The Effects of Carbohydrate Supplementation on Fatigue during Intermittent Cycling in Healthy Young Adult Men

Honors College Senior Thesis
HONRS 499

By:
Lisa Marie Guth

Advisor:
Anthony D. Mahon, Ph.D.

Ball State University
Muncie, IN
August 17, 2007
The Effects of Carbohydrate Supplementation on Fatigue during Intermittent Cycling in Healthy Young Men

Guth, L.M., Mahon A.D. Human Performance Laboratory, Ball State University, Muncie, IN

Aim: The effect of carbohydrate (CHO) supplementation of fatigue was examined in six healthy young men (22.8 ± 1.0 yrs) during an intermittent cycling protocol. Methods: Subjects consumed either a 22% CHO beverage or a placebo (PL) beverage thirty minutes before exercise and immediately before each set of exercise. The exercise consisted of three 12-min sets of four three-minute cycles. Each three-minute cycle was as follows: 60 seconds at 150 W, a 10-second sprint against 5% of the subject’s body weight, 50 seconds at 100 W, and 60 seconds of active rest with no resistance. Results: Mean power decreased from 729.2 ± 80.9 W to 638 ± 135.3 W in the CHO trial and from 722 ± 76.7 W to 657 ± 131.0 W in the PL trial. There were no significant effects for trial or interaction, but MP tended to decline over time (P = .055). RPE significantly increased (P =0.000) over time, but was similar between trials. There was a significant time by trial interaction effect for blood glucose. The difference in pre-exercise blood glucose between the CHO and PL trials approached significance (P = 0.056). Blood glucose was significantly higher in the PL trial after Set C (P = 0.007). Within the CHO trial, sets A, B, and C were all significantly decreased from the pre-exercise measure (P = 0.036, 0.005, and 0.045, respectively), but were not significantly different from each other. There were no significant differences between sets in the PL trial. Pre-exercise blood lactate concentration was similar for CHO and PL trials (1.6 ± 0.4 mmol/L and 1.5 ± 0.4 mmol/L, respectively). Blood lactate concentration increased significantly after the first set in both trials (9.7 ± 3.5 mmol/L in the CHO trial and 9.1 ± 2.8 mmol/L in the PL trial) and remained close to those levels for the remained of the protocol. There were no significant differences for trial and there was no interaction effect. Conclusions: Carbohydrate supplementation does not appear to affect high-intensity intermittent cycling performance.
Acknowledgements

First and foremost, I would like to thank my advisor, Dr. Anthony Mahon, for his invaluable guidance in completing my first research project. I would also like to thank Lauren Hanna, Jonah Lee, and Nathan Ashworth for their assistance in data collection and proofreading. I would also like to thank Bill Fink for his help in the analysis of blood lactate samples. Lastly, I would like to acknowledge Gatorade Sport Science Institute for providing all of the beverages for this project.
CHAPTER I
INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Physical activity brings about many physiological changes in the human body. Cardiorespiratory activity increases, muscles become more active, and energy in the form of adenosine triphosphate (ATP) is consumed (Gastin, 2001). A person’s entire quantity of stored ATP in skeletal muscle could be used in as little as two seconds of peak activity, so it must be continually replenished (Houston, 2001). A variety of energy transfer systems in the body work together to ensure that ATP synthesis is matched to demand in order to fuel exercise requirements.

The primary resources available for ATP resynthesis are phosphocreatine (PCr), glucose, and free fatty acids. PCr is stored in the muscles. Glucose is found in the blood and is also stored as glycogen in the muscles and the liver. Most of the body’s lipid is stored in adipose tissue, but additional lipid is stored in the muscle as intramuscular triglyceride (Coyle, 1995). These three substrates are generally used in combination with one another, but the ratio of utilization is largely dependent on the intensity of the exercise being performed (Christmass, 1999). The pathways available for ATP resynthesis are the PCr pathway, glycolysis, and oxidation. The PCr pathway and glycolysis are anaerobic pathways, and rely on PCr and glucose, respectively, as energy sources. Oxidation is aerobic, and can use both glucose and lipid as energy sources (McArdle, 2000).

The PCr pathway produces energy almost immediately at the onset of activity by combining a phosphate from a stored molecule of PCr with a molecule of ADP. Glycolysis also begins at the onset of muscular activity and produces ATP from glucose molecules through substrate-level phosphorylation (Hargreaves, 1995). These two systems will regenerate the majority of the ATP for about 30 seconds of activity, though after the first 10 seconds, PCr stores become depleted, reaching their lowest levels by
the end of 30 seconds (Spriet, 1995). Activity that is longer than 30 seconds must be fueled by oxidation. In oxidation, more ATP can be produced per mole of nutrient compared with glycolysis alone (Houston, 2001).

Low-intensity activity (<50% of VO$_2$max) will tend to be reliant on predominantly aerobic metabolism, with a high percentage of lipid serving as the fuel source, especially as duration increases. As exercise intensity increases (from 50 – 100% of VO$_2$max), the reliance on carbohydrate as an energy source will also increase. In high-intensity activity, lipid alone cannot act as a fuel source if carbohydrate sources become depleted. Maximal exercise (100 – 150% of VO$_2$max) must be supplemented by anaerobic energy production (Layzer, 1990). Regardless of intensity, as fuel resources, especially PCr and glucose become depleted, fatigue will set in and performance will decline.

**Review of Literature**

*Energy Resources / Pathways*

The concentration of ATP in the muscle at rest is between 20-25 mmol/kg dry muscle (Glaister, 2005). During intense exercise, this amount would be used up in only a few seconds if ATP were not continually replenished. ATP is resynthesized in two ways: anaerobically and aerobically. Anaerobic metabolism takes place in the cytoplasm, while aerobic metabolism occurs within the mitochondria.

One anaerobic pathway is the PCr/ATP system. This pathway is a lactic, meaning that lactate is not formed. In the PCr pathway, a high-energy phosphate group is split from a molecule of PCr and is transferred to adenosine diphosphate, creating a molecule of ATP (Layzer, 1990). This reaction is catalyzed by creatine phosphate kinase, also known as creatine kinase (Spriet, 1995; Houston, 2001).

\[
\text{ADP} + \text{PCr} \leftrightarrow \text{ATP} + \text{Cr}
\]
Approximately 80 mmol/kg dry muscle of PCr is stored in the muscle (Gaitanous, 1993). Energy from PCr is available immediately at the onset of activity. However, the rate of ATP production from the PCr pathway peaks immediately and starts to decline in as little as 1.3 seconds (Gastin, 2001). The rate at which PCr levels decrease is proportional to the intensity of the exercise being performed (Houston, 2001). After approximately 10 seconds of all-out exercise, intramuscular PCr is almost completely depleted and is no longer capable of resynthesizing ATP at a high rate.

The reaction above is fully reversible. When muscles are contracting, the reaction proceeds in the forward direction and produces ATP. During periods of recovery, the reaction reverses, meaning that the creatine rejoins with a free inorganic phosphate (from ATP produced via oxidation) to reform PCr (McArdle, 2000). PCr stores are replenished with a half-time of about 30 seconds, meaning that half of the used up PCr will be replenished during 30 seconds of recovery following high-intensity activity. This rate is, however, dependent on fitness level. For example, an endurance-trained athlete with a superior capacity to produce ATP aerobically will be able to replenish PCr stores more quickly, because more ATP will be available during rest periods. The increased ATP availability is due to a superior muscle oxidation capacity in endurance-trained athletes (Houston, 2001).

