ABSTRACT

Effects of Carbohydrate Supplementation on Resistance Exercise Performance, Blood Glucose, Endocrine and Metabolite Responses, Immediately Before Exercise and During Recovery

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The positive effects of carbohydrate supplementation on aerobic exercise has been studied extensively. However, less information is known on carbohydrate supplementation before resistance exercise and its effects on post exercise glycogenesis. It has been proposed that supplementing carbohydrate before resistance exercise may improve performance and foster glycogenesis to better prepare an individual for another exercise bout. Serum glucose and insulin, muscle glycogen, and angled leg press exercise performance were assessed before, immediately after, and 1hr post exercise with or without carbohydrate supplementation. Participants completed 4 sets to failure at 70% of 1-RM with 45s between sets. There was no significant difference in resistance exercise performance or relative glycogen content between conditions. Serum insulin and glucose displayed a greater decrease during exercise in the supplementation condition. This shows that carbohydrate supplementation before resistance exercise will not improve resistance exercise performance when completed to fatigue despite alterations in glucose and glycogen utilization.

Effects of Carbohydrate Supplementation on Resistance Exercise Performance, Blood Glucose, Endocrine and Metabolite Responses, Immediately Before Exercise and During Recovery

by

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CHAPTER ONE

Introduction

Carbohydrate and Sports Performance

Glycogen and Athletic Performance

Those involved with athletic development are constantly trying to find ways to maximize performance through training, nutrition, rest, mechanical function, and endocrine responses in the human body. (Drain et al., 2015; Wagner et al., 2017; Lee et al., 2017; Hernández Davó, Botella Ruiz, and Sabido, 2017; Hammond et al., 2010; Charlton, Hammond, Cochrane, Hatfield, and Hunt, 2017; Taylor et al., 2017). Typically specific training adaptations are ultimately limited by the genetic potential of the athlete. However, few genes have been frequently linked to elite level performance (Guth and Roth, 2013). There have been numerous theories on what governs athletic performance and many studies have found that performance is limited with depleted or low glycogen levels in muscle (Nicholas, Green, Hawkins and Williams, 1997; Lima-Silva, De-Oliveira, Nakamura, and Gevaerd, 2009). Decreased glycogen content in skeletal muscle fibers is linked to decreased force of contractions in the muscle which can be detrimental to sports performance (Ørtenblad, Nielsen, Saltin, and Holmberg, 2011). Glycogen is a storage form of glucose that can be found in large quantities in the liver and skeletal muscle. During exercise, the energetic demands increases substantially and demand for ATP is markedly increased for muscle contractions. Glycogen serves as the main substrate for ATP synthesis during moderate- to high-intensity exercise (Van Loon et al.,

2001). It is also known that creatine phosphate (CrP) is utilized for immediate ATP synthesis during intense muscle contractions and supplementation can increase 1-RM strength (Wang, Lin, Hsu, Yang, and Chan, 2017). During submaximal exercise to fatigue, there are large decreases in skeletal muscle glycogen that are also seen with decreases in creatine phosphate (Sahlin, Tonkonogi, and Söderlund 1998; Norman, Sollevi, and Jansson, 1988). This suggests that both glycogen stores and CrP stores may also be decreased substantially from high-intensity, fatiguing exercise. Many of the theories connecting fatigue and glycogen depletion and link both by extrapolating low metabolic fuel leads to decreased performance. However, Ørtenblad, Nielsen, Saltin, and Holmberg (2011) found that low glycogen levels decreased the sarcoplasmic reticulum's ability to release Ca^{2+} which alters contractions in muscle fibers. The altered release of Ca^{2+} from the sarcoplasmic reticulum by reduced level of glycogen is associated with a faster decrease of tetanic Ca^{2+}during repeated contractions (Ørtenblad, Westerblad, and Nielsen, 2013). Strength and power athletes may have compromised contractile abilities if muscle glycogen levels are low from both a metabolic and excitation contraction coupling standpoint.

The majority of sports teams aim to improve physical ability by utilizing some form of periodization with resistance training. The primary bioenergetic pathways involved with anaerobic exercise are intramuscular ATP stores, CrP, and anaerobic glycolysis from intramuscular glycogen (Gastin, 1994; Jacobs et al., 1982). Many teams use multiple training sessions in a single day to maximize adaptations to increase performance. Utilizing multiple training sessions in a 24-hour period requires a substantial need to refuel glycogen stores to allow for continued exercise intensity in

subsequent training bouts. The amount of glycogenesis possible is directly related to the total availability of carbohydrates that can be used to create glycogen post-exercise (Bergstrom and Hultman, 1966). Carbohydrate ingestion with aerobic- based exercise has been thoroughly investigated over the past 100 years (Hearris, Hammond, Fell and Morton, 2018). Slater and Philips (2011) have expressed concern that maximal fueling and nutritional timing guidelines for power athletes, other than for post-exercise hypertrophic goals, are not readily available and need further investigation. The inability to contract forcefully and quickly during training, due to depleted glycogen content, can decrease productivity of training. It has been proposed that researchers investigating carbohydrate supplementation for resistance exercise should measure blood glucose, insulin, and catecholamine levels if carbohydrate is ingested during exercise (Tsintzas and Williams, 1998). The ability to decrease glycogen breakdown through the potential influence of supplementation relies mainly on hormonal mechanisms. This has practical application in exercise performance due to the ability to resynthesize muscle glycogen stores post-exercise, and also partially dictate substrate utilization during exercise. To adequately investigate the effects of carbohydrate supplementation during resistance training, endocrine response and metabolite changes before, during, and post should be examined.

Blood Glucose Utilization at Rest

At rest, blood glucose is tightly regulated with in a range of 4-5 mM by the actions of the hormones insulin and glucagon (Röder, Wu, Liu, and Han, 2016). When blood glucose levels are low, the pancreas secretes glucagon from α cells, promoting gluconeogenesis and glycogenolysis in the liver to increase plasma glucose levels. When

blood glucose levels are high, such as after a meal, insulin is secreted from β cells in the pancreas, causing increased glycogen synthesis in the liver and skeletal muscle decreasing plasma glucose levels. Regulating blood glucose is extremely important for normal central nervous system functions and this homeostasis is challenged during exercise.

Blood Glucose Utilization and Intense Exercise

The regulation of blood glucose is controlled by synergistic hormonal mechanisms that are altered during exercise and after exercise (James and McFadden, 2004). Increases in blood glucose levels have been shown to have a positive relationship with exercise intensity (Romjin et al. 1993). During exercise, the demand to resynthesize ATP in the muscle is very high and requires the metabolism of various substrates from their respective metabolic systems. There is a limited amount of ATP, around 8 mmol/kg wet weight of muscle that must be resynthesized in order to sustain cellular function (Baker, McCormick, and Robergs, 2010). Anaerobic glycolysis is a large contributor of ATP synthesis when intense exercise lasts longer than a few seconds (Baker, McCormick, and Robergs, 2010). This energy pathway predominately uses glycogen to create ATP for muscle contractions when intensity of contractions is high, even though glucose is mobilized into blood circulation (Katz et al., 1991; Romjin et al. 1993). Cortisol is known to block the uptake of glucose into tissue beds and has been found to be at elevated levels during moderate to high intensity exercise (Hill et al., 2008). Insulin levels have also been shown to decrease at the onset of exercise which may also influence glucose clearance (Brooks, Fahey, and Baldwin, 2005). The decreased intake of blood glucose, and increased reliance on glycogen during intense exercise, may be a protective

mechanism to maintain blood glucose levels in a safe range for neurological function. The alteration in these hormones during exercise due to the supplementation of carbohydrate may promote glycogen sparing.

Blood Glucose Homeostasis Following Intense Exercise

The increased hepatic production of glucose coupled with decreases in glycogen in muscle during exercise can also be thought of as a feed-forward mechanism to promote glucose transport into the muscle post-exercise (Katz et al., 2016). This idea can also be supported due to large increases in insulin being secreted post-exercise due to hyperglycemic effect of the bout (Pascoe and Gladden, 1996). The understanding of the hormones in circulation post-exercise can be used to decrease recovery time for subsequent exercise bouts. These situations are found in multi-event track athletes, basketball, soccer tournaments, and other sports that have multiple intense exercise bouts in the same day. A large majority of the studies evaluating acute changes in hormone response to resistance exercise have investigated their effects on muscle protein synthesis or hypertrophy (Kraemer et al., 1990; Griggs et al., 1989). The interaction of hormonal responses and skeletal muscle metabolite changes both during and following exercise have been studied in primarily aerobic, endurance exercise (Näveri, Kuoppasalmi, and Härkönen, 1985; Ivy 2004). Insulin, epinephrine, and cortisol are hormones that help regulate substrate mobilization and euglycemia during and following intense exercise (Institute of Medicine, 1994; Shizamu, 1992). These may also be the hormones that are released during intense resistance training and have been speculated to be useful for interpreting supplementation effects for glycogenesis (Tsintzas and Williams, 1998). Understanding dynamic hormonal fluctuations, changes in blood glucose, and muscle

metabolite changes during intense resistance exercise has potential to enhance athletes' ability to recover, decrease risk of injury, improve training stimulated effects, and performance.

Purpose

In response to either placebo or carbohydrate supplementation, the purpose of this study was to investigate the changes in serum hormone and muscle metabolite concentrations before and after supplementation and immediately after and 1 hour after an intense bout of resistance exercise. The specific aims of the study were to determine if: 1) skeletal muscle glycogen content before and immediately post and 1 hr post-exercise differ with and without carbohydrate supplementation, 2) serum levels of glucose, and insulin before and after supplementation, immediately post and 1 hr post-exercise differ with and without carbohydrate supplementation, 3) resistance exercise performance is different between baseline, carbohydrate, supplementation and placebo conditions.

Hypotheses

H1: There were no significant differences in muscle glycogen concentrations between carbohydrate and placebo conditions (pre, post-exercise, 1 hr post).

H2: There were no significant differences in muscle glycogen concentrations within the carbohydrate or placebo conditions (pre, post-exercise, 1 hr post).

H3: There were no significant differences between the levels of serum glucose or insulin between the carbohydrate and placebo conditions (pre, post-supplement, post-exercise, 1 hr post).

H4: There were no significant difference in levels of serum glucose or insulin, within each of the carbohydrate and placebo conditions (pre, post-supplement, post-exercise, 1 hr post).

H5: There were no significant differences between multiple reps performed on the angled leg press machine between the baseline, placebo, and carbohydrate conditions.

Delimitations

1. Apparently healthy males between the ages of 18 to 30 who are familiar with intense resistance training. (Participants who have been resistance training for more than a year)

2. Participants were recruited from Baylor University and within the surrounding Waco, TX area by flyers and online advertisements.

3. Participants were excluded from the study if they consumed any dietary supplement (except a multivitamin) or any pharmaceutical that is used as a potential ergogenic aid for three months prior to the study.

4. All participants were considered low risk for cardiovascular disease, with no contraindication to exercise as outlined by the American College of Sports Medicine (ACSM).

5. All participants were tested at the Baylor Laboratory for Exercise Science and Technology (BLEST) and Exercise Nutritional Biochemical Laboratory (EBNL) location under the supervision of the investigator in accordance with Helsinki Code after signed university approved informed consent documents.

Limitations

1. Inferences may have been limited to the time points at which samples were collected. Hormone and metabolite concentration could have been affected with the intervals of collection.

