EFFECTS OF FATIGUE INDUCED BY INTERMITTENT RUNNING ON
MUSCULAR STRENGTH, POWER, AND GLYCOGEN CONTENT IN FEMALE
SOCCER PLAYERS

by

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A majority of ACL injuries in female soccer players occur during the later stage of a game when fatigue is likely present. In a fatigued condition, reductions in the strength ratio of hamstrings to quadriceps and the lower extremity muscular strength and power can cause altered landing techniques that predispose female athletes to a higher risk of ACL injuries. Additionally, a significant reduction in the muscle glycogen content has been reported after a simulated soccer game. The current study investigates a role of the muscle glycogen content with knee strength and power in the presence of fatigue.

Seventeen female subjects participated in the study (age: 21.5±2.9yrs, height: 166.9±7.2cm, and weight: 63.7±6.6kg). Before and after an intermittent running protocol, subjects completed a battery of testing including maximal isokinetic knee flexion and extension muscular strength normalized to their body weight (%BW), a depth-jump onto a force plate to measure reactive strength index (RSI), and non-invasive ultrasound-based muscle glycogen content of six lower limb muscle groups. Based on normality, paired t-tests or Wilcoxon signed-rank test were performed to compare the strength, RSI, and muscle glycogen content pre- and post-fatigue. Additionally, correlation analyses were used to examine the relationships between the baseline muscle glycogen level and the changes (post/pre-fatigue values) in muscle glycogen content with the changes in muscular strength and power. Significance was set at $p<0.05$ a priori.
After the fatigue protocol, knee flexion strength, knee extension strength, and the flexion/extension strength ratio were significantly decreased while RSI was significantly increased. There were no significant differences in muscle glycogen content before and after the fatigue protocol (p>0.05). For correlational analyses, the baseline vastus medialis muscle glycogen content was significantly correlated in the positive direction with the changes in the changes in knee flexion strength and knee extension strength. There were no significant correlations in any other comparisons (p>0.05). A lack of significant findings on muscle glycogen content indicates potential limitations with the current noninvasive ultrasound-based system. Future studies should continue exploring methodologies to measure muscle glycogen content and its relationship with other neuromuscular characteristics during a fatigued condition.
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1.0 INTRODUCTION

Soccer is a sport characterized by intermittent running, causing increased physiological demands. Over the last 30 years, high school soccer participation has increased nearly 30-fold among girls. As a consequence of increased soccer participation, a high prevalence of musculoskeletal injuries have been reported. Particularly, female soccer players suffer anterior cruciate ligament (ACL) injuries up to six times higher rates than male soccer players. Previous studies have revealed that female athletes tend to have diminished knee proprioception, altered landing techniques with less knee flexion angle, and weaker muscular strength than male counterparts, predisposing them to a higher risk of ACL injuries. It is also reported that a majority of ACL injuries occur through a non-contact mechanism and during the later stage of a game when fatigue is most likely present. In a fatigued condition, it has been shown that hamstring strength decreases more than quadriceps strength, causing further muscular imbalance between two muscle groups. This disparity of the strength ratio of hamstrings to quadriceps in addition to decreased muscular strength and power caused by fatigue may contribute to altered landing techniques. Therefore, it is important to investigate a role of fatigue induced by intermittent running on muscular strength and power.

From the perspective of energy demands of soccer, the anaerobic energy systems and muscular glycogen are believed to be the most important systems and substrate for energy production, respectively. In fact, a 36-43% reduction in the muscular glycogen content in the
lower extremity in addition to a significant decline in sprint performance has been reported after a simulated soccer match.\textsuperscript{61, 96} Although it is important to understand a role of muscular glycogen content in relation to fatigue, there have been fewer studies have been conducted in the past, largely due to methodological limitations: invasive nature of muscle biopsy or a limited access to nuclear magnetic resonance (NMR) spectroscopy or magnetic resonance spectroscopy (MRS) to assess muscle glycogen content.\textsuperscript{61, 96} Recently, a new technique has been developed to utilize diagnostic musculoskeletal ultrasound imaging to estimate muscle glycogen content and validated by comparing ultrasound-based muscle glycogen content with muscle glycogen content of muscle biopsy before and after a steady-state cycling over 90 minutes.\textsuperscript{52, 82} Since few studies have included this technology (ultrasound-based muscle glycogen content assessment), basic research questions have not been addressed yet. The current investigation will help to answer some questions: 1) Is this technology sensitive enough to differentiate muscle glycogen content before and after an intermittent running fatigue protocol? 2) Does a decline in muscle glycogen content correlate with a reduction in knee strength and power? 3) Does the baseline muscle glycogen content correlate with the changes in knee strength and power after the fatigue protocol? Based on the outcomes from the current study, this ultrasound-based muscle glycogen measurement might be included in the future studies to examine a role of muscle glycogen content on musculoskeletal injuries, neuromuscular risk factors, nutrition, and performance in soccer.
Female athletes are at greater risk than male athletes in sustaining serious knee ligament injuries.\textsuperscript{1, 12, 37, 54, 74, 83, 95} A longitudinal study reported that per 1,000 collegiate athlete exposures, female soccer players had a significantly greater incidence of ACL ruptures at a rate of 0.32 than did male players at a rate of 0.12.\textsuperscript{76, 83} It is generally accepted that 70-84\% of all ACL injuries have a non-contact mechanism.\textsuperscript{60, 89} Additionally, non-contact ACL injuries have been reported to occur when athletes are under fatigued conditions; therefore, a fatigued state can be considered as a risk factor.\textsuperscript{23, 94} Risk factors are often categorized into two groups: intrinsic and extrinsic factors. Intrinsic risk factors include anatomical, hormonal, physiological, biomechanical, and neuromuscular characteristics.\textsuperscript{1} Extrinsic risk factors include intensity level of competition, footwear, playing surface, protective, weather, and referee involvement.\textsuperscript{95} Extrinsic factors may influence intrinsic factors and game specific skill involvements that include passing, shooting, dribbling, tacking, trapping, high speed running, and sprinting.\textsuperscript{27} For the purpose of this thesis, intrinsic neuromuscular risk factors and fatigue are investigated.

Several intrinsic neuromuscular risk factors for ACL injury have been identified.\textsuperscript{66, 99, 107} Female athletes have demonstrated high ground reaction forces, decreased proprioception, decreased knee flexion angles and decreased lower leg internal rotation when landing, increasing risk of injury by causing increased stress on the ACL.\textsuperscript{66} ACL injury may result as a combination of knee valgus, anterior tibial translation force, and decreased time to peak knee flexion, especially during a side-cut maneuver.\textsuperscript{1, 79} Another risk factor for injury is a decreased relative hamstring to quadriceps strength ratio, and female athletes have been shown to be quadriceps dominant, altering proper dissipation of high impact forces.\textsuperscript{66, 79} Hamstring to quadriceps
strength imbalance may be due to low hamstring strength and/or high quadriceps strength.\textsuperscript{13} Female athletes have demonstrated lower peak hamstring torque and decreased activation of the hamstrings during dynamic movements such as running, cross-cutting, and side cutting, and side-cutting compared to male athletes.\textsuperscript{20, 79} It is reported that the ability to stability joint may be compromised in a fatigued state.\textsuperscript{16, 105} Fatigue would likely contribute and exacerbate altered landing kinematics with decreased knee flexion angles, increased knee valgus angles, and increased hip abduction, contributing to a higher risk of ACL injury.\textsuperscript{1, 10, 12, 70, 74}

1.2 ENERGY DEMANDS AND SUBSTRATE UTILIZATION IN SOCCER

The energy systems that contribute to short duration sprinting common in team sports (2-3 seconds) are phosphocreatine (PCr) metabolism and anaerobic glycolysis.\textsuperscript{108} Phosphocreatine and adenosine triphosphate (ATP) are depleted with repeated sprinting, and have the capacity to partially resynthesize during recovery periods.\textsuperscript{7} There are contributions of PCr metabolism and anaerobic glycolysis in a soccer match, yet the activity profile relies more on aerobic metabolism.\textsuperscript{5} Energy required for activity is dependent on the form and availability of fuel for metabolism, which are carbohydrates, fats, and protein. In particular, ingested carbohydrate is stored in muscles and the liver in a more complex form, glycogen.\textsuperscript{58}

Glycogen, stored within the muscle, is a readily available fuel for power production in under both aerobic and anaerobic conditions, with regard to adenosine triphosphoate-phosphocreatine (ATP-PCr), which contributes only several seconds of energy output.\textsuperscript{58} Prolonged exercise can deplete glycogen stores in the liver and skeletal muscle.\textsuperscript{58} Carbohydrate ingestion has been shown to offset muscle glycogen depletion during a soccer match and after
intermittent shuttle running.\textsuperscript{18, 113} Glycogen availability determines the rate of ATP regeneration, if levels are inadequate a subsequent drop in force production follows.\textsuperscript{85} The physiological demands of intermittent activity may deplete glycogen stores at a different rate than endurance exercise, and contribute to decreased motor control, decreased power output, and increased rate of fatigue.\textsuperscript{113}

Reductions in glycogen content in the lower extremities have been correlated with decreased work rate and power output in soccer players.\textsuperscript{91, 94} The rate of glycogen depletion and glycolytic rate influence the power output over repeated sprints.\textsuperscript{108} A soccer match has been shown to deplete muscle glycogen by 85-90\%, and even after 45 minutes of soccer, some players have shown marked depletions of glycogen.\textsuperscript{108} Soccer athletes with lower muscle glycogen content were shown to sprint less and walk more, as opposed to players with higher muscle glycogen content.\textsuperscript{102} Limitations to energy supply, metabolite accumulation, and mechanical stress during high intensity intermittent running can cause athletes to fatigue, characterized by decreased power output or maintenance of energy output, and negatively influence neuromuscular characteristics.\textsuperscript{28, 64, 65}

\section*{1.3 \textbf{EFFECTS OF FATIGUE ON NEUROMUSCULAR CHARACTERISTICS AND RISK FACTORS}}

Fatigue is defined as a decrease in force output attributed to reduced muscle fiber recruitment,\textsuperscript{43} displayed with a decrease in power and performance.\textsuperscript{91} Muscular fatigue is a risk for ligament injuries, as joint stability is comprised of static and dynamic components.\textsuperscript{79} As stated earlier, a large percentage of noncontact knee injuries occur in the last 15 minutes of the first half and in
the last 30 minutes of the second half of soccer matches. These events most likely correspond to times during which athletes succumb to the mental and physiological demands of the game. Fatigued muscles are less capable of dissipating destabilizing forces, which may compromise the integrity of elastic joint components.

Fatigue has been shown to cause proprioceptive deficiencies and delayed motor responses, both of which decrease joint control in the lower limbs. Fatigue is believed to play a major role in the musculotendinous mechanoreceptors (muscle spindles and golgi tendon organs) and articular mechanoreceptors and negatively influence the proprioceptive feedback and the regulation of muscle stiffness during dynamic tasks. Female athletes have different muscle-activation strategies than male athletes during the landing phase of jumping and cutting movements, characterized by the quadriceps dominance/activation and a lack of effective muscle agonist and antagonist co-activation. Fatigue can influence landing biomechanics such as increased initial and peak knee abduction and internal rotation angles, increased peak knee internal rotation, adduction, and abduction moments, with the abduction being more prominent in female athletes. For the purpose of this thesis, the effects of fatigue on muscle glycogen, strength, and power are described further below.

1.3.1 Effects of Fatigue on Muscle Glycogen Content

A previous investigation investigated muscle glycogen degradation in elite male soccer athletes during a simulated fatiguing soccer match. Using NMR to assess the muscular glycogen content, it was found that the muscular glycogen was decreased from 135 mmol/kg to 87 mmol/kg after a simulated soccer match. Furthermore, data analysis also revealed moderate correlation (r = 0.62) between net muscle glycogen to time to exhaustion. This study utilized
NMR technology that may be impractical for coaches, and subjects included male athletes. Similarly, glycogen content in lower limb muscles was shown to decrease progressively from the start of a soccer match to the conclusion of the match. Muscle biopsies after a soccer match revealed that muscle glycogen was drained, and the force production was affected with fatigue and changes in muscle glycogen content. It is important to examine muscle glycogen content, strength, and power and to explore the relationship among those variables.

1.3.2 Effects of Fatigue on Muscular Strength

Strength capabilities of the quadriceps and hamstrings is important for running acceleration and deceleration, sprinting, and other sport specific movements. In addition to the major role of strength in performance, the quadriceps muscles, or strength imbalances between the quadriceps and hamstrings, play a role in the ACL strain and possible injuries. Female athletes tend to display a greater peak torque of quadriceps than the hamstrings, as well as being quadriceps dominant, and have decreased proprioception which may influence strength balance and stabilization strategies.

The type of exercise that induces fatigue has an effect on the capacity of the knee flexors and extensors to resist fatigue, and the disparity of strength losses influences the imbalances of strength relationship of these two muscle groups. As fatigue causes strength deficits, the length-tension relationship of the hamstrings shifts towards extension, causing decreased hamstring force and stabilization strategies. Powerful changes in direction or landing mechanics as an athlete fatigues may lead to poor biomechanics, thus increasing the risk of injury. Aerobic fatigue paired with localized lower extremity fatigue has resulted in increased peak anterior tibial shear force and decreased knee flexion angles. This may put the knee joint
at risk for anterior tibial displacement and lack of knee stabilization. Strength imbalances in the presence of fatigue during may result in increased risk of injury during dynamic sport specific tasks.

1.3.3 Effects of Fatigue on Power

For soccer athletes, power is an important characteristic due to acceleration, explosive changes in direction, jumping, and cutting demands of the sport. Leg strength has been associated with power output, as triple hop distance has been correlated with the vertical jump and strength of the quadriceps and hamstrings during concentric contractions. Power can be measured by vertical jump and kicking distance, and improved through plyometric movements. Plyometric movements utilize rapid, powerful movements that are preceded by a preloading countermovement which activates the stretch shortening cycle. Assessing the stretch shortening cycle that an athlete possesses can be measured by the squat jump and the countermovement jump. An athlete who utilizes the stretch shortening cycle effectively should have a short time to takeoff during a countermovement jump but still have the ability to achieve a high just height.

Stretch shortening cycle has been used as a model to study fatigue. It is generally accepted that fatigue induced by intermittent running or jumping to exhaustion result in immediate reduction in strength and power performance through three mechanisms: structural changes of the musculoskeletal system, metabolic changes (a lack of available energy substrate), and neural changes (presynaptic inhibition). Particularly, this type of fatigue protocol can have a negative influence in drop -jump power as well as the neuromuscular characteristics during landing and jumping. Therefore, it is important to include a measurement of power
during a depth-jump in addition to muscular strength. A specific power parameter of depth-jump evaluated in the current study is the reactive strength index (RSI), and describes an athlete’s explosive capabilities in dynamic jumping activity.\(^{38}\) Reactive strength index has not been evaluated in reference to fatigue, muscle glycogen content, and strength.

### 1.4 DEFINITION OF PROBLEM

A simulated soccer match (intermittent running) relies on intramuscular glycogen stores to meet energy demands. Although depletion of glycogen has been speculated to be a cause of fatigue,\(^7\),\(^61\) there is a lack of evidence if the activity of soccer results in depletion of glycogen to elicit fatigue. The effect of fatigue on muscular glycogen, and corresponding strength and power characteristics is yet to be determined. Similarly, relationships among muscle glycogen content, strength, and power after fatigue are still largely unknown.

Also, there is little research regarding non-invasive muscular glycogen content in lower limbs compared before and after intermittent high intensity running. It is largely unknown if ultrasound-based muscle glycogen measurement is sensitive enough to detect significant changes after intermittent running. Lately, it is largely unknown if baseline muscle glycogen content may be related to the rate of reductions in strength and power characteristics.
1.5 PURPOSE

The primary purpose of this study was to evaluate and compare strength, power, and muscle glycogen content before and after intermittent running. A secondary purpose was to evaluate relationships between change in strength, power, and muscle glycogen content. Lastly, another purpose of the study was to evaluate the relationship between the baseline muscle glycogen content and changes in strength and power after the fatigue protocol.

1.6 SPECIFIC AIMS AND HYPOTHESES

Specific Aim 1: To examine the peak isokinetic knee flexion and extension torque and ratio, reactive strength index of a depth-jump task, and ultrasound-based muscle glycogen content of thigh/calf muscles and compare these variables before and after a fatigue protocol (intermittent running).

Hypothesis 1: It was hypothesized that knee strength, power, and muscle glycogen content would significantly decrease after the fatigue protocol.

1a. The peak isokinetic knee flexion and extension torque would be significantly reduced after the fatigue protocol. Also, the peak knee flexion/extension strength ratio would be significantly lower after the fatigue protocol.