The second pathway for anaerobic ATP resynthesis is glycolysis. As with the PCr pathway, glycolysis commences immediately at the onset of muscle contraction (Hargreaves, 1995). A 70 kg man with a normal diet has approximately 400 g of carbohydrate stored as glycogen. The liver will store 40-50 g, and the remaining 350 g are stored intramuscularly. Approximately 4.5 g of additional glucose can be found circulating in the blood under normal conditions (Houston, 2001).

In glycolysis, glucose from the blood or from muscle glycogen is broken down into pyruvate during a series of 10 reactions. ATP is formed from ADP and inorganic phosphates using the free energy released from these reactions, a process called substrate-level phosphorylation. Each molecule of
glucose provides enough free energy to produce a net two ATP (Houston, 2001). The ATP is used for energy purposes and the pyruvate is converted to lactate in an eleventh step in the pathway.

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{Pi} \rightarrow 2 \text{lactate}^- + 2 \text{H}^+ + 2 \text{ATP}
\]

There are three basic stages to glycolysis: priming, splitting, and ATP formation. In the priming stage, the glucose molecule is phosphorylated twice, with the second phosphorylation producing Fructose 1,6-bisphosphate. Each of these reactions requires splitting ATP in order to obtain a free phosphate group. The production of Fructose 1,6-bisphosphate is catalyzed by phosphofructokinase, and after this step, the molecule cannot revert back to glucose. The splitting stage separates the Fructose 1,6-bisphosphate into two three-carbon molecules. These molecules are not identical, but are easily transformed into the other by an isomerase enzyme. This ensures that all of the carbons from the original glucose molecule are in the same form for the remaining steps of glycolysis. From this point onward, all of the remaining steps occur twice, once for each three-carbon molecule of Glyceraldehyde 3-phosphate. In the next reaction, each molecule is transformed into 1,3-bisphosphoglycerate by adding an inorganic phosphate. This reaction is catalyzed by the enzyme glyceraldehyde phosphate dehydrogenase, reducing NAD\(^+\) to NADH and beginning the ATP production stage. Next, a phosphate group is removed, forming 3-phosphoglycerate. The removed phosphate group is transferred to ADP to produce ATP. At this point, there is a gross yield of 2 ATP, but no net yield, due to the two ATP used in the earlier reactions. The 3-phosphoglycerate is mutated to 2-phosphoglycerate by phosphoglycerate mutase. Next, the 2-phosphoglycerate is dehydrated to phosphoenolpyruvate by the enzyme enolase. Then a phosphate is removed from each phosphoenolpyruvate and transferred to ADP to form two additional ATP in the process of forming pyruvate. This reaction is catalyzed by pyruvate kinase. There is now a net yield of two ATP as shown in the reaction above, plus two molecules of pyruvate. When
pyruvate is produced at a high rate, such as during anaerobic exercise, the final step is the transformation of pyruvate to lactate and is catalyzed by lactate dehydrogenase (Houston, 2001).

In aerobic metabolism, the pyruvate from glycolysis can enter the mitochondria and undergoes oxidative phosphorylation. A primary factor affecting the fate of pyruvate is the rate at which it is produced. If the rate of pyruvate production exceeds the rate of mitochondrial uptake of pyruvate, lactate formation occurs. However, if the rate of pyruvate production is less than the rate of mitochondrial uptake, pyruvate enters the mitochondria (Houston, 2001). Free fatty acids can also undergo oxidative phosphorylation. The ability to oxidize substrate has several advantages. First, the oxidation of pyruvate results in additional ATP not obtained in glycolysis. Second, fat is an invaluable fuel source, as the average human body stores enough fat to produce 75,000 kcals of energy (as compared to the 2,000-3,000 kcals worth of energy stored as carbohydrate). Fat is stored as triacylglycerol, or triglyceride, in adipose tissue and in skeletal muscle, and is mobilized by lipolysis into free fatty acids as needed (Houston, 2001).

Oxidative phosphorylation is the process by which ATP is created from ADP and Pi. The process is fueled by free energy released from the transfer of electrons from the fuel source (carbohydrate or fat) to coenzymes, then to oxygen, the final electron acceptor. Pyruvate and fatty acids are converted into acetyl units, attached to enzyme CoA, and fed into the tricarboxylic acid (TCA) cycle. The TCA cycle oxidizes the acetyl groups, and creates reduced coenzymes (NADH and FADH$_2$) by attaching electrons to NAD$^+$ and FAD. The free energy released from this transfer of electrons is used to pump protons across the inner membrane of the mitochondrial into the intermembrane space, creating an electrochemical gradient. The proton pumping process occurs in the electron transport chain. After being pumped out of the mitochondrial matrix, protons reenter by moving down the electrochemical gradient, releasing the free energy that is used to create ATP from ADP and Pi (Houston, 2001). The equations below illustrate the ATP potential from the oxidation of glucose and fatty acid molecules.
\[
C_6H_{12}O_6 + 6 O_2 + 30 (ADP + Pi) \rightarrow 6 CO_2 + 30 ATP + 6 H_2O
\]

\[
C_{16}H_{32}O_2 + 23 O_2 + 106 (ADP + Pi) \rightarrow 16 CO_2 + 129 ATP + 16 H_2O
\]

Note: Glucose oxidation reaction does not account for glycolytic ATP production.

Through aerobic energy transfer, submaximal physical activity may be sustained for a prolonged period of time. The primary limiting factor is carbohydrate availability. At rest, energy production is completely aerobic, and uses lipid almost exclusively as fuel (Layzer, 1990). During low to moderate intensity exercise, energy is predominately produced aerobically using a mixture of intramuscular glycogen and free fatty acids. Relatively more fat than carbohydrate is used as fuel at rest and during low intensity physical activity. As exercise intensity increases, carbohydrate use increases and fat use decreases. Fat utilization also increases as exercise duration increases and glycogen stores start to become depleted (Hargreaves, 1995). However, when glycogen stores approach complete depletion, ATP can no longer be produced from lipid alone (Coyle, 1995). In contrast, short-duration maximal intensity exercise is fueled mostly through anaerobic energy production.

**Fatigue**

The standard definition of fatigue is a progressive deterioration in physical performance. Several variables contribute to the onset and severity of the fatigue. Some of these variables cannot be manipulated, such as age, gender, and genetic makeup. However, many other variables play into fatigue, such as the type and intensity of exercise. Additionally, environmental conditions such as temperature, humidity, and altitude can influence fatigue (Green, 1995).

One type of fatigue is correlated with depleted glycogen levels in the liver and muscles, a “nutrient-related” fatigue. Regardless of oxygen and lipid availability, ATP can no longer be replenished at a high rate in this state. However, the exact mechanism is unclear. It may be due to a negative effect
on the central nervous system, resulting from lowered blood glucose. Another theory is that ATP regeneration slows due to a possible role of muscle glycogen as a primer to lipid metabolism (McArdle, 2000). Lipid oxidation is only capable of producing half of the ATP needed to exercise at 70% VO₂max and less than a third of exercise at intensities greater than 70% of VO₂max (Coyle, 1995).

Anaerobic fatigue is the percentage decrease in power relative to peak power. The amount of anaerobic fatigue illustrates the ability of the PCr and aerobic glycolysis pathways to regenerate ATP at an acceptable rate. Anaerobic or metabolic fatigue includes an accumulation of lactate, inorganic phosphate and hydrogen ions in the muscle fibers (McArdle, 2000). Lactate is thought to interfere with Ca²⁺ release from the sarcoplasmic reticulum (Allen, 2004). Likewise, hydrogen ion concentration decreases the affinity of calcium by troponin, disrupting the muscle fiber contraction mechanism (Hultman et al., 1986).