2. Each participant's meals, sleep, and activity level 24hr prior to each resistance training bout may have influenced the results of the study.

3. The psychological factors surrounding each participant such as the motivation to finish the resistance training test to the best of their ability may potentially have affected the results of the training study.

4. The results of this training study may possibly carry external validity to only non-diabetic male participants within the age group of 18-30.

Assumptions

1. All laboratory equipment was functioning properly to produce valid and reliable measurements. Proper calibration and the use of trained research staff minimized any potential for error.
2. Participants put forth maximal effort during the maximal strength testing sessions.
3. All participants compliantly carried out the respective resistance training protocol, at the desired intensity throughout the study.
4. All participants maintained their normal dietary habits throughout the study.
5. All participants took their respective supplement given by the investigator before the resistance training program.

6. All participants will not undergo any resistance training and also abstain from aerobic activity during the study.

Definition of Terms

1-repetition maximum (1-RM) the maximum amount of weight able to be lifted for one repetition

Lactate – A byproduct of fast glycolysis that results from conversion of pyruvate and lactic acid

Glycolysis – An energy system that utilizes glycogen or glucose to create ATP

Creatine Phosphate (CrP) – A metabolite that is used by muscle to create ATP

Cortisol – A hormone secreted during intense exercise to maintain blood glucose levels

Insulin – A hormone that increases the uptake of glucose into muscle tissue during resting conditions

CHAPTER TWO

Review of Literature

Hormonal Control of Glucose Metabolism

Endocrine System

Hormones are chemical substances that are released into the blood that relay regulatory messages to maintain homeostasis within the body. The release of hormones into the blood to impact another part of the body is one example of long-range cellular communication (Röder, Wu, Liu, and Han, 2016). There are three main types of hormonal release used for cellular communication: endocrine, paracrine, and autocrine signaling (Iliodromiti et al., 2012). Endocrine signaling includes secretion of hormones into the blood stream that travel bound to their specific binding proteins and act on specific target cells in other parts of the body (Bartalena, 1990). Paracrine signaling is local chemical secretion that triggers a response on the surrounding target cells. Autocrine signaling is the release of chemicals that cause a change in the cell that released the hormone. Hormones only act on target cells that have the specific matching receptors, which signals changes in cellular function. Some of the influence hormones have on the body include glucose blood pressure, and gene expression regulation, energy metabolism, and altered behavior (Tata, 2005; Chopra, Baby, and Jacob, 2011, Röder, Wu, Liu, and Han, 2016; Pfaff, 1997).

Types of Hormones

Hormones fall into three main classes: polypeptides, amines, and steroids. Polypeptide and amine hormones are water soluble, meaning they do not freely move through cell membranes and require cell-surface receptors to cause altered gene regulation or cytoplasmic responses. Insulin is a peptide hormone that acts through endocrine signaling and requires a functioning surface cell receptor to cause cellular glucose influx (De Meyts, 2016). Steroid hormones are lipid soluble and can pass through a cell membrane easily where it binds to its receptor in the cytoplasm of the cell causing altered cellular function. Cortisol is a lipid soluble steroid hormone that is able to freely diffuse through the cellular membrane, bind to its glucocorticoid receptor in the cytoplasm, and cause a signaling cascade leading to altered gene expression (Walker, 2011).

Hormonal Blood Glucose Control at Rest

Blood glucose, under resting conditions, is tightly regulated by the antagonistic effects of insulin and glucagon. Both of these hormones are secreted by the pancreas in response to altered euglycemic conditions. When blood glucose levels are high, such as after a meal, insulin is secreted from β cells in the pancreas. Insulin is a peptide hormone that requires a functioning surface cell receptor to cause cellular glucose influx (De Meyts, 2016). Skeletal muscle is the largest glucose-consuming tissue in the body and relies heavily on insulin for glucose transport (Garvey et al., 1998). Specifically, insulin binds to the α subunit of the cell membrane receptor causing the autophosphorlyation of the tyrosine residue of the β subunit, leading to signaling cascade provoking the translocation glucose transporting protein 4 (GLUT-4) to the cell membrane, that

facilitates glucose influx (De Meyts, 2016; Brooks, Fahey, and Baldwin, 2005). The glucose that enters into skeletal muscle is either stored as glycogen or utilized in glycolysis depending on the energetic needs of the cell.

Between meals or during sleep, our body is readily consuming blood glucose for energy. This constant uptake causes lower levels of blood glucose to stimulate the release of glucagon from α cells in the pancreas (Röder, Wu, Liu, and Han, 2016). Glucagon stimulates glycogenolysis in the liver, while also increasing gluconeogenesis from amino acids, allowing hepatic glucose secretion to continuously regulate blood glucose (Rui, 2014). Under long-term fasted conditions or exercise, glucocorticoids are released by skeletal muscle to promote proteolysis. Amino acids are then released into the circulation from muscle proteolysis for uptake by the liver to be used by gluconeogenesis to produce glucose. This glucose is then released into circulation in order to stabilize euglycemia (Simmons, Miles, Gerich, and Haymond, 1984). Similarly, norepinephrine is released during short term starvation periods to promote hepatic glucose production and fatty acid breakdown (Zauner et al., 2010). It is clear that blood glucose is tightly regulated by hormones and environmental factors. Some of these environmental factors, specifically during exercise periods, can impact the endocrine system and alter blood glucose regulation.

Blood Glucose Utilization during Aerobic Exercise

During the onset of exercise there is an increase in demand for ATP that is primarily met by glycolysis. Some of the substrate being used for exercise is met by blood glucose, which causes signals in the body to increase hepatic glucose production, which helps regulate continued central nervous system and organ function in an increased

state of glucose demand. The largest increases in blood glucose are seen after 20 minutes before returning to normal resting parameters (Zinker 1990). Many studies that examine blood glucose change and/or uptake during exercise look at prolonged aerobic, submaximal, steady states between 30 and 60 minutes (Warhen et al., 1984, Van Loon et al., 2001, Zinker et al., 1990). It has been shown that blood glucose becomes more of a fuel source during extended exercise (Coggan, 1991; Katz et al., 1991; Peric, Meucci, & Nikolovski, 2016; Ahlborg, Felig, Hagenfeldt, Hendler, and Wahren, 1974). Plasma insulin has been shown to decline during exercise which decreases the insulin stimulated translocation of GLUT-4. (Brooks, Fahey, and Baldwin, 2005) However, muscle contractions stimulate GLUT-4 translocation to the cell surface and allow glucose uptake despite decreased insulin concentrations (Yang and Holman, 2005; Lauritzen, 2013). The decrease in glucose levels stimulate glucagon to increase hepatic glucose production (Goodwin, 2010). Hepatic glucose output increases due to increased glucagon and catecholamines while insulin concentration decreases (Lauritzen, 2013; Brooks, Fahey, and Baldwin, 2005). If submaximal exercise is sustained for hours, skeletal muscle glucose uptake can outpace hepatic glucose production and lead to hypoglycemia (Ahlborg, Felig, Hagenfeldt, Hendler, and Wahren, 1974). Similar to resting conditions, submaximal intensity aerobic exercise blood glucose is controlled mainly by the interactions of insulin and glucagon (Goodwin, 2010). Low-intensity aerobic exercise has been shown to decrease circulating cortisol levels indicating that cortisol plays a minor role in glucose homeostasis at intensities around 40% (Hill et al., 2008).

Anaerobic Exercise and Blood Glucose Control

Increases in blood glucose levels have shown to have a positive relationship with exercise intensity (Romjin et al. 1993). It has been shown that the increases in blood glucose level rise faster during high-intensity exercise with the peak being reached at 15 minutes (Guelfe, Ratnam, & Smythe 2007). This seems to imply that blood glucose is mobilized faster so that it can be metabolized with higher intensities of exercise, which is paired with rapid decreases in muscle glycogen. However, the proportion of glucose uptake is relatively small when compared to the total energy supply (Katz et al., 1991). This was also found in another study where participants exercised at 75% of where the main fuel source metabolized was muscle glycogen (Van Loon et al., 2001).

The increase in blood glucose levels can be partly accounted for by changes in hormonal secretions influencing hepatic glucose production and tissue absorption. Increases in catecholamine and cortisol levels are both known to stimulate hepatic glucose production and cause increases in blood glucose (Hill et al., 2008; Zouhal, Jacob, Delamarche, and Gratas-Delamarche 2008). Cortisol has also been shown to cause insulin resistance-like effects in skeletal muscle, which influence glucose clearance (Gathercole, Bujalska, Stewart, and Tomlinson, 2007). Epinephrine has been shown to increase dramatically in plasma concentrations after intense exercise (Kjaer, Farrell, Christensen, Galbo, 1986). Both of these changes in cortisol and epinephrine are likely the main glucose-regulating hormones during intense exercise, not insulin and glucagon (Kjaer, Farrell, Christensen, and Galbo, 1986; Gathercole, Bujalska, Stewart, and Tomlinson, 2007).

The amount of blood glucose utilized during exercise is also dependent upon the existing muscle glycogen levels (Richter et al., 2001). The surface membrane content of GLUT 4 after muscle contractions was negatively associated with increasing muscle glycogen levels, indicating glycogen levels mediate GLUT 4 glucose transport from the blood to the muscle (Derave et a., 1999). Similarly, one study suggests that glucose transport behaves in a gradient- like fashion between intracellular and extracellular glucose, which mediates part of the diffusion into the muscle, and is controlled by molecular signaling mechanisms (Richer & Hargreaves 2013). Blood glucose uptake during exercise is influenced by glycogen availability in the muscle due to increased rates of glycogenolysis filling the muscle cell with glucose 6 phosphate. The increase in glucose 6 phosphate being supplied from muscle glycogenolysis may cause hexokinase inhibition, which limits blood glucose uptake and blood glucose metabolism (Katz, Sahlin, and Wahren 1986; Wasserman, 2008). The hyperglycemic effects of short high-intensity bouts of exercise may be due to the inhibition of both GLUT 4 glucose translocation due to high concentrations of muscle glycogen, hexokinase inhibition, circulating hormone changes that increase hepatic glucose production, and decrease glucose uptake in skeletal muscle (Katz, Sahlin, and Wahren 1986; Wasserman, 2008; Gathercole, Bujalska, Stewart, and Tomlinson, 2007).

Resistance Training and Blood Glucose

It appears that hepatic blood glucose production occurs with any intensity of exercise, but the mechanisms involved in glucose regulation during exercise depends on the intensity (Goodwin, 2010; Lauritzen, 2013; Kjaer, Farrell, Christensen, Galbo, 1986; Gathercole, Bujalska, Stewart, and Tomlinson, 2007). Many of the endocrine response

studies centered on resistance exercise evaluate anabolic hormone release and muscle protein synthesis post-exercise (Morton, McGlory, and Philips 2015; Burd et al., 2010; Damas et al., 2016). Many of the studies that investigate these changes use intense aerobic-based exercise (Ivy, Katz, Cutler, Sherman, and Coyle, 1988). Intense resistance training involving short rest periods and high repetitions, have been shown to increase cortisol and catecholamine levels similar to intense aerobic or anaerobic training (Szivak et al., 2013; French et al., 2007). Also, resistance training has been shown to partially decrease glycogen stores in muscle and depletion is associated with decreased performance (Aragon and Schoenfeld, 2013; Ørtenblad, Westerblad, and Nielsen, 2013). It has been proposed that supplementation before exercise may increase glycogen synthesis rates and increase performance outcomes by maintaining metabolic fuel (Haff, Lehmkul, McCoy, and Stone, 2003). These changes would likely be reflected in the endocrine responses to glucose management and how it influences glycogenesis.