1b. The reactive strength index would be significantly reduced after the fatigue protocol.
1c. The ultrasound-based muscle glycogen content of thigh and calf muscles (rectus femoris, vastus medialis, vastus lateralis, lateral hamstring, medial hamstring, and gastrocnemius/soleus) would be significantly reduced after the fatigue protocol.

Specific Aim 2: To examine the relationships between the change (calculated as a proportion of post-fatigue/pre-fatigue values and expressed in percentage) in the peak isokinetic knee flexion torque, extension torque, torque ratio, and reactive strength index and the change in muscle glycogen content.

Hypothesis 2: It was hypothesized that the changes in knee strength and power would be significantly correlated in the positive direction with the changes in muscle glycogen content.

2a. The change in the peak isokinetic knee flexion, extension, and torque ratio would be significantly correlated to the change in muscle glycogen content in the positive direction.

2b. The change in the reactive strength index would be significantly correlated to the change in muscle glycogen content in the positive direction.

Specific Aim 3: To examine the relationship between baseline (pre-fatigue protocol) muscle glycogen content to the change in knee strength and power.

Hypothesis 3: It was hypothesized that the baseline muscle glycogen content would correlate negatively to the change in strength and power.

3a. The change in the peak isokinetic knee flexion, extension, and torque ratio would be significantly correlated to the baseline muscle glycogen content in the positive direction.

3b. The change in the reactive strength index would be significantly correlated to the baseline muscle glycogen content in the positive direction.
This research study would help to determine the relationship among knee strength, power, and muscle glycogen content before and after an intermittent running fatigue protocol. It would also aid in determining if the presence of higher muscle glycogen content affects the onset of fatigue (as quantified in reductions in the peak knee torque and power). Results of this study could be utilized to design interventions to minimize the negative impact of fatigue on female soccer players and maintain performance over time. For example, recovery strategies before and after training and competition could incorporate methods to replenish muscle glycogen stores. Incorporating strategies that replenish glycogen stores could enable an athlete to recover from sport activity that might have damaged muscle fibers. The utilization of ultrasound-based muscle glycogen measurement (if it could detect significant changes in glycogen content before and after the fatigue protocol) would have implications for field settings, and could be useful for determining the concentration of available fuel (muscle glycogen).
2.0 LITERATURE REVIEW

In this chapter, topics covered in the introduction were further described in detail, including ‘musculoskeletal injuries and neuromuscular risk factors in soccer’, ‘energy demands and substrate utilization in soccer’, and ‘effects of fatigue on neuromuscular characteristics and risk factors’. Additionally, methodological considerations were described.

2.1 MUSCULOSKELETAL INJURIES AND NEUROMUSCULAR RISK FACTORS IN SOCCER

Among women’s sports, soccer accounted for the highest estimated number of injuries per year and the highest competition injury rate in the NCAA Injury Surveillance Program. Lower extremity injury is common among women’s soccer, distribution of injuries occurring in games and practices included 16.7% of injuries reported being ankle ligament sprains, 3.7% of injuries being ACL injuries, and 5.3% of injuries being concussions. The severity of these injuries is varied, however 88% of the ACL injuries reported by various athletes across several sports resulted in over ten days of time lost from sport. Long-term health implications are associated with ACL injuries that influence the injured athlete’s ability to return to their pre-injured level of sport participation. Mechanisms of ACL injury may be either contact or non-contact, involving sudden deceleration, jumping, cutting, or player collision. Female athletes
are at higher risk of ACL injury than their male counterparts. Female athletes have a threefold increase of injury in games compared to practices. Approximately 70% of the injuries to female soccer athletes reported to the NCAA Injury Surveillance System included the lower extremity during practices and games. Ankle ligament sprains, knee internal derangements, concussions, and leg contusions were most prevalent during games. Upper leg muscle strains, ankle ligament sprains, knee internal derangements, and hip muscle strains were most prevalent during practices. Ankle ligament sprains are the most common injury seen in both practices and games, however knee internal derangement resulted in the greatest time loss. Most of the ACL injuries occurred from non-contact mechanisms.

Hamstring injuries are also a contributor to loss of time from sport, with 12% of injuries over the course of two seasons. Some predisposing factors for hamstring injuries include poor flexibility, muscle imbalances, muscle weakness, neural tension, fatigue, and previous injury. Hamstring strains are more likely to occur than quadriceps strain. The common occurrence of hamstring strains is accepted, however the pathophysiology and diagnostic investigation is still limited. The proper function of the hamstrings may influence injury risk, and a low hamstrings to quadriceps strength (both concentric and eccentric) ratio may be the greatest predictor for hamstring strains and knee instability.

The role of the hamstrings is to decelerate the tibia and apply posterior shear force that aids to decrease strain on ligaments that stabilize the knee. Stabilization of the knee is increased with greater knee flexion angles and co-activation of the hamstrings and quadriceps during landing tasks. These mechanisms help to protect against knee abduction, dynamic knee valgus, and high loads on the ACL. Medial knee movement is associated with femoral adduction, ankle eversion, knee abduction, femoral internal rotation in relation to the hip, tibial
external rotation in relation to the femur, and contributes to knee valgus and frontal plane movement. Strength and activation strategies of hip adductors, hip abductors, ligaments of the knee, gluteus medius, and hamstrings reflect knee abduction movement in female athletes. Hip abduction control influences femoral internal rotation and adduction, indicating that the gluteus medius strength and activation strategies have a role in frontal plane knee movement. Muscle strength or activation patterns that lead to increased joint load limit the effectiveness of the active muscular control system working with the passive joint restraints to create dynamic knee stability. Due to injuries occurring in the second half of matches, fatigue has a role in injury risk. Fatigue has been shown to increased quadriceps contraction, decreased hamstring activation, or a combination of factors that predispose an athlete to injury. The following sections describe the energy demands and substrate utilization in soccer first; then, basic information about fatigue is described.

2.2 ENERGY DEMANDS AND SUBSTRATE UTILIZATION IN SOCCER

Pathways for energy metabolism are determined by intensity and duration of exercise, as well as the fitness level of an athlete. Anaerobic and aerobic metabolism are explored in later sections, but both have implications over glycogen storage and utilization. Anaerobic metabolism involves energy production without the presence of oxygen. Aerobic metabolism involves energy production in the presence of oxygen, but takes longer to produce ATP. ATP is the only fuel that can be used directly for muscle force generation, and available ATP will fuel about 2 seconds of maximum intensity exercise. Anaerobic metabolism is short in duration with a high rate of force production over a very short period of time, lasting several seconds. As rate of
energy production decreases and the demand for force production remains high, aerobic metabolism contributes to energy production.\textsuperscript{57, 58} Together, these processes are responsible for energy production, and can be modified with training.\textsuperscript{57, 58}

Ingested carbohydrate, or exogenous carbohydrate, is broken down into glucose,\textsuperscript{57, 58} synthesized into glycogen in a process called glycogenesis.\textsuperscript{58} Glycogen content in skeletal muscle at rest is between 12-16g/kg.\textsuperscript{57} At high intensities, muscle glycogen is broken down very rapidly and is depleted in a relatively short period of time, especially when high intensity exercise is performed intermittently.\textsuperscript{57} High intensity exercise requires high rates of ATP resynthesis from anaerobic glycolysis, causing rapid breakdown of muscle glycogen.\textsuperscript{57} The liver is responsible for releasing glucose in the bloodstream when blood glucose concentration drops.\textsuperscript{57} During high intensity exercise, liver glucose output increases dramatically.\textsuperscript{57} High intensity exercise causes a mismatch between glucose uptake and glucose production by the liver.\textsuperscript{57} At 80% VO\textsubscript{2}max or greater, the liver produces glucose at higher rate than the rate that it is taken up by the muscle due to neural feedforward mechanisms.\textsuperscript{57} Unless an athlete’s resting glycogen levels are below 25mmol, muscle glycogen availability may not be explanation for fatigue in high intensity sports.\textsuperscript{57}

Low carbohydrate availability may be an explanation for metabolic disturbances in instances of low energy availability if an athlete is attempting to lose weight.\textsuperscript{18} However, when intensities of exercise reach 95-100% VO\textsubscript{2}max, very low carbohydrate diets may limit performance.\textsuperscript{57} Low glycogen levels during training and competition will decrease performance and cause muscle damage, leading to impaired glycogen storing capacity.\textsuperscript{24} Muscle fibers that are recruited most frequently may become depleted more quickly, and reduces the number of fibers recruited to compensate for loss in muscle force.\textsuperscript{91}
Dietary interventions that manipulate muscle glycogen have implications over performance, amount of ground covered, velocity of ground covered, and ability of the muscle to resynthesize intramuscular glycogen. It has been shown that in an intermittent sport such as soccer, more ground was covered at higher velocities when athletes consumed a high carbohydrate diet, compared with a low carbohydrate diet.

### 2.2.1 Anaerobic Metabolism

Adenosine triphosphate (ATP) is the most immediately available source of energy that can be utilized by the body. Cells can only hold limited amounts of ATP, and new ATP must generated for metabolism and muscle contraction. ATP is generated through the ATP-PCr system, glycolysis, and the oxidative (aerobic) system. The first two forms of energy production occur without the presence of oxygen and comprise the components of anaerobic metabolism.

The ATP-PCr system is the simplest of energy systems, and utilizes a high energy molecule called phosphocreatine, or PCr. The breakdown of stored PCr by the enzyme creatine kinase within a muscle cell contributes to regenerating ATP. Available ATP within the cell, and the activation of the ATP-PCr system to produce energy is rapid and requires no additional structures to produce energy. As PCr levels decline due to powerful muscle contraction or rapid force production, levels of ATP become depleted and cannot provide the energy for contraction and relaxation of muscle. ATP and PCr stores account for about 3-15 seconds of a sprint. The role of PCr as a source of energy to resynthesize ATP after bouts of high intensity activity is very important as an energy buffer for anaerobic metabolism during a soccer match. Intramuscular stores of glycogen and PCr responsible for anaerobic metabolism can be drained after sprints...
over a long duration, causing decrease in power. Exercise of high energy demand for longer than 15 seconds cause anaerobic glycolysis to become primary source for energy production.  

Anaerobic glycolysis requires the breakdown of glucose, a substrate that accounts for 99% of all the sugars circulating in the blood. Glucose, similar to the role PCr plays in energy production, goes through a series of enzymatic reactions to produce energy. Glycogen, which is synthesized from glucose, can also enter the glycolysis pathway, which begins once glycogen or glucose is broken down into glucose 6-phosphate. The 10-12 enzymatic reactions that follow this initial conversion results in pyruvic acid, which is broken down to form lactic acid. For each molecule of glycogen broken down, 3 moles of ATP are generated; whereas 2 moles are generated if glucose is used, due energy being needed to convert glucose to glucose 6-phosphate. This system does not produce large amounts of ATP itself, but combined with the ATP-PCr system, accounts for the energy requirements in the early minutes of high intensity exercise. Without oxygen, lactic acid is formed due to anaerobic glycolysis, which disassociates to lactate. High energy events lasting 1-2 minutes cause high demands on the glycolytic system. This results in high concentrations of lactic acid. The decreasing pH of muscle cells cause a decreased rate of glycolysis due to inhibition of glycolytic enzymes. In addition, the decreasing pH causes the muscle fibers’ calcium-binding capacity to be inhibited. Hydrogen ion buildup due to increase concentrations of lactate, causes decreased pH within the muscle cell, and has been previously thought to inhibit anaerobic metabolism. The availability of glycogen, as well as the ability of the muscles to utilize and resynthesize glycogen as activity continues are determinants of high intensity exercise performance. Blood glucose concentrations have been shown to be higher during a soccer match than at rest, due to duration of soccer athletes spent at high intensity running and number of sprints performed. It is
important to understand that energy systems overlap, and that they do not act independently to produce energy. During a six second sprint, muscle glycolysis contributes 50% to energy production, 48% from PCr, and 2% from ATP. In addition, resistance training of repeated 30 second maximal bouts of knee extensions caused greater adaptations to the anaerobic glycolysis system than to the ATP-PCr system. After about 2 minutes of high intensity effort, the aerobic metabolism system contributes greatly to energy production.

2.2.2 Aerobic Metabolism

Aerobic metabolism requires oxygen and has a slower rate of energy production and anaerobic systems, but has a much larger energy–producing capacity. Oxidative energy can come from carbohydrates or fats. As intensity and duration of exercise drains PCr and glycogen, aerobic metabolism increases in contribution to energy production. Glycolysis can occur for both anaerobic and aerobic metabolic processes, but the end product is different between the two. Lactic acid that is produced from anaerobic glycolysis leaves the cell through the bloodstream to the liver, where it becomes oxidized and can enter the aerobic pathway in the presence of oxygen. Aerobic glycolysis results in pyruvic acid, which is converted to Acetyl CoA that is further broken down in series of chemical reactions known as the Krebs cycle. The Krebs cycle is paired with another series of chemical reactions known as the electron transport chain, that buffers the H+ ions that accumulate throughout glycolysis and the Krebs cycle. This process involves enzymes, coenzymes, and proteins within the mitochondria that are utilized to maintain cell pH and produce more ATP.

One molecule of glycogen results in 33 molecules of ATP while one molecule of glucose yields 32 molecules of ATP. The presence of oxygen allows the oxidation of glycogen to
occur, and also allows triglycerides to be utilized for energy.\textsuperscript{58} Triglycerides must be broken down into glycerol and free fatty acids (FFAs), fatty acids being the preferred energy source for fat metabolism.\textsuperscript{58} FFAs enter the muscle fibers through diffusion, mediated by concentration of FFAs in the bloodstream; the higher the concentration in the blood, the higher the rate of diffusion.\textsuperscript{58} Once FFAs enter the muscle cell, they are prepared for breakdown and converted to Acetyl CoA. Acetyl CoA is broken down through the Krebs cycle and, similarly to oxidative glycolysis, the byproducts are utilized by the electron transport chain.\textsuperscript{58} A given amount of fat results in a greater number of units of Acetyl CoA that enter the Krebs cycle compared to the same given amount of carbohydrates, thus making fat oxidation of greater energy-producing capability than glycogen.\textsuperscript{58} FFA oxidation produces about twelve times more ATP than anaerobic glycolysis.\textsuperscript{115}

The mitochondria are essential components of the cell that utilize oxygen to produce energy from glycogen, fats, and even proteins.\textsuperscript{58} The number and size of mitochondria may be increased with training, and have been known to be more prevalent in type I fibers compared to type II muscle fibers.\textsuperscript{58} Intramuscular buffering capacity, after high intensity anaerobic exercise associated with oxidative metabolism, could influence the maintenance of performance of intermittent exercise despite individual peak anaerobic power, rate of anaerobic glycolysis, and percentage of fast twitch muscle fibers.\textsuperscript{87} Endurance exercise at moderate intensities has been shown to deplete type I fibers to a greater extent than type II fibers.\textsuperscript{58}

\subsection{2.2.3 Muscle Glycogen Content}

The importance of proper nutrition for any sport has been generally accepted, and nutritional habits of athletes have an effect over their overall performance. Carbohydrate intake has been of
special interest due the key component glucose, which stored in the muscle and liver as glycogen.\textsuperscript{57, 58} Glycogen is a molecular part of carbohydrate, and is a molecule that is stored predominantly within muscle cells and the liver,\textsuperscript{57, 58} and can be readily used for energy production both under anaerobic and aerobic conditions.\textsuperscript{57, 58} Maintaining adequate levels of intramuscular glycogen and blood glucose can reduce glycogen depletion and delay fatigue.\textsuperscript{113} However, different forms of exercise and different durations of exercise affect the dependence of force production on glycogen as well the rate of glycogen use and synthesis.

