competitive soccer match. By utilizing a continuous VO_2max protocol, the researcher is eliminating the importance of an athlete's ability to recover from short, high-intensity exercise. It is possible that a continuous protocol, because of the absence of recovery periods; limits the soccer athlete's ability to achieve a high enough intensity that elicits a VO_2max.

The purpose of the present study is to: determine the difference in aerobic capacity (maximum oxygen uptake) measurements between a continuous and discontinuous GXT protocol performed by soccer players; and to determine whether a true VO_2max is achieved during the discontinuous protocol.

METHODS

Participants

The eleven collegiate soccer players were all participating in championship season training selected during the study. All subjects in the study reported at least twelve years of competitive soccer experience at the amateur level. Prior to the testing all soccer players trained for three consecutive months without interruption. Current training patterns include three to five skill/team sessions per week and two or three resistance training sessions. All participants participate in approximately 20 hours per week of intermittent soccer related activities. Training focused on intermittent, match-like simulations, skill sessions, resistance training, and conditioning. The skill and team training averaged eight to ten hours per week during the season period. Any additional aerobic training or sprint work was considered voluntary and was not able to be documented for the purpose of this study. Conditioning protocols included, but were not limited to, high-intensity repetitive bouts of sprinting with active recovery or interval training. Continuous aerobic running sessions were reserved for post-match recovery training days, and were limited to less than one time per week. All participants were active in resistance training programs approximately 2-3 times per week, each session lasting 1-2 hours.

At the onset of the study the 11 soccer players convened for an informational meeting to learn about the exercise protocols. Each participant read and signed a written informed consent approved by Barry University's Internal Review Board prior to participation. At this meeting, participants were instructed to not consume a large meal within three hours before any of the test sessions, as well as to not consume any beverage with measurable amounts of caffeine for at least eight hours before any of the testing protocols (Bangsbo, Mohr, & Krustrup, 2006). Other guidelines for the participants was to maintain a daily diet that is high in carbohydrates to make sure that all subjects have maximized their glycogen stores at test time, and all participants were directed to abstain from any drug or alcohol consumption the week prior to testing. At the conclusion of the informational portion of the meeting each participant completed a sub-maximal treadmill protocol to familiarize them with the equipment.

The sub-max protocol consisted of the participant be outfitted with a Hans Rudolph two-way breathing valve apparatus. The subject completed two to three stages at increasing intensities perceived to be a 14-15 on the RPE (Rate of Perceived Exertion) Borg Scale. VO_2max and heart rate measures were not taken during this protocol; this was utilized to familiarize the subjects with the headgear, mask, and changes of intensity during the protocol.

Maximal Oxygen Uptake (VO_2 max) Testing Procedures

All subjects active in this experiment were familiar with the testing protocols to insure stable and reliable results. All assessments were executed at a similar time of day to maintain consistency for all participants during protocols. The laboratory graded exercise tests were executed on an electronic treadmill (Quinton Med-Track SR60). Upon arrival to the laboratory body weight and height were measured. Body weight was measured with a calibrated SECA electronic scale (± 0.1 kg), and height was measured with the SECA wall-mounted measurement ruler (± 0.1 cm). Each participant then executed a five-minute warm-up run on the treadmill at seventy-five percent of the participant's maximal perceived intensity (approximately 13 or 14 on the Perceived Exertion (RPE) Borg Scale).

After the completion of the warm-up each participant was fitted with a Hans Rudolph two-way valve as described above. A Polar T-31 electrode heart monitor was then fitted to the participant's chest to obtain heart rate readings during the testing protocol.

VO_2max was measured continuously during each stage, using open circuit spirometry (Parvomedics TrueOne 2400 Metabolic Measurement Gas Analyzer, Sandy, Utah). Expired gases were collected and analyzed for volume and fractional oxygen and carbon dioxide. Calibration measurements were performed prior to each test. A two-point calibration of gases was performed with an exact compound mixture (16.01% Oxygen, 4.01% Carbon Dioxide, BAL% Nitrogen) and room air. A 3-L syringe was used for the flow-meter calibration. Data was collected continuously for VO_2, ventilation (VE) and respiratory exchange ratio (RER). All values were retrieved in breath-by-breath measurements.