Hormonal and Non-Hormonal Influences on Skeletal Muscle Glycogenolysis during Intense Exercise

During exercise, norepinephrine and epinephrine levels increase and bind to adrenergic receptors in the liver respectively (Chu et al., 2000). The binding of these catecholamines increase the rates of gluconeogenesis and glycogenolysis to increase blood glucose levels in the blood which can then be cleared into tissue for subsequent use. However, during intense exercise blood glucose uptake and utilization is minimal (Van Loon et al., 2001). Skeletal muscle also contains adrenergic receptors and the binding of epinephrine can cause increases in glycogenolysis in muscle. Intense exercise has been shown to cause larger increases in catecholamine response than moderate

intensities (Kjaer, Farrell, Christensen, Galbo, 1986). The increased glycogenolysis is caused by epinephrine induced cell signaling causing a specific membrane-attached G protein to become activated, which then interacts with adenylyl cyclase. Adenylyl cyclase activation causes an increase in cyclic AMP formation from ATP, which leads to the activation of protein kinase A. This causes the activation of phosphorylase kinase, leading to the activation of glycogen phosphorylase (Exton, 1987). This leads to glycogen breakdown for glycolysis and may also contribute to hexokinase inhibition, limiting blood glucose uptake that is seen in other studies (Katz, Sahlin, and Wahren 1986; Wasserman, 2008; Van Loon et al., 2001).

Glycogenolysis can be activated by non-hormonally mediated mechanisms during exercise. Muscle contractions can lead to the activation of a protein called calmodulin. The Ca^{2+} used to bind with calmodulin is mainly released from the sarcoplasmic reticulum during the excitation contraction coupling process. The activation of calmodulin causes the activation of phosphorylase kinase leading to the activation of glycogen phosphorylase for glycogen metabolism. (Picton, Klee, and Cohen, 1981). Also, during intense anaerobic exercise, ATP stores and CrP are primarily used for energy which leads to a larger concentration of ADP, AMP, and Pi. These three substances are allosteric activators of phosphofructokinase (PFK) which is the rate limiting enzyme in glycolysis (Berg, Tymoczko and Stryer, 2002). Both the non-hormonally mediated breakdown of glycogen and increased activation of PFK from immediate contraction energy sources are feedforward mechanisms for continued contraction capabilities. These mechanisms likely account for the changes in glycogen during short term resistance exercise or short maximal sprints lasting under 5 seconds due to the reliance of alactic

energy sources. These responses may also influence glycogen change in muscle if hormonal concentrations changes are not seen before and after brief exercise periods.

Normal Metabolite Levels

Normal glycogen content in skeletal muscle is around 80-120 mmol/kg wet weight (Bussau et al., 2002). A short-term carbohydrate loading protocol was shown to double muscle glycogen stores in individuals from 95 ±5 mmol/kg wet weight to 180 ±15 mmol/kg wet weight when fed 10 grams of high-glycemic carbohydrates per kg body mass over a 24-hour period after high intensity exercise (Bussau et al., 2002). Twenty-four hours of fasting has the opposite impact on glycogen concentration, but the loss of glycogen occurs in the liver and can decrease by 65% (Magnusson et al., 1992). Muscle glycogen concentrations do not rapidly decrease due to fasting because skeletal muscle does not contain the enzyme glucose 6 phosphatase. This is needed to create free glucose that can flow through the circulatory system and is involved with steps directly related to blood glucose homeostasis (Jensen, Rustad, Kolnes, and Lai, 2011). During an overnight fast, hepatic glycogen content decreases and may need to be replenished before exercise. After ingesting food, the intestine will extract the glucose from the meal and transport it to the liver through the portal vein. The glucose transported to the liver is then converted to glycogen and utilized to regulate blood glucose concentrations. It was found that hepatic glycogen stores are replenished around 180 minutes after ingesting a meal (Petersen et al., 2001).

Glycogenesis in Fasted State and with CHO Supplementation Following Intense Exercise

Without ingesting carbohydrates following exercise, humans have the capability to replenish a portion of the glycogen stores mainly from blood glucose (Bangsbo, Gollnick, Graham, and Saltin, 1991). During a fasted state it was proposed that amino acids serve as the precursor for gluconeogenesis, that is then released into the blood, to be used for glycogenesis in the skeletal muscle after sustained aerobic exercise (Favier et al., 1987). In a fasted state, lactate, after its conversion in the Cori cycle, has also been speculated to serve as a large substrate for glycogen synthesis after intense exercise (Fournier et al., 2002). However, controversy on this exists between older and new studies involving rat models showing no glycogenesis from lactate following exercise (Brooks et al., 1973). What is clear is that glycogenesis following exercise does occur with eating and is very important for maintaining muscle performance (Bangsbo, Gollnick, Graham, and Saltin, 1991; Burke, Kiens, and Ivy, 2004; Ørtenblad, Nielsen, Saltin, and Holmberg, 2011). Hyperglycemia during intense exercise is prolonged into the post-exercise stages and is coupled with increased insulin secretion (Pascoe and Gladden, 1996). The role of insulin is of paramount importance while trying to rapidly replenish glycogen stores for a repeated bout of intense exercise (Pascoe and Gladden, 1996). The paralleled increases in insulin from increased blood glucose post-exercise can be manipulated with carbohydrate supplementation, thereby increasing glycogen synthesis rates (Pascoe and Gladden, 1996). Haff et al., (1999) found that carbohydrate supplementation before, during, and 4 hours post resistance exercise can increase squat to fatigue performance in a second training session. Supplementing during an exercise bout

should better prepare an athlete for the performance requirements that may occur within the same 24-hour period.

Timing, Type, and Amount of Carbohydrate Supplementation

Glycogen synthesis rates are known to occur in rapid and slow phases post-exercise, and timing of supplementation can affect glycogenesis (Ivy, Katz, Cutler, Sherman, and Coyle, 1988). Immediate supplementation of carbohydrates following aerobic exercise have been shown to produce the highest rates of glycogen synthesis (Ivy, Katz, Cutler, Sherman, and Coyle, 1988). In another study, it was found that intravenous delivery of glucose and oral consumption of glucose resulted in similar amounts of glycogenesis post-exercise (Blom, 1989). Similarly, liquid and intravenous carbohydrates result in more glycogen produced during recovery than solid carbohydrates provided during the same time frame (Reed, Brozinick, Lee and Ivy, 1989). High glycemic carbohydrates result in more glycogen synthesis than low glycemic carbohydrates (Burke, Collier, and Hargreaves, 1985). It has also been proposed that more than 1 gram of carbohydrate per kilogram body weight needs to be consumed every two hours to maximally stimulate glycogenesis (Ivy, 1998). During post-exercise recovery, in order to maximize the amount of glycogenesis in a short time frame, immediate supplementation with a liquid high-glycemic carbohydrate at quantities greater than 1 gram per kg body weight are optimal.

CHO Supplementation Before Resistance Exercise

Supplementation of carbohydrates immediately before exercise has been shown to increase maximal voluntary force of contractions post-consumption (Gant, Stinear,

Byblow, 2010). These changes in force output are thought to be due to corticomotor activation by oral taste sensory mechanisms because endocrine responses had not changed at corresponding time frames (Gant, Stinear, Byblow, 2010). Another study found that similar sweetness between carbohydrates did not alter activation in brain reward centers, but potentially some form of unidentified caloric sensory mechanism that plays a role in a feed-forward mechanism for exercise (Gant, Stinear, Byblow, 2010; Chambers, Bridge, and Jones, 2009). The fluctuations in blood glucose level and insulin were seen 20 minutes post consumption and were not thought to contribute to these changes (Gant, Stinear, Byblow, 2010). Another study had mixed results showing that carbohydrate ingestion before and during anaerobic exercise, including resistance training, did not decrease performance (Krings et al., 2016). Supplementing carbohydrate in doses of 0.3 g/kg body mass before resistance exercise was shown to not have an impact on performance (Kulik, Touchberry, Kawamori, Blumert, Crum, and Haff, 2018). However, carbohydrate doses at 1g/kg body mass before combined with 0.17 g/kg body mass during resistance exercise every 6 minutes was shown to increase force output and time to exhaustion with superimposed electromyostimulation (Wax, Brown, Webb, and Kavazis 2012). The methodological differences between the studies make it difficult to determine if pre-carbohydrate ingestion can increase performance. It may be possible that increased resistance exercise performance may be seen with a higher total volume of carbohydrate supplementation before exercise. Two g/kg was the total amount of carbohydrate used for supplementation by Wax, Brown, Webb, and Kavazis (2012) that elicited increases in resistance exercise performance. Carbohydrate supplementation before resistance training has shown to have a positive influence on glycogen stores in

the post exercise state (Leat and Jacobs, 1989). The increased glycogen content post exercise is thought to be due to glycogen being resynthesized during the rest periods between resistance exercise lifts and has been postulated to partially maintain glycogen stores (Leat and Jacobs, 1989; Haff et al., 2000). The increase in circulating glucose from supplementation may increase insulin levels during resistance training and improve glycogenesis between sets by facilitating blood glucose uptake during exercise.

Concluding Ideas

The world of athletic development is constantly evolving and ways to enhance athletic ability by manipulating various environmental and internal factors are continually being sought. A large portion of exercise performance revolves around the ability to provide substrate for continued powerful muscle contractions. Substrate that is utilized in both extended aerobic and short anaerobic exercise and recovery is controlled by hormonal responses related to blood glucose regulation. Maximizing performance that is related to the substrate bioenergetics needs to be viewed with the various endocrine responses in mind. When supplementing with any potential nutritional ergogenic aid, the physiological state that the body is currently in will be substantially responsible for the conditions that nutrient supplementation will be applicable.

CHAPTER THREE

Methods

Approach, Methods, and Statistical Analysis

Experimental Approach

Participants visited the laboratory on 4 separate occasions in the following manner: visit 1 = entry/familiarization, visit 2 = baseline one repetition maximum (1-RM) and multiple repetition muscle performance testing, visit 3 = multiple repetition muscle performance testing with or without carbohydrate, and 7 to 10 days later, visit 4 = multiple repetition muscle performance testing with or without carbohydrate. In a randomized, double-blind, cross-over experimental design, participants performed a resistance exercise session involving the angled leg press after ingesting either placebo or carbohydrate in order to collect measurements on muscular strength and muscular endurance. Each session involved the gathering of data for the analysis of muscular strength, biochemical markers of muscle metabolite change and blood hormone changes. This experimental approach is based on the premise that resistance exercise is known to stimulate endocrine changes in the body and influence metabolite changes in muscle. The proposed experimental model also allowed for the determination of whether carbohydrate supplementation can decrease glycogen expenditure during exercise and increase glycogen synthesis post-exercise.

Participants

Ten apparently healthy, recreationally resistance-trained [regular, consistent resistance training (i.e. thrice weekly) for at least 1 year prior to the onset of the study], men between the ages of 18 and 30 volunteered to serve as participants in this study. Enrollment was open to men of all ethnicities. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM), and who have not consumed any nutritional supplements (excluding multi-vitamins) one month prior to the study were allowed to participate. This study was approved by the Institutional Review Board for Human Subjects at Baylor Universit and all eligible participants signed university-approved informed consent documents. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Helsinki Code.