Carbohydrate Supplementation

Fatigue due to glycogen depletion is often thought of as the feeling of “hitting the wall.” This is the point where it feels like continuation of exercise is impossible. As previously mentioned, the exact reasons why this occurs are not clear. However, the “hitting the wall” feeling is closely correlated with severe glycogen depletion (McArdle, 2000). If glycogen depletion can be prevented, exercise performance may be able to be maintained.

An obvious solution to “nutrient-related” fatigue would be to keep nutrients, particularly carbohydrate, in good supply during exercise. This can be accomplished by providing carbohydrate before and/or during exercise. Most studies agree that carbohydrate supplementation during prolonged exercise can improve exercise performance. This topic has been studied for the majority of the preceding century, with conclusions supporting the positive effect of carbohydrate supplementation during exercise appearing in the early 1920s.
Levine and his colleagues (1924) studied 11 runners who competed in the 1924 Boston Marathon. They found that runners who consumed carbohydrate during the race did not exhibit the negative effects of some of the other runners who did not consume carbohydrate during the race. They also found a strong correlation between blood glucose post-race and physical condition, where runners with normal glucose levels presented in good condition, while those with low to very low levels presented poorly, with symptoms comparable to those of insulin shock. This was one of the first studies to suggest that normal blood glucose is insufficient to perform a marathon run, and that the ingestion of glucose during exercise of long duration might prevent hypoglycemia and fatigue.

Over the following decades, the practice of consuming carbohydrate during activity led to the appearance of sports drinks in the 1980s. Many studies have since demonstrated the ergogenic effects of carbohydrate supplementation on endurance performance (Coombes & Hamilton, 2000). There are a variety of theories concerning the mechanism by which exogenous carbohydrate improves performance. In a study done by Coyle et al. (1986), ingestion of exogenous carbohydrate prevented a drop in glucose when cycling at 71% of VO₂max (to exhaustion) when compared to water ingestion. In this study, when the subjects ingested the carbohydrate, they cycled at the above intensity for four hours, compared to three hours in the placebo trial. This study supports a theory that maintenance of blood glucose levels can improve endurance performance.

Another study by Coggan and Coyle (1987), concluded that fatigue is partially caused by a drop in plasma glucose concentration. Their study showed that when carbohydrate was ingested or given intravenously, fatigue could be reversed. A protocol of cycling to fatigue at 70% of VO₂max was employed as in the previous study. However, in this study, participants received either a placebo, glucose polymers by mouth, or intravenous glucose twenty minutes after the point of exhaustion and then asked to cycle to exhaustion again. Subjects who received either glucose solution were able to cycle significantly longer during this second bout. With the intravenous administration of glucose,
euglycemia was maintained. Subjects also continued the second bout of cycling for the longest duration with the glucose infusion (43 minutes, compared to 26 minutes with glucose ingestion and 10 minutes with placebo), suggesting that the resulting euglycemia was instrumental in the improved performance.

Finally, a study by Mitchell et al. (1989) showed that ingesting of a 12% carbohydrate solution improved performance during a 15-minute isokinetic performance ride following either 105 minutes of cycling at 70% of VO$_2$max or seven 15-minute rides at 70% of VO$_2$max with three minutes of rest between each ride. Performance was measured by total work performed during the final 15-minute ride. Three different concentrations of carbohydrate solution (6%, 12%, and 18%) as well as placebo were given during separate trials, though significant effects were not seen in the 6% or 18% trial. Drinks were given every 15 minutes during the exercise protocol. In the 12% trials (one continuous and one intermittent), total work was significantly increased compared to the placebo trial. Although the 6% and 18% trials were not significantly higher in total work, total work was consistently higher than in the placebo trial. There were no significant differences between any of the drink trials. This study was unique because glycogen was measured from muscle biopsies before the exercise and after 105 minutes of exercise using mixed muscle analysis. No evidence of glycogen sparing was seen, nor were any discrepancies in glycogen usage between the placebo and carbohydrate trials. The researchers concluded that the improved performance therefore must be due to blood glucose level maintenance.

Although some studies, such as the one above have not shown evidence of glycogen sparing, some researchers still believe this is a possibility and attribute improved exercise performance to the sparing of muscle and liver glycogen. They propose that the positive effects (superior performance, delayed fatigue) seen when exogenous carbohydrate is supplied is the fact that the extra carbohydrate can serve as an additional fuel source. This is thought to spare the depletion of glycogen from the muscle and liver. Sufficient muscle glycogen is necessary to perform high intensity exercise, particularly
during activities of a long duration, so glycogen sparing may be the key to improved performance (Shi & Gisolfi, 1998).

Evidence supporting this theory is sparse, but this may be due to the analysis of mixed muscle tissue versus fiber-specific analysis. A recent study by DeBock et al. (2007) found that carbohydrate ingestion before and during endurance exercise results in the sparing of glycogen, but only in type IIa muscle fibers. In this study, eight subjects completed a carbohydrate trial (in the form of a carbohydrate-rich breakfast) and a fasted trial of exercise. This exercise was two hours in duration and at approximately 180 Watts (W), a moderate- to high-intensity workload for these subjects. Muscle and blood samples were taken before and after the exercise. Glycogen content was significantly reduced in type IIa muscle fibers in the post-exercise biopsy for the fasting trial, but not for the carbohydrate trial.

Support for the benefits of carbohydrate ingestion for exercise of short duration (≤ one hour) is less common than exercise bouts of longer duration. However, Anantaraman and colleagues (1995) produced a paper on the positive effects of carbohydrate supplementation on power output during high-intensity exercise that was one hour in duration. Their subjects were instructed to begin pedaling at 80 revolutions per minute at a resistance that would equal the work rate associated with 90% of VO₂max. Over the course of the hour, power output was continuously recorded based on pedal rate. Subjects consumed either glucose pre-exercise and placebo during exercise, glucose both pre-exercise and during exercise, or placebo both pre-exercise and during exercise. Drinks were consumed every 15 minutes during exercise. Subjects were able to maintain power at a significantly higher level during the final 20 minutes of the protocol when they consumed glucose prior to exercise. There did not appear to be any added benefit from consuming additional glucose during the exercise bout. In summary, glucose ingestion resulted in a smaller decline in power output over one hour of high-intensity cycling. The researchers suggest that the glucose from the carbohydrate drink may have served as a substrate as muscle glycogen may have been somewhat depleted after 40 minutes.
The mechanism by which carbohydrate ingestion improves exercise performance (blood glucose maintenance or decreased muscle glycogen use) is dependent on exercise type and intensity, subjects' nutritional and fitness status, and the type, amount, and timing of carbohydrate ingestion. Maintenance of blood glucose appears to play more of a role during continuous, moderate-intensity cycling, while running and intermittent exercise appear to utilize the glycogen-sparing mechanism (Tsintzas & Williams, 1998).

**Intermittent Exercise**

An area that has not been studied as extensively is intermittent exercise. However, this is an important area of study. Intermittent exercise is often characterized by periods of high-intensity exercise interspersed with periods of lower-intensity exercise or rest. The high-intensity periods often require a much higher effort than continuous exercise does, and therefore may deplete glycogen more quickly.