When the participant was ready to begin testing, the speed was raised to the pre-determined 65% to 75% percent intensity and the protocol began. For the continuous protocol speed was maintained and the percent grade was increased 2.5% every two minutes. This test was terminated when the participant reached volitional exhaustion.

For the intermittent protocol a 1-min active recovery period between each 2-min work stage was included during which the speed was decreased to 4 mph, and grade returned to zero. After the completion of the active recovery stage, the speed was returned to the seventy-five percent intensity speed and the grade was then increased 2.5% from the last stage. This pattern is followed until volitional exhaustion. At the end of the test, the speed is decreased to 2.5 mph without grade. This active recovery intensity was maintained for 10-minutes. With the completion of the 10-minute active recovery the treadmill was raised to one stage intensity higher than the last completed stage of the protocol. The participant ran at this intensity for two-minutes to complete the verification phase. All oxygen uptake values were measured by breath-by-breath analysis through the Parvomedics system. The criteria utilized for the determination of plateau during the verification phase was an increase of less than $2.1 ml \cdot kg^{-1} \cdot min^{-1}$ with an increase in stage intensity (Taylor et al., 1955).

Statistical Analyses

Analyses of data were completed using the Statistical Package for the Social Sciences (SPSS), version 16 for Windows. Statistical significance was set at $p \leq 0.05$ and data were described as mean ± standard deviation. For the VO_2 max tests paired samples t-test was applied to determine the significant difference between the continuous protocol, intermittent protocol, and verification phase for each protocol. Dependent variables explored were VO_2max, maximum heart rate, RER, and maximum ventilation values. Maximum values for each dependent variable are determined by taking the average of the last two 15-second values in the final stage of the test protocol, whether the stage was completed or not.

RESULTS

The purpose of the present study is to determine if the implementation of a 1-minute active recover stage in the intermittent graded exercise test protocol better replicates soccer training, and results in a higher stage intensity being reached, as well as, higher heart rate, minute ventilation, respiratory exchange ratio, and maximum oxygen uptake. It was predicted that the intermittent protocol was allow the participants to achieve a higher stage intensity, as well as, achieve higher maximum heart rate, minute ventilation, respiratory exchange ratio, and maximum oxygen uptake.

Descriptive Data

There were eleven participants that completed both protocols. All participants were able to complete both of their protocols at the same time of day, and all protocols were executed between noon and five o'clock in the afternoon to assure consistency in the participants eating habits prior to the protocols.

Table 1. Descriptive characteristics of participants.

	Men	Women	Total
N	6	5	11
Age	21.8 ± 1.7	20.6 ± 2.0	21.3 ± 1.9
Height, cm	180.3 ± 7.3	164.9 ± 6.4	173.3 ± 10.3
Body mass, kg	74.8 ± 10.0	60.5 ± 6.1	68.3 ± 34.9

Continuous VO_2max Protocol and Intermittent VO_2max Protocol

From the eleven participants that were able to complete both protocols, 8 of the 11 achieved higher oxygen uptake values during the intermittent protocol. From those 8 participants, 7 of them achieved values outside of the plateau criteria range. There were similar values measured for ventilation and respiratory exchange ratio, but there was a significant difference in the total maximum heart rate average of the eleven participants. All but three participants achieved a higher heart rate and minute ventilation during the intermittent protocol

Table 2 displays the difference between the maximum oxygen uptake achieved during the continuous and intermittent protocols. 8 out of 11 participants achieved a higher VO_2max during the intermittent protocol compared to the continuous protocol. From those 8 participants, 7 had an increase that was outside of the 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ range to satisfy the plateau (Taylor et al., 1955). Furthermore, 6 out of the 8 achieved a value that was at least 5% higher than their measured VO_2max during the continuous protocol. Combined, the 11 participants displayed a 3.8% increase from their continuous maximum oxygen uptake ($p < 0.05$).

