THE INFLUENCE OF A WHEY PROTEIN PRELOAD PRIOR TO CARBOHYDRATE CONSUMPTION ON CYCLING PERFORMANCE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF KINESIOLOGY

COLLEGE OF HEALTH SCIENCES

BY

CHRISTHOPER IRVINE B.S. M.ED.

DENTON, TEXAS

DECEMBER 2019

DEDICATION

For my wife, Courtney Irvine, thank you for continually support and love, this journey would not have been possible without you by my side. Also for my family, my father Tim, my mother Rebecca, and my brother Ben. Throughout my academic career, they have supported me and encouraged me to pursue my goals.

ACKNOWLEDGEMENTS

I would like to acknowledge Ryan Gordon and Matthew Sokoloski for their friendship and support throughout the last two years at Texas Woman's University. Their support and kinship throughout the dissertation process was a pivotal part of its success. Thank you both very much for your time and for dedication. I would like to thank my dissertation committee chair Dr. Vic Ben-Ezra for all your hard work in assisting me throughout the dissertation process. I would also like to thank my other committee members, Dr. David Nichols, Dr. Mindy Patterson, and Dr. Anthony Duplanty for their input and assistance. I would also like to thank Isaac Rowland for his contribution and willingness to learn during the data collection process. In addition, I am very grateful to the Texas Woman's Kinesiology department for funding part of my supplies required to complete my analysis. I would also like to thank the National Strength and Conditioning Association for awarding me with the 2019 Sport Nutrition Grant funded by GNC to support my research endeavors.

ABSTRACT

CHRISTOPHER J. IRVINE

THE INFLUENCE OF A WHEY PROTEIN PRELOAD PRIOR TO CARBOHYDRATE CONSUMPTION ON OVERALL CYCLING PERFORMANCE

DECEMBER 2019

The addition of whey protein co-ingested with a carbohydrate source during aerobic exercise has been theorized to augment insulin secretion, invoke a muscle glycogen sparing effect, improve glycemic control, and ultimately improve endurance performance. However, previous literature has reported a discrepancy between the performance measures outcomes. This study aimed to examine if 0.7 g/kg/LBM of whey protein isolate administered as a preload to a glucose bolus of 0.9 g/kg/LBM influences cycling performance and the metabolic profile during a 60 min cycling performance trial. Ten recreationally trained cyclists and triathletes (age 32.2 ± 8.7 years; weight 81.4 ± 11.5 kg; body fat 23.7 ± 5.1%; lactate threshold 203 ± 37.6 W) completed two experimental trials. Each trial was assigned in a counter-balanced order and separated by at least one week. For each experimental trial, participant reported to the exercise physiology lab in a 10-12 hour fasted state. Each participant was required to perform a cycling performance test, which consisted of cycling for 30 min at 90% LT, followed by a 30 min time trial. Prior to the cycling performance test, participants consumed either a whey protein isolate preload (0.7 g/kg/LBM) or a placebo 20 min prior to the consumption of a glucose beverage (0.9 g/kg/LBM). The glucose beverage was

consumed 10 min prior to the cycling performance test. Following the completion of both experimental trials, results indicated there were no significant differences in overall time trial performance (WP 16.8 ± 0.34 km; PL 17 ± 0.4 km; $p = .346$). The whey protein stimulated a significant increase in plasma insulin concentrations at time point 0 (WP = 222.88 ± 45.1 pg/ml; PL = 85.95 ± 45.1 pg/ml; $p = .047$) compared to the placebo trial. The increase in insulin during WP stimulated a significant interaction effect for plasma glucose concentrations ($p = 0.009$) between the two trials, however, there were no significant differences. The whey protein stimulated a significant increase in plasma glucagon concentrations for timepoint -10, 0, 15, 30, 45, and 60 when compared to the PL trial (all values $p < .05$). There were no reported differences in RER, NEFA, or any other variables between the two experimental trials. Although there were significant metabolic alterations due to the consumption of the whey protein preload, this did not influence overall cycling performance or substrate utilization.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The digestion of dietary carbohydrates begins in the mouth by mastication and interacting with salivary α-amylase. Through the interaction with salivary α-amylase, carbohydrates are broken down into various monosaccharides, starch dextrins, isomaltose, and maltotrioase. When entering the stomach, the high pH content inhibits the activation of salivary α-amylase and delays the breakdown of carbohydrates. Once inside the small intestine, carbohydrates interact with pancreatic α-amylase to aid in the digestion process. The final location of digestion occurs in the upper jejunum and duodenum were various disaccharides cleave the monosaccharides; fructose, glucose, or galactose that are absorbed and feed into glucose production in the liver (Ferrier, 2014). Glucose can be transported into the skeletal muscle and stored as glycogen. Stored glycogen content is a critical energy substrate that effects exercise intensity and overall aerobic performance.