Study Site

All supervised testing and sample analyses were conducted in the Exercise and Biochemical Nutrition Laboratory (EBNL) at Baylor University.

Independent and Dependent Variables

The independent variable was the supplementation of placebo or carbohydrate before exercise and time. The dependent variables included serum insulin, glucose, glycogen stores, and maximal repetitions to fatigue.

Participant Entry Protocol

Participants expressing interest in participating in this study were interviewed to determine whether they appeared to qualify to participate in this study. Participants

believed to meet eligibility criteria were invited to attend an entry/familiarization session. Once reporting to the laboratory, participants were familiarized to the study protocol via a verbal and written explanation outlining the study design and then read and signed a university-approved informed consent document. Participants completed a medical history questionnaire and underwent a general physical examination to determine whether they further meet eligibility criteria. At the conclusion of the familiarization session, participants were then given an appointment in which to attend their first testing session. In addition, each participant was instructed to refrain from exercise for 24 hours prior to each testing session, eat a light, low carbohydrate meal 3 hours prior to reporting for each testing session, and record their dietary intake for two days (including the light meal the morning of testing) prior to each of the three testing sessions (Lippi et al., 2010; Clinical and Laboratory Standards Institute, 2009). They were informed that when they report to the laboratory for their testing sessions (visits 2-4), they would undergo assessments for lower-body muscle strength assessments involving the angled leg press. To ensure that participants who enrolled in the study are resistance trained the one-rep maxes of each participant were compared to normal strength to body weight ratios. The minimum age specific strength to body weight ratio for males that were allowed to enter into the study were set in the superior category as adapted and outlined by the Cooper Institute for Aerobics Research in Table 3.1 below (1997).

Table 3.1

Strength to Body Weight Ratio

Age	<20 yrs	20-29 yrs	30-39 yrs
Superior	>2.82	>2.13	>1.93

Note: Age Specific Strength - Body Weight Ratio from Cooper Institute for Aerobics Research (1997)

Hydration and Dietary Analyses

Participants were required to record their dietary intake for two days prior to each of the three testing sessions. The participants' diets were not be standardized but participants were asked not to change their dietary habits during the course of the study. The dietary recalls were evaluated with the My Fitness Pal mobile application (Under Armor Inc.) to determine the average daily macronutrient consumption of fat, carbohydrate, and protein in the diet for the duration of the study.

Packed cell volume (PCV) is a measure of the ratio between volume of red blood cells to the volume of whole blood and can be expressed as a decimal or percentage fraction. This measure was used to determine the proportion of hematocrit in the blood and used as an indicating measure of dehydration (Pagana and Pagana, 2013; Jimenez, Melin, Koulmann, Allevard, Launay, and Savourey, 1999). At the beginning of testing sessions 3 and 4, blood was drawn into microhematocrit tubes by capillary action and then sealed using a small amount of clay material at a 90-degree angle. The microhematocrit tube was placed into the centrifuge with its position recorded, balanced, spun for 2 minutes, then removed and read on a hematocrit reader card. Normal ranges for this ratio in adult males are between 42-52% (Pagana and Pagana, 2013; Billet, 1990). Participants with PCV ratios over 54% were deemed dehydrated and asked to rehydrate before further steps of the testing session would performed.

Muscle biopsies

Percutaneous muscle biopsies (~30 mg) were obtained from the middle portion of the vastus lateralis muscle of the dominant leg at the midpoint between the patella and the greater trochanter of the femur at a depth between 1 and 2 cm. After the initial biopsy, the

remaining biopsies attempts were made to extract tissue from approximately the same location as the initial biopsy by using the pre-biopsy scar, depth markings on the needle, and a successive incision that was made approximately 0.5 cm to the former from medial to lateral. After removal, adipose tissue was trimmed from the muscle specimens and immediately frozen in liquid nitrogen and then stored at -80°C for later analysis. Three muscle samples were obtained at visit 3 and 4 for a total of 6 muscle biopsies performed during the course of the study. Biopsies were taken before, immediately after, and 1-hour post-exercise for visits 3 and 4.

Blood Sampling

Venous blood samples were obtained into 10 ml vacutainer tubes from a 21-gauge phlebotomy needle inserted into the antecubital vein. Blood samples were allowed to stand at room temperature for 10 min and then centrifuged. The serum was removed and frozen at -80°C for later analysis. Four bloods samples were obtained at visit 3 and 4 for a total of 8 blood samples obtained during the course of the study. The blood samples were collected before supplement ingestion, immediately before resistance exercise (30 minutes following supplement ingestion), immediately post-exercise, and 1-hr post exercise.

Muscle Strength Evaluation

In order to determine muscular strength, at session 2, participants performed 1-RM tests in accordance with the National Strength and Conditioning Association (NSCA) recommendations on the angled leg press exercise. Similarly, the foot placement of the participants were recorded and held constant over all testing conditions in order to

maintain biomechanical consistency. To standardize and ensure that participants were moving through the indicated range of motion during each repetition, a goniometer was used to establish 90 degrees of knee flexion while seated on the leg press machine before the exercise bout. The safety catches on the leg press were then adjusted just below this point for all tests to standardize range of motion. The participants were instructed to lower the weight just above the catches during the eccentric period before contracting through the range of motion.

Participants warmed up by completing 10 repetitions at approximately 50% of the estimated 1-RM. Then participants rested for 1 minute, and then completed 5 repetitions at approximately 70% of the estimated 1-RM. The weight was then increased conservatively, and the participants attempted to lift the weight for one repetition. If the lift is successful, the participant rested for 2 minutes before attempting the next weight increment. This procedure was continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition. 30 minutes following the 1-RM test, participants completed 4 sets of maximal reps as outlined in the resistance exercise protocol below. This value served as a baseline performance measure to be compared to placebo and carbohydrate conditions.

Resistance Exercise Protocol

During testing sessions 2, 3, and 4, participants performed 4 sets of maximum repetitions with 70% of the 1-RM on the angled leg press. During sessions 3 and 4, participants completed two warm up sets of 10 reps and 5 reps at 50% and 70% of the testing load respectively. Each set was followed by two minutes of rest and then the exercise testing session began. When muscle fatigue/failure occurred during a set, study

personnel provided assistance to help re-rack the weight safely. In all cases, 45 seconds of rest was allowed between sets during the maximum reps to fatigue protocol. The total number of repetitions performed at each testing session was recorded individually.

Nutrient Supplementation Protocol

A period of 7 to 10 days separated sessions 3 and 4 to allow for adequate supplement washout and muscle recovery. At session 3 and 4, 30 minutes prior to resistance exercise, in a randomized, doubled-blind fashion, participants ingested either a placebo or carbohydrate supplement. The placebo supplement consisted of a flavored, non-caloric beverage (Crystal Light) mixed with 12 ounces of water and orally ingested. The carbohydrate supplement consisted of maltodextrin (Pure Karbolyn, Pro Supplements, Inc., Allen, TX, USA) at a dose of 2g/kg body mass, that was mixed with Crystal Light in 12 ounces of water, and orally ingested (Wax, Brown, Webb, and Kavazis 2012).

Serum Glucose Assessment

Serum glucose was assessed with a colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) which uses the glucose oxidase-peroxide reaction for the determination of glucose concentration. In this assay, glucose is oxidized to δ-gluconolactone with concomitant reduction of the flavin adenine dinucleotide (FAD)-dependent enzyme glucose oxidase. The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide. The absorbances was read at a wavelength of 514 nm and unknown concentrations determined by linear regression against

a known standard curve using commercial software (Microplate Manager, Bio-Rad, Hercules, CA.

Serum Hormone Assessment

The concentrations of serum insulin and epinephrine was determined using commercially-available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI, USA) with a microplate reader (X-Mark, Bio-Rad, Hercules, CA, USA). The absorbances were read at a wavelength of 450 nm and unknown concentrations determined by linear regression against known standard curves using commercial software (Microplate Manager, Bio-Rad, Hercules, CA, USA).

Intramuscular Metabolite Assessment

Muscle glycogen was assessed with a colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA). In this assay, glycogen is hydrolyzed by amyloglucosidase to form β-D-glucose, which is then specifically oxidized to D-glucono-δ-lactone by glucose oxidase forming hydrogen peroxide in the process. The absorbances were read with a microplate reader (X-Mark, Bio-Rad, Hercules, CA, USA) at a wavelength of 540 nm unknown concentrations determined by linear regression against a known standard curve using commercial software (Microplate Manager, Bio-Rad, Hercules, CA, USA).

Statistical Analyses

Statistical analyses for blood hormone concentrations were performed by utilizing separate 2 x 4 [Condition (CHO, Placebo) x Time (Pre, post-consumption, post-exercise, 1-hr post-exercise)] factorial analyses of variance (ANOVA) with repeated measures for serum glucose, epinephrine, and insulin. Muscle glycogen concentration analyses were

performed by utilizing a 2 x 3 [Condition (CHO, Placebo) x Time (Pre, post-exercise, 1-hr post-exercise)] factorial analyses of variance (ANOVA) with repeated measures. If a significant interaction between the independent variables was found, simple effects analysis was conducted to determine where the interaction occurred. Additionally, if a significant interaction was present, analysis of main effects was conducted using the simple effects, pairwise comparisons with a Bonferroni adjustment used to compare dependent variables within each independent variable condition. If no interaction was present between variables, then normal pairwise comparisons were used with a Bonferroni adjustment for main effects analysis with collapsed groups. An *a-priori* power calculation was completed with studies of similar methodological design to establish the necessary number of participants (Green et al., 2007; Filho, Gobbi, Gurjão, Gonçalves, Prado, and Gobbi, 2013; Senna, Salles, Prestes, Mello, and Roberto, 2009). The calculation indicated that 24 participants are adequate to detect a significant difference between conditions based on repetitions to failure in response to resistance exercise given a type I error rate of 0.05, a 95% confidence interval, and a power of 0.80. The index of effect size utilized was partial Eta squared ($\eta 2$), which estimates the proportion of variance in the dependent variable that can be explained by the independent variable. Partial Eta squared effect sizes determined to be: weak = 0.17, medium = 0.24, strong = 0.51, very strong = 0.70. For all statistical analyses, an alpha level of 0.05 will be adopted throughout.

CHAPTER FOUR

Results

Analysis of Data

Dietary Analysis

The mean (±SD) for relative macronutrient dietary intake recorded over the duration of the testing visits for each testing condition is displayed in the table below.

Table 4.1

Dietary Intake.

Macronutrient	Preliminary Visit	Placebo t	Carbohydrate
Carbohydrate	3.3 g/kg (±.98)	2.8 g/kg (±1.18)	2.7 g/kg (±1.11)
Fat	1.3 g/kg (±.5)	1.1 g/kg (±0.44)	1.08 g/kg (±0.48)
Protein	2.1 g/kg (±.72)	1.5 g/kg (±.38)	1.3 g/kg (±.59)
Total kcal	2267kcal(±463)	2404kcal(±560)	2261(±514)

Note: Shows that mean(±SD) of macronutrient and kcal intake across all the testing visits

The results of the separate one way repeated measures ANOVAs indicated that there was no difference in carbohydrate intake ($F= 1.051$, $p=.370$, $\eta^2=.105$), fat intake ($F= .087$, $p=.917$, $\eta^2=.01$), protein intake ($F= .576$, $p=..572$, $\eta^2=.06$) or total kcals ($F=.48$, $p=.646$, $\eta^2=.047$).