Table 2. Intermittent and Continuous VO$_2$max achieved

Participants	Continuous	Intermittent	Difference (%)
	VO$_2$	VO$_2$	
1	50.5	52.6 $^{\beta}$	4.2
2	49.3	52.5 $^{\beta}$	6.5
3	56.7	53.9	-4.9
4	47.9	51.4 $^{\beta}$	7.3
5	54	56	3.7
6	58.9	61.9 $^{\beta}$	5.1
7	51.2	54.5 $^{\beta}$	6.4
8	64.7	64.7	0.0
9	63.9	70.1 $^{\beta}$	9.7
10	55.7	58.5 $^{\beta}$	5.0
11	59.4	59	-0.7
Total	55.7 ± 5.7	57.7 ± 5.8**	3.8

Ventilation Comparison Continuous vs. Intermittent

Table 3 displays the ventilation values achieved during the continuous and intermittent protocols. There was no significant difference ($p > 0.05$) between the maximum minute ventilation values of the continuous and intermittent protocol.

Table 3. Max ventilation achieved during continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	100.7	110.4	9.6
2	110.9	119.4	7.7
3	144.8	145.8	0.7
4	105.3	106.1	0.8
5	109.6	109.0	- 0.5
6	193.5	185.5	- 4.1
7	140.3	160.7	14.5
8	127.7	128.8	0.9
9	163.8	162.0	- 1.1
10	128.2	136.1	6.2
11	180.8	176.0	- 2.7
Average	136.9 ± 31.3	140.0 ± 28.0	2.9

Table 4 displays the respiratory exchange ratio (RER) averages of the last 30-seconds of each protocol. There was no significant difference ($p > 0.05$) in the RER values of the continuous and intermittent protocols.

Table 4. Respiratory exchange ratio values for continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	1.17	1.17	0.0
2	1.11	1.13	-1.8
3	1.23	1.24	-0.8
4	1.27	1.12	11.8
5	1.18	1.16	1.7
6	1.15	1.04	9.6
7	1.21	1.14	5.8
8	1.08	1.11	-2.8
9	1.26	1.17	7.1
10	1.11	1.11	0.0
11	1.19	1.17	1.7
Average	1.17 ± 0.06	1.14 ± 0.05	2.9

Table 5 maximum heart rate values of the continuous and intermittent protocols. Although the average increase in heart rate was only 1.6% for the entire population there was an increase of at least 1.5% for 7 of the 11 participants. The 1.6% difference in maximum heart rate during the intermittent protocol equals an increase of 4 beats per minute higher. This was another significant difference ($p < 0.01$).

Table 5. Maximum heart rate values for continuous and intermittent protocol

Participants	Continuous	Intermittent	Difference (%)
1	180	181	0.6
2	192	199	3.6
3	188	192	2.1
4	201	201	0.0
5	188	192	2.1
6	182	186	2.2
7	191	190	-0.5
8	184	188	2.2
9	188	187	0.5
10	182	185	1.6
11	179	184	2.8
Average	186 ± 6	190 ± 6	1.6

Table 6 displays the values calculated from the last completed stage of the intermittent protocol and the last 30-seconds completed of the verification phase following the intermittent protocol. Four out of the five female participants were unable to achieve a higher value on the verification phase following the intermittent protocol. Five of the six male participants achieved a lower VO_{2max} value during the verification phase compared to the last completed stage of the intermittent protocol. The two participants that did achieve a higher value were within the 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ plateau criterion. The two participants that achieved 5.8 and 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ less than the intermittent completed approximately 60 seconds of the verification phase.

Table 6. VO_2max values for participants last completed stage of intermittent protocol and Verification Phase

Participants	Inter VO_2max	Verification VO_2max	Difference
Female			
1	52.6	50.8	-1.8
2	52.5	52.3	-0.1
3	53.9	52.2	-1.7
4	51.4	45.0	-6.3
5	56.0	56.8	0.7
Male			
1	61.9	56.0	-5.8
2	54.5	53.9	-0.3
3	64.7	66.5	1.9
4	70.1	67.4	-2.5
5	58.5	58.3	-0.1
6	59.0	58.8	-0.1
Total	57.7 ± 5.8	56.2 ± 6.6	-1.5 ± 2.6

VO_2max values are expressed as $ml \cdot kg^{-1} \cdot min^{-1}$.