During prolonged moderate-to-high intensity aerobic exercise (60-85% VO_2max), the primary substrate utilized to produce energy is α-D-glucose (Brooks, Fahey, & Baldwin, 2005). Skeletal muscles predominantly rely upon stored glycogen and supplemental exogenous carbohydrate intake to fuel the work required. Exogenous sources of carbohydrates that are commonly consumed before or during an aerobic exercise bout include gels, bars, and liquid sport drinks (Pfeiffer et al., 2010a; Pfeiffer et al., 2010b). The primary sources of carbohydrate consumed during endurance events are

high in glucose (commonly labeled dextrose), fructose, or as the disaccharide sucrose (glucose: fructose; Jeukendrup, 2004; Jeukendrup, 2008). The need for exogenous carbohydrate intake during exercise is dictated by intensity and duration (Brooks & Mercier, 1994).

As exercise intensity progresses from low- to high-intensity, there is a curvilinear relationship with carbohydrate oxidation (Brooks et al., 2005). As aerobic exercise intensity increases, the oxidation of carbohydrate to produce adenosine triphosphate (ATP) increases. The importance of ATP production is critical to maintain muscular contraction and exercise intensity. The amount of carbohydrate available to be oxidized also has an inverse relationship with fatigue (Alghannam et al., 2016). During prolonged moderate-to-high intensity aerobic exercise, the amount of glycogen available for energy production is reduced and can be depleted if exercise intensity is maintained. Glycogen depletion will promote a shift in the hormones insulin and glucagon to reduce the reliance of glucose as an energy source. The increase in glucagon causes an increase reliance upon free fatty acid (FFA) oxidation leading to a decrease in overall exercise intensity (Hearris, Hammond, Fell, & Morton, 2018). Therefore, it is vital to maximize glycogen stores prior to a prolonged exercise bout. The use of exogenous carbohydrate sources before and during endurance events can increase glycogen stores (prior to the event), or induce a glycogen sparing effect (during the event), thus delaying the onset of fatigue (Sherman, Peden, & Wright, 1991; Tsintzas & Williams, 1998).

Recommendations for the use of carbohydrates before and during prolonged aerobic exercise to improve glycogen stores and subsequent performance (e.g., time to

exhaustion, time trial, power output) have been strongly established (Jeukendrup, 2004; Jeukendrup, 2008; Ormsbee, Bach, & Baur, 2014). Given the underlying importance of carbohydrate utilization during moderate-to-high intensity bouts of aerobic exercise, researchers have explored additional supplements that may improve exogenous carbohydrate utilization and induce a glycogen sparing effect (Hansen, Bangsbo, Jensen, Bibby, & Madsen, 2015; Ivy, Res, Sprague, & Widzer, 2003; Saunders, Luden, & Herrick, 2007, Saunders, 2007)