Carbohydrate (CHO) intake has been shown to improve performance reflected through time spent during high intensity activity.\textsuperscript{113} Delayed time to fatigue with ingestion of CHO during high intensity activity and maintenance of cognitive and motor skill performance has been the rationale for sports drink consumption.\textsuperscript{113} Sports drinks have been used before, during, and after exercise to replenish and to maintain glycogen levels of various modes of exercise.\textsuperscript{22} Intermittent activity increases both the work of skeletal muscle and the removal of glucose, while low intensity prolonged exercise causes a decreased rate of glycogen depletion.\textsuperscript{22} Muscular concentration of glycogen reflects the capability of ATP regeneration. With decreased levels of glycogen, the muscle is unable to maintain adequate levels of ATP, leading to compromised force production due to decreased excitation-contraction coupling capability.\textsuperscript{85}

Glycogen has been found to reside in pools within muscle fibers, not evenly distributed throughout the muscle.\textsuperscript{85} Decreased glycogen has been shown to have an effect on sarcoplasmic reticulum release of calcium. Tetanic $\text{Ca}^{2+}$ has been shown to decrease at a faster rate when muscle fiber glycogen content is low.\textsuperscript{85} Even when global myoplasmic ATP levels are high, glycogen content affects the excitation contraction coupling in single muscle fibers.\textsuperscript{85} Each
glycogen granule within the muscle fiber has its own regulatory proteins and enzymes, which is also dependent on the location of these pools within the cell.85

Glycogen pools have been located in three areas: directly beneath the sarcolemma, between the myofibrils lying close to the sarcoplasmic reticulum and the mitochondria, and in the myofibril.85 The glycogen pool located between the myofibrils accounts for the largest distribution of glycogen, although distribution may be partially dependent on training status and fiber type.85 The pool of glycogen located within the myofibril may have an effect on Na, K-ATPase activity. The conversion of the action potential to SR Ca\(^{2+}\) release is partially dependent on Na, K-ATPase activity, which may be influenced by the amount of glycogen between the t-tubules and the sarcoplasmic reticulum.85

The concentration of glycogen pools varies depending on fiber type and exercise type, as revealed by type II fibers becoming depleted of glycogen to a greater extent than type I after intermittent high-intensity exercise.113 Both intermittent high intensity exercise and longer duration steady state exercise causes a decrease in blood glucose and higher free fatty acid concentrations.113 This decreases CNS function and elevate brain serotonin, which decreases motor control skills and perceived mood.113 Peripheral fatigue is attributed to muscle damage, metabolic disturbances, glycogen depletion, and physiological processes involving the movement of calcium.2, 98 Due to intermittent exercise causing greater peripheral fatigue than central fatigue, muscular glycogen decrement due to fatigue may decrease performance characteristics and muscular contraction capabilities compared to pre-fatigue.
2.3 FATIGUE

Fatigue is defined as a decrease in force output attributed to reduced muscle fiber recruitment, displayed with a decrease in power and performance. Fatigue, both central and peripheral, has been shown to have adverse effects on movement coordination, reaction times, motor and postural control, proprioception, muscle power output, and biomechanical properties such as lower extremity kinematics. Physical fatigue reduces neuromuscular characteristics and sensorimotor control, which contributes to decreased performance and increased risk of injury. Fatigue induced by exercise compromises distal mechanisms such as the neuromuscular junction, sarcolemma excitability, and muscular contractile properties. Proximal mechanisms such as descending pathways, motivation, spinal synapses, and motoneuron pool excitability are also negatively affected. New motor units are activated under fatigued conditions so that force production may continue as both high intensity and low intensity exercise may change frequency of fatigue. Fatigue frequency refers to occurs alteration in the action potential propagation or the excitation-contraction coupling failure. It is excitation-contraction coupling failure that has been observed with muscle twitch contractile properties and isometric strength changes during exercise such as high intensity uphill running.

Intermittent activity mimicking the profile of soccer has been shown to elicit greater physiological responses of fatigue compared to steady state exercise, although laboratory protocols cannot properly replicate the multidirectional nature of soccer, the varying durations of high intensity to low intensity velocities simulate match play. Racinais, Girard, Micallef, and Perrey compared continuous versus intermittent modes of running exercise on central fatigue, and it was found that exercise intensity and duration had influenced the effect on central fatigue. It was determined that both intermittent and continuous running caused decreased motoneuron
pool excitability due to presynaptic inhibition, as both subject groups completing the exercise performed similar durations of work. In a study performed by Miura et al.\textsuperscript{77}, it was found that proprioception was decreased to a greater extent under central fatigue rather than peripheral fatigue. Grieg, McNaughton, and Lovell\textsuperscript{43} found that the ratio of low intensity work to high intensity work dissipated the cumulative nature of physiological stress, yet intermittent activity that replicated the movement patterns of a soccer match are likely to impose greater mechanical stress on the musculoskeletal system.\textsuperscript{43} Due to the varying intensity of movements during a soccer match, it appears as if peripheral fatigue causes more limitations to performance than central fatigue.\textsuperscript{11}

Mechanical fatigue has been shown to be more influential to injury risk and decreased performance.\textsuperscript{43} Mechanical fatigue refers to decreased biomechanical function and inhibited musculoskeletal response to perturbations.\textsuperscript{43} Altered kinematics as a result of fatigue highlights the mechanical implication of injury incidence.\textsuperscript{43, 47} The mechanical stress of soccer may increase peripheral stress such as the decrease in contractile strength of the muscle, changes in intracellular environment and within the muscle fibers, and impaired excitation-contraction coupling.\textsuperscript{17} The intramuscular buffering capacity and the ability to recover after high intensity anaerobic exercise associated with oxidative metabolism could influence the resistance to fatigue of this kind of intermittent exercise.\textsuperscript{87} Fatigue protocols yield various effects depending on the nature of the protocol. The mode of exercise, duration, frequency, and physical demands have varied effects on central or peripheral fatigue mechanisms, and the extent that force output is influenced.\textsuperscript{17} Injury prevention programs that include fatigue elements should use protocols that replicate the aspect of fatigue associated with the sport and the joints most affected.\textsuperscript{105}
2.3.1 Central Fatigue

Central fatigue refers to a decline in voluntary activation of the muscle, caused by the number and the rate of discharge of motor units to generate force. Decreases in motoneuron excitation due to spinal or supraspinal influence is classified as central fatigue. Decreases in force output may also involve neurotransmitter and hormonal changes due to exercise. Neurotransmitter depletion and accumulation cause a decrease in corticospinal descending excitation. Increased serotonin levels due to increased concentrations of the transport enzyme tryptophan during prolonged exercise result in decreased recruitment of motor units. Afferents at the muscular level may limit voluntary activation by acting upstream to the motor cortex, while motoneuron and motor cortex excitation remain unaffected. Decreased motoneuron activity may be caused by peripheral reflexes due to extracellular increase in lactate and potassium caused by exercise. The impairment of voluntary muscle activation seems to occur during low intensity muscle contractions due to muscle afferents.

There are four groups of afferents within skeletal muscle. Groups Ia/II are muscle spindle afferents, group Ib and Golgi tendon organs, group III are non-spindle afferents, and groups IV are unmyelinated afferents. Group Ia and II afferents are located parallel to muscle fibers and signal the neuromuscular system with changes in muscle length. The decreases or changes of discharge rate of afferent signaling may limit motoneuron activity. Inhibitory afferents stimulated under local changes of the muscle may cause decreased motoneuron function at the spinal level. Lactic acid accumulation or mechanical changes to the intramuscular environment may cause increased afferent activity. The Golgi tendon organs’ (group Ib afferents) inhibit neuronal activity as they signal the central nervous system with feedback on intramuscular tension. Under conditions of fatigue, the Golgi tendon organs and muscle spindles may inhibit
motoneuron firing. Progressive decrease in the number of firing motor units or number of recruited motor units results in a decreased force generation.

Other alterations in force generation that may be a result of central fatigue include propagation to the motoneurons from the central nervous system and activation of the motor units and muscles. Preserved motoneuron excitability can be linked to consistent afferent activity, even after repeated sprint activity. However, it has been shown that exercise intensity and duration have varying effects on central fatigue and the recovery of the central nervous system.

2.3.2 Peripheral Fatigue

Peripheral fatigue refers to changes with the excitation-contraction coupling, availability of metabolic substrates, propagation of the action potential, intracellular environment, and performance of contractile properties. Neuron signaling and transmission may be altered by ion movement and concentration, specifically hydrogen, sodium, potassium, and calcium. Intramuscular calcium concentrations, movement of calcium within the sarcoplasmic reticulum, and permeability of the sarcoplasmic reticulum to calcium influence the regeneration of ATP and the efficiency of the cell to produce energy.

Anaerobic and aerobic energy production influences neurotransmitter quantities and release, actin and myosin cross-bridge action, the availability of substrate, and the overall function of cellular components. Neuron firing at the neuromuscular junction occurs as the nerve action potential transforms into muscle action potential. Speed of action potential transmission into muscle action potential has a role in force production and muscle metabolism. Energy production occurs as ATP is synthesized, and regeneration of ATP allows force
production to continue.\textsuperscript{57} The rate of energy production and demand may cause changes to the intracellular environment and limit muscle contraction. When oxygen supply is limited or under conditions where glycolytic activity exceeds mitochondria’s oxidative capacity, increases in hydrogen ions and a drop in muscle cell pH result.\textsuperscript{17} This reliance on anaerobic glycolysis may increase the concentration of inorganic phosphate, which may impact force production to a greater extent than decreased pH.\textsuperscript{17} The accumulation of inorganic phosphate may indirectly decrease the action of the cross-bridges, decrease the muscle cell sensitivity to calcium, decreased stores of calcium capable of being released, and subsequent levels of available ATP.\textsuperscript{17} Shifts in ATP concentrations and inorganic phosphate determine the quantities of calcium available.\textsuperscript{17} The efficiency of regulatory mechanisms of calcium, buffering capacity of hydrogen and inorganic phosphate, capacity of mitochondria for aerobic metabolism, and other local factors influence the maintenance of force production and resistance to fatigue. High demand and rate of force output necessary for high intensity short duration forms of running have been shown to cause substantial peripheral fatigue.\textsuperscript{86} Markers of peripheral fatigue commonly associated with fatigue include decreased intracellular pH, increased concentration of hydrogen ions, and increased concentrations of inorganic phosphates.\textsuperscript{64} Peripheral parameters that inhibit the excitation contraction coupling by influencing the release of Ca\textsuperscript{2+} are of interest with the onset of fatigue.\textsuperscript{64} Impairment in muscle function following repeated sprints was mainly due to peripheral alterations, as high intensity short duration exercise induces peripheral fatigue.\textsuperscript{86}

2.3.3 Physiological Responses to Fatigue

In a study performed by Dittrich et al.\textsuperscript{32}, an intermittent running protocol elicited greater mean running velocity, greater VO\textsubscript{2}, and greater lactate response compared to a continuous running
protocol, with each corresponding with the subjects’ maximal lactate steady state. Time to exhaustion increased for the continuous mode of exercise compared to the intermittent mode, implying a greater metabolic demand with the intermittent nature of exercise. Although soccer match play involves a greater reliance on aerobic contribution over anaerobic, metabolic processes and metabolic byproduct accumulation tend to cause impaired excitation contraction-coupling.\textsuperscript{17, 28}

Rapid force development may increase byproduct accumulation, as muscle lactate was shown to be four times higher after intense bursts of activity during a soccer match compared to pre-match.\textsuperscript{61} The anaerobic component of soccer causes an increase in hydrogen ion concentration, decrease in available ATP, increase in inorganic phosphate by the breakdown of phosphocreatine, and decrease of glycogen stores.\textsuperscript{28} The increases in inorganic phosphates and hydrogen ions as a byproduct of anaerobic glycolysis slow calcium release and calcium sensitivity, thus decreasing the rate of muscle fiber contraction and the number of contracting fibers.\textsuperscript{17} Additional chemical changes such as sodium and potassium concentrations fluctuating as a result of repeated depolarization may also cause decreased contractile capabilities.\textsuperscript{17, 86}

Force reduction is commonly associated with intracellular acidosis, but recent studies have revealed that accumulation of inorganic phosphates has a larger role than acidosis.\textsuperscript{64} Lactate accumulation and lowered pH have low correlation to cause of fatigue.\textsuperscript{61} High concentrations of inorganic phosphates reduce calcium release in the sarcoplasmic reticulum, the number of involved cross-bridges, and myofibrillar sensitivity to calcium.\textsuperscript{64} Repeated uphill running was shown to cause some loss in voluntary isometric torque of the knee extensors which could be attributed to excitation-contraction coupling failure, although central fatigue may have played a small role in fatigue.\textsuperscript{64, 65} As opposed to sprint training, high intensity interval training has been
shown to elicit greater accumulation of pH buffering,\textsuperscript{28} therefore high intensity intermittent training can increase metabolic capabilities of an athlete who is required to possess anaerobic endurance and power. Greater accumulation of pH buffering enzymes can delay fatigue by increasing synthesis of PCr between high intensity activity bouts during periods of recovery, and by maintaining lactate concentrations or by increasing clearance capability of lactate.\textsuperscript{28}

2.4 EFFECTS OF FATIGUE ON NEUROMUSCULAR CHARACTERISTICS AND RISK FACTORS

2.4.1 Effects of Fatigue on Muscle Glycogen Content

Numerous studies have investigated the relationship between anaerobic and aerobic metabolism influences on performance, measured by intermittent running.\textsuperscript{5-8, 61} Anaerobic metabolism contributes to rapid rates of force production without the presence of oxygen, utilizing glycogen to produce ATP. Aerobic metabolism contributes to lower rates of force production with the availability of oxygen, utilizing the breakdown of triglycerides to free fatty acids to produce ATP. Together, these two systems account for force production, and the reliance on these systems becomes evident with concentrations of plasma glucose and glycerol. After a high intensity soccer-specific running protocol until exhaustion, there was a significant increase in plasma FFAs and glycerol, indicating a greater contribution of aerobic metabolism as high intensity work was no longer able to be maintained.\textsuperscript{96}

The use of glycogen and the availability of glycogen are important for the maintenance of high intensity energy output and high rate of force production. Several studies have evaluated
glycogen availability and decrement as well as the influence of glycogen content on performance during a soccer match.\textsuperscript{61, 62, 96} Soccer is characterized by sprints of 3-4 seconds with intermittent periods of lower intensity before the next bout of high intensity, reflecting the alternating metabolic systems contributing to exercise.\textsuperscript{115} Saltin\textsuperscript{102} observed differences between two samples of soccer players, one with low pre-match glycogen levels and one with normal pre-match glycogen levels. The low glycogen group had almost depleted glycogen stores by half time, while the normal glycogen group had comparatively high glycogen stores at halftime and but were significantly lowered towards the end of the game. This is supported by the relationship of resting glycogen content and muscle glycogen utilized during specific exercise modes.\textsuperscript{96}

A fatiguing soccer simulated protocol caused resting muscle glycogen to be decreased by 36\% at exhaustion.\textsuperscript{96} This indicates a high correlation between muscle glycogen and muscle degraded during the test, as well as a possible role for an athletes’ muscle glycogenolytic capacity in fatigue during soccer specific performance.\textsuperscript{96} Research has revealed that glycogen levels towards the end of a game may not be high enough to maintain maximal glycolytic rate, while others argue that glycogen is still available.\textsuperscript{7} Blood glucose levels and muscle glycogen concentrations may vary due to individual player position, style, level of play, and other factors.\textsuperscript{7} However, it has been shown that individual muscle fibers may be depleted of glycogen to an extent that prevents maximal effort sprints and decreases the amount of high intensity activity.\textsuperscript{7}

Previous research has shown that high intensity running decreases towards the end of the match, regardless of player position.\textsuperscript{5-8, 78} Soccer involves short periods of high intensity running with periods of low intensity activity and sport-specific movements. It has been shown that oxidation of FFAs contributes to energy production more so in the second half matches,\textsuperscript{7} which means that rate of glycolysis is higher in the first half. As muscle glycogen is lowered, lipolysis
increases in proportion to the utilization of glycogen.\textsuperscript{7} This is supported by findings of decreased lactate, elevated catecholamine concentrations, lowered insulin concentrations, and elevated glycerol concentrations at the end of a match as a result of high intensity and long in duration demands of energy production.\textsuperscript{5,7} A high intensity soccer-specific test to fatigue caused glycogen decrement of 36\% from pre-exercise to post-exercise, specifically 135 mmol/kg to 87 mmol/kg in elite soccer players.\textsuperscript{96} It is important to note that in this study, noninvasive glycogen content was quantified; however the reliability and validity of the noninvasive data collection was not specified.