DISCUSSION

The purpose of this study was to examine the incidence of VO_2max achievements in soccer players during a continuous and intermittent treadmill graded exercise test. There have been a number of previous studies that have examined the achievement of an individual's maximum oxygen uptake. The necessary criteria to determine a maximum effort and the incidence rate of a plateau at the completion of the classic continuous graded exercise test have been disputed for many decades. This study demonstrates that a continuous GXT protocol may limit some athlete's ability to achieve true VO_2max and that an intermittent protocol maybe more appropriate for intermittently trained athletes.

Hypothesis 1: A higher intensity will be achieved during the intermittent protocol compared to the continuous protocol. As a result, a higher heart rate, RER and minute ventilation will be achieved.

All participants on the intermittent protocol compared to the continuous protocol achieved higher intensity. Although there was a slightly higher average for the intermittent ventilatory response, there is no evidence that this was the leading factor in the attainment of a higher VO_2max. There was also no significant difference between the respiratory exchange ratio during the last completed stage of the continuous and intermittent protocol ($p > 0.05$). The RER was higher in the continuous protocol compared to the intermittent protocol. However, this did not result in a significant difference ($p > 0.05$) a higher maximal ventilation or RER for the continuous and the intermittent protocols. The maximum heart rate attained during the last minute of the completed stage of the intermittent protocol was significantly higher ($p < 0.05$) than during the continuous protocol. Higher heart rate is directly correlated with the higher intensity. Increased heart rate during the intermittent protocol is an attempt to maintain sufficient cardiac output at a higher intensity.

It is assumed that because of the intermittent protocol's active recovery stage that there is the opportunity for re-synthesis of PCr, muscle glycogen, and ATP. Therefore, the RER is not expected to be as high as a continuous exhaustive bout of increasing intensity exercise. The ventilation measures were not significantly different either because there is more time during the active recovery to maintain lower levels of CO_2 in the blood. Therefore the ventilation demands are not as high at specific intensities because there has been more time to expel CO_2 during the active recovery.

Of the eight participants that achieved a higher maximum oxygen uptake during the intermittent protocol compared to the continuous, six demonstrated an increase greater than the 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ plateau criterion. Of these participants only four achieved a higher heart rate, and average increase was less than 4 beats per minute. Similarly, 4 participants who achieved higher oxygen uptake values during the intermittent protocol also achieved a higher maximum ventilation value. In general, there were too many inconsistencies between the eleven participant's ventilation and maximum respiratory exchange ratios to conclude a significant difference between the two protocols. The participant's ability to achieve a higher maximum heart rate is hypothesized to aid in maintaining sufficient cardiac output at higher intensity to aid in the delivery of oxygen to the working muscle.

Unlike the Duncan, Howley, and Johnson (2007) study of 10 males, this study did show a significantly higher heart rate during the intermittent protocol than during the continuous protocol. The RER was, however, higher in the discontinuous protocol when compared to the continuous protocol for Duncan, Howley, and Johnson (2007). Duncan, Howley, and Johnson (2007) also did not show a significant difference in the ventilation measurements of either protocol (150.7 ± 16.0 vs. $149.5 \pm 17.5 \cdot min^{-1}$ BTPS). The participants in the Duncan, Howley, and Johnson study were recreationally active males who described their training as structured running, bicycling, and weightlifting, but reported nothing "intermittent-like" in their training. Also, the discontinuous protocol was spread across 2-3 days, and therefore was more like continuous protocol with the objective of reaching steady state at each progressive exercise intensity. The progression during the continuous protocol was a speed progression instead of an increase in grade like the present study. This means the reasoning for termination may also have been due to participant's inability to maintain stride length during a particular stage (Duncan, Howley, and Johnson, 2007). Due to the numerous differences in protocol and participant training status it is difficult to accurately compare the results. The specificity of the intermittent protocol in the present study, compared to Duncan, Howley, and Johnson's multiple day discontinuous protocol better emphasizes the training adaptations of soccer players.