The addition of whey protein co-ingested with a carbohydrate source during aerobic exercise has been theorized to augment insulin secretion, invoke a muscle glycogen sparing effect, improve glycemic control, decrease the onset of central fatigue, improve fluid availability and ultimately improve endurance performance (Betts & Stevenson, 2011; Ivy et al., 2003; Saunders et al., 2007). One of the key mechanisms to invoke a glycogen sparring effect and increase exogenous carbohydrate oxidation during exercise is an increase in insulin concentration. During moderate-to-high intensity aerobic exercise (> 70% of VO_2max), exogenous carbohydrate intake has a limited effect on insulin secretion (Coyle et al., 1983; Hargreaves & Briggs, 1985). Whey protein has been shown to elicit a significant insulin response in healthy individuals when co-ingested or consumed prior to carbohydrate consumption (Akhavan, Luhovyy, Brown, Cho, & Anderson, 2014; Claessens, Saris, & van Baak 2008; Nilsson, Holst, & Björk, 2007; Petersen et al., 2009; Spiller et al., 1987). Whey protein has been shown to significantly increase the plasma concentrations of the incretin hormones, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The incretin

hormones may account for 50-70% of total insulin secretion following glucose or mixed meal consumption in healthy individuals (Baggio & Drucker, 2007). The addition of whey protein with a carbohydrate solution may further augment insulin secretion during exercise when compared to the consumption of carbohydrates alone. The larger insulin secretion from the whey protein should induce a greater translocation of glucose transporter 4 protein (GLUT4) within the skeletal muscle. (Carnagarin, Dharmarajan, & Dass, 2015; Ivy et al, 2003; Ritcher & Hargreaves, 2014; Saunders et al., 2007). Therefore, allowing greater uptake of exogenous carbohydrates to be utilized by the skeletal muscle during exercise and spare muscle glycogen. This notion has led researchers to explore the effects of whey protein co-ingested with a carbohydrate source during an acute bout of aerobic exercise.

The current body of literature examined small doses of whey protein co-ingested with carbohydrates (most commonly in form of sucrose) consumed intermittently throughout a prolonged moderate-to-high intensity exercise bout. Primary outcome variables assessing the influence of whey protein co-ingested with carbohydrates include: time to exhaustion, time trials, metabolic responses (e.g., glucose and insulin), substrate utilization, rating of perceived exertion (RPE) using the Borg rating of perceived exertion scale and level of discomfort (Breen, Tipton, & Jeukendrup, 2010; Ivy et al., 2003; Oosthuyse, Carstens, & Millen, 2014; Saunders et al., 2007; Van Essen & Gibala, 2006). The majority of studies incorporating whey protein with a carbohydrate solution administer the supplement immediately before exercise and every 15 to 20 min throughout an exercise trial (McLellan, Pasiakos, & Lieberman, 2014). The amount of

protein added to the carbohydrate solution ranges from 5.6 – 22 g/hr during exercise and the amount of carbohydrate ranges from 36.5 – 77.5 g/hr (McLellan, Pasiakos, & Lieberman, 2014). Due to the wide range of protein and carbohydrate consumption (grams/hour), results regarding performance measure outcomes are conflicting (Betts & Stevenson, 2011). Ivy, Res, Sprague, and Widzer (2003) examined the influence of a whey protein (1.94%) plus carbohydrate (7.75%) solution intermittently consumed during a glycogen depleting protocol followed by a time to exhaustion test at 85% VO_2max. Compared to the carbohydrate (7.75%) only solution, the protein plus carbohydrate solution improved time to exhaustion by 36%. Saunders et al. (2007) also reported a 13% increase in time to exhaustion when whey protein (0.038 g/kg of body mass) plus carbohydrate (0.15 g/kg) was provided every 15 min during the cycling trial. Research has also reported no significant differences in endurance performance outcomes when whey protein was co-ingested with a carbohydrate compared to the consumption of carbohydrates alone. Breen et al. (2010) reported no significant difference in time to completion between a carbohydrate (65 g/hr) solution and a carbohydrate plus protein (65g/hr + 19 g/hr) solution. Van Essen and Gibala (2006) reported no significant changes in performance following an 80 km time trial between a whey protein plus carbohydrate (20 g/hr + 60 g/hr) solution and a carbohydrate (60 g/hr) only solution. One speculation as to why certain studies reported an improvement in performance with the addition of whey protein was due to the sub-optimal delivery of carbohydrates (Breen et al., 2010; McLellan, Pasiakos, & Lieberman, 2014; Van Essen & Gibala, 2006). Optimal carbohydrate delivery for aerobic exercise is defined as > 60 g/hr (McLellan et al., 2014).