In relation to high intensity running and intermittent activity, Saltin\textsuperscript{102} revealed that players with low muscle glycogen covered less ground and performed activity at lower intensities than players with high muscle glycogen. A low carbohydrate diet of 30\% daily intake compared to an intake of 65\% carbohydrate in soccer players resulted in the higher carbohydrate diet participating in 30\% more high intensity running during a match.\textsuperscript{115} More recent studies have also revealed that low carbohydrate diets are associated with decreased glycogen concentrations and reductions in work performed during high intensity intermittent exercise.\textsuperscript{18} Intermittent exercise, jump ability, and sprint ability have been shown to decrease after a soccer match.\textsuperscript{61}

Muscle fiber contribution to activity depends on fiber type, exercise intensity, and duration of force output. Muscle fiber composition and contribution to exercise is partially reliant on the specificity of training. Individuals that ran on a treadmill positioned uphill, downhill, and level at 70\% of their VO\textsubscript{2} max for a total of 2 hours were evaluated for glycogen content in the vastus lateralis, gastrocnemius, and soleus.\textsuperscript{58} Regardless of treadmill position, the gastrocnemius displayed the greatest extent of glycogen depletion, suggesting that lower limb muscles may
become fatigued before thigh muscles in distance running.\textsuperscript{58} Before a soccer match, muscle biopsy of lower limb muscle revealed 73\% of muscle fibers were full of glycogen while only 17\% of fibers were full post-match.\textsuperscript{61} Further fiber analysis revealed that 54\% of type I, 46\% of type IIa, and 25\% of type IIx fibers were almost depleted after the soccer match.\textsuperscript{61} Faster depletion rates of glycogen in type II fibers than type I fibers may indicate a decrease in power output or the capability of certain muscles to exert maximal force output in the presence of fatigue. Muscle biopsies have revealed that muscle glycogen stores were slightly above 50\% of their resting values 42 hours post-match for an athlete consuming a high carbohydrate diet.\textsuperscript{7} High carbohydrate diet can enhance glycogen recovery after exercise, and restricted or low carbohydrate intake can cause decreased ability to recover and store muscle glycogen.\textsuperscript{24} Damaged muscle fibers, such as from fatiguing eccentric exercise, display diminished glycogen resynthesis.\textsuperscript{24} Sprint performance was decreased temporarily after periods of intense exercise during a match, and was decreased at the end of the game compared to pre-match.\textsuperscript{61} Glycogen utilization between fiber types and glycogen concentrations at the start of a match or training bout may be indicative of amount of high intensity activity performed.

\textbf{2.4.2 Effects of Fatigue on Muscular Strength}

Soccer-specific intermittent running is more likely to cause peripheral fatigue rather than central fatigue. Decline in squat jump performance and decreased sprint speed by halftime of a match reveals the strength reduction of the quadriceps muscles due to soccer activity.\textsuperscript{98} Anaerobic metabolism, paired with decreased ATP re-synthesis, may limit force production caused by a decrease in calcium release by the sarcoplasmic reticulum due to an increase in inorganic phosphates.\textsuperscript{17}
Increases in work intensity during an intermittent protocol is likely to elicit an anaerobic cellular environment.\textsuperscript{43} Energy production becomes restricted without the presence of oxygen to clear lactate and resynthesize ATP, as intracellular conditions become increasingly acidic. As the intracellular environment changes, the excitation-contraction coupling capability decreases along with a decrease in neurotransmitter release. As these factors accumulate, the efficiency of working muscle to produce force decreases. If muscular imbalances are present before the onset of peripheral fatigue, the decline of muscle efficiency may increase the chance of injury or incorrect movement patterns.\textsuperscript{104} The amount of tibial shear and anterior tibial displacement during landing may predict injury risk, both of which are influenced by strength of surrounding elastic components and landing strategies.\textsuperscript{20, 49, 79}

A study by Mendez-Villanueva et al.\textsuperscript{75} investigated neuromuscular fatigue in recreationally active males during a series of repeated sprints. They found that power output and EMG amplitude decreased throughout exercise protocol, implying a decline in motor unit activity reflected through decreased EMG amplitude. The disparity between agonist and antagonist muscle strength may become not only a hindrance to performance and athletic efficiency but also a risk factor for injury especially during the latter moments of a soccer match. After about a half period of a soccer match, it has been shown that squat jump height and quadriceps and hamstrings maximal voluntary isometric torque were significantly decreased.\textsuperscript{98} Soccer match activity involving velocities determined by Bangsbo et al.\textsuperscript{8} revealed that by halftime, decreases in concentric maximal voluntary torque of the quadriceps muscle were evident.\textsuperscript{98} In addition, isometric contractions of the hamstrings declined by halftime compared to baseline, and concentric contractions revealed significant declines by the end of the match.\textsuperscript{98} Squat jump height, sprint speed, and stride frequency were reduced at halftime of a soccer match,
and were farther decreased at the end of the match.\textsuperscript{98} It is important to note that torque of the knee flexors in the eccentric condition is more prominent than the concentric condition, especially in regard to the nature of soccer activity.

During an intermittent soccer shuttle test, eccentric hamstring peak torque has been shown to decrease.\textsuperscript{29} Although eccentric contractions reflects the involvement of the hamstrings during various activities common with soccer,\textsuperscript{29} other authors have found that the concentric hamstrings peak torque decreases due to soccer activity.\textsuperscript{42} Fatigue has a greater influence on the hamstrings, which are predominantly type II fibers, compared to the type I muscle fibers that compose the quadriceps.\textsuperscript{29,30,104}

Due to the nature of soccer, the eccentric hamstring strength to concentric quadriceps strength ratio ($H_e:Q_c$) is an appropriate screening tool for injury risk.\textsuperscript{30} A higher $H_e:Q_c$ ratio can reflect the ability of the hamstrings to counteract the force of the quadriceps, and therefore be of importance in determining strength imbalances and injury risk.\textsuperscript{30} In addition, fatigue resistance is greater in the quadriceps potentially due to muscle fiber composition of the quadriceps compared to the hamstrings.\textsuperscript{104} High quadriceps peak torque may reveal a low $H:Q$ ratio, thus increasing the risk of hamstring injuries.\textsuperscript{13} A high hamstrings to quadriceps peak torque ratio may protect individuals from ACL injuries.\textsuperscript{1} This ratio can indicate muscle group strength imbalances.\textsuperscript{79} Tibial shear force is influenced by the orientation of the patellar tendon.\textsuperscript{1} The orientation of the patellar tendon is influenced by knee flexion angle during landing and magnitude of pull exerted from the quadriceps.\textsuperscript{1}
2.4.3 Effects of Fatigue on Power

The influence of power, rapid contraction causing high rate of force production, is important to intermittent sports. Rapid changes in direction, jumping, deceleration, and sprinting ability are influenced by the rate of contraction and force output. Mathematically the equation of power is as follows: work \times time = power. A component of muscle power is the stretch shortening cycle which involves a combination of eccentric and concentric muscle actions. The length changes of muscle fibers before contraction activates muscle spindle, therefore increasing force production. Muscle spindles are of importance in the stretch reflex, by sending sensory information to the spinal to elicit activation of a-motor neurons of motor units in the muscle group of the muscle spindle, causing force production. The sensory capability of the muscle spindles sense the changes in muscle tension and allow the length to change to increase tension in the subsequent contraction. Stretch shortening cycle exercise utilizes the stretch reflex to facilitate recruitment of motor units, and stores energy in the elastic and contractile components of muscle during the eccentric contraction that can be recovered during the concentric contraction. It has been shown that stretch shortening cycle exercise such as a depth jump or countermovement jump induces myofibril damage. Muscle damage may be caused by eccentric exercise by increases in creatine kinase, revealing a relationship between decreases in stretch shortening cycle performance and stretch reflex sensitivity. The stretch shortening cycle is characterized by a stretch reflex and possible recruitment of fast type motor units. Motor unit recruitment may differ in concentric and eccentric muscle activation, and stretch shortening cycle action may be influenced with concentric or eccentric contractions. Possible mechanisms to stretch shortening cycle fatigue and damage may involve pre-synaptic inhibition or reductions in muscle spindle sensitivity. Concentric muscle function is more affected by acute metabolic
fatigue after stretch shortening cycle exercise. Motor recruitment strategies may differ between concentric and eccentric exercise, as fast motor units may be recruited preferentially during eccentric contractions. Motor recruitment will also reflect metabolism for force production. Power output is dependent on rate of motor unit recruitment and energy availability. Anaerobic power output is dependent predominately on the ATP-PCr and anaerobic glycolysis metabolic pathways. Maximal efforts lasting 6 seconds place the greatest demands on the breakdown and re-synthesis of ATP and PCr. Anaerobic power tests include the Wingate anaerobic test, vertical jump test, triple hop test, squat jump test, and depth jump test. The Wingate test is a test in which subjects pedal on a cycle ergometer at maximal speed for 30 seconds against a high braking force. This test is longer in duration than the other maximal power tests listed, as it stresses the anaerobic glycolytic system for energy. The other tests utilize the ATP-PCr test, due to the short duration of the tests. The vertical jump test has been established as an accurate measure of lower body power. The squat jump and countermovement jump have also been used to measure power. Both of these tests examine jump height as the outcome variable, expressing an athlete’s plyometric abilities. A commonly used plyometric exercise to utilize the stretch shortening cycle is the depth jump, in which an athlete steps from a measured drop height onto the ground, and immediately performs a maximal vertical jump. A depth jump onto a force plate reveals jump height, ground contact time, and reactive strength index. The stretch shortening cycle involves stored energy of the eccentric action enhances the concentric action, and the reactive strength calculation is an indirect method to examine an athlete’s use of the stretch shortening cycle. Effective stretch shortening cycle utilization should result in high jump height and short time to
takeoff. Time to takeoff has been theorized to be an indicator of the rate of force development for reaching maximal jump height, but is not a reliable measure for reactive strength index. Depth jumps have been shown to affect the stretch shortening cycle due to fatigue caused by muscle damage during the eccentric phase of the movement. Fatigue caused by repeated depth jumps caused peak power decreases in both the eccentric and concentric contractions of lower limb muscles. Joint power production and stretch shortening cycle efficiency is influenced by acute metabolic fatigue and muscle damage.

### 2.5 METHODOLOGICAL CONSIDERATIONS

#### 2.5.1 Muscle Glycogen Content Measurements

Muscle tissue biopsy with direct biochemical measurement has been the predominant method of assessing metabolite concentrations and muscle structure, including muscle glycogen content. Much of the previous literature evaluating muscle glycogen has been gathered by muscle biopsies. Muscle biopsies cause discomfort to the subject, have limitations on number of measurements, and are difficult to carry out accurate analyses on small samples. Therefore, noninvasive ways to measure muscle glycogen content have been investigated, including NMR spectroscopy, MRS, and diagnostic musculoskeletal ultrasound.

Nuclear magnetic resonance (NMR) spectroscopy is a noninvasive way to measure intramuscular glycogen concentration, using a spectrometer technology for scan collection. Intramuscular glycogen measurements through NMR technology are in agreement with muscle biopsy measurement at both high and low glycogen levels. There was a strong relationship
between muscle biopsy measurements and NMR measurements of intramuscular glycogen \( (r = 0.95; \ P < 0.001) \).\textsuperscript{110}

Similarly, magnetic resonance spectroscopy (MRS) technology can be used to assess glycogen content.\textsuperscript{97} A major limitation of this study and technique is that investigators\textsuperscript{97} did not conduct a validation study comparing between the MRS-based muscle glycogen and muscle biopsy measurements. Instead, the authors\textsuperscript{96} utilized this MRS-based technique to evaluate the effects of fatigue induced a simulated soccer game on muscle glycogen content and reported significant reductions in muscle glycogen content after exhaustion (before: 135 mmol/kg; after: 87 mmol/kg, \( p < 0.001 \)), supporting utilization of MRS-based muscle glycogen content measurement. Although it is noninvasive, limitations of these techniques include expensive cost of NMR/MRS equipment and accessibility to facility.

Diagnostic musculoskeletal ultrasound is a noninvasive diagnostic tool commonly used to assess skin, muscle, tendon, nerves, blood vessels, bone, joint, and subcutaneous tissue structure and appearance.\textsuperscript{11} More recently, new technology has been developed to estimate muscle glycogen content based on musculoskeletal ultrasound imaging with grey-scale analysis software.\textsuperscript{52, 82} Ultrasound-based muscle glycogen content has been shown to correlate with muscle biopsy muscle glycogen content of the rectus femoris and vastus lateralis muscles \( (r = 0.87 – 0.92, \ p < 0.001) \).\textsuperscript{82} Another study that evaluated glycogen content changes in trained cyclists utilized muscle biopsy and ultrasound methodology found high correlations between both techniques \( (r = 0.93 – 0.94; \ P < 0.001) \).\textsuperscript{52} Because this methodology is non-invasive and easy to carry and use virtually anywhere (inside or outside), it will be used to measure muscle glycogen content in this study.
2.5.2 Muscular Strength Measurement

In order to assess the imbalance between the knee flexors and extensors, conventional hamstrings: quadriceps ratio ($H_C:Q_C$) is used.\textsuperscript{30, 53} This calculates the maximal concentric knee flexion strength divided by the maximal concentric knee extension strength at the same angular velocity.\textsuperscript{30} A high $H_C:Q_C$ ratio has been shown to decrease chance of injury, while a low $H_C:Q_C$ ratio may increase the risk of injury.\textsuperscript{53} With increasing velocities, higher $H_C:Q_C$ ratios tend to result due to decreased force production during concentric contractions.\textsuperscript{53} The hamstrings to quadriceps strength ratio is important to consider, especially in the presence of fatigue when decreased force capability hinders motor unit firing.\textsuperscript{42} Due to the hamstrings being type II muscle fibers, force production tends to decrease faster than the quadriceps, as well as having a lower capability to restore muscle glycogen.\textsuperscript{30, 42, 91, 104} The hamstrings’ role to eccentrically contract repeatedly due to the nature of soccer enables the knee joint to regain stabilization.\textsuperscript{29}

Leg dominance, soccer specific movements, and the differences in muscle metabolism are reasons for decreases in $H_C:Q_C$ ratio in the presence of fatigue.\textsuperscript{104} Female athletes have been shown to be leg dominant, resulting in muscular imbalances and stabilization changes.\textsuperscript{49, 66, 79} Under fatigued conditions, the dominant leg displays greater decreases in force production, and the hamstrings fatigue to a greater extent than the quadriceps on the dominant leg,\textsuperscript{73} especially at high velocities.\textsuperscript{53 30, 104} Increases in fatigue during a soccer match cause increases in muscle soreness and muscular damage, which may be a result of reliance on the hamstrings to stabilize the knee and ACL during sprinting, jumping, and kicking a ball.\textsuperscript{29} Comparisons of $H_C:Q_C$ ratios before, at halftime, and after a soccer specific protocol revealed highest values at the start of the protocol, but progressively decreasing ratio values both at halftime and at the conclusion.\textsuperscript{34, 91} Greco, da Silva, Camarda, and Denada\textsuperscript{42} found that after a soccer-specific protocol, $H_C:Q_C$ and
H_{eccentric}:H_{concentric} were affected due to fatigue. Rate of force development decreased after the soccer-specific intermittent protocol. Muscle breakdown markers such as plasma creatine kinase and loss of strength after a soccer specific protocol revealed the relative strength loss was dependent on contraction type.\textsuperscript{42} Previous testing of conventional H_{C}:Q_{C} ratios has revealed that a value of 0.60 and below should elicit a hamstring training program, and that a healthy H_{C}:Q_{C} ratio should be above 0.60.\textsuperscript{13, 53} Overly strong knee extensors compared to the hamstrings may cause a low H_{C}:Q_{C}, which does not reflect weak hamstrings.\textsuperscript{13} Muscle imbalances between the hamstrings and quadriceps may predispose an athlete to injury, and the corresponding risk of injury may be increased in the presence in fatigue. It has been shown that both conventional and functional H:Q ratios were similar in division 1 and division 2 soccer players.\textsuperscript{13} At an angular velocity of 60°/s\textsuperscript{-1}, players with a low H:Q ratio may have higher quadriceps peak torque compared to players with a high H:Q, rather than low hamstring strength.\textsuperscript{13} Isokinetic strength testing has been previously shown to be reliable ICC range= 0.914-0.943) according to the methods of Nagai et al.\textsuperscript{80}