Hypothesis 2: The intermittent protocol will elicit significantly larger relative VO_2 max values compared to the continuous protocol

During the intermittent protocol, compared to the continuous protocol, all soccer completed. 1 additional stage at a higher intensity. Although the mean duration of the intermittent protocol (16.26 ± 0.05 minutes) was 6.5 minutes longer than the continuous protocol (9.75 ± 0.05 minutes) soccer players were able to achieve a higher maximum oxygen uptake value, as previously hypothesized. The duration of both test still satisfied the time interval of Buchfurer et al. (1983) that the VO_2max was significantly higher on tests where the increment magnitude was large enough to induce test durations of 8-17 minutes. This is evidence that there are limitations in the progression of oxygen kinetics when performing the continuous protocol for intermittently trained athletes.

Intermittently trained participants (i.e. soccer players) present different physiological adaptations (Iaia et al., 2009; Bangsbo et al., 1992a; Balsom et al., 1999; Roberts et al., 1982; Shepley et al., 1992) to training that may affect their performance in a continuous protocol and result in early termination before the achievement of their maximum oxygen uptake. The model that states the limits of exercise are due to the cardiorespiratory system were first presented by Hill and Lupton (1923). Noakes (2000) has more recently presented four other likely models that explain the termination of exercise at maximal exercise according to one of the following models:

- The energy supply/energy depletion model
- The muscle power/muscle recruitment model
- The biomechanical model
- The psychological model

From the four models presented by Noakes it seems from the present study that the second of the four models is the most likely. The muscle power/muscle recruitment model states that it is the process in skeletal muscle recruitment, excitation and contraction that limits maximal exercise. More specifically, it is local muscle fatigue, including failure of sarcoplasmic reticulum calcium release (Allen et al., 2007), impaired sodium/potassium pump activity (McKenna et al., 2007), and slowed cross-bridge cycling (Fitts, 2008) that may directly limit the rate of muscle contraction and force production in the muscle that would hinder one's ability to exercise maximally.

Na^-, K^+ pump subunits

For the purpose of this study, soccer players are defined as speed endurance trained athletes. There are many physiological adaptations as a result of speed endurance training that have been documented (Iaia et al., 2009; Bangsbo et al., 1992a; Balsom et al., 1999; Roberts et al., 1982; Shepley et al., 1992). An important adaptation for prolonged performance may be the increase NA^+, K^+ pump subunits as a result of speed endurance training (Green et al., 2004). The increase in NA^+, K^+ pump subunits is believed to be the cause for the participant's achieving a higher work intensity, as well as, higher maximum oxygen uptake during the intermittent protocol.

There are many factors that are believed to contribute to fatigue during maximal exercise. Sarcolemmal depolarization due to extracellular K^+ accumulation has been suggested to be a primary contributor to fatigue development during maximal intensity exercise (Sejersted & Sjogaard, 2000). Potassium is an essential intracellular ion used to actively convert stored glucose into glycogen in the muscle. Thus, with increased exercise there is a significant potassium flux from the intracellular space of the working muscle (Sejersted & Sjogaard, 2000). This influx has the potential to decrease the membrane potential of the cell to half of its resting value. Sejersted and Sjogaard (2000) describe the flow of potassium as being directed out of the cell, resulting in an outward electro diffusion of K^+. This is normally opposed by K^+ uptake mediated by the Na^+-K^+ pump. Under certain circumstances, the net electro potential driving force for K^+ may be directed into the cell, which means that there may be an inward K^+ flux through the Na^+-K^+ pump. This hypothesis is based on observations that during maximal intensity exercise contracting muscles lose K^+ which progressively accumulates in the extracellular space of the cell (Juel, 1988; Cairns et al., 1995). Speed endurance training has shown to reduce interstitial K^+ accumulation (Nielsen et al., 2004) in response to an increase in the amount of Na^+, K^+ pumps (Clausen, 2003). Increase in Na^+, K^+ subunits allows for increase efficiency in K^+ clearance out of the cell resulting in the continued depolarization of the cell.