Research that provides sub-optimal carbohydrate delivery plus whey protein may elicit an increase in performance due to the increase in energy availability. In contrast, when carbohydrate was delivered at optimal levels, the addition of whey protein during aerobic exercise yields no significant benefits when compared to carbohydrate alone (McLellan et al., 2014). However, one of the speculative mechanisms of whey protein improving exercise performance stems from its ability to augment insulin secretion. The doses administered in the aforementioned studies were likely too low to stimulate a significant rise in plasma insulin when compared to the carbohydrate alone trials (Breen et al., 2010; Ivy et al., 2003; Van Essen & Gibala, 2006). Therefore, providing a larger bolus of whey protein prior to an aerobic exercise bout may stimulate a superior insulin response.

Currently, there is no human research regarding the ingestion of whey protein as a preload to a bolus of carbohydrates prior to an acute bout of aerobic exercise. Morifuji, Kanda, Koga, Kawanaka, & Higuchi (2011) examined the influence of a whey protein preload prior to aerobic exercise in rodents. Male Sprague-Dawley rats where divided into three groups, the first group (n = 7) received 1.0 ml/100 g of body weight solution with only water, the second group (n = 7) received 1.0 ml/100 g body weight solution with 30% glucose and the last group (n = 7) received 1.0 ml/100 g body weight solution with 30% glucose plus 10% whey protein hydrolysate. All groups were given their test solution 10 min prior to a glycogen-depleting bout consisting of a 60 min swim with a load equivalent to 3% of their body weight. Although this was not a test for performance, the rats that consumed the glucose plus protein solution significantly preserved skeletal muscle glycogen content compared to the glucose only and the water only interventions.

An increase in skeletal muscle glycogen content following exercise may have been due to the increase in insulin concentration in the glucose plus protein group when compared to the other test groups (glucose + protein = 1.92 ± 0.39 ng/ml; to glucose = 0.75 ± 0.33 ng/ml; water = 0.75 ± 0.17 ng/ml). The increase in insulin concentration may have increased exogenous carbohydrate oxidation; therefore, promoting a glycogen sparing effect. However, it is unknown if this response in rats similar in human physiology.

Aims of the Study/ Problem Statement

This study aimed to investigate the effects of 0.7 g/kg/LBM body mass of whey protein consumed 30-min prior to the consumption of 0.9 g/kg/LBM of glucose preceding a 60-min performance trial on a cycle ergometer. The specific amount of whey protein isolate and glucose chosen was based on previous literature and previous research conducted in our laboratory that stimulated a significant insulin response when compared to the consumption of carbohydrate alone (Ma, Stevens, & Cukier, 2009; Rayner, Ma, Jones, Clifton, & Horowitz, 2014). The secondary outcome of this study investigated the metabolic responses invoked by the consumption of a whey protein preload during a performance cycling trial. Outcomes from this study may provide further insight for implementing a whey protein preload to improve aerobic performance and the metabolic profile during prolonged, submaximal cycling in trained individuals.