Body Mass, Body Fat Percentage, Total Body Water, and Packed Cell Volume Analysis

The mean (±SD) for body mass, body fat percentage, total body water, and packed cell volume over the two supplement testing visits is displayed in the table below.

Table 4.2

Body Mass and Hydration

Anthropometric Measure	Placebo Visit	Carbohydrate Visit
Body Mass	90.2 kg (±17.5)	89.6 kg (±18.7)
Body Fat Percentage	20.7 % (8.5)	20.6 % (±8.27)
Total Body Water	49.1 kg (±5.96)	49.3 kg (±49.3)
Packed Cell Volume	47.8 % (±2.44)	47.2 % (±2.57)

Note: Shows the mean(±SD) body mass, body composition, and hydration variables for each testing visit.

The results of the separate pair samples t-tests indicated no significant differences in body mass (t= .429, p=.678), body fat percentage (t= 1.083, p=.307), total body water (t= -.298, p=.773), or packed cell volume (t= .58, p=.576).

Baseline Testing Measures

All participants had similar macronutrient intakes across all scheduled testing visits. All participants were similarly hydrated and had been similarly fasted for 3 hours before each testing visit. When the baseline concentrations of glycogen, glucose, and insulin were compared there were no statistical differences in any variable between testing condition. This indicates that participants complied with the regulations outlined in the study and to the best of our knowledge the changes seen in each dependent variable were altered as a result of the independent variables.

Completed Repetitions to Fatigue Analysis

The mean (±SD) for completed repetitions to fatigue for each condition and completed reps to fatigue during each set in each condition are indicated in the table below.

Table 4.3

Performance Measures.

Performance Measure	Placebo Visit	Carbohydrate Visit
Total Repetitions to Fatigue	53.8 (±7.8)	51.8 (±6.9)
1st Set to Fatigue	29.9 (±5.9)	29.5 (±4.9)
2nd Set to Fatigue	11.3 (±1.7)	10.1 (±3.3)
3rd Set to Fatigue	6.9 (±2.7)	6.4 (±2.1)
4th Set to Fatigue	5.7 (±2.5)	5.8 (±2.8)

Note:Shows the mean(±SD) of total reps to fatigue for each set and testing condition

The results of the pair samples t-test indicated that there were no significant differences between total completed repetitions to fatigue between placebo and carbohydrate conditions (t= 1.473, p=.175). The results of the 2 x 4 [Condition (CHO, Placebo) x Set (1st Set, 2nd Set, 3rd Set, 4th Set)] factorial ANOVA with repeated measures indicated that there was no main effect for supplement on completed repetitions to fatigue during each set between supplement conditions (F= 2.169, p=.175, η^2=.194). There was a significant main effect for the number of sets completed, which showed as number of sets completed increased number of completed repetitions decreased (F= 126.18, p<.001, η^2=.933). Pairwise comparisons indicated that there was a significant difference between all set comparisons except sets 3 and 4 (p=.212). There was not a significant interaction found between set and supplement on repetitions to fatigue (F= .337, p=.799, η^2=.036).

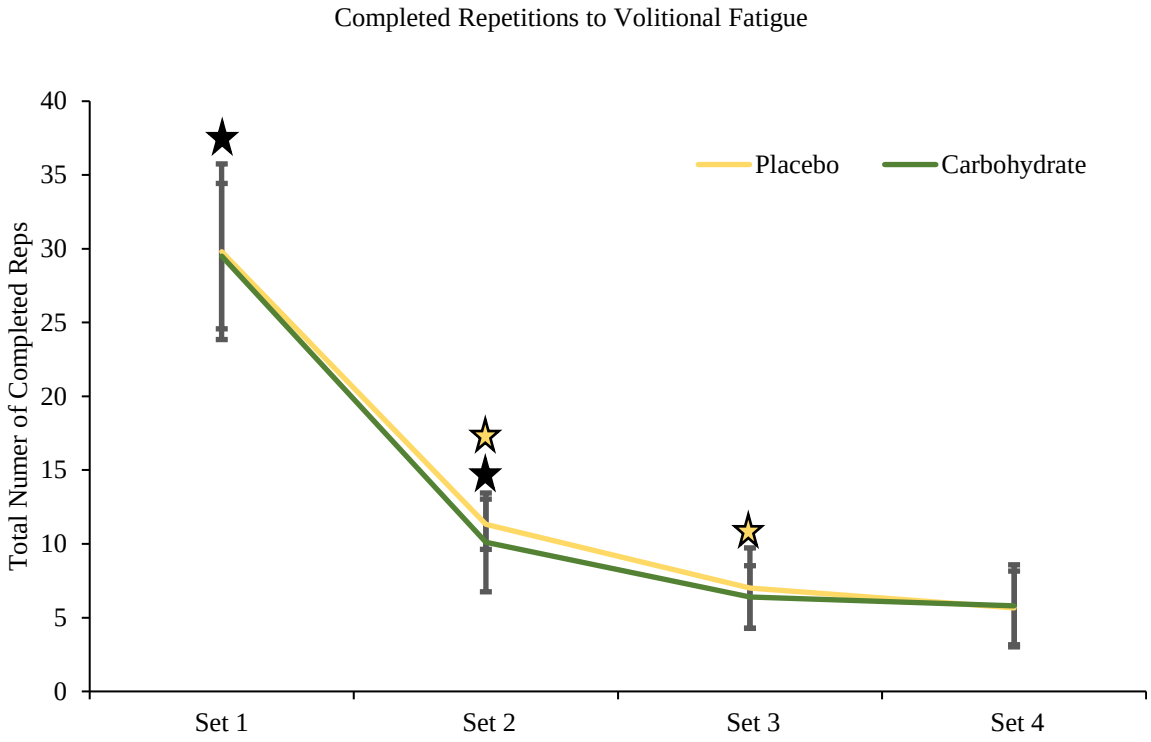

Figure 4.1. Above shows the repetitions that were completed during each set for both supplement conditions. There was no overall difference in repetitions during each set between each condition. There was a significant decrease in repetitions completed in both conditions in sequential sets 1 to 2, and 2 to 3.

Intramuscular Glycogen

The mean (±SD) for intramuscular glycogen relative to total muscle protein content for each time point and condition are indicated in the table below.

Table 4.4

Intramuscular Glycogen

Condition	Pre-Exercise	Immediate Post	1hr Post	Total Average
Placebo	0.00725 (±0.0042)	0.00423 (±0.0019)	0.00418 (±0.0022)	0.00522(±0.003)
Carbohydrate	0.00536 (±0.0011)	0.00376(±0.00084)	0.00396 (±0.0011)	0.0043(±0.001)
Total Average	0.0063(±0.00265)	0.0039(±0.00137)*	0.0041(±0.00165)*	.00475(±.0019)

Note: Shows the mean (±SD) for intramuscular glycogen for each condition during the study The "" indicate the that there was a statistical decrease in intramuscular glycogen in both post exercise time points from the pre exercise values at a significance level of 0.05.*

The results of the 2 x 3 [Condition (CHO, Placebo) x Time (Pre, post-exercise, 1-hr post-exercise)] factorial ANOVA with repeated measures indicated that there was not a main effect of supplement condition on glycogen measures (F= 3.416, p=.102, η^2=.299). There was a significant main effect for time (F= 34.379, p<.001, η^2=.62). Pairwise comparisons revealed that there was a significant decrease in intramuscular glycogen between pre exercise and immediately post exercise (p=.001), pre exercise and 1hr post exercise (p=.03), but no differences between immediately post and 1hr post exercise (p=1.00). There was not a significant interaction between condition and time (F = 1.495, p=.254, η^2=.157).

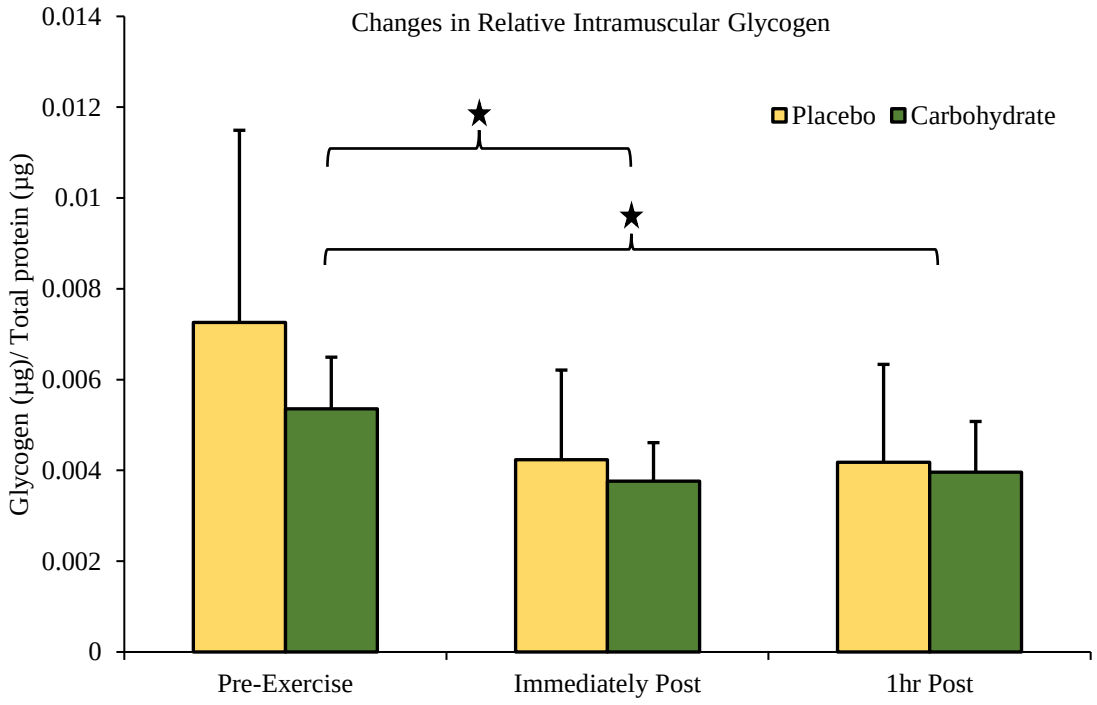

Figure 4.2. Above shows the changes in relative intramuscular glycogen over time between conditions. There was no overall difference in reps during each set between each condition. The stars indicate that there was a significant decrease in relative intramuscular glycogen in both groups between pre-exercise and both post exercise time points.

Serum Glucose

The Shaprio-Wilks test of normality indicated that there were violations of normality in the immediately post-exercise time point for both placebo (p=.003) and carbohydrate conditions (p=.042). The two outlier that were disrupting the data set were removed to meet the needed assumptions to run the appropriate statistical analysis. The mean (±SD) for serum glucose concentration in mg/dl at each time point and condition are indicated in the table below.