Without the intracellular build up of potassium the cell is able to continue depolarization and contraction of the working muscle. With respect to the present study, the continuous protocol was highlighted with lower VO_{2max} values possibly because the participants were being limited by the interstitial K^+ accumulation. With the implementation of the one-minute active recovery period between each working stage the soccer players were able to use the increased Na^+, K^+ pump subunits to reduce the interstitial K^+ concentration and allow for the continued depolarization of the working muscle.

There are other possible training adaptations that also contributed to the success of the participants achieving higher work intensity during the intermittent protocol. There are many physiological benefits to implementing the 1-minute recovery periods between each work stage. During these active recovery stages it is assumed that there is increased lactate clearance, as well as, some energy substrate (i.e. PCr and ATP) re-synthesis that will aid in the achievement of a higher intensity during the end of the protocol.

Effects of Recovery on Performance

The increased concentration of interstitial potassium during the continuous protocol is one assumed variable that causes the early termination of the soccer players VO_2max protocol before reaching their true maximum. The implementation of the 1-minute recovery stage in between each working stage is hypothesized to assist in the regulation of the interstitial potassium concentration, thereby, assisting in the continued depolarization of the muscle for contraction. Other variables hypothesized to play a role in the achievement of a higher intensity during the intermittent protocol are the re-synthesis of ATP and phosphocreatine (PCr) in the muscle and increased lactate clearance to the blood. Results from Bogdanis et al (1995) muscle biopsies at the end stages during repeated sprints tests on a cycle showed the re-synthesis effects of recovery on PCr in the muscle. After the first sprint the PCr and ATP contents were 19.7 ± 1.2% and 70.5 ± 6.5% of the resting values, respectively. During recovery, PCr increased rapidly to 65.0 ± 2.8% of rest after 1.5 min, but reached only 85.5 ± 3.5% of rest after 6 min of recovery. The population for the Bogdanis (1995) study was described as male cyclists and there was no indication of the nature of the participant's previous training.

A second investigation by Bogdanis et al. (1996) attempted to study the contribution of phosphocreatine and aerobic metabolism during multiple repeated bouts of sprint exercise. Eight male had muscle biopsies obtained before the sprints, immediately after the first sprint, 15 seconds before the second sprint, and after the second sprint. At the end of the first sprint the PCr level was 16.9 ± 1.4%. Fifteen seconds before the second sprint PCr re-synthesis had raised stores to 78.7 ± 3.3% of resting values. Most of the PCr during the second sprint was utilized during the first 10 seconds and remained unchanged until its completion. Bogdanis once again highlights the regeneration capabilities of the muscle during repeated bouts of high-intensity exercise.

Similar to the protocol that the current study explored, the cyclists experienced alternating high and low-intensity stages. The low intensity stages offered an opportunity to replenish PCr stores in the muscle, which would aid in the continued performance at the next higher intensity. Evident in both Bogdanis studies (1995 & 1996) the stores were never completely regenerated, but a re-synthesis of 40 – 80% of resting values is going to provide a significant contrast in energy metabolism at the next intensity. With respect to the current study, the active recovery between high-intensity work stages, allowed for increased blood flow to the working muscles. This consistent blood flow provided oxygenated blood to aid in aerobic metabolism during the low-intensity active recovery stages. Increased aerobic metabolism aids in the replenishing of PCr, which is going to aid in anaerobic metabolism at the next subsequent working stage.