### 2.5.3 Power Measurement

There have been various ways to measure the power capability of athletes, which includes the squat jump, vertical jump, triple horizontal hop, short sprints, and the Wingate Anaerobic Test.\textsuperscript{45, 106} An athlete’s ability to maintain power during the Wingate test may be a predictor of running speed in female soccer athletes.\textsuperscript{71} The standard 30-second Wingate Anaerobic Power Test may not be applicable to evaluate stretch shortening cycle, as its outcome measures (mean power output and peak power output) are more applicable for short distance running performance.\textsuperscript{44} The vertical jump test has been widely used as an effective measurement of power of athletes.\textsuperscript{100}
Lower body power has been linked to an athlete’s ability to accelerate and move efficiently in sport specific attacking or defending positions.\textsuperscript{71} The ability of an athlete to change from eccentric to concentric muscular contraction can express their explosive capabilities.\textsuperscript{38} The effectiveness of the stretch shortening cycle (SSC) has implications over power output expressed through jumping and plyometric movements.\textsuperscript{96} Actions such as sprinting, backwards and sideways running, tackles, vertical jumps, and side-cutting demands intense force demands from the SSC and measuring the SSC has implications over an athlete’s capability to produce power.\textsuperscript{56} The combination of eccentric and concentric actions that are unique to the SSC involves different impact loading and metabolic loading compared to pure concentric and eccentric exercise.\textsuperscript{81} Loading of braking and push-off phases of a SSC action can be measured by ground contact time.\textsuperscript{81} Intense and exhaustive SSC exercise may be related to changes in mechanical behavior and structural modifications of the muscle-tendon unit.\textsuperscript{81} Variables such as jump height, ground contact time, mean and peak power output, and rate of force development have been measured as indicators of power.\textsuperscript{38,56} It has been shown that jump height and contact time determined from depth jumps are highly reliable, revealed by high single-measure intraclass correlations (>0.9).\textsuperscript{38,67} Intraclass correlation values for jump height, peak power, and time to takeoff ranged from 0.89-0.99 during squat jumps compared and countermovement jumps.\textsuperscript{109} Men’s soccer players have displayed high efficiency of the SSC due to reactive strength jump height and peak power values compared to other sports.\textsuperscript{109} Reactive strength can be measured several ways; relationship between squat jump height and countermovement jump height, relationship between countermovement jump time to takeoff and jump height,\textsuperscript{109} and relationship between depth jump height and ground contact time.\textsuperscript{38} Unlike countermovement jumps and squat jumps, the reactive strength index gathered from depth jumps involves higher loaded braking and push-off actions,
which are more characteristic of sport activity with the inclusion of ground contact time data.\textsuperscript{81} The Triple Hop test has been correlated with countermovement jump height (r = 0.695; P < 0.1),\textsuperscript{45} however, the reactive strength index includes ground contact time, which provides coaches and clinicians with additional information concerning stress placed on the musculotendinous complex during plyometric exercises.\textsuperscript{38}
3.0 METHODOLOGY

3.1 RESEARCH DESIGN

The first aim of the current research project was to examine muscular strength, power, and ultrasound-based muscle glycogen content and compare pre- and post-fatigue protocol (intermittent running). An intermittent running fatigue protocol consisted of two sessions: the first and second session lasting about 45 minutes and about 12 minutes, respectively. The same battery of testing (muscular strength, power, and ultrasound-based muscle glycogen content) was repeated three times: before, during and, after the intermittent running fatigue protocol. For the purpose of this investigation, only two time points (pre- and post-intermittent running fatigue protocol) were used to address the first specific aim and hypotheses. Therefore, a research design was repeated measures (within-subjects) design. The independent variable was a time with two levels (pre/post-intermittent running protocol). The dependent variables included knee strength (peak isokinetic knee flexion torque, extension torque, and torque ratio), power (reactive strength index during a depth jump task), and muscle glycogen content in thigh/calf muscles (rectus femoris, vastus lateralis, vastus medialis, lateral hamstring, medial hamstring, and gastrocnemius/soleus).

The second specific aim was to examine the relationships between the change in the strength and power variables and the change in muscle glycogen content. The third specific aim
was to examine the relationships between the baseline muscle glycogen content (pre-intermittent running fatigue protocol) and the change in strength and power variables. Research design for both aims was correlational analyses.

3.2 SUBJECTS

Subjects included recreationally active female (age 18-30) athletes. The female participants must have been capable of participating in a full range of dynamic activities required to compete in athletic events. Subjects were currently physically active individuals performing moderate to high intensity exercise training at least 3 times per week for 60 minutes at a time, or performing programmed soccer fitness training. Subjects must meet the following inclusion criteria. Subjects with any one of exclusion criteria would be excluded from the current investigation.

Inclusion Criteria:

- Healthy female recreational/college athletes
- Cleared for full athletic competition by team physician or athletic trainer
- No history injury within the two months of competition requiring medical attention or requiring more than loss of two consecutive games or training
- Currently able to fully participate in training and competition

Exclusion Criteria:

- Currently on special eating plans that restrict calorie intake and carbohydrate intake
- Concussion or neuromuscular injury diagnosed by medical professional or clinician within the last twelve months
- Pre-existing condition that corresponds with inability, pain, or compromised health condition to complete testing procedures
- Lower extremity surgery or injection, currently participating in rehabilitation
- Previous history of grade III or ankle sprain
- Currently pregnant

### 3.2.1 Power Analysis

To calculate an effective number of subjects, a power analysis was performed using G*Power 3.1.9.2 (Franz Faul, Universität Kiel, Germany). A power of 0.80 was calculated at a two-tailed alpha of 0.05 to determine the number of subjects, with an effect size of 0.79 (dz). Under the condition of statistical test as the difference between two dependent means (matched pairs), sample size was determined to be 15 subjects. To account for attrition, an increase in sample size was applied, increasing the sample size to 17 subjects. Demographic data is represented in Table 2.

### 3.3 INSTRUMENTATION

#### 3.3.1 Biodex Isokinetic Dynamometer

Peak torque of the hamstrings and quadriceps muscles, as well as hamstrings to quadriceps ratio were measured using the Biodex System 3 Multi Joint Testing and Rehabilitation System.
(Biodex Medical Inc, Shirley, NY). The torque measurements on the Biodex System 3 is reported to be very reliable (ICC = 0.99 – 1.00).33

3.3.2 Force Plate

Force plates (Kistler 9286A, Amherst, NY) were used to quantify peak ground reaction forces during the depth jump task to gather jump height, flight time, and ground reaction force for RSI calculation.

3.3.3 Heart Rate Monitor

Each subject’s heart rate (beats per minute) was monitored throughout the intermittent running fatigue protocol. A Polar heart rate monitor strap (Polar USA, Lake Success, NY) was used to collect heart rate data.

3.3.4 Treadmill

A treadmill (Woodway USA Inc, Waukesha, WI) was used for the intermittent running fatigue protocol.

3.3.5 Ultrasound

A portable diagnostic ultrasound, Philips Lumify (Koninklijke Philips N.V, Eindhoven, Netherlands) with L12-4 broadband linear array 12 MHz transducer, was used to capture ultrasound images. Aperture size of this device was 34mm. MuscleSound software
3.3.6 Anthropometrics

Height was measured using a stadiometer (Seca North America, East Hanover, MD), and mass was measured using a weight scale (Seca North America, East Hanover, MD). A tape measure was used to measure aspects of the lower extremities to locate muscles of interest. All anthropometric measurements were taken prior to testing.

3.4 PROCEDURES

3.4.1 Order of Testing

All procedures were conducted in the morning, when subjects were in the fasted state. Testing procedures took 2-2.5 hours to complete. Before the day of testing and upon review of subjects’ eligibility, subjects were informed of all testing procedures and were asked to refrain from eating the day of testing. Informed consent approved by the University’s IRB was taken before testing procedures began. During the subjects’ visit to the laboratory after informed consent, subjects were taken through a familiarization process, subjects were fitted with a Polar heart rate monitor, and anthropometric data was collected. Anthropometric data was collected and recorded in kilograms (weight) and centimeters (height). Subjects were free to ask questions concerning the
testing during this time. After anthropometric data was collected, baseline testing began. Order of testing procedures is listed in Figure 1.

Figure 1. Order of Procedures

3.4.2 Average Peak Knee Flexion and Extension Torque

The Biodex System 3 Pro Isokinetic Dynamometer was used for strength testing for knee flexion and extension using concentric/concentric reciprocal contractions at $60^\circ/s$. The system was calibrated prior to testing on each day according to instructions in the Biodex manual. Biodex testing velocity at $60^\circ/s$ of $H_C/Q_C$ strength ratios has been used previously. Subjects were seated in the Biodex chair, with dominant limb secured with the appropriate Biodex attachment for the knee flexion and extension. Subjects were secured at the chest, waist, thigh, and lower limb to ensure proper stabilization. Limb weight was taken prior to testing, and range of motion limits were set with the knee in both flexion and extension. Strength testing was described to
subjects as a maximal test in which the subject should move as fast and as hard as possible in a continuous motion without stopping, and was instructed to continue breathing throughout the entire test.

Peak knee flexion and extension torque protocol was similar to that used by Nagai and colleagues. Tests began with the knee fully flexed, and subjects were given 3 practice trials performed at 50% maximal effort. Instructions were repeated to the subject for the test, and questions from subjects (if any) were answered. An additional set of 3 practice trials were performed at 100% effort. After a 60-second recovery period, testing began. Subjects performed 5 maximal effort repetitions as fast and as hard as possible for knee extension and flexion. Only the dominant limb was tested for peak torque values.

3.4.3 Non-Invasive Glycogen Content Measurement

The Philips Lumify portable ultrasound transducer was utilized with a portable electronic tablet, and integration and processing of image data was accomplished by Lumify Mobile App v1.2 and MuscleSound technology. Subjects were informed of the muscle groups of interest: rectus femoris (RF), the vastus lateralis (VL), vastus medialis (VM), lateral hamstring (LH) and medial (MH), and gastrocnemius/soleus (GS) muscles. Three images for each muscle group per set of scans (5 sets of scans for each muscle group, taken pre- during, and post- fatigue) were taken for consistency in location and image quality. Locations of each muscle of interest are described below:

- Rectus Femoris (RF): The midpoint distance between the top of the kneecap and the ASIS using a measuring tape and a surgical marker to mark the RF point of reference.
• Vastus Lateralis (VL): The VL was found by using the RF mark as a reference point, and sliding the transducer laterally. Using the RF image reference medially on the screen, the VL was located as the RF muscle could no longer be seen.

• Vastus Medialis (VM): Using the RF reference point, the transducer was moved medially. Fascia brightness indicated the disparity of the vastus medialis apart from the rectus femoris. The transducer was moved medially just until the rectus femoris was no longer visible.

• Lateral Hamstring (LH): The belly of the lateral hamstring was defined as the location halfway between the greater trochanter and the head of the fibula. To aid in location specificity, subjects flexed their knee and raised their heel toward the ceiling, thus causing the belly of the muscle to be more prominent. The intersection of the medial and lateral hamstring was located with the short axis of the transducer. The transducer was moved laterally until the intersection was no longer in view. The transducer was then shifted long axis for image capture.

• Medial Hamstring (MH): Returning to the intersection of the medial and lateral hamstring that was found in locating the hamstring muscle group, the transducer was moved along the bicep femoris. Both heads of the bicep femoris muscle were moved into view the long head thickness being about 2 cm. When the long head of the biceps tendon reached about 2 cm in thickness, the transducer was moved medially until the bicep femoris was just lateral to the image on the screen.

• Gastrocnemius/Soleus (GS): The GS was found by the subject plantarflexing at the ankle, allowing the medial and lateral aspect of the calf to be prominent. Once the division between the inside and outside of the calf was located, the probe was moved upwards
about a half inch towards the mid-belly of the muscle. This area revealed continuous fascia that formed an apex as the division between gastrocnemius lateral and medial muscle fibers. The apex of the lateral and medial gastrocnemius also revealed the soleus, deep to the gastrocnemius.

Subjects were asked to lie supine on a treatment table for ultrasound image capture of the RF, VL, and VM. For scans of the LH, MH, and GS, subjects laid face down on treatment table. The transducer was oriented short axis for images collected for all muscles except for the hamstring muscles. Due to the linear orientation of the hamstring muscle group, image capture occurred with the transducer oriented on the long axis. Ultrasound gel was applied on the skin for each image capture. Each muscle was scanned three times consecutively before a new muscle was located. Preliminary intra-rater test-retest reliability with 95% confidence interval (95% CI) and precision data is summarized in Table 1. Based on the preliminary results, this procedure is reliable (ICC = 0.716 – 0.907) and precise (SEM = 1.822 – 2.958) for each muscle group. Image capture occurred in the order of capture was RF, VL, VM, LH, MH, GS. Standard error measurement was calculated by the following mathematical equation using standard deviation and ICC value: \[ SEM = SD \sqrt{(1-ICC)} \]. MuscleSound technology relied on the shading of the scans and linear striations of muscles to calculate the amount of glycogen in the muscle under observation. Examples of muscle groups scanned by ultrasound are shown in Figure 2. Scan depth was consistent among subjects and muscle groups, set at 3.5cm, as well as transducer frequency of 12Hz.
Table 1. Intrarater Test-Retest Reliability, 95% Confidence Interval (95% CI) and Standard Error Measurement (SEM)

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>ICC (2,1)</th>
<th>95% CI</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectus Femoris</td>
<td>0.725</td>
<td>0.220, 0.924</td>
<td>2.232</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>0.744</td>
<td>0.118, 0.935</td>
<td>1.822</td>
</tr>
<tr>
<td>Vastus Medialis</td>
<td>0.888</td>
<td>0.632, 0.971</td>
<td>2.792</td>
</tr>
<tr>
<td>Lateral Hamstring</td>
<td>0.716</td>
<td>0.233, 0.926</td>
<td>2.958</td>
</tr>
<tr>
<td>Medial Hamstring</td>
<td>0.907</td>
<td>0.674, 0.976</td>
<td>2.225</td>
</tr>
<tr>
<td>Gastrocnemius/Soleus</td>
<td>0.764</td>
<td>0.296, 0.936</td>
<td>2.497</td>
</tr>
</tbody>
</table>
Figure 2. Scanned Images (from top left/right to bottom): Rectus Femoris, Vastus Lateralis, Vastus Medialis, Lateral Hamstring, Medial Hamstring, and Gastrocnemius/Soleus
3.4.4 Reactive Strength Index

Reactive strength index was collected through a depth jump protocol that was similar to the validated protocol by Flanagan and colleagues.\textsuperscript{38} Force plate sampling frequency of 1500 Hz remained constant throughout all testing procedures. Collected data were passed from analog to digital signal, and corresponding software was utilized to process depth jump data. Subjects began by standing on a box 30cm high. They were instructed to step off the box and down onto the force plate. On contact with the force plate, subjects were asked to jump up as high as possible, minimizing the time spent on the force plate. For accurate data collected, subjects were asked to stick the landing of the jump and regain stabilization by standing as still as possible on the force plate for 7 seconds after the jump.

The reactive strength index (RSI) was calculated by dividing jump height (JH) by the time on the ground required to make the vertical jump. Prior to initial data collected, subjects were given as many practice trials necessary to feel comfortable with the procedures and maximize consistent performance of the protocol, similar to the study performed by Wikstrom and colleagues\textsuperscript{114} as referenced in the work of Flanagan and colleagues.\textsuperscript{38} Jump height and ground contact time (CT) were shown to be highly reliable with this protocol from trial to trial (Cronbach $\alpha > 0.95$), high single measure ICCs, ($>0.9$) and high average measures ICCs ($>0.95$).\textsuperscript{38} Due to the above variables being highly reliable, RSI therefore was a reliable measure gathered through this protocol.
3.4.5 Fatigue Protocol

An intermittent soccer-specific treadmill protocol was performed on a motorized treadmill. Subjects performed isokinetic strength testing, depth jumps, and ultrasound glycogen quantification before and after the soccer-specific intermittent running protocol. Prior to testing, subjects performed a self-paced warm up on the treadmill for 5 minutes. After the 5 minute warm up, subjects were taken through the treadmill speeds used for the intermittent running protocol. The speeds and duration of the intervals were previously used by Drust et al. and described in Figure 3 below. Speeds described in that protocol were 6 km/h (walking), 12 km/h (jogging), 15 km/h (cruising), and 21 km/h (sprinting). The intermittent running described in the above research was divided into two equal parts: 22 minutes in duration separated by a 1 minute rest period. The intermittent running protocol that simulated soccer activity was based off video analysis of an elite men’s soccer match. Speeds in the current study were decreased in order to account for the lower fitness levels of the subjects participating in the study. Each subject performed the intermittent running protocol for nearly 45 minutes in duration.