Table 4.5

Serum Glucose

Condition	Pre-Supplement	Pre-Exercise	Immediate Post	1hr Post	Total Avg.
Placebo	103.1 (±7.3)	107.8 (±6.6)	107.0 (±1.31)	103.9 (±3.8)	105.45(±4.8)
Carbohydrate	103.0 (±3.9)	111.6 (±14.1)*	98.4 (±12.0)*	105.6 (±7.7)	104.7(±9.4)
Total Average	103.05(±5.6)	109.7(±10.35)+	102.7(±6.7)+	104.75(±5.75)	105(±7.1)

Note: Shows that mean(SD) for serum glucose at each time point in both conditions. The "" indicates a statistical difference between the pre-exercise and immediate post exercise time points for the carbohydrate condition. Further, "+" indicates that there was a significant interaction occurring between these time points. Both tests had an p-value less than 0.05.*

The results of the 2 x 4 [Condition (CHO, Placebo) x Time (Pre, post-consumption, post-exercise, 1-hr post-exercise)] factorial ANOVA with repeated measures indicated that there was a significant interaction between supplement and time (F= 3.791, p=.026, η^2=.351). Pairwise comparisons with a Bonferroni adjustment of the simple effects, indicated that the interaction caused serum glucose to be utilized more between the pre-exercise and the immediately post exercise time points for the carbohydrate condition. There was a statistical difference between serum glucose content between the pre-exercise and immediately post exercise time points in the carbohydrate condition (p=.033). Further, there was not a main effect for supplement condition on blood glucose

(F= .072, p=.796, η^2=.01). The initial statistical analysis indicated that there was a significant main effect for time (F= 4.254, p=.017, η^2=.378). However, pairwise comparisons with a Bonferroni adjustment indicated this was a false main effect and that there was not a statistical difference between any time points when groups were compared (p>.05).

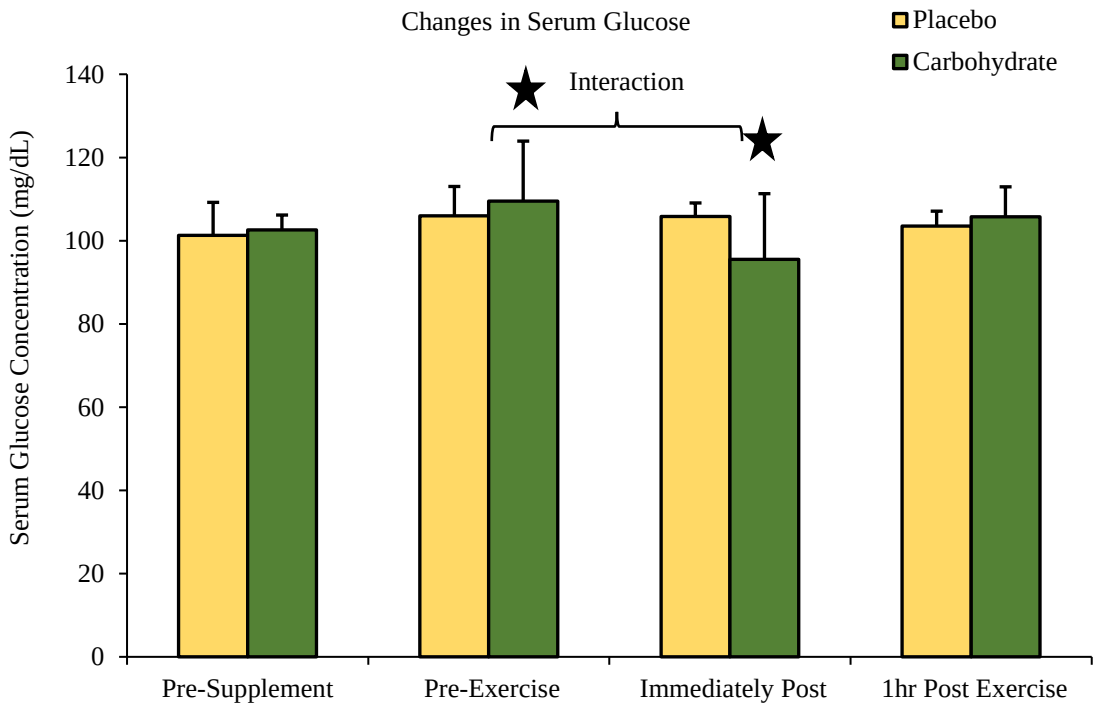

Figure 4.3. Above shows the changes in serum glucose over time for both placebo and carbohydrate conditions. There was no overall difference in serum glucose between conditions. There was a significant interaction that occurred between the pre-exercise and immediately post exercise time points that led to a significant difference in serum glucose being used during exercise in the carbohydrate group.

Insulin Analysis

The mean (±SD) for serum insulin concentration for each time point and condition are listed in the table below.

Table 4.6

Serum Insulin Content

Condition	Pre-Supplement	Pre-Exercise	Immediate Post	1hr Post	Total Avg.
Placebo	1.67 (±1.0)	1.77 (±.92)*	1.18 (±.81)**	1.71 (±.89)	1.58(±.91)M
Carbohydrate	1.45 (±.64)	9.54 (±6.11)*	3.67 (±1.49)**	6.84 (±6.59)	5.38(±3.7)M
Total Average	1.56(±.82)	5.65(±3.52)+	2.52(±1.15)+	4.275(±3.74)	3.48(±2.3)

Note: Shows the mean(±SD) serum insulin between each time point and condition. The "" denotes that there was a statistical difference between supplement conditions at the pre-exercise time point. The "**" indicates that there was a statistical difference between supplement conditions at the immediate post time point. The "+" indicates that there was a significant interaction occurring between these time points. The "M" indicates a main effect between supplement conditions.*

The results of the 2 x 4 [Condition (CHO, Placebo) x Time (Pre, post-consumption, post-exercise, 1-hr post-exercise)] factorial ANOVA with repeated measures indicated that there was there was a significant interaction between supplement and time ($F= 47.14$, $p=.003$, $\eta^2=.479$). Simple effects analysis indicated that the carbohydrate condition significantly increased insulin at pre-exercise ($p=0.13$) and immediately post exercise ($p=.006$) time points when compared to placebo conditions. There was a statistically significant main effect of the carbohydrate supplement increasing serum insulin content between conditions ($F= 7.72$, $p=.027$, $\eta^2=.525$). The initial statistical analysis indicated that there was a significant main effect for time ($F= 9.373$, $p<.001$, $\eta^2=.572$). However, pairwise comparisons with a Bonferroni adjustment indicated that this was a false main effect and no differences in serum insulin were found at any time point within each condition ($p>.05$).

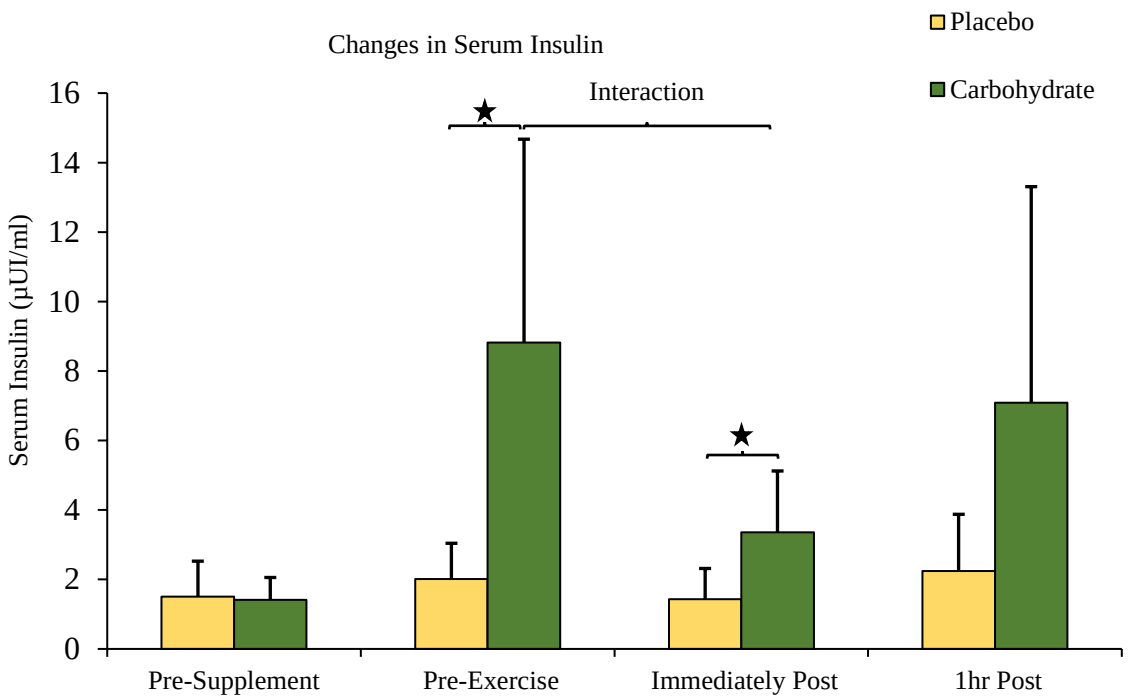

Figure 4.4: Above shows the changes in serum insulin over time between conditions. There a statistical difference in insulin between conditions at the pre-exercise time points and immediately post exercise time points indicated by the black stars. There was a significant interaction between pre-exercise and immediately post exercise time points that led to a greater amount of insulin being removed from circulation than in the placebo group.

CHAPTER FIVE

Discussion

Discussion and Conclusion

Performance Changes

It is widely accepted that carbohydrate ingestion before endurance exercise increase the aerobic work capacity and performance of individuals (Helge, 2017). A large portion of endurance exercise performance is governed by the total amount of metabolic substrate that can be mobilized for ATP synthesis. Increasing the total amount of glucose that can be utilized for the creation of ATP for endurance exercise is likely one of the major causes for improved work capacity. It has been shown that glycogen can be depleted after an exhaustive bout of endurance exercise and performance is limited by total glycogen content (Hermansen, Hultman, and Saltin, 1967, Murray and Rosenbloom, 2018). However, resistance exercise to fatigue may not be limited by the total amount of substrate available as seen in aerobic exercise under normal conditions. Glycogen is typically only depleted by 20-40% of the initial values following a single bout of resistance exercise (Robergs et al., 1991). Similar changes in relative glycogen content were seen in the present study with the placebo and carbohydrate groups depleting roughly 34% and 28% of the initial glycogen content, respectively. This indicates that even when multiple sets are completed to fatigue, muscle glycogen was not significantly depleted in either group. Additionally, the carbohydrate supplementation before exercise did not increase the total number of reps completed. This similarity in performance

between groups may have been seen because the total amount of carbohydrate that was normally ingested was already enough to sustain the workload. Many sources have similar ranges for moderate to high dietary carbohydrate intakes between 3-10 g/kg/d and is thought to prevent the gradual depletion of glycogen over days or weeks of training and facilitate increases in performance and adaptation (Slater and Phillips, 2011, Haff, Lehmkul, McCoy, and Stone, 2003, Sherman and Wimer, 1991, Thomas, Erdman, and Burke, 2016, Krieder, et al., 2010). The recommendations for carbohydrate intake was recently called into question by Escobar, VanDusseldorp, and Kersick, (2016) indicating that the recommendations for adequate carbohydrate intake for recovery from resistance exercise may be higher than needed. Participants in this study normally consumed approximately 2.9 g/kg/d of carbohydrate, close to moderate consumption by the guidelines above. This may have been keeping their glycogen stores full enough to not see changes in performance between the exercise bouts. Adding a carbohydrate supplement to an adequately carbohydrate-fed individual will not increase resistance exercise performance to fatigue in a similar manner that it does with endurance exercise. It appears when there is adequate glycolytic metabolic substrate available, resistance exercise performance and fatigue are predicated by other mental and physiological factors.