Many team sports are examples of intermittent exercise, including soccer, hockey, and basketball, among others. Soccer is likely the most extensively studied of the intermittent team sports with regard to fuel usage. As reported by Ekblom (1986), a study by Karlsson in 1969 found that glycogen stores (in vastus lateralis) are rapidly depleted during a soccer game. Out of six subjects, four had less than 0.5 g/100g wet muscle remaining at half-time, and all six were severely depleted by the end of the game. An accompanying video analysis showed that the players with low glycogen at half-time were slower and covered less distance in the second half.

Leatt and Jacobs (1989) demonstrated that muscle glycogen was spared in players who consumed a carbohydrate drink before and during a soccer match versus a placebo. Biopsies of the vastus lateralis muscle before and after the game showed a significantly greater decrease in muscle glycogen in the players who had consumed the placebo beverage.
Carbohydrate supplementation has been repeatedly shown to benefit intermittent exercise in the laboratory setting as well. A study by Mitchell et al. (1988) claimed a significantly improved performance with carbohydrate supplementation of varying concentrations during a cycling protocol consisting of seven 12-minute bouts at 70% of VO₂max followed by a 12-minute sprint. Unfortunately, exact data were not published. Another sprint performance study by Ball et al. (1995) showed an improvement in performance on the Wingate Anaerobic Test following a simulated 50-minute time trial when carbohydrate was ingested periodically during the 50 minutes.

Several studies have shown an increase in time to fatigue after ingestion of carbohydrate. Davis et al. (1997) used a protocol of intermittent cycling alternating between one minute at 120-130% of VO₂max and 3 minutes of rest until exhaustion. They found that subjects significantly increased time to exhaustion when the carbohydrate drink was ingested compared to a placebo. The average increase in time to fatigue was 27 minutes, including rest. Therefore, the subjects completed an average of seven additional cycles of the exercise protocol. The researchers suggested the delay in fatigue might be due to selective glycogen sparing in Type II muscle fibers, or an increase in muscle glycogen resynthesis during the three-minute rest periods. Other researchers have offered the explanation that the body can oxidize a large percentage of the exogenous carbohydrate, providing 16-20% of the energy required for the activity (Peronnet et al. 1992).

A study performed by Yaspelkis et al. (1993) showed a positive correlation between muscle glycogen concentrations after 190 minutes of variable-intensity exercise and time to fatigue during a subsequent cycling bout. This study also found that blood glucose levels were maintained during the carbohydrate trial, indicating that muscle glycogen sparing and blood glucose maintenance were likely responsible for the increased time to fatigue.

Performance improvements have been observed in other forms of laboratory-based intermittent activity, as well. Specifically, a study performed by Nicholas et al. (1995) examined the
effects of carbohydrate ingestion on shuttle running. The protocol consisted of 15-minute segments. Each segment started with walking, then a sprint, followed by a jog at 55% of VO$_2$max, followed by faster running at 95% of VO$_2$max. This protocol was repeated 5 times for a total of 75 minutes, and then subjects alternated the jog and fast running until exhaustion. Subjects consumed either a carbohydrate drink or placebo immediately before exercise and every 15 minutes during exercise. The subjects who consumed the carbohydrate were able to do the final run to fatigue for 2.2 minutes longer, or 33%, which was significant. The researchers attribute this increase in endurance capacity to reduced use of muscle glycogen during the first 75 minutes of the protocol.

A study by Welsh et al. (2002) also found improvements in a simulation study utilizing shuttle running. In this study, four 15-minute quarters separated by a 20-minute half-time included varying intensities of shuttle running, followed by a run to fatigue. Increased time to fatigue was seen following carbohydrate ingestion. Sprint times in the fourth quarter were also faster in the carbohydrate trial. In addition, subjects performed better on a motor skill test and reported significantly less fatigue during the carbohydrate trial when compared with the placebo trial.

However, there are numerous studies on intermittent exercise that have not shown positive effects, particularly when the exercise protocol is very short in duration (< 30 minutes). For example, a study by Kerksick et al. (2005) involved the use of five WAnTs interspersed with three-minute rest periods and did not show improvements in power with ribose supplementation. Similarly, no effect on peak anaerobic performance was seen in a study by Wouassi et al. (1997), despite metabolic changes following carbohydrate ingestion.

A recent study from this laboratory also found no significant effects of carbohydrate supplementation on WAnT performance in boys. Marjerrison et al. (2007) examined peak and mean power outputs of four WAnTs interspersed with two minutes of recovery time. The researchers
suggested that the exercise protocol may not have been fatiguing enough, and that more intense and/or longer duration activity may be necessary to observe positive effects due to carbohydrate ingestion.

Summary

Based on the information present in this chapter, the following summary points can be made:

• ATP must be continually replenished during exercise.

• There are three main pathways to replenish ATP: the PCr pathway, anaerobic glycolysis, and oxidation. The PCR pathway regenerates ATP very quickly, but cannot sustain production very long. Anaerobic glycolysis also generates ATP quickly, but also cannot sustain a high rate for longer than about 30 seconds. Oxidation replenishes ATP during aerobic exercise and rest. This pathway can produce ATP for much longer duration exercise, but at a slower rate compared to anaerobic ATP production.

• Fatigue is defined as a progressive deterioration in performance and may be caused by depleted glycogen levels in the body, as well as acidosis and depletion of PCr.

• Carbohydrate supplementation has been shown to benefit aerobic exercise by increasing time to fatigue or improved power data. This may be due to the maintenance of blood glucose or to the sparing of muscle glycogen.

• There is some evidence that carbohydrate supplementation may improve performance during intermittent activity as well. Both lab and field studies have shown improvements in performance following carbohydrate ingestion. However, the research in this area is not as conclusive as research involving prolonged aerobic exercise, as some studies have not demonstrated a correlation. Thus, more research is needed.
Purpose

Research has shown it is possible to observe performance enhancements on exercise that is shorter in duration (< one hour), and in intermittent or variable-intensity exercise. However, sufficient research has not been done while combining these two factors. The present study looks to bridge these two areas to determine whether carbohydrate feedings are beneficial in delaying or reducing fatigue during variable intensity intermittent exercise. It was hypothesized that ingestion of carbohydrate (vs. placebo) would result in a smaller decline in mean power over the course of the protocol.
Chapter II

METHODS

Subjects

Ten healthy, physically active young (ages 18-26) men volunteered for this study. All subjects were pre-screened via questionnaire to determine health status, and were informed about the possible risks involved with the experimental procedures before consenting to participate. Subjects also completed a Health History Questionnaire after consenting to the study. The experimental protocol was reviewed by the Ball State University Institutional Review Board and approved for use with human subjects.

Study Design

The study consisted of three separate visits to the laboratory per subject. The first visit was a familiarization session. The second and third visits were experimental trials. Carbohydrate (CHO) or placebo (PL) beverages were given only during the experimental trials. Likewise, blood samples were collected only during the experimental trials. This study was randomized, double-blinded, and used a crossover design.

Familiarization Session

Prior to the experimental trials, the subjects completed the familiarization session to become accustomed to the exercise protocol and equipment. During this session, height and mass were measured. The subjects completed four three-minute cycles on the cycle ergometer consisting of 60 seconds at 150 Watts (W), a 10-second sprint against a resistance of 0.05 kiloponds/kilogram body weight, 50 seconds at 100 W, and 60 seconds of pedaling against no resistance. Heart rate (HR) and rate
of perceived exertion (RPE) were recorded, as was power data (mean power (MP), and fatigue index (FI)) from each sprint. Subjects were given a list of acceptable breakfast choices for Trials 2 and 3 and a sheet on which to record the selected breakfast.