During prolonged, moderate-to-high intensity aerobic exercise, there is an increased reliance upon stored muscle glycogen to provide fuel for the work required (Brooks & Mercier, 1994). Sustained moderate-to-high intensity aerobic exercise can

lead to a gradual decline in muscle glycogen levels, promoting an increased reliance upon FFA oxidation if exogenous carbohydrates are absent. Moderate-to-high intensity exercise cannot be sustained on FFA oxidation alone; thus, exercise intensity will decrease (Hearris et al., 2018). Therefore, it seems vital for endurance athletes performing moderate-to-high intensity bouts of effort to maximize muscle glycogen stores prior to exercise and promote a glycogen sparing effect by increasing exogenous carbohydrates utilization. The addition of whey protein with a carbohydrate source during exercise may be a viable intervention to augment insulin secretion and increase exogenous carbohydrate utilization (Morufuji et al., 2011). However, many of the research studies incorporating whey protein during aerobic exercise have reported inconsistent results. The issue may be due to the small dose of whey protein consumed during exercise. Whey protein doses incrementally consumed by participants within research designs range from 2-5 g every 15-20 min (McLellan et al., 2014). This dose of whey protein may not be large enough to elicit a significant increase in insulin concentration during exercise. A larger dose of whey protein may be warranted to examine the effects. Therefore, this crossover design study investigated the influence of whey protein consumed prior to carbohydrate consumption on cycling performance and metabolic profile alterations in recreational trained cyclist and triathletes. Three specific aims were analyzed in this study:

Aim 1) to test the hypothesis that a 0.7 g/kg/LBM whey protein isolate preload prior to the ingestion of 0.9 g/kg/LBM glucose would improve endurance

performance (30 min time trial) compared to the ingestion of the glucose only beverage of 0.9 g/kg/LBM plus a non-caloric placebo.

Aim 2) to test the hypothesis that a 0.7 g/kg/LBM whey protein isolate preload prior to the ingestion of 0.9 g/kg/LBM glucose would increase carbohydrate utilization as indicated by an increased respiratory exchange ratio and decrease in plasma FFA compared to the ingestion of the glucose only beverage of 0.9 g/kg/LBM plus a non-caloric placebo.

Aim 3) to test the hypothesis that a 0.7 g/kg/LBM of body mass whey protein isolate preload prior to the ingestion of 0.9 g/kg/LBM glucose would increase plasma insulin concentration during exercise compared to the ingestion of the glucose only beverage of 0.9 g/kg/LBM plus a non-caloric placebo.

Definitions

Whey Protein Isolate - The composition of milk protein can be divided into two different proteins, casein and whey (Adams & Broughton, 2016; Stevenson & Allerton, 2017). Whey protein accounts for 20% of the total protein in milk, while casein accounts for the remaining 80% (Stevenson & Allerton, 2017). During the cheese manufacturing process, the casein protein is strained and separated into curds, while the remaining whey protein remains in a liquid state (Adams & Broughton, 2016; Krissansen, 2007). Whey protein can be further separated into whey protein isolate with a higher concentration of whey protein (90-95%) and can be virtually lactose free (Gangurde, 2011).

Glucose Transporter Protein 4 (GLUT4) – a key determinant of blood glucose disposal from the blood stream into the skeletal muscle tissue. GLUT 4 translocates to the plasma membrane in an insulin-dependent manner to uptake glucose into the cell. GLUT4 proteins are highly concentrated in human skeletal muscle and adipose tissue (Huang & Czech, 2007).

Incretins – incretin hormones are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These peptides are labeled incretins due to their ability to augment the intestinal secretion of insulin following the consumption of glucose or a mixed meal (Baggio & Drucker, 2007).

Maximal Oxygen Consumption **–** A quantitative measurement of aerobic capacity. This value represents the maximal amount of oxygen (L/min or ml/kg/min) that is taken up and utilized to fuel the aerobic energy requirement during exercise (Joyner & Coyle, 2008).

Respiratory Exchange Ratio (RER) **–** is the ratio of the amount of carbon dioxide produced (VCO_2) over the amount of oxygen consumed (VO_2). This value indicates the percentage of carbohydrates and fat being utilized to produce energy. The RER scale ranges from .70 (100% fat oxidation) to 1.00 (100% carbohydrate oxidation). This ratio is measured using an indirectly calorimeter from expired pulmonary gases (Brooks et al., 2005).

Time Trial **