Serum Glucose and Insulin After Supplementation

Serum glucose levels at baseline between conditions were nearly identical at 103 mg/dL, indicating that the participants followed the 3hr fasting requirement at each testing session. The normal fasting levels of blood glucose are between 70-110 mg/dL and in both conditions the participants were within this range. Although not statistically

significant, both groups had slight elevations in blood glucose during the pre-exercise time point. Surprisingly, the maltodextrin supplement at 2 g/kg body mass did not elevate serum glucose to similar levels that were previously reported at a 30min time period in collegiate female soccer players (Pannoni, 2011). The peak in blood glucose in this study was 166 mg/dL at 30mins, with the maltodextrin supplement dosed at 1 g/kg body mass ingested after an overnight fast (Pannoni, 2011). It has also been reported that ingestion of 75 g of glucose during an oral glucose tolerance test causes large spikes in serum glucose that mostly occur in individuals with prediabetes and diabetes with peak ranges being close to 150 mg/dL and 250 mg/dL respectively at 30min (Pagana and Pagana, 2013). However, it is also stated that individuals with the ability to respond to this glucose load with the proper insulin response will experience a minimal rise in serum glucose (Pagana and Pagana, 2013). When the participants' insulin response is plotted with the rise in blood glucose, there is a clear increase in insulin to the ingested blood glucose. This increase in insulin may have dampened the peak in serum glucose that would be expected to be seen in a normal post prandial state, causing serum glucose to be held at a relatively constant amount. These values have been plotted in Figure 5.1 below.

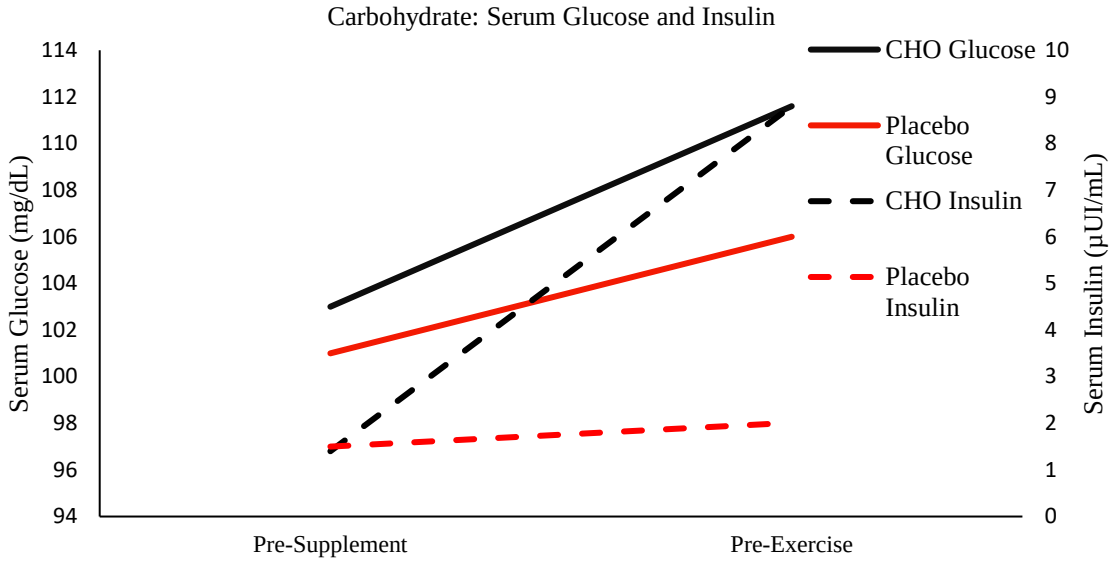

Figure 5.1 Above shows the changes in both glucose and insulin plotted for both conditions at baseline before supplementation and 30min after pre-exercise.

This change in glucose and insulin between the supplement conditions plotted at the same time points clearly indicates that glucose was stimulating insulin release. The large release in insulin that is seen likely contributed to the lack of statistical difference in serum glucose that was seen between conditions. This also indicates that the glucose was entering circulation and was able to be utilized for the subsequent exercise bout at the 30min mark post ingestion.

Alterations in Serum Glucose, Insulin, and Muscle Glycogen During Exercise

The significant interaction that was present between the pre-exercise and immediately post exercise time points shows that there was a larger decrease in serum glucose in the carbohydrate group than in the placebo group. Insulin concentration was also shown to decrease when exercise began but on average remained more elevated than at baseline. The decrease in insulin during exercise is caused by an altered metabolism of

blood glucose. In this study the increased glucose uptake in the carbohydrate group is likely caused by the both insulin-dependent and insulin-independent signaling pathways increasing GLUT-4 translocation at the same time. Insulin is known to bind to its tyrosine kinase receptor and stimulate GLUT-4 translocation to the membrane by phosphorylating AKT substrate 160 (AS160) through the activation of the insulin receptor substrate-1 (IRS-1)/phosphatidylinositol 3 kinase (PI3-K)/Protein kinase B (Akt) signaling pathway (Larance et al., 2005, De Meyts, 2016). During exercise, AS160 can be phosphorylated by other molecules, not dependent on the insulin signaling pathway, that will allow GLUT-4 translocation and subsequent glucose entry into the muscle. AS160 may be a downstream target of activated AMP- activated kinase (AMPK) which is allosterically activated during contractions when the AMP:ADP ratio is high (Treebak et al., 2006). Additionally, Ca^{2+} calmodulin-dependent protein kinase II (CaMKII) is activated during muscle contractions and has been postulated to phosphorylate AS160 increasing GLUT-4 translocation (Funai, & Cartee, 2008, Wiczak et al., 2010). Liver Kinase B1 (LKB1) has also been shown to activate AMPK during exercise increasing its activity and facilitating glucose uptake (Sakamoto et al., 2005). AMPK activity was significantly elevated in participants immediately after completing 10 sets of 10 repetitions of leg extensions at 70% of their 1-RM (Dreyer, Fujita, Cadenas, Chinkes, Volpi, and Rasmussen, 2006). One study has also shown that exercise increases CaMKII activity; however, it was after 40 min of aerobic exercise (Rose & Hargreaves, 2003). This indicates that during this specific acute resistance exercise bout in this study, LKB1 stimulated AMPK activation was likely the main insulin independent signaling molecule that increased GLUT-4 translocation. Since there was statistical difference in insulin concentration between

conditions before exercise, it is likely that the insulin-dependent GLUT-4 translocation was occurring during the onset of exercise. Further, the exercise bout was completed to exhaustion during each set, which would likely increase AMPK activation, stimulating GLUT-4 translocation, increasing glucose influx. Both signaling pathways likely caused the interaction effect in blood glucose by impacting membrane GLUT-4 concentration. The larger increase in glucose uptake in the carbohydrate group indicates that it will be preferentially used as an energy source during exercise and may have a sparing effect on muscle glycogen. Although not statistically significant, there was a 5% lower amount of glycogen used in the carbohydrate group during exercise when compared to baseline measures. These findings suggest that ingestion of carbohydrate may slightly decrease overall glycogen utilization in a single acute bout of exercise which is corroborated by other research (Haff, Lehmkul, McCoy, and Stone, 2003).

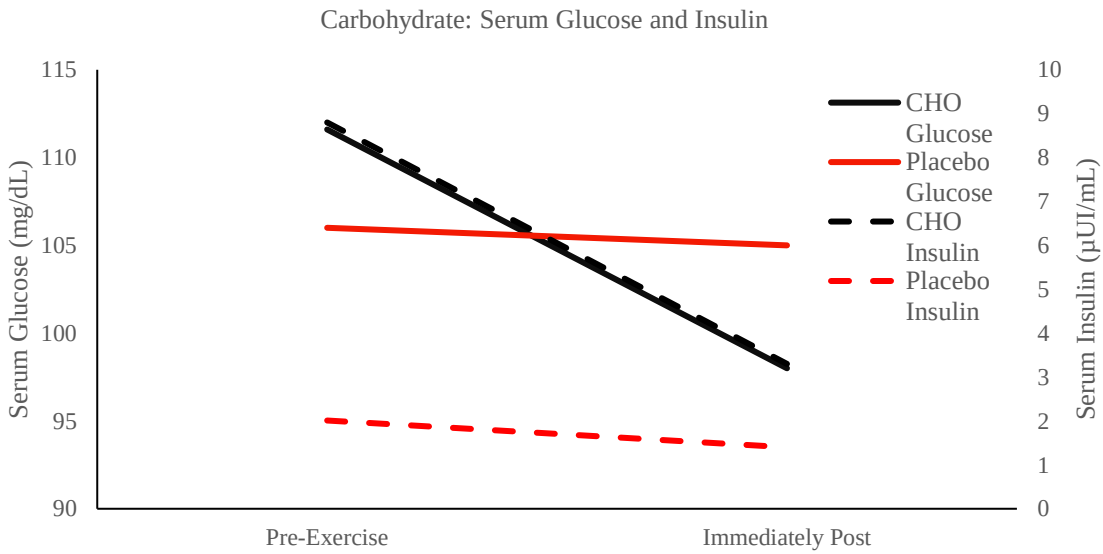

Figure 5.2 Above shows both the changes in glucose and insulin during exercise. There was a larger decrease in both serum glucose and insulin during exercise with the carbohydrate supplement. There were significant interactions in both glucose and insulin between these time points.

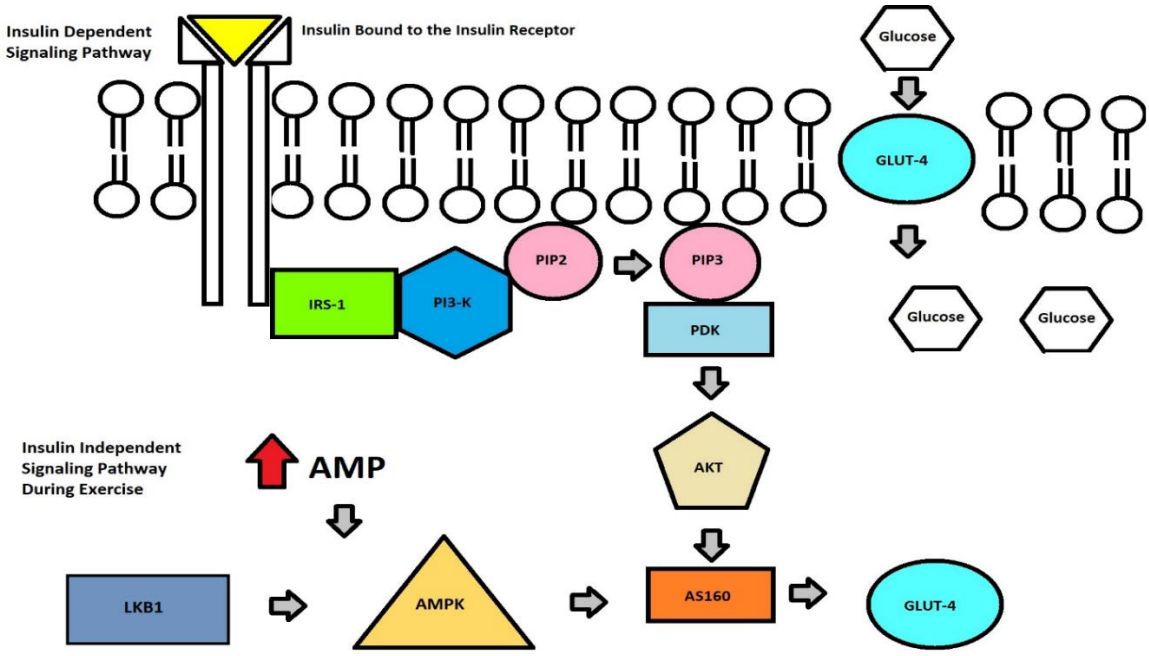

Figure 5.3 Above shows both the insulin dependent and insulin independent signaling pathway that stimulates GLUT-4 translocation to the membrane for glucose influx. The insulin dependent pathway was likely activated pre-exercise. Insulin Receptor Substrate 1 (IRS1), phosphatidylinositol 3 kinase (PI3-K), Phosphatidylinositol (4,5)-bisphosphate (PIP2), phosphatidylinositol (3,4,5)-trisphosphate (PIP3), Protein kinase B (Akt), Akt Substrate 160 (AS160), Glucose transporter type 4 (GLUT-4), Liver Kinase B1 (LKB1), AMP activated protein kinase (AMPK).