**Experimental Sessions**

Trials 2 and 3 were the experimental sessions. These sessions were separated by a minimum of 48 hours to allow for recovery. On these days, the subject reported to the laboratory one hour after consuming a controlled breakfast. The subject chose their breakfast for Trial 2 from a list of options and recorded that choice. A breakfast similar in size and content was consumed for Trial 3. Upon arrival to the lab, the subject was fitted with a HR monitor. The subject consumed 1.6ml/kg body weight of either a 22% carbohydrate beverage or a placebo beverage (Gatorade Sport Science Institute, Barrington, IL). The drinks for each experimental session were administered in a double blind and counterbalanced manner. Each subject received both drinks. Half of the subjects received the PL drink first and half received the CHO drink first. After the drink consumption, the subject rested in a seated position for 30 minutes, after which a fingerstick blood sample was taken and a second beverage of equal size and content to the first was consumed. Following the blood sample, the subject began the exercise protocol on the cycle ergometer.
The exercise protocol began with three minutes of warm-up at 50 W, after which testing commenced. The protocol (see Figure 1) consisted of three 12-min sets. Each set consisted of four 3-minute cycles. Each 3-minute cycle was as follows: 60 seconds at 150 W, a 10-second sprint against 5% of the subject’s body weight, 50 seconds at 100 W, and 60 seconds of active rest with no resistance. After each 12-minute set, the subject was given a three-minute recovery period in which they got off the bike to walk around. During this recovery period, a fingerstick blood sample was taken and a drink identical in volume and content to the previous drinks was consumed. After the final set, a final fingerstick blood sample was taken. The subjects were given strong verbal encouragement during each set and throughout the protocol. Throughout the exercise bouts, HR and RPE were recorded after each sprint, and MP and FI were determined from each 10-second sprint.

Instrumentation

Height was measured to the nearest millimeter using a wall-mounted stadiometer (Seca 222, Hanover, MD). Mass was measured to the nearest gram with a digital scale (Toledo 1D1 Multirange Scale, Worthington, OH). The mass recorded during Trial 1 was used as the subject mass for drink
administration and sprint resistance for Trials 2 and 3 in all subjects. HR was recorded using a Polar Heart Rate Monitor. The OMNI (0-10) RPE scale was used to assess RPE. Exercise was performed on an Excalibur Sport (Lode BV, Groningen, The Netherlands). Power data was recorded using the Wingate for Windows software (Version 1, Lode BV, Groningen, The Netherlands).

**Blood Analysis**

Capillary blood samples were collected using a fingerstick procedure. Prior to the first fingerstick, the subject soaked their hand in warm water to encourage blood flow. Each additional fingerstick was taken from a different finger. Upon puncture of the skin, the first drop of blood was wiped away and the second drop was collected and analyzed using an automated glucose meter (BD Logic, Waltham, MA). Two 30-µl capillary tubes were then filled and deposited into 1.5-ml Eppendorf tubes containing 100 µl of perchloric acid. The samples were subsequently frozen for later lactate analysis.

After data collection was completed, the previously frozen blood samples were thawed to perform the lactate assay. Each sample was centrifuged (IEC Micromax RF ultracentrifuge) at 21000 RCF for one minute. A cocktail of distilled water, glycine hydrazine buffer, NAD⁺, and lactate dehydrogenase was mixed and one ml of the solution was pipetted into a series of test tubes. Twenty-five µl of supernatant liquid from each centrifuged blood sample was then added to the cocktail solution. The test tubes were vortexed and placed in a water bath at 37°C for 45 minutes. After the incubation period, samples were analyzed with a spectrophotometer (Backman Coulter Inc, DU 530 Life Science UV/Vis, Fullerton, CA) with a wavelength of 340 nanometers. The blank used to calibrate the spectrophotometer consisted of 25 µl of perchloric acid and one ml of the cocktail. Duplicate samples were tested for each time point. Lactate concentration (mmol/L) was calculated from the absorbance using a conversion factor.
Statistical Analysis

A two-way ANOVA (trial by time) was used to analyze the data. The dependent variables were MP, FI, HR, RPE, blood glucose concentration, and blood lactate concentration. In the event of a significant interaction or time effect, a Bonferroni post hoc test was performed to determine specific differences. Statistical significance was set at a p-value of ≤ 0.05.
CHAPTER III

RESULTS AND DISCUSSIONS

Results

Subjects

Ten male subjects volunteered to participate in this study. Of these ten, six subjects completed all three trials. Three subjects withdrew from the study due to gastric distress. The fourth subject did not complete the study due to scheduling conflicts. Subject characteristics (n=6) were as follows: age 22.8 ± 1.0 years, height 178.6 ± 10.5 cm, and mass 80.7 ± 14.5 kg.

Performance Data

Power data (MP and FI) was recorded for each sprint and averaged within each set. There were three sets (A, B, and C); each set included four sprints. In the CHO trial, MP was 729.2 ± 80.9 W in Set A, 659.5 ± 110.8 W in Set B, and 638 ± 135.3 W in Set C. MP in the PL trial was 722 ± 76.7 W in Set A, 686.5 ± 137.9 W in Set B, and 657 ± 131.0 W in Set C (Figure 2). There were no significant differences between trials and there was no interaction, but MP tended to decrease over time (P = 0.055).
Figure 2. Mean Power ± SD for carbohydrate and placebo trials.

In the CHO Trial, FI was 42.1 ± 7.3% in Set A, 39.6 ± 11.5% in Set B, and 37.9 ± 13.5% in Set C. In the PL trial, FI was 40.7 ± 9.3% in Set A, 39.2 ± 14.4% in Set B, and 39.0 ± 13.5% in Set C (Figure 3). There were no significant effects for time or trial, and there was no interaction.
HR and RPE were recorded at the end of each sprint. These measures were also averaged according to set and are shown in Table 1. There were no significant effects for HR. RPE was significantly increased \( (P = 0.000) \) with each set (Set A to Set B, \( P = 0.031 \); Set A to Set C, \( P = 0.013 \); Set B to Set C, \( P = 0.023 \)), but the trial effect and interaction effect were not significant.
Table 1. Heart rate and Rating of Perceived Exertion for carbohydrate and placebo trials.

<table>
<thead>
<tr>
<th></th>
<th>Set A</th>
<th>Set B</th>
<th>Set C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>CHO 158 ± 22</td>
<td>163 ± 20</td>
<td>164 ± 17</td>
</tr>
<tr>
<td></td>
<td>PL 157 ± 21</td>
<td>164 ± 14</td>
<td>165 ± 14</td>
</tr>
<tr>
<td>RPE (0-10)</td>
<td>CHO 5.1 ± 1.7*</td>
<td>6.6 ± 1.2*</td>
<td>7.7 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>PL 4.8 ± 1.5*</td>
<td>6.2 ± 1.7*</td>
<td>7.8 ± 1.0*</td>
</tr>
</tbody>
</table>