![Figure 3. Intermittent Running Protocol Simulating 22 Minutes of Soccer Match Play](image-url)
The fatigue protocol was timed, from the start of the uphill treadmill running protocol until volitional exhaustion. The purpose of the exhaustive running protocol was to ensure fatigue, due to varying fitness of the athletes. The initial speed of the treadmill was set at 5 km/h\(^{-1}\) at 18\% grade and increased by 1 km/h\(^{-1}\) every two minutes until volitional exhaustion.\(^6^4\) Heart rate, rating of perceived exhaustion (RPE) using the OMNI scale, and lactate measurement were recorded to assess fatigue.\(^{15, 88, 112}\) Lactate measure was taken during baseline testing, after the intermittent running protocol, and immediately after the uphill running protocol until fatigue. Rating of perceived exertion was taken during the intermittent treadmill protocol, at each 2 minute stage of the fatigue protocol, and at the time of exhaustion. This uphill running protocol was originally developed as a tool to evaluate neuromuscular recovery after peripheral lower extremity fatigue.\(^6^4\)

3.4.6 Ultrasound Glycogen Measurement

MuscleSound software was used for image interpretation and objectification of glycogen content to numerical score. Muscles of interest were scanned pre-, during, and post-intermittent running fatigue protocol. MuscleSound processing cropped images so that only ultrasound images remained. The ultrasound images were based on reflected sound waves from the transducer. The reflective nature of bone, ligaments, tendons, muscles, and other soft tissue is based on the degree of absorption versus reflection of the sound waves.\(^8^4\) Areas of tissue that have little reflection of the sound waves are revealed in darker shaded scans, which are considered as being hypoechoic. Images that are brighter in appearance (bone, cortical surfaces, fascial layers) are
considered hyperechoic. During image processing, MuscleSound software highlighted the area that it analyzed as the muscle of interest. This highlighted area was a component of the MuscleSound software, and the area inside the highlighted region to be processed for glycogen quantification could not be altered by the researcher. Areas within a scan that appeared hypoechoic signified a higher concentration of glycogen, while areas that were hyperechoic signified areas that were depleted of glycogen. Gaussian blur was applied via image smoothing technique, so that the ultrasound image could be converted to binary black and white image. Morphing techniques were applied to fill in holes, which connected portions of the muscle scans. Connective tissue is considered a critical artifact and was digitally subtracted. The image then was then returned to gray scale. Subsequent cropping and filtering visually removed skin, fat, connective tissue, and artifact. Connective tissue that remained was portrayed at 255 pixel intensity, and the muscle glycogen score was determined as a measure of pixel intensity. Pixels were averaged and linked across images automatically, and the pixel intensity was proportionally reduced. Pixel intensity determined through subjective image converted to objective value by pixel intensity ranging from 1-255 pixel intensity. For simplicity of interpretation, scale of intensity was simplified to a scale of 0-100 (higher values represented higher muscle glycogen content).103

3.4.7 Biodex Peak Torque Measurement

The Biodex Isokinetic Dynamometer System 3 was used to measure peak knee flexion and extension torque (Nm). The average of five torque values for each direction was calculated by the Biodex. The average peak knee flexion and knee extension torque (Nm) was normalized to body weight (kg) and expressed in percentage of body mass (%BM) for statistical analyses. After
the average peak torque values for knee flexion and extension were calculated, torque ratio was calculated by dividing the average peak knee flexion torque by the average peak knee extension torque for statistical analyses.

### 3.4.8 Force Plate Measurement for RSI

Force plate data determined ground reaction force from which initial foot contact to landing.\(^{38}\) The time points between takeoff and landing signified flight time. Flight time was used to calculate RSI \([(9.81 \times \text{flight time}^2)/8]\). Jump height, flight time, initial contact, and landing forces were converted from analog to digital signal through BioWare force plate technology.

### 3.4.9 Dietary Recall

Subjects performed an online dietary recall using the ASA 24 recall questionnaire. After the testing procedures, each subject reported their dietary intake 24 hours prior to testing. The ASA 24 software generated macronutrient and micronutrient data based on subjects’ reported dietary intake.

### 3.5 STATISTICAL ANALYSIS

Data analyses were conducted using SPSS (version 22.0; IBM Corp, Armonk, NY). Descriptive statistics were calculated for all dependent variables (means and standard deviations). Each variable was checked for normality using the Shapiro-Wilk test. For the first specific aim, paired
t-tests or Wilcoxon signed rank test (non-parametric test) was used to compare each dependent variable before and after the intermittent running fatigue protocol. For the second specific aim and hypotheses, changes in strength, power, and muscle glycogen before and after the fatigue protocol were calculated and checked for normality using the Shapiro-Wilk test. Then, correlation analyses were used to examine the relationship between the change in strength and power and muscle glycogen content using Pearson’s correlations or Spearman’s rank tests (non-parametric test). Similarly, for the third specific aim and hypotheses, the same correlation analyses were used to examine the relationship between the baseline muscle glycogen content and the change in strength and power variables. Statistical significance was set a priori at alpha equal to 0.05.
4.0 RESULTS

4.1 SUBJECT DEMOGRAPHICS

Due to feasibility in recruitment, subjects recruited for testing were recreationally active females who participated in moderate to intense exercise a minimum of three times per week for 60 minutes. A majority of subjects (15 subjects) were current soccer players at the college level or active members of an amateur soccer league; training for this league included twice a week sessions each lasting two hours, and one weekly match. All athletes were between the ages 18-30, fitting the inclusion criteria. Two potential subjects were denied participation into the study due to a history of lower limb surgery (ankle surgery and knee surgery) within the past year. Demographic information is included in Table 2. The dominant limb was left in three subjects while the rest of subjects were right limb dominant.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Means ± SDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.5 ± 2.9</td>
</tr>
<tr>
<td>Height (centimeters)</td>
<td>166.9 ± 7.2</td>
</tr>
<tr>
<td>Mass (kilograms)</td>
<td>63.7 ± 6.6</td>
</tr>
</tbody>
</table>
All subjects completed the intermittent running protocol, and each subject performed the uphill running bout until volitional failure. Blood lactate was taken immediately before and after the intermittent running protocol (Table 3). The rating of perceived exertion in the OMNI scale (0-10) and heart rate results were taken at the end of the intermittent running bout #1-3 (Table 3).

### Table 3. Blood Lactate, Heart Rate, and RPE during the Intermittent Running Protocol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Means ± SDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Blood Lactate (mmol/l)</td>
<td>2.9 ± 2.1</td>
</tr>
<tr>
<td>Baseline Heart Rate (bpm)</td>
<td>84.0 ± 16.8</td>
</tr>
<tr>
<td>RPE at the end of bout #1</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>Heart Rate at the end of bout #1 (bpm)</td>
<td>161.8 ± 17.2</td>
</tr>
<tr>
<td>RPE at the end of bout #2</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the end of bout #2 (bpm)</td>
<td>168.8 ± 15.0</td>
</tr>
<tr>
<td>RPE at the end of bout #3</td>
<td>7.0 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the end of bout #3 (bpm)</td>
<td>173.3 ± 14.7</td>
</tr>
<tr>
<td>Post-Protocol Blood Lactate (mmol/l)</td>
<td>7.0 ± 4.7</td>
</tr>
</tbody>
</table>

RPE = rating of perceived exertion; bpm = beats per minute; mmol/l = millimoles per liter

After the intermittent running protocol, subjects completed the knee strength, power, and ultrasound muscle glycogen content assessments. The results of these assessments were not a part of the current thesis proposal and therefore not included in the current document. Immediately after the assessments, subjects were asked to complete the uphill running bout until
volitional failure. The RPE in the OMNI scale and heart rate results taken at the end of each 2-minute stage and at the volitional failure during the uphill running protocol (Table 4). Additionally, blood lactate was measured immediately after the uphill running protocol (Table 4). Two athletes finished in the stage #3 while 14 athletes finished in the stage #4. Only one athlete finished in the stage #5. After the blood lactate was taken, subjects were asked to complete the last/final set of the knee strength, power, and muscle glycogen content assessments.

Table 4. Blood Lactate, Heart Rate, and RPE during the Uphill Running Protocol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Means ± SDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE at the end of stage #1</td>
<td>4.7 ± 1.6</td>
</tr>
<tr>
<td>Heart Rate at the end of stage #1 (bpm)</td>
<td>159.8 ± 16.0</td>
</tr>
<tr>
<td>RPE at the end of stage #2</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the end of stage #2 (bpm)</td>
<td>172.0 ± 20.3</td>
</tr>
<tr>
<td>RPE at the end of stage #3</td>
<td>8.1 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the end of bout #3 (bpm)</td>
<td>182.1 ± 10.7</td>
</tr>
<tr>
<td>RPE at the end of stage #4</td>
<td>8.7 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the end of bout #4 (bpm)</td>
<td>184.1 ± 11.1</td>
</tr>
<tr>
<td>RPE at the volitional failure</td>
<td>8.4 ± 1.4</td>
</tr>
<tr>
<td>Heart Rate at the volitional failure (bpm)</td>
<td>185.7 ± 9.5</td>
</tr>
<tr>
<td>Post-Protocol Blood Lactate (mmol)</td>
<td>11.3 ± 5.0</td>
</tr>
</tbody>
</table>

RPE = rating of perceived exertion; bpm = beats per minute; mmol/l = millimoles per liter
4.3 EFFECTS OF FATIGUE PROTOCOL ON STRENGTH, REACTIVE STRENGTH INDEX, AND MUSCLE GLYCOGEN CONTENT

4.3.1 Effects of Fatigue Protocol on the Knee Flexion and Extension Strength and Flexion/Extension Ratio

Average peak torque over the five trials performed on the Biodex System 3 at 60°/sec$^{-1}$ was collected pre- and post- fatigue protocol. Average peak strength and peak strength ratio between hamstrings and quadriceps were compared pre- to post- fatigue to examine changes in force output. All strength variables were screened for normally using Shapiro-Wilk tests. They were normally distributed (p = 0.089 – 0.908) except post-fatigue flexion/extension strength ratio (p = 0.011).

In order to address specific aim 1 and hypothesis 1a, the average peak knee flexion, extension, and flexion/extension strength ratio were compared before and after the fatigue protocol. Descriptive data (means and standard deviations) is shown in Table 5. Average peak knee flexion and extension and ratio of knee flexion to extension were significantly decreased from pre-post fatigue protocol (p < 0.05), supporting hypotheses 1a.

Table 5. Effect of Fatigue on Strength

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Pre-Fatigue</th>
<th>Post-Fatigue</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion (%BW)</td>
<td>129.1 ± 22.7</td>
<td>115.9 ± 25.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Extension (%BW)</td>
<td>231.9 ± 28.5</td>
<td>218.8 ± 39.6</td>
<td>0.016</td>
</tr>
<tr>
<td>Flex/Ext Ratio (%)*</td>
<td>55.8 ± 8.5</td>
<td>53.4 ± 10.2</td>
<td>0.039</td>
</tr>
</tbody>
</table>

* Wilcoxon Signed Rank test; %BW = percent of body weight
4.3.2 Effects of Fatigue Protocol on Reactive Strength Index

All subjects performed three trials of the depth jump task pre and post-fatigue protocol, and the RSI was calculated. The RSI pre- and post-fatigue protocol was tested for normality using Shapiro-Wilk normality test, and the pre-fatigue RSI was significant (p = 0.006). Therefore, non-parametric test (Wilcoxon signed rank test) was used to compare the RSI pre and post-fatigue. In order to assess hypothesis 1b, pre-fatigue RSI and post-fatigue RSI were compared using Wilcoxon signed rank test. Significant increases in RSI were found from pre-fatigue to post-fatigue shown in Table 6 (pre-fatigue RSI = 0.671 ± 0.236, post-fatigue RSI = 0.749 ± 0.276, p = 0.006). Contrary to hypothesis 1b, RSI was significantly increased from pre- to post-fatigue.

Table 6. Effect of Fatigue on Reactive Strength Index

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Pre-Fatigue</th>
<th>Post-Fatigue</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Strength Index*</td>
<td>0.671 ± 0.236</td>
<td>0.749 ± 0.276</td>
<td>0.006</td>
</tr>
</tbody>
</table>

*Wilcoxon Signed Rank test

4.3.3 Effects of Fatigue Protocol on Muscle Glycogen Content

Glycogen content was analyzed per muscle group using MuscleSound technology. Average score of 15 ultrasound images per muscle were analyzed pre- and post-fatigue protocol. Average glycogen score per muscle group was analyzed for normality using Shapiro Wilk normality test. The post-fatigue lateral hamstring was significant (p = 0.001).
In order to address hypothesis 1c, paired t-tests (Wilcoxon Signed Rank test for lateral hamstring) were performed to compare the muscle glycogen content before and after the fatigue protocol. Descriptive statistics and t-test results are shown in Table 7. Contrary to hypothesis 1c, no significant changes were observed in glycogen content in any muscle group examined pre- to post-fatigue protocol.

**Table 7. Effect of Fatigue on Muscle Glycogen Content**

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Pre-Fatigue</th>
<th>Post-Fatigue</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectus Femoris</td>
<td>44.3 ± 1.3</td>
<td>45.1 ± 1.4</td>
<td>0.079</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>46.0 ± 1.3</td>
<td>45.8 ± 1.3</td>
<td>0.261</td>
</tr>
<tr>
<td>Vastus Medialis</td>
<td>46.3 ± 1.4</td>
<td>46.3 ± 1.5</td>
<td>0.787</td>
</tr>
<tr>
<td>Lateral Hamstring*</td>
<td>48.3 ± 1.6</td>
<td>47.9 ± 1.6</td>
<td>0.177</td>
</tr>
<tr>
<td>Medial Hamstring</td>
<td>46.3 ± 1.6</td>
<td>46.4 ± 2.2</td>
<td>0.733</td>
</tr>
<tr>
<td>Gastrocnemius/Soleus</td>
<td>47.1 ± 1.5</td>
<td>46.9 ± 1.1</td>
<td>0.471</td>
</tr>
</tbody>
</table>

* Wilcoxon Signed Rank test

### 4.4 RELATIONSHIP BETWEEN THE CHANGE IN STRENGTH AND REACTIVE STRENGTH INDEX AND THE CHANGE IN MUSCLE GLYCOGEN CONTENT

Knee flexion and extension peak torque expressed as a ratio from pre- to post-fatigue, as well as flexion to extension ratio pre- to post-fatigue, were analyzed for normality using Shapiro-Wilk. Similarly, the pre- to post-fatigue reactive strength index and muscle glycogen content of six muscle groups were also screened for normality. Based on Shapiro-Wilk normality tests, the
post/pre-fatigue knee extension value was significant (p = 0.008); and the assumption of
normality was violated. The post/pre-fatigue reactive strength index was also significant, and the
assumption of normality was violated (p = 0.001). All muscle groups were normally distributed
except the post/pre-fatigue in the medial hamstring (p = 0.014).

Descriptive statistics of pre/post-fatigue strength variables, reactive strength index, and
muscle glycogen content were shown in Table 8. As previously observed in specific aim #1,
systematic declines in strength variables were observed (89.3 – 95.5%) while an increase in the
reactive strength index was observed (112.6%). However, only small changes were observed in
muscle glycogen content (99.3 – 101.7%).

Table 8. Descriptive Statistics of Post/Pre-Fatigue Values in Knee Strength Variable, Reactive
Strength Index, and Muscle Glycogen Content

<table>
<thead>
<tr>
<th>Strength and RSI Variables</th>
<th>Means ± SDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post/Pre-Fatigue Flexion Strength (%)</td>
<td>89.3 ± 8.5</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Extension Strength (%)</td>
<td>93.9 ± 9.5</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Strength Ratio (%)</td>
<td>95.5 ± 7.7</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Reactive Strength Index (%)</td>
<td>112.6 ± 23.8</td>
</tr>
<tr>
<td>Muscle Glycogen Content</td>
<td>Statistics</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Rectus Femoris (%)</td>
<td>101.7 ± 3.7</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Vastus Lateralis (%)</td>
<td>99.5 ± 1.8</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Vastus Medialis (%)</td>
<td>99.9 ± 1.4</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Lateral Hamstring (%)</td>
<td>99.3 ± 2.8</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Medial Hamstring (%)</td>
<td>100.2 ± 3.0</td>
</tr>
</tbody>
</table>
In order to examine hypotheses 2a and 2b, the post/pre-fatigue values in muscle glycogen content for each muscle group were evaluated for correlations with the post/pre-fatigue values in the knee strength variables and reactive strength index. Correlations were analyzed using two-tailed Pearson’s correlations for normally distributed values while Spearman’s correlations were used for non-parametric analyses (Table 9). Contrary to hypotheses 2a and 2b, there were no significant correlations between the post/pre-fatigue muscle glycogen content and post/pre-fatigue knee strength and reactive strength index.