Alterations in Serum Glucose, Insulin, and Glycogen During Recovery

During the 1hr recovery period there was an increase in serum glucose and insulin in the carbohydrate group, but it was not statistically significant. This indicates that not all the ingested carbohydrate had been released into circulation at the 30 min time point before exercise. Additionally, there was not a hyperglycemic effect immediately after the short intense exercise in either supplement condition which is contradictory to the 120-160 mg/dL range that was reported by Pascoe and Gladden, 1996. Additionally, despite serum glucose and insulin concentrations increasing during the 1hr recovery period, there was not a significant increase in glycogen content after 1hr. The changes in glucose and glycogen during and after exercise can be seen in the figures below.

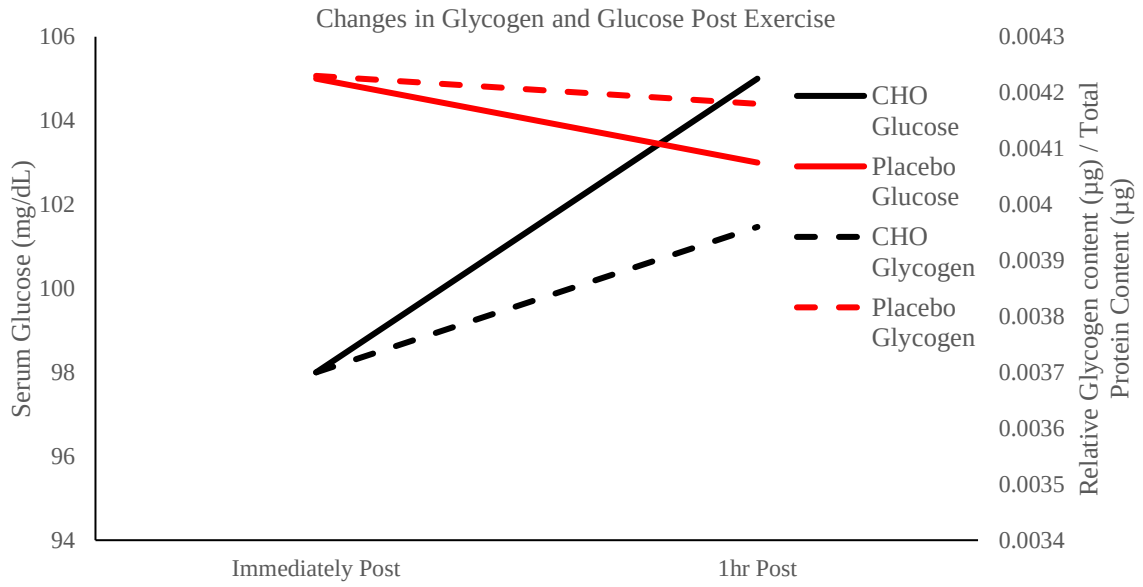

Figure 5.4 Above shows both the changes in glucose and glycogen immediately post and 1hr post exercise. There was a larger decrease in both serum glucose and insulin during exercise with the carbohydrate supplement. There were significant interactions in both glucose and insulin between these time points.

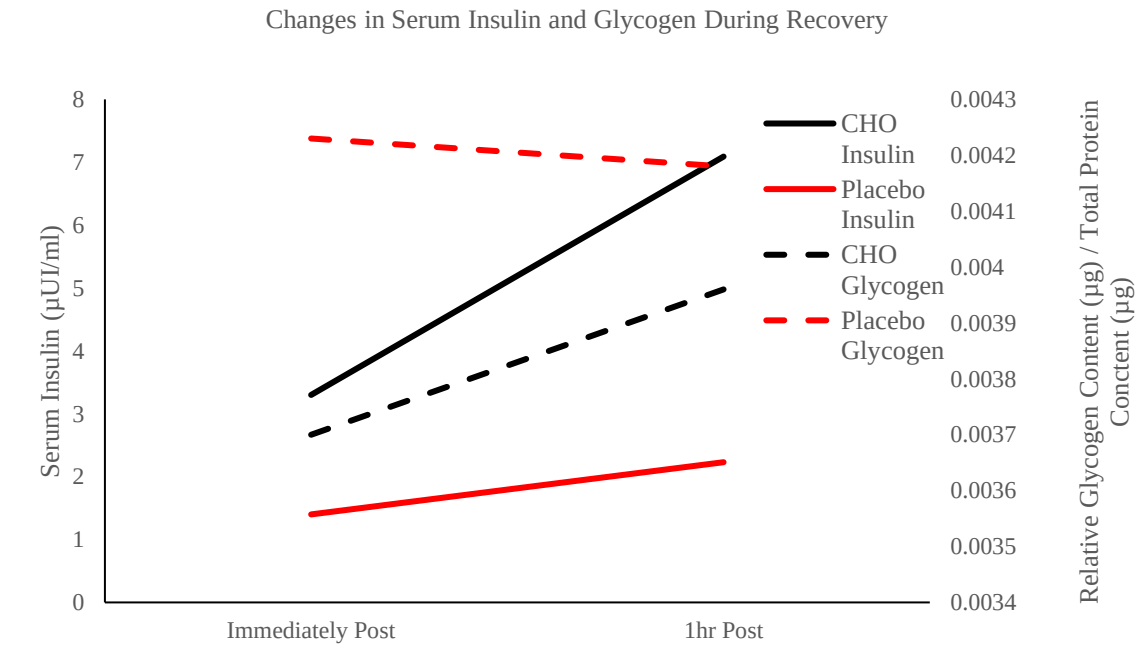

Figure 5.5 Above shows both the changes in glycogen and insulin immediately post and 1hr post exercise. There was a larger increase in glycogen and serum insulin in the carbohydrate group but was not statistically significant.

It has been suggested that glycogen synthesis post-exercise occurs in a bi-phasic manner that has both insulin-independent and insulin-dependent phases, respectively (Price, Rothman, Taylor, Avison, Shulman, and Shulman, 1994). The insulin-independent phase has been reported to be linked with increases in GLUT-4 translocation to the cell membrane increasing glucose permeability (Jentjens and Jeukendrup, 2003). In a rat model, GLUT-4 translocation was shown to increase 1.8 and 1.6 times higher immediately post and 30min post-exercise with increased glucose uptake that had returned to normal after 2hr (Goodyear, Hirshman, King, Horton, Thompson, and Horton, 1990). It has also been suggested that this first phase of glucose uptake and glycogen synthesis may be directly influenced by glycogen content post-exercise (Jentjens and Jeukendruup, 2003). This resistance exercise bout only decreased the initial glycogen content by 33% in the placebo condition and even less in the carbohydrate condition. The resistance exercise bout completed in this study may not have elicited a substantial decrease in glycogen content to cause the insulin independent mechanisms to rapidly accelerate glycogen synthesis. As seen in figure 5.5, the placebo condition showed no increase in glycogen content or insulin content post-exercise which indicates that there was not a significant insulin independent increase in glycogen synthesis. There is a possibility that more glycogen needed to be depleted in order to see rapid increases in glycogen synthesis post-exercise. However, this threshold of glycogen depletion is somewhat speculative and is more likely that short term rapid glycogenesis post-exercise is not noticeable without some form of carbohydrate ingestion. It has been proposed the glycogen content and glucose availability exert a regulatory role in the synthesis of glycogen post-exercise (Richter, Derave, and Wojtaszewski 2001). When viewing the

recovery results of the carbohydrate group, serum glucose, insulin, and muscle glycogen content were all rising at the 1hr time point but were not statistically significant from the immediately post-exercise values. The immediate post-exercise and 1hr post-exercise relative glycogen values for the placebo were 66% and 65% of the baseline values while carbohydrate groups were 72% and 76% respectively. This indicates that insulin-dependent glycogen synthesis was occurring during the 1hr recovery period, but a longer sampling time frame would be needed to possibly see significant results. AMPK is known to phosphorylate glycogen synthase, causing a decrease in glycogen synthesis during exercise (Jorgensen, Richter, and Wojtaszewski, 2006). These inhibitory effects may be prolonged into the recovery period until the needed homeostatic metabolic conditions of a cell are met. One study has shown that AMPK activity is significantly elevated for up to an hour following a resistance exercise bout (Dreyer, Fujita, Cadenas, Chinkes, Volpi, and Rasmussen, 2006). This may have been one of the many signaling molecules that was active after exercise regulating metabolic fluctuation. AMPK has also been shown to allosterically activate glycogen synthase by increasing glucose uptake post exercise, eventually leading to glycogen synthesis during recovery (Hunter, Treebak, Wojtaszewski, and Sakamoto, 2011). Insulin has been shown to also allosterically activate glycogen synthase activity through the increase in glucose availability (Bouskila, et al., 2010). Insulin has also been shown to phosphorylate glycogen synthase kinase 3, increasing glycogen synthesis (Wojtaszewski, Nielsen, Kiens, and Richter, 2001). It is possible that the increase in serum glucose seen after exercise in the carbohydrate supplement group was entering the muscle more rapidly due to the separate effects of AMPK and insulin GLUT-4 mediate translocation. This increased glucose entry into the

cell would increase glucose concentration and lead to the allosteric activation of glycogen synthase immediately post-exercise. Both mechanisms would help facilitate faster recovery of glycogen and possibly an increase preparedness for an additional exercise bout. The non-significant differences between supplement conditions may have been caused by a lingering inhibitory effect on glycogen synthase activity that was not overcome until later in the recovery period.

Conclusion

Carbohydrate ingestion before resistance exercise did not increase the overall reps that were completed to failure when compared to a placebo. Blood glucose in the carbohydrate group was utilized for metabolic fuel more so than in the placebo group during exercise and was likely caused by AMPK and insulin mediated GLUT-4 translocation. There was a slight decrease in the amount of glycogen that was used during exercise in the carbohydrate condition but not enough to be statistically relevant. Similarly, on average there was an increase in glycogen synthesis post exercise with carbohydrate supplementation but was not statistically significant. However, there was a trend in the carbohydrate group during the recovery period that indicated that there may have been increased ability to resynthesize glycogen but was not able to be detected within an hour. Practically, it appears if normal dietary consumption of carbohydrate is already adequate then an additional supplement will not influence maximum resistance exercise performance when completed to fatigue. However, there is a small amount of evidence that suggests carbohydrate ingestion before exercise may slightly benefit

individuals during the acute recovery phase 1hr after exercise. Further research would be needed to assess the timing and amount of carbohydrate that would be needed to see these changes in recovery.

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