* P < 0.05 for time

Blood Data

Blood glucose was measured immediately before exercise (30 minutes after the first drink administration) and after each set. In the CHO trial, blood glucose was 137.2 ± 27.8 mg/dL pre-exercise and 98.2 ± 29.7 mg/dL, 89.2 ± 14.6 mg/dL, and 94.0 ± 8.4 mg/dL in sets A, B, and C, respectively. In the PL trial, blood glucose was 105.5 ± 15.7 mg/dL pre-exercise and 89.7 ± 12.9 mg/dL, 98.2 ± 10.3 mg/dL, and 107.5 ± 13.8 mg/dL in sets A, B, and C, respectively (Figure 4). Blood glucose significantly decreased over time. Specifically, there was a decrease between pre-exercise and Set A (P = 0.007) and between pre-exercise and Set B (P = 0.005). There was also a significant time by trial interaction effect. The difference in pre-exercise blood glucose between the CHO and PL trials approached significance (P = 0.056). Blood glucose was significantly higher in the PL trial after Set C (P = 0.007). Within the CHO trial, sets A, B, and C were all significantly decreased from the pre-exercise measure (P = 0.036, 0.005, and 0.045, respectively), but were not significantly different from each other. There were no significant differences between sets in the PL trial.
Blood lactate was also measured immediately before exercise (30 minutes after the first drink administration) and after each set. In the CHO trial, blood lactate was $1.6 \pm 0.4$ mmol/L pre-exercise and $9.7 \pm 3.5$ mmol/L, $9.6 \pm 2.8$ mmol/L, and $9.7 \pm 2.4$ mmol/L in sets A, B, and C, respectively. In the PL trial, blood lactate was $1.5 \pm 0.4$ mmol/L pre-exercise and $9.1 \pm 2.8$ mmol/L, $9.1 \pm 2.6$ mmol/L, and $9.7 \pm 1.8$ mmol/L in sets A, B, and C, respectively (Figure 5). Lactate was significantly higher than the pre-exercise value for all sets, ($P = 0.005$, $P = 0.003$, $P = 0.001$ for sets A, B, and C, respectively). There were no significant differences for trial and there was no interaction effect.

Figure 4. Blood glucose ± SD for carbohydrate and placebo trials.
Discussion

The aim of the present study was to determine whether carbohydrate supplementation could delay or reduce fatigue during an intermittent exercise protocol. It was hypothesized that the exogenous carbohydrate ingestion would cause a lesser decline in power output over time (representing a lesser amount of fatigue) when compared to ingestion of a placebo. In addition, measures of blood lactate and glucose concentrations were made, as well as HR and RPE. However, no differences in power output were observed between trials. Blood glucose tended to be higher pre-exercise in the CHO trial and decreased over time, particularly between pre-exercise and Set A and between pre-exercise and Set B. Blood glucose was higher in the PL trial after Set C. Within the CHO trial, sets A, B, and C

Figure 5. Blood lactate ± SD for carbohydrate and placebo trials.
were all significantly decreased from the pre-exercise measure, but were not different from each other. Blood lactate concentration increased significantly after the first set in both trials and remained close to those levels for the remained of the protocol. There were no differences between trials. HR was elevated after the first set and post-sprint HRs remained stable throughout the protocol. RPE increased with each set in both trials.

Performance Data

There was no effect on performance with carbohydrate supplementation, however there was a time effect as MP tended to decrease over time in both trials \( (P = 0.055) \). This would suggest that the subjects did start to fatigue in both the CHO and PL trials. FI was stable over time, evidencing that, although power declined between sprints, within-sprint effort was the same throughout the protocol.

Several studies have found a performance benefit with carbohydrate ingestion. Coggan (1986), Coggan and Coyle (1987), and Mitchell et al. (1989) all found improved performance in continuous prolonged cycling with carbohydrate supplementation. Improvements have also been observed in exercise protocols of shorter duration. Ball et al. (1995) found improved peak anaerobic performance after a 50-min bout of aerobic activity was improved with carbohydrate supplementation. Anantaraman also found improved performance during a 1-hour protocol when carbohydrate was consumed.

The effect of carbohydrate supplementation in intermittent exercise is not yet clearly determined. Some studies, such as Yaspelkis et al. (1993), Davis et al. (1997), and Nicholas et al. (1995) have found superior performance with carbohydrate supplementation using varied protocols. However, there are also several published studies that found no effect on performance due to carbohydrate ingestion, such as Kersick et al. (2005), Wouassi et al. (1997), and Marjerrison (2007).

The decrease in MP demonstrates that the protocol was sufficient to fatigue the subjects. As there was no benefit seen from the carbohydrate supplementation, it may be possible that the exercise...
was too strenuous and that fatigue may have been caused by something other than nutrient depletion, such as acidosis caused by anaerobic glycolysis. Acidosis can interfere with the affinity of calcium by troponin and disrupt muscle fiber contraction (Hultman et al., 1986). If this was the case, any positive effect of the exogenous carbohydrate may have been masked by the unavoidable muscle fatigue generated by the protocol. Regardless of glycogen availability, if the muscle fiber mechanism is not working properly, physical activity cannot continue at an optimal level.

Blood Data

Although not statistically significant, blood glucose prior to exercise tended to be higher in the CHO trial \( (P = 0.056) \). This difference is substantial enough to show that glucose from the drink was absorbed during the 30-minute rest period. There were no statistical differences between trials after Set A or B, though blood glucose was significantly lower in each set compared to starting values. There was a significant difference after Set C between the CHO and PL trials, with blood glucose statistically higher in the PL trial. In the CHO trial, blood glucose significantly decreased with the commencement of exercise, suggesting that the exercising muscles absorbed glucose from the blood.

The increase in blood glucose with carbohydrate ingestion is consistent between studies. Marjerrison et al. (2007) observed a very similar response to the present study, as glucose increased with carbohydrate consumption and was decreased following exercise. However, due to the short duration of the protocol, blood glucose was not measured at additional time points. In the study by Davis et al. (1997), glucose increased in both trials throughout the protocol. Glucose concentration was greater in the CHO trial, as were the increases in concentration. However, there was no decrease during the exercise protocol in either trial from pre-exercise levels, as was seen in both Marjerrison et al.'s and the present study.
During exercise, muscle contraction activates the GLUT-4 transporters with the elevated Ca\(^{2+}\) concentration caused by the motor neuron’s activation of the muscle fiber (Houston, 2001). Additionally, glucose uptake is moderated by insulin, which is released when blood glucose concentration increases. This process also employs the GLUT-4 transporters (Houston, 2001). The additive effect of both of these mechanisms may explain why blood glucose concentration was slightly lower in the later stages of exercise in the present study. There were no significant changes throughout the protocol in the PL trial.

Lactate values were statistically identical at rest between trials and for all exercise samples, regardless of time or trial. This is an additional indication that the subjects started each trial in the same lactic condition and that the amount of anaerobic work was similar throughout both trials. The consistently high lactate levels during the exercise protocol show that lactate was being produced much faster than could be cleared, an indication of increased reliance on anaerobic glycolysis due to the intense nature of the exercise protocol.

In the study by Davis et al. (1997), lactate increased from resting levels to 6-8 mmol/L by 15 minutes, and continued to increase to about 10 mmol/L at fatigue. Interestingly, in the present study, lactate increased from similar resting levels to about 9.5 mmol/L after the first set, and remained at that level for the remainder of the protocol. Thus lactate increased much more quickly in the present study compared to Davis et al. This is interesting, because Davis’s study required far more supramaximal work. However, the present study also included moderate work between sprints and did not include passive rest periods.

The increase in blood lactate concentration indicates an increased reliance on glycolysis. The increased dependency on anaerobic metabolism can be due to a number of factors. Catecholamines (epinephrine and norepinephrine) increase with exercise. The concentration of both hormones generally increases linearly with duration and exponentially as intensity increases (Kjaer, 1992).
Increased catecholamine levels may lead to an increased rate of glycolysis. A study by Cheetham et al. (1986) found a correlation between catecholamine levels and estimated glycolytic ATP production, which they attributed to epinephrine's effect on glycogenolysis in the muscle tissue. In addition, an increase in the ADP/ATP ratio will also affect the rate of glycolysis. Increases in the ADP/ATP ratio occur when ATP demand is high and seems to activate a number of enzymes in the glycolytic pathway. It is imperative that the ratio is maintained to ensure that ATP availability is maintained. If the ratio increases, ATP synthesis from stored fuel sources will increase (McArdle, 2000). Lastly, the intense nature of the sprint bouts the necessitated the recruitment of type II muscle fibers, which are glycolytic in nature.