<table>
<thead>
<tr>
<th></th>
<th>Rectus Femoris</th>
<th>Vastus Lateralis</th>
<th>Vastus Medialis</th>
<th>Lateral Hamstring</th>
<th>Medial Hamstring*</th>
<th>Gastrocnemius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Flexion</td>
<td>r = -0.202</td>
<td>r = -0.187</td>
<td>r = -0.032</td>
<td>r = -0.171</td>
<td>r = -0.162</td>
<td>r = -0.146</td>
</tr>
<tr>
<td>p</td>
<td>p = 0.438</td>
<td>p = 0.473</td>
<td>p = 0.903</td>
<td>p = 0.511</td>
<td>p = 0.535</td>
<td>p = 0.577</td>
</tr>
<tr>
<td>Knee Extension</td>
<td>r = -0.328</td>
<td>r = -0.208</td>
<td>r = 0.132</td>
<td>r = -0.213</td>
<td>r = -0.363</td>
<td>r = -0.020</td>
</tr>
<tr>
<td>p</td>
<td>p = 0.198</td>
<td>p = 0.422</td>
<td>p = 0.613</td>
<td>p = 0.411</td>
<td>p = 0.152</td>
<td>p = 0.940</td>
</tr>
<tr>
<td>Knee Flex/Ext</td>
<td>r = 0.023</td>
<td>r = 0.180</td>
<td>r = 0.040</td>
<td>r = -0.177</td>
<td>r = 0.152</td>
<td>r = 0.017</td>
</tr>
<tr>
<td>p</td>
<td>p = 0.930</td>
<td>p = 0.490</td>
<td>p = 0.879</td>
<td>p = 0.496</td>
<td>p = 0.560</td>
<td>p = 0.947</td>
</tr>
<tr>
<td>Reactive</td>
<td>r = -0.098</td>
<td>r = -0.140</td>
<td>r = 0.213</td>
<td>r = -0.181</td>
<td>r = -0.056</td>
<td>r = -0.064</td>
</tr>
</tbody>
</table>
Table 9. Correlations of Post/Pre-Fatigue Muscle Glycogen Content and Post/Pre-Fatigue Strength and Reactive Strength Index

| Strength Index | p = 0.708 | p = 0.593 | p = 0.411 | p = 0.486 | p = 0.830 | p = 0.808 |

Table 9. Correlations of Post/Pre- Fatigue Muscle Glycogen Content and Post/Pre-Fatigue Strength and Reactive Strength Index

a Units of measurement are percentages (%).
*Spearman’s correlation analyses were used.

4.5 RELATIONSHIP BETWEEN THE CHANGE IN STRENGTH AND REACTIVE STRENGTH INDEX AND THE BASELINE MUSCLE GLYCOGEN CONTENT

The post/pre-fatigue knee flexion, extension, flexion/extension ratio, and reactive strength index were already assessed for normality previously. Similarly, the pre-fatigue muscle glycogen content was already assessed for normality previously with the Shapiro-Wilk normality test. Post/pre-fatigue extension strength and post/pre-fatigue reactive strength index violated the assumption of normality. Therefore, Spearman’s correlational analyses were used. Descriptive statistics of pre-fatigue muscle glycogen content and post/pre-fatigue strength variables and reactive strength index are shown in Table 10.

Table 10. Descriptive Statistics of Pre-Fatigue Muscle Glycogen Content and Post/Pre-Fatigue Values in Knee Strength Variable and Reactive Strength Index

<table>
<thead>
<tr>
<th>Strength and RSI Variables</th>
<th>Means ± SDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post/Pre-Fatigue Flexion Strength (%)</td>
<td>89.3 ± 8.5</td>
</tr>
<tr>
<td>Post/Pre-Fatigue Extension Strength (%)</td>
<td>93.9 ± 9.5</td>
</tr>
</tbody>
</table>
Post/Pre-Fatigue Strength Ratio (%) 95.5 ± 7.7
Post/Pre-Fatigue Reactive Strength Index (%) 112.6 ± 23.8

Muscle Glycogen Content Statistics
Pre-Fatigue Rectus Femoris 44.3 ± 1.3
Pre-Fatigue Vastus Lateralis 46.0 ± 1.3
Pre-Fatigue Vastus Medialis 46.3 ± 1.4
Pre-Fatigue Lateral Hamstring 48.3 ± 1.6
Pre-Fatigue Medial Hamstring 46.3 ± 1.6
Pre-Fatigue Gastrocnemius/Soleus 47.1 ± 1.5

SDs = standard deviations; % = percent

In order to examine hypothesis 3a and 3b, the pre-fatigue muscle glycogen content for each muscle group was assessed during baseline measures; average score was quantified as an average of fifteen scans per muscle group. The pre-fatigue muscle glycogen content values were correlated to the post/pre-fatigue knee flexion strength, post/pre-fatigue knee extension strength, post/pre-fatigue flexion/extension strength ratio, and post/pre-fatigue reactive strength index.

Table 11. Correlations of Post/Pre- Fatigue Ratio (%) Between Strength, RSI, and Baseline Glycogen Content

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Rectus Femoris</th>
<th>Vastus Lateralis</th>
<th>Vastus Medialis</th>
<th>Lateral Hamstring</th>
<th>Medial Hamstring</th>
<th>Gastrocnemius/Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee Flexion Strengtha</td>
<td>r = -0.020</td>
<td>r = 0.180</td>
<td>r = -0.616</td>
<td>r = -0.201</td>
<td>r = -0.243</td>
<td>r = 0.278</td>
</tr>
<tr>
<td></td>
<td>p = 0.940</td>
<td>p = 0.490</td>
<td>p = 0.008</td>
<td>p = 0.440</td>
<td>p = 0.348</td>
<td>p = 0.280</td>
</tr>
</tbody>
</table>
It was hypothesized that pre-fatigue muscle glycogen content per muscle group would be negatively correlated with the post/pre-fatigue strength variables (hypothesis 3a) and reactive strength index (hypothesis 3b). However, only post/pre-fatigue knee flexion and knee extension strength had moderate negative correlation to the pre-fatigue glycogen measures of the vastus medialis (knee extension: \( r = -0.616, p = 0.008 \); knee extension: \( r = -0.603, p = 0.010 \)) as shown in Table 11. As mentioned previously in the methods section (Section 3.4.6), measurement for baseline glycogen content is quantified as a value 0-100 without a unit (higher values represent more muscle glycogen in the muscle). Contrary to hypothesis 3b, there was no significant relationship (in the negative direction) between RSI and baseline muscle glycogen content for any of the muscle groups (Table 11).
5.0 DISCUSSION

5.1 EFFECTS OF FATIGUE PROTOCOL ON STRENGTH, POWER, AND MUSCLE GLYCOGEN CONTENT

The primary purpose of this study was to evaluate the changes in strength, power, and muscle glycogen content before and after an intermittent running protocol and uphill running bout. It was hypothesized that knee strength, power, and muscle glycogen content would significantly decrease after the fatigue protocol. For hypothesis 1a, it was hypothesized that the peak isokinetic knee flexion and extension torque would be significantly reduced after the fatigue protocol. Also, it was hypothesized that the peak knee flexion/extension ratio would be significantly lower after the fatigue protocol. The current results support the hypothesis 1a as the knee strength and strength ratio were significantly decreased after fatigue protocol.

Strength and power of the lower limbs have been shown to be decreased due to soccer simulated exercise, as well as lower limb intramuscular glycogen content. The current study revealed that knee extension and flexion strength significantly declined pre- to post- fatigue protocol. As expected in hypothesis 1a, average peak torque of the hamstrings and quadriceps decreased pre- to post- fatigue in agreement with previous studies. Peak torque of the knee extensors and flexors has been shown to decrease due to fatigue induced by exercise simulating a full soccer match.
Additionally, the peak torque ratio of the hamstrings to quadriceps significantly declined after the fatigue protocol. It has been shown that fatigue caused by soccer simulated exercise decreases the \(H_c:Q_c\) ratio.\(^{30}\) The current findings support previous research that soccer play causes concentric maximal torque of the hamstrings and quadriceps to decline.\(^{98}\) Previous research using soccer match modeling revealed that even by halftime, decreases in hamstring and quadriceps strength were evident.\(^{98}\) Protocols utilized in the literature vary in duration and mode to analyze soccer activity and strength relationships, \(^{98,111}\) while the protocol used in the current study was short in duration (~45 minutes), significant decreases in quadriceps and hamstring peak torque and \(H_c:Q_c\) ratio were found, suggesting that a short duration (~45 minutes) intermittent running fatigue protocol was sufficient to observe subjects’ fatigue. The findings of the current study are in agreement with previous research described above with changes peak torque of knee flexors, extensors, and peak torque ratio caused by fatigue and should be considered in terms of athlete performance in the presence of fatigue.

For hypothesis 1b, it was hypothesized that RSI would be significantly reduced after the fatigue protocol. The RSI was in fact significantly increased after the fatigue protocol, rejecting hypothesis 1b. The explanation for the rejection of hypothesis 1b may be multifaceted. The RSI calculation is dependent on rate of force development and expression of the SSC. Short contact times and high jump heights will result in a high RSI output, reflecting rapid rate of force development and efficient function of the stretch shortening cycle. Unlike the isokinetic dynamometer, the subject’s ability to properly carry out the testing measure has a direct implication to the RSI output. Descriptive data shown in Appendix A show changes in ground contact time (CT) and jump height (JH), which are components of the RSI output. The descriptive data, although not statistically analyzed, shows that there is a decrease in CT from
pre- to post- fatigue, and an increase in JH pre- to post- fatigue. This may indicate a learning curve, as movement efficiency potentially increased. As subjects became more accustomed to the movement, they were able to perform the sport specific movement pattern with greater efficiency. Previous research indicates that rate of force development decreases due to fatigue induced by soccer activity, and that SSC is decreased in the presence of fatigue. The current findings revealed that reactive strength values increased from pre-fatigue to post fatigue, partially attributable to a learning curve.

The athletic nature of the task may be the reason for the confounding results, and may also be cause for the development of the RSImod, a modified method of the RSI calculation used in the current study and validated previously. Due to the calculation of RSI being dependent on ground reaction forces and contact times, manipulation of the ground contact time and jump height will cause RSI values to shift, and improper execution of the task can cause varied results due to eccentric and concentric phases of the movement. Therefore, it is likely that the explanation for the rejection of hypothesis 1b is improper task execution.

For hypothesis 1c, it was hypothesized that the ultrasound-based muscle glycogen content of thigh and calf muscles (rectus femoris, vastus medialis, vastus lateralis, lateral hamstring, medial hamstring, and gastrocnemius/soleus) would be significantly reduced after the fatigue protocol. Based on the current results, muscle glycogen content was not significantly reduced after the fatigue protocol, rejecting hypothesis 1c. The current findings are not consistent with previous research involving fatigue and glycogen quantification. For example, it was found that glycogen stores decline pre- soccer match to post- soccer match, and some muscle fibers can be almost completely depleted of glycogen at the end of a match.
It has been shown that both distance running and soccer match play causes muscle glycogen decrement.\textsuperscript{58, 61} The different intensities of continuous and intermittent running cause metabolic contributions to shift. The higher intensity of soccer match play causes a greater reliance on muscle glycogen, as research has revealed decreased running velocities and higher FFA utilization towards the end of a soccer match.\textsuperscript{5, 7} Muscle glycogen concentration and utilization may be dependent on training status and running mechanics.\textsuperscript{25} Fatigue caused by running at \(\sim 80\% \text{VO}_2\text{max}\) was not attributed to decline in glycogen content, however caution should be taken due to small sample size of the study and the varied training status of the subjects.\textsuperscript{25} However, training status, intensity and duration of exercise, and utilization of glycogen has a role on glycogen depletion. One study revealed that glycogen levels were depleted by 36\% after a soccer simulated protocol, and that there was a moderate relationship between glycogen utilized and time to exhaustion.\textsuperscript{97} Degradation of glycogen is associated with both central and peripheral fatigue. Contributions to fatigue during a soccer match may be attributed to both central and peripheral factors,\textsuperscript{7} thus creating a relationship between fatigue and glycogen utilization.

The sensitivity to detect changes may be a methodological challenge of the MuscleSound technology, as the shading of the scans determined pixel intensity. Scans that appeared dark (hypoechoic) indicated a muscle area being concentrated with glycogen, while lighter areas (hyperechoic) indicated regions that were depleted of glycogen. Ligament and bone are characterized by their hyperechoic nature.\textsuperscript{51} Scans analyzed by MuscleSound software were highlighted for areas corresponding with the muscle of interest. However, the highlighted area previously mentioned and described in Section 3.4.6 may have included various types and concentrations of tissue that altered the hypoechoic or hyperechoic nature of the scan. Muscle
size, bone, and fascia may have been factors that affected the shading of the scans and thus the quantification of glycogen.

Athletes in the current study were allowed to drink water ad libitum, as previous research has indicated that water intake does not affect glycogen utilization, or the reflective nature of the scans. It has been shown that as muscle glycogen is utilized and leaves the cell, water also leaves the cell. As exercise induces fatigue and depletes glycogen stores, it has also been shown that blood vessel dilation and decreases in muscle size result from intense exercise. Physiological changes and metabolic changes that are evident with fatiguing exercise may be cause for changes in the reflective nature of the scans. Structures such as bone and artifact being included in the MuscleSound quantification of the muscle region scanned may have been a potential cause for the lack of changes pre- to post- fatigue.

In addition, the correlations between MuscleSound glycogen score and muscle biopsy were utilized in the current study to convert the glycogen score to a numeric value. The previous study conducted by Hill found that RF MuscleSound glycogen score to be 59.8 ± 15.9 pre-exercise and 39.8 ± 13.9 post-exercise. Muscle biopsy revealed glycogen levels to be 97.2 ± 34.1 mmol*kg⁻¹ pre-exercise to 62.4 ± 22.8 mmol*kg⁻¹ post-exercise. In the current study, average MuscleSound RF muscle glycogen score was 44.3 ± 1.3, and using the correlation found in the previous study, the RF glycogen score would be around 84.73 mmol*kg⁻¹. The correlation equation between muscle biopsy and MuscleSound score was found in the study by Hill as follows: MuscleSound Score = 0.4324 (muscle biopsy glycogen level) + 17.719. It’s important to note that this relationship changed pre-exercise to post-exercise. The data presented in the previous study reveals that there are two separate relationships of MuscleSound quantification to muscle biopsy pre- to post-exercise, which may indicate some methodological shortcomings of
the noninvasive technology to detect changes in muscle glycogen score. The correlation of the baseline RF muscle biopsy glycogen level of the current study is over 10 mmol*kg\(^{-1}\) less than the previous findings.\(^{52}\) The disparity between resting RF levels of glycogen might be attributed to the demographic differences of the subjects (female soccer players in the current study and male cyclists in the previous study), the fasted state of the athletes in the current study, and the differences in fatigue protocols (intermittent repeated run and uphill run in the current study compared to steady pace long-duration cycling on stationary bicycles in the previous study).

Performance variables such as knee strength and running velocity have been shown to decrease from the first to second half of matches, elucidating the onset of fatigue before the match has concluded.\(^{91}\) The intermittent running protocol utilized in the current study, although modified for females, has been previously shown to be reliable and repeatable.\(^{34,\ 91}\) Significant decreases in quadriceps and hamstrings peak torque were found halfway through the soccer simulated running bout used in the previous study, confirming that effects of fatigue on performance even at halftime are evident.\(^{91}\) In addition, the previously mentioned running bout has been shown to cause significant changes in the H\(_{Ci}\):Q\(_C\) ratio after ~45 minutes of soccer simulated running.\(^{91}\) The participants of the previous study were highly trained male soccer players. Due to the inclusion criteria of subjects recruited for the current study, an additional uphill running bout was included to increase peripheral fatigue. The uphill running protocol included in the current study was utilized previously by Lattier et al.\(^{64}\) to evaluate maximal aerobic velocity (MAV) and neuromuscular fatigue.\(^{64,\ 65}\) Volitional fatigue (mean MAV = 9.4 ± 0.9 km*h\(^{-1}\)) during the previous study have shown that this protocol resulted in decreased neuromuscular activation due to central and peripheral fatigue.\(^{64}\) In the current study, the
performance decline following the intermittent running protocol and subsequent uphill running bout can be evidence that those protocols used in the current study did indeed cause fatigue.

5.2 RELATIONSHIP OF CHANGES OF KNEE STRENGTH AND POWER TO CHANGE IN MUSCLE GLYCOGEN CONTENT DUE TO FATIGUE PROTOCOL

Another aim of the current study was to evaluate the relationships of the changes in knee strength and power (expressed as a proportion of post-fatigue/pre-fatigue values) to the changes in muscle glycogen (also expressed as a proportion of post-fatigue/pre-fatigue values) due to the fatigue protocol. It was hypothesized that fatigue at the peripheral level caused by intermittent running would cause positive correlations between the changes in knee strength and power with changes in muscle glycogen. For hypothesis 2a, it was expected that changes in knee flexion, extension, and torque ratio would be significantly correlated to muscle glycogen in the positive direction. However, the current results show no correlation between changes in knee strength and strength ratio to changes in muscle glycogen, therefore we reject hypothesis 2a. It was also hypothesized in hypothesis 2b that there would be a positive correlation with changes in power to the changes in muscle glycogen content. Similar to the findings for hypothesis 2a, no positive relationship was found between changes in power post-fatigue/pre-fatigue and changes in muscle glycogen post-fatigue/pre-fatigue. Possible explanations for the current findings are discussed below.