Another important aspect of the active recovery during the intermittent protocol is the ability to shuttle the lactate from the muscle into the blood and/or buffer the lactate within the muscle to convert it into usable energy. The main metabolic pathway for lactate elimination is oxidation in the tricarboxylic acid to end products CO_2 and H_2O (Rontoyannis, 1988). It has been reported the approximately 23-27% of lactate is converted to glycogen during recovery (Bangsbo et al., 1991). Coffey et al (2004) studied running performance with respect to blood lactate concentrations. Coffey reported that there are positive effects of active recovery on the clearance of lactate, and also agreed that the increased blood flow, theoretically, should enhance lactate clearance from the muscle, as well as, aid with the delivery of oxygenated blood to the muscle for aerobic metabolism which aids in the conversion of lactate to pyruvate via lactate dehydrogenase enzyme. Bonen and Belcastro (1976) studied 6 individuals recovery, with respect to lactate clearance, between three different recovery methods. Their findings showed an increase clearance of lactate when the 6 individuals performed an active recovery compared to no recovery, or passive recovery.

The 1-minute active recovery at a low-intensity in between each working stage allows for the increased clearance of lactate from the muscle and/or the conversion of lactate to pyruvate in the muscle. The conversion of lactate to pyruvate makes it readily available for aerobic metabolism. The increased blood flow to the working muscle allows the delivery of oxygen to continue aerobic metabolism at the lower intensities, which also promotes the re-synthesis of PCr. These variables coupled with the increased sodium-potassium pump subunits are assumed to be key variables in the achievement of a higher intensity during the intermittent protocol.

Plateau Prevalence during Intermittent Verification vs. Intermittent Protocol

Table 7 displays the attained values from the last 30 seconds of the completed stage from the intermittent protocol and the last completed 30 seconds of the verification phase. During the intermittent protocol plateau criteria was satisfied in 10 of the 11 participants with an increase of less than 2.1 $ml\cdot kg^{-1}\cdot min^{-1}$ oxygen uptake with a 2.5% grade increase (Taylor et al., 1955). Two of the participants achieved values much lower than the last completed stage of the intermittent protocol because they were unable to complete more than 1-minute of the verification phase. Of the 9 participants that were able to complete the verification phase, only 2 achieved values higher than the maximum oxygen uptake measured at the end of the intermittent protocol. One of these participants was on 0.7$ml\cdot kg^{-1}\cdot min^{-1}$ higher, and the other was 1.9 $ml\cdot kg^{-1}\cdot min^{-1}$. Both of these participants are within the plateau criteria of 2.1 $ml\cdot kg^{-1}\cdot min^{-1}$ (Taylor et al., 1955). Most of the participants were unable to achieve the same value as they had achieved at the last stage of the intermittent protocol.

Reasoning for this lies in the oxygen kinetic hypotheses. Xu and Rhodes (1999) stated the two hypotheses as, one suggesting that the rate of the increase in oxygen uptake at the onset of exercise (i.e. verification phase after ten minutes of walking) is limited by the capacity of oxygen delivery to the active muscle. The other hypothesis suggests that the ability of the oxygen utilization in exercising muscle (i.e. during the last stage of the intermittent protocol) acts as the rate-limiting step. Therefore, by the verification phase only being two minutes in duration, and the participants returning to near resting values after 10 minutes of slow walking, the oxygen kinetics during the verification phase were not fast enough to display the same values as the end of the intermittent protocol. The active recovery serves as an ideal time for lactate clearance from the muscle into the bloodstream, but with the low-intensity walking it is hypothesized that there was not total clearance of the lactate from the blood. As a result, increases in blood lactate levels, accompanied with elevation in body temperature, and increased ventilatory work have been shown to increase the magnitude of the slow component (Xu and Rhodes, 1999).

Summary

The hypotheses that there are training adaptations in intermittently trained athletes that are restricted by a continuous graded exercise test are supported by the values achieved in the present study. There was a significant difference in maximum heart rate and maximum oxygen uptake achieved during the intermittent protocol compared to the continuous protocol. The increased ability to clear the interstitial potassium by means of the increased Na^+, K^+ pump subunits is believed to play a key role in the achievement of a higher intensity during the intermittent protocol. Performance increases are also due to increased efficiency in lactate clearance, re-synthesis of energy substrates, as well as, regulation of energy metabolism byproducts (i.e. hydronium ions, lactic acid, etc.)

Further exploration of interstitial potassium accumulation, blood lactate levels, and electromyography muscle readings during maximal intensities for both protocols is necessary to defend the present study.

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