Heart Rate and Ratings of Perceived Exertion

HR was considerably elevated after each sprint, roughly 80% of age-predicted maximum on average, demonstrating moderate- to high-intensity exercise. HR was similar throughout the protocol, even though power decreased. RPE increased significantly ($P = 0.000$) with each set from a rating of about 5 after the first set to near 8 after the final set, indicating that subjects felt more tired with each sprint set, despite a lack of change in HR and a decrease in total work over time.

Limitations

Out of an initial ten subjects, three experienced significant gastric distress during this study. One subject became ill during the familiarization trial. The other two subjects became ill during the CHO trial. It is not known whether the subjects would have experienced gastric symptoms in the PL trial, as all subjects experiencing gastric distress withdrew from the study after the problematic trial.

The volume and concentration of carbohydrate drink used in the present study deviated from ideal. According to Tsintzas and Williams (1998), a previous study has shown a fast rate of gastric
emptying after a bolus of carbohydrate beverage immediately prior to exercise and intermittent ingestion of smaller amounts of beverage during exercise. The concentration of the drink in that study was not reported by Tsintzas and Williams, but subjects in the present study did not consume a large bolus before exercise due to the high concentration of the drink. Prior research has shown that glucose absorption cannot generally occur faster than 1.8 g/min. On average, the amount of glucose consumed would take 62 minutes to be absorbed, assuming this rate (Jeukendrup, 2004).

Gastric emptying is influenced by gastric volume. Therefore, gastric emptying occurs more quickly when gastric volume is greater. However, gastric emptying is also influenced by beverage concentration and decreases as concentration increases (Shi & Gisolfi, 1998). It is therefore likely that the absorption occurred at a slower than optimal rate due to the high concentration and small volume of the beverage. In addition, gastric emptying may have slowed as a result of the exercise intensity, as was demonstrated by Leiper et al. (2001).

Overall, the ideal concentration of carbohydrate beverage has been determined to be between 5% and 7% carbohydrate (Shi & Gisolfi, 1998). Additionally, the timing of the drink may have been less than ideal. There is evidence that pre-exercise glucose ingestion may actually be detrimental to exercise performance by causing a hyperinsulinaemic response and ensuing hypoglycemia (Hargreaves, 1985). No evidence of hypoglycemia was seen in the present study, however. This effect is now known to be dependent on a number of variables.

There is no definitive way to know whether the additional glucose was, in fact, taken up by the exercising muscle without performing muscle biopsies. Glucose can be transported without regulation into some cells, such as the brain and red blood cells. Additionally, glucose can be taken up by the liver to be stored as glycogen (Houston, 2001). It is possible that the exogenous glucose could have been taken up by one of these tissues instead of by the muscle, as is assumed.
Clearly, an exercise protocol in the laboratory will never be equivalent to game-play. Game-play exercise is intermittent in nature, but workloads and work/rest time periods are neither constant nor controllable. This study attempted to maximize the similarities with game-play by using a variety of intensities to simulate sprinting, running, jogging, and walking. However, typical realistic game-play intervals between these different speeds are not easily simulated, as each action may occur for only a few seconds or as long as several minutes (Bangsbo, 1994). Unfortunately, research involving actual game-play is also very limited by natural breaks in the game, as well as the inconsistencies inherent in game-play due to position, skill level, environment, and the opposing team, among other things.

Future Studies

It may be beneficial to examine the effect of carbohydrate supplementation on a protocol similar to the one used in this study, but longer in duration. A typical soccer game is 90 minutes in duration, with a half-time break after the first 45 minutes (Bangsbo, 1994). Therefore, this study’s protocol simulated only one-half of the average soccer game. Benefits due to exogenous carbohydrate may be evident in the second half of the game, as muscle glycogen may be more depleted. Additionally, the examination of muscle tissue to determine glycogen levels before, during, and after intermittent activity is necessary to determine the mechanism for improved exercise performance.

Summary

Based on the above information, the following summary points can be made:

• It was hypothesized that the exogenous carbohydrate ingestion during an intermittent exercise protocol would cause a lesser decline in power output over time. However, this hypothesis was not supported by the data.
• Mean power declined over time, but FI remained stable, evidencing that within-sprint effort did not decrease. The decline in mean power shows the fatiguing nature of the protocol. However, as no benefit was seen due to carbohydrate ingestion, the dominating cause of the fatigue must have been a variable other than nutrition.

• Blood glucose varied between trials. The elevated glucose in the CHO trial following exercise may have been due to additive effects of insulin and muscle contraction encouraging glucose uptake by the muscle.

• Lactate values were statistically identical between trials, showing that the level of anaerobic glycolysis was similar between trials. A similar response was seen in a similar study that also did not find a difference between trials.

• Heart rate was elevated, which shows that the exercise protocol was of moderate to high intensity. RPE also increased significantly with exercise, indicating that the subjects perceived the exercise to increase in difficulty.

• Some subjects experienced significant gastric distress during this study. The exact reasons for this distress are unknown, but all subjects experienced the distress during the CHO trial.

• The drink volume and concentration were different from a previously reported optimal level, which may have influence gastric emptying and carbohydrate absorption.

• There are several differences between laboratory trials and game-play that are not controllable such as workloads and work/rest time periods. This study attempted to minimize the differences by using varied intensities.

• Additional research is needed in this area. Specifically, more research involving direct examination of muscle glycogen levels in intermittent exercise should be performed to achieve a better understanding of the mechanisms involved with carbohydrate ingestion and utilization during this type of exercise.
Appendices

Appendix A: Pre-Screening Form

Subject’s Name __________________________ Date of Birth ____________

Address ________________________________

Phone (H) ____________________________ (W) ______________________

This study is restricted to healthy persons who have no known physical disorder.

Do you have any known presence of a disorder? YES NO
If YES, explain ______________________________

Do you regularly take any medications? YES NO
If YES, explain ______________________________

a) Do you have?:

Restrictions to physical activity YES NO
Cardiovascular disease (heart murmurs, etc.) YES NO
Pulmonary disease (asthma, etc.) YES NO
Skeletal or Muscle disorders YES NO
Endocrine disorders YES NO

b) If you answered YES to any item above, please explain below ______________________________

__________________________________________________________

Any other pertinent health information ______________________________

__________________________________________________________

Best time to call: __________________________ Email ______________
Appendix B:   Health History Questionnaire

Subject’s Name ___________________________ Date of Birth ____________

Address ___________________________

Phone  (H) _______________________

(W) _______________________

1. Do you participate in any sports and/or regular physical activities?   YES    NO

If YES, please list the activity below and indicate extent of participation (days/week).

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

2. Do you have any known endocrine, metabolic, or cardiorespiratory disorder?  YES    NO

If YES, explain:

________________________________________________________________________

________________________________________________________________________

3. Please list any medication(s) that you are presently taking.

<table>
<thead>
<tr>
<th>Medications</th>
<th>Purpose</th>
<th>Date of Initial Prescription</th>
<th>Amount taken per day (ie. mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Times when medication is not used ____________________________________________

Signature ______________________ Date __________
Subject: Research Subjects Needed

Researchers in the Human Performance Laboratory at Ball State University need men between the ages of 18 and 26 years to be participants in a research study titled “The effects of carbohydrate supplementation on fatigue during intermittent cycling in healthy young men”. This study will require 3 separate visits to the laboratory. The first visit is a familiarization and practice session. The second and third visits will require subjects to consume a carbohydrate or placebo beverage upon arrival. Exercise will consist of varying series of high-intensity cycling exercise over a 45 minute period of time.