The proportion of decline in knee strength and strength ratio was hypothesized to be related in the positive direction to proportion of decline in muscle glycogen content. It was shown in the current study that there was a decline in proportion of muscle strength output due to fatigue. Statistics in Table 7 and Table 8 describe the lack of positive relationship between
changes in strength and changes in muscle glycogen. It is shown that there is a decline in knee
strength and strength ratio, however muscle glycogen changes are minimal. Surprisingly, there
was an increase in muscle glycogen content in the RF from pre-fatigue to post-fatigue, which
does not coincide with previous research regarding glycogen fuel utilization and simulated
soccer activity.96 A predominant contributing factor to fatigue is depletion of glycogen,61,96
consequently leading to the hypothesis of strength decrement to be related to glycogen depletion.
The findings of the current study may reveal the sensitivity of the MuscleSound technology to
detect changes may not be precise enough to cause trends in glycogen utilization, which is
supported by the descriptive pre-/post- fatigue data in Table 7 and the proportion of glycogen
utilization post-/pre- fatigue in Table 9.

Similar reasoning for the lack of relationship between power changes post-/pre- fatigue to
glycogen changes post-/pre- fatigue can explain the current findings. Descriptive data in Table 8
reveals that there was highly variable individual data, and that this may be that the RSI protocol
was not executed similarly for each subject. Each subject was given similar instructions,
however subjects’ ability to perform short contact time with initial touch onto the force plate and
maximal vertical jump into the air was subjective. It was expected that there would be a decline
in power measures pre-fatigue to post-fatigue, due to glycogen content decline. As muscular
strength declines due to fatigue, fatigue induces rate of force decrements which may lead to
impaired ability to exert explosive actions necessary for high intensity activity in soccer.111
Decreases in force output are related to changes in contractile properties caused by metabolic
changes as a result of fatigue.64 Fatigue response at the metabolic and neural level is very
individual and thus a fixed exercise duration may not reveal true effects of fatigue,81 thus
intermittent activity could cause power decrement. It has been shown that both contractile
properties and sprint ability declines pre- to post- soccer match,\textsuperscript{92} as does vertical jump height,\textsuperscript{98} attributed to fatigue and likely linked to glycogen availability. It has been shown that both resistance training and plyometric training cause increased rate of force of development, increased area of type-II muscle fibers, increased motor unit recruitment, and increased motor unit firing frequency.\textsuperscript{56} The training status of the athletes (e.g. college athletes, recreational athletes) in the current study, the difficulty in proper execution of the power protocol (which has been previously validated), and the potential lack of sensitivity of the glycogen measurement are likely contributors for the lack of correlation between proportion of changes in power and glycogen content due to fatigue.

\section*{5.3 RELATIONSHIP OF CHANGES OF KNEE STRENGTH AND POWER TO BASELINE GLYCOGEN CONTENT}

An additional area of investigation of the current study was to evaluate any relationship to changes in knee strength and power output as a proportion post-/pre-fatigue to baseline glycogen content. As the intermittent running bout caused fatigue, it was hypothesized that resting glycogen levels would have a relationship to the changes (expressed as a proportion post-fatigue/pre-fatigue) in knee strength and power. Specifically, hypothesis 3 was that the changes in strength and power would have a positive relationship with baseline glycogen. That is, it was expected that the higher the resting level of muscular glycogen, the less the change post-fatigue/pre-fatigue knee strength and power output (higher proportion of post-/pre-fatigue values). Addressing peak torque, it was expected in hypothesis 3a that the changes in knee extension, knee flexion, and torque ratio would have a positive relationship with baseline
glycogen levels. In addition, it was hypothesized in hypothesis 3b that the changes in power would also have a positive relationship to baseline glycogen levels. The current research however has revealed no significant relationship between knee strength, strength ratio, or power to baseline glycogen content, except between the knee flexion and extension strength changes and baseline the VM glycogen content. Therefore, hypothesis 3 was largely rejected.

It has been shown that resting intramuscular glycogen levels influence performance variables, especially in the presence of fatigue. Baseline glycogen levels have an impact over high intensity activity over the duration of a soccer match, and may inhibit maximal effort in repeated sprint performance and high intensity running. Although the level of depletion of glycogen tends to vary on an individual basis, decline in high intensity running as increases of free fatty acid concentrations in the blood indicates the decline of glycogen availability and utilization. It has been shown that soccer athletes who ingest carbohydrate before a 90 minute soccer simulated exercise had an increased intermittent running capacity and elevated performance. It has also been found that there may be a threshold glycogen concentration that is an indicator of performance decline. Glycogen levels above ~200 mmol/kg dry weight has been shown to be an unlikely factor that influences the onset of transient fatigue. Therefore, if glycogen levels are not depleted sub-threshold, performance decrement may be attributed to various other factors influenced by the onset of fatigue.

The current study reveals that there was a decline in knee strength output and knee strength ratio, however there seems to be little relationship between the changes in strength and baseline glycogen content. Table 11 reveals that baseline glycogen content in the vastus medialis was significantly related to changes in knee extension strength and knee flexion strength. It appears curious that baseline glycogen content of the VM was the only muscle group that had a
relationship to changes in knee strength. Baseline glycogen in the other muscle groups showed no relationship to knee strength or to power output (Table 11). Although baseline glycogen in most of the muscle groups revealed no relationship to changes in strength and power, there may be an explanation to the significance of baseline glycogen in the VM to changes in knee extension and knee flexion strength. It has been found that glycogen is not uniformly distributed in a cell, and does not provide average concentrations within the cell. Distribution of glycogen within pools is largely dependent on factors such as fiber type and training status. One study found that the soleus muscle appeared to phosphorylate more glycogen than the vastus lateralis and gastrocnemius and could store more glycogen, which would reduce the likelihood of isolated muscle fatigue at the soleus. It has also been shown that muscle fiber type is a factor in glycogen utilization, as some fibers were nearly depleted post- soccer match and some fibers still had glycogen available. Although glycogen depletion occurs in both fiber types, specific depletion in type II muscle fibers may result in power decrement. Thus it appears as though muscle fiber type and glycogen availability within the cell may influence glycogen utilization. The rate of glycogen depletion may be related to glycogen availability.

Baseline glycogen values have also been correlated with the onset of fatigue as well as the muscle glycogen degradation during a simulated soccer fatigue protocol. The previous study revealed a high correlation (r = 0.87) between resting muscle glycogen and glycogen utilized during a soccer-specific running test. There was a moderate correlation (r = 0.62) between net muscle glycogen used and time to exhaustion of the soccer-specific running test. It is understood that just half a soccer match can impair knee flexor and extensor strength output. The onset of central and peripheral fatigue may cause these changes in strength output, as well as muscular damage that may occur with eccentric contractions and repetitive explosive
movements occurring in a match. The efficiency of the stretch shortening cycle and rate of force development may also decline during a soccer match due to fatigue. Depletion of glycogen at the cellular level, specifically within the sarcoplasmic reticulum, hinders the release of calcium which reduces peak power output. However, it has been shown that peripheral fatigue that causes contractile impairments is attributed to several factors including muscle damage, metabolic disturbances, and muscle glycogen depletion. Other research has indicated that the baseline glycogen level has implications on muscle power output, due to changes within the sarcoplasmic reticulum, and that power decrement due to glycogen depletion is explained by decrease in the rate of glycogenolysis. The current study reveals that there were significant changes in knee strength and strength ratio, and that baseline glycogen content in the VM was moderately correlated to knee extensor and knee flexor strength. The current findings may highlight various mechanisms contributing to fatigue as well as the questioned sensitivity of the noninvasive glycogen quantification using ultrasound-based technology. This explanation also applies to the lack of relationship between the changes in power and baseline glycogen pre-/post-fatigue. An additional explanation to the lack of relationship with changes in power and baseline glycogen can be attributed to the difficulty of the power protocol and the varied efficiency in task execution.

In order to understand the relationship between baseline glycogen measurement by MuscleSound and subjects’ nutritional intake, each subject reported their diet recall by means of the ASA 24 online dietary recall. Data analysis of the subject’s nutritional intake was not a specific aim for the current study, however, descriptive statistics of subjects’ macronutrients and caloric intake are included in Appendix B. Means and standard deviations of absolute values and macronutrients intake g/kg are included in Appendix B. A future aim using the current study as
framework could be to understand absolute values of nutritional components to performance variables in the presence of fatigue. In addition, strength, power, and glycogen data collected between the intermittent running protocol and the uphill running bout until fatigue are included in Appendix A. Although not a specific aim of the current study, trends in the data collected between exercise bouts could be an area of potential research to investigate the relationships between the rate of fuel usage and performance changes.

5.4 LIMITATIONS

The confounding factors and limitations of this study may be cause for the lack of support for some of the hypotheses. A limitation of the current study may be the duration and type of exercise protocol utilized to induce soccer simulated fatigue. Much of the previous research involving soccer play and glycogen utilization included protocols such as the LIST, an actual soccer match, or other protocols that simulated soccer play for durations ~90 minutes. These protocols were of various modes and durations, participants were of varied skill level and training status, and there were methodological differences in dependent variables collection. Another limitation as described in the study is the difficult execution of the RSI measurement. The RSI measurement has previously demonstrated reliability, however the current study reveals that the precision of execution is vital to an accurate measure of power. This is due to the multifaceted approach to assess both rate of force development and the stretch shortening cycle. The RSI protocol was developed in order to describe an individual’s ability to change from an eccentric to concentric muscular contraction and express explosive capabilities. This measure implies an athletes’ plyometric performance, as rate of force development (short force plate
contact times) and maximal vertical jump (expression of the SSC) together comprise the power
capability of an athlete assessed by reactive strength index. A recently developed modified RSI
measurement (RSImod) may be a more feasible option for a power measurement that still
includes rate of force development and stretch shortening cycle efficiency. Another
methodological consideration that may have influenced the current findings is the use of the
noninvasive glycogen measurement and use of glycogen analyzing software. The portable
ultrasound has demonstrated high intrarater reliability in the current study. Previous research has
also established that the portable ultrasound and MuscleSound glycogen software to be valid. The
previous study revealed high correlations between muscle biopsy muscle glycogen
quantification and Musclesound quantification in lower limb muscles of trained cyclists before
and after a steady state cycling bout. Although the results in that study show high
correlations between MuscleSound glycogen measurements to muscle biopsy quantification,
results must be interpreted carefully. Several measures of MuscleSound glycogen from pre-
post- exercise actually increased, causing questioning of the sensitivity of MuscleSound to detect
precise changes in muscle glycogen concentrations. Alternatives to noninvasive MuscleSound
technology to assess glycogen with decreased likelihood of subjective instrument are 13C-MRS
technology, or nuclear magnetic resonance.

5.5 FUTURE RESEARCH

The current study has the potential to serve as the framework for future research to investigate
the effect of glycogen depletion due to soccer play. An area of investigation may be the
relationship of nutritional intake to glycogen availability and utilization. A number of studies
have investigated carbohydrate intake, recovery strategies, fasted state exercise, and nutritional halftime strategies of soccer matches to understand the role of glycogen and performance decrement. Although not analyzed in the current study, caloric intake and macronutrient components of pre-participation diet were collected. Energy intake and contribution from carbohydrates, fats, and protein may have an effect on glycogen availability, performance variables, and onset of fatigue. For example, carbohydrate proportion to total caloric intake may be related to baseline glycogen levels and subsequent exercise or sport performance. Another area of future research may include comparing the muscle glycogen utilization in lower limb muscles across varied modes of exercise such as running, cycling, and sport play. The validation of the ultrasound-based muscle glycogen quantification technology utilized only trained cyclists, as other glycogen studies have also used cyclists for data collection. Running mechanics differ from cycling mechanics and therefore may cause dissimilar glycogen utilization based on muscle group contribution to exercise. Understanding the contribution of glycogen utilization among various exercises can be helpful in understanding the various rates of glycogen depletion. The current study has a number of applications that can be built upon to grow the body of pertinent literature to decrease injury risk and influence performance characteristics.

## 5.6 Conclusion

The current study investigated the effect of fatigue on strength, power, and noninvasive measurement of intramuscular glycogen. Simulated soccer activity and uphill running bout to cause aspects of central and peripheral fatigue were hypothesized to be related to glycogen
utilization and baseline glycogen levels, as well as have an influence on strength and power. Knee extension, knee flexion, $H_C:Q_C$ ratio, and power were all significantly altered due to fatigue. However, changes in these variables as a proportion post-/pre- fatigue showed little correlation to with changes in glycogen content or baseline glycogen levels. Changes in glycogen content were not significant, and baseline glycogen content had no relationship to the changes in knee strength and power observed due to the fatigue protocol. Contradictory findings in previous research that relate glycogen to performance variables may lead to methodological considerations as cause for error in the current study. Decline in knee strength and strength ratio after only ~45 minutes of intermittent running may reveal that rate performance decrement may be affected by factors such as fitness level and recovery strategies.
APPENDIX A

DESCRIPTIVE DATA PRE-, DURING-, AND POST-FATIGUE STRENGTH, POWER,
GROUND CONTACT TIME, JUMP HEIGHT, AND MUSCLE GLYCOGEN CONTENT

<table>
<thead>
<tr>
<th>Strength and Power Variables</th>
<th>Pre-Fatigue</th>
<th>During-Fatigue</th>
<th>Post-Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion (%BW)</td>
<td>129.1 ± 22.7</td>
<td>119.1 ± 24.0</td>
<td>115.9 ± 25.7</td>
</tr>
<tr>
<td>Extension (%BW)</td>
<td>231.9 ± 28.5</td>
<td>224.2 ± 35.6</td>
<td>218.8 ± 39.6</td>
</tr>
<tr>
<td>Flex/Ext Ratio (%)</td>
<td>55.8 ± 8.5</td>
<td>53.5 ± 10.3</td>
<td>53.4 ± 10.2</td>
</tr>
<tr>
<td>Reactive Strength Index</td>
<td>0.671 ± 0.236</td>
<td>0.741 ± 0.237</td>
<td>0.749 ± 0.276</td>
</tr>
<tr>
<td>Contact Time</td>
<td>0.547 ± 0.012</td>
<td>0.377 ± 0.070</td>
<td>0.330 ± 0.030</td>
</tr>
<tr>
<td>Jump Height</td>
<td>0.275 ± 0.024</td>
<td>0.319 ± 0.013</td>
<td>0.315 ± 0.007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle Glycogen Content</th>
<th>Pre-Fatigue</th>
<th>During</th>
<th>Post-Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectus Femoris</td>
<td>44.3 ± 1.3</td>
<td>44.9 ± 1.3</td>
<td>45.1 ± 1.4</td>
</tr>
<tr>
<td>Vastus Lateralis</td>
<td>46.0 ± 1.3</td>
<td>46.0 ± 1.2</td>
<td>45.8 ± 1.3</td>
</tr>
<tr>
<td>Vastus Medialis</td>
<td>46.3 ± 1.4</td>
<td>46.1 ± 1.5</td>
<td>46.3 ± 1.5</td>
</tr>
<tr>
<td>Lateral Hamstring</td>
<td>48.3 ± 1.6</td>
<td>48.1 ± 1.2</td>
<td>47.9 ± 1.6</td>
</tr>
<tr>
<td>Medial Hamstring</td>
<td>46.3 ± 1.6</td>
<td>46.3 ± 1.8</td>
<td>46.4 ± 2.2</td>
</tr>
<tr>
<td>Gastrocnemius/Soleus</td>
<td>47.1 ± 1.5</td>
<td>46.7 ± 1.1</td>
<td>46.9 ± 1.1</td>
</tr>
</tbody>
</table>
APPENDIX B

DESCRIPTIVE DATA OF TOTAL CALORIE INTAKE AND TOTAL CARBOHYDRATE, PROTEIN, AND FAT INTAKE

<table>
<thead>
<tr>
<th>Dietary Recall Variable</th>
<th>Means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Calorie Intake</td>
<td>2068.08 ± 747.61</td>
</tr>
<tr>
<td>Total Carbohydrate Intake (g)</td>
<td>241.01 ± 79.50</td>
</tr>
<tr>
<td>Normalized Total Carbohydrate Intake (g/kg)</td>
<td>3.77 ± 1.18</td>
</tr>
<tr>
<td>Total Protein Intake (g)</td>
<td>90.61 ± 52.34</td>
</tr>
<tr>
<td>Normalized Total Protein Intake (g/kg)</td>
<td>1.42 ± 0.80</td>
</tr>
<tr>
<td>Total Fat Intake (g)</td>
<td>84.55 ± 39.35</td>
</tr>
<tr>
<td>Normalized Total Fat Intake (g/kg)</td>
<td>1.34 ± 0.68</td>
</tr>
</tbody>
</table>