If you are interested in participating in this study or have any further questions, please contact:

**Principal Investigator**  
Lisa M Guth  
Human Performance Laboratory  
Ball State University  
Muncie, IN 47306  
(765) 210-3320  
Imguth@bsu.edu

**Faculty Supervisor**  
Anthony D Mahon PhD  
Human Performance Laboratory  
Ball State University  
Muncie, IN 47306  
(765) 285-8693  
tmahon@bsu.edu

---

Appendix D: *BSU Email / Class Announcement Script*

Subject: Research Subjects Needed

Researchers in the Human Performance Laboratory at Ball State University need men between the ages of 18 and 26 years to be participants in a research study titled “The effects of carbohydrate supplementation on fatigue during intermittent cycling in healthy young men”. This study will require 3 separate visits to the laboratory. The first visit is a familiarization and practice session. The second and third visits will require subjects to consume a carbohydrate or placebo beverage upon arrival. Exercise will consist of varying series of high-intensity cycling exercise over a 45 minute period of time.

If you are interested in participating in this study or have any further questions, please contact:

**Principal Investigator**  
Lisa M Guth  
Human Performance Laboratory  
Ball State University  
Muncie, IN 47306  
(765) 210-3320  
Imguth@bsu.edu

**Faculty Supervisor**  
Anthony D Mahon PhD  
Human Performance Laboratory  
Ball State University  
Muncie, IN 47306  
(765) 285-8693  
tmahon@bsu.edu
Appendix E: **Informed Consent**

THE EFFECTS OF CARBOHYDRATE SUPPLEMENTATION ON FATIGUE DURING INTERMITTENT CYCLING IN HEALTHY YOUNG MEN

Informed Consent

The purpose of this research project is to investigate the effect of carbohydrate supplementation on repetitive anaerobic performance in young men.

You will be required to report the Human Performance Laboratory on three separate occasions (1 preliminary and 2 experimental sessions). Prior to the preliminary visit, you will be asked to complete a health history questionnaire. Your height and weight will be measured during the preliminary visit and you will practice exercises on the bicycle to become familiar with the testing procedures. During this visit, you will complete 12 minutes of exercise at varying intensities. During this visit you will be given instructions about selecting and recording a breakfast option for the morning of the exercise trials. This visit should last approximately 30 minutes.

On the day of the second visit, you will be required to record the timing and content of your breakfast choice from the breakfast option sheet given to you. You will be asked to consume this breakfast one hour prior to your arrival at the Human Performance Laboratory. You will be required to replicate the breakfast prior to the third visit. You are required to refrain from any other eating/drinking (with the exception of water) aside from your breakfast before arriving for your second and third visits. Upon arrival your weight will be measured. You will then be asked to consume either a carbohydrate or placebo beverage followed by 30 minutes of rest. After this rest period, a finger stick blood sample will be taken and you will be given a second carbohydrate or placebo beverage. After a brief warm-up, you will then complete 12 minutes of intermittent cycling exercise followed by 3 minutes of rest. During this recovery period, a second finger stick blood sample will be taken and you will be given a third carbohydrate or placebo beverage. You will then perform another 12 minutes of intermittent cycling exercise followed by 3 minutes of rest. During this recovery period, a third finger stick blood sample will be taken and you will be given a final carbohydrate or placebo beverage. You will then perform a final 12 minutes of intermittent cycling exercise. A final finger stick blood sample will be taken following this last exercise bout. Each blood sample will be collected in a capillary tube. The second and third visits should last approximately 90 minutes, each.

There are always risks associated with exercise testing in any age group. The maximal effort required during anaerobic exercise can lead to lightheadedness, syncope, nausea, muscle soreness, chest discomfort, and stomach distress. Risk statistics in adults provided by the American College of Sports Medicine for graded exercise testing to maximal effort are as follows: “The risk of death during or immediately after an exercise test is less than or equal to 0.01%; the risk of acute heart attack during or immediately after an exercise test is less than or equal to 0.04%; the risk of complication requiring hospitalization (including heart attack and/or serious arrhythmias) is less than or equal to 0.2%.” Researchers are not aware of risk statistics for maximal-effort anaerobic testing. Risks of taking a blood sample include a pricking sensation and possible bruising or risk of infection. The risks associated with this study are no greater than those present during high-exertion play and intense sports training.

To minimize these risks subjects will be familiarized with testing procedures and will also perform a warm-up prior to testing. Based on subject report, only healthy persons will eligible to participate in this
study. Sterile procedures will be used in handling the blood samples in accordance with universal precautions. Testing sessions will be supervised by a qualified member of the research team.

Your participation in this study is voluntary. You may withdraw from the study without prejudice from the investigator at any time. Your name will not be used when reporting results from this study and data will be treated with strict confidentiality.

Benefits for participation in this study include knowledge of anaerobic performance, and power output using a bicycle ergometer and the educational experience of participating in scientific research. Other benefits include information regarding anaerobic exercise capacity and the effects of carbohydrate beverages, contributing to a further understanding of the body's metabolic regulation to exercise.

For research subjects' rights, the following person can be contacted: Ms. Melanie Morris, Coordinator of Research Compliance, Office of Academic Research and Sponsored Programs, Ball State University, Muncie, IN 47306 (765) 285-5070.

Emergency medical treatment is available if you become injured or ill during your participation in this research project. You will be responsible for the costs of any medical care that is provided. It is understood that in the unlikely event of an injury or illness of any kind as a result of your participation in this research project that Ball State University, its agents, and employees will assume whatever responsibility is required by law. Participants are responsible to notify the Principal Investigator if any illness or injury occurs during this study.

I, ______________________ (PRINT), believe that I am in good physical condition and agree to participate as a subject in the research study entitled "The effects of carbohydrate supplementation on fatigue during intermittent cycling in healthy young men." I have had the study explained to me and my questions have been answered to my satisfaction. I understand that I may quit at any time. I have read a description of the procedures of the study and agree to participate. I understand that I will receive a copy of this form to keep for future reference.

__________________________  __________________________
Signature of Subject  Date

__________________________  __________________________
Name of Subject (Printed)  Date

Principal Investigator  Faculty Supervisor
Lisa M Guth  Anthony D Mahon PhD
Ball State University  Human Performance Laboratory
Muncie, IN 47306  Ball State University
(765) 210-2230  Muncie, IN 47306
lmguth@bsu.edu  (765) 285-8693
tmahon@bsu.edu
Appendix F: Breakfast Option/Recording Sheet

Name: ___________________________ Date: ___________________________

You will be recording the breakfast option that you consume the morning of your second visit into the Human Performance Laboratory. Check the appropriate box for the breakfast you chose. Remember that you will be asked to repeat this meal in regards to both timing and content for your third visit.

☐ 2 pieces wheat toast with 1 tsp. jam, water to drink

☐ 1 small bowl of Cheerios with low-fat milk, water to drink

☐ 1 small bagel, 1 small cup low-fat yogurt, water to drink
References


Levine SA, Gordon B, and Derick CL. Some changes in the chemical constituents of the blood following a marathon race. *JAMA* 82: 1778-1779, 1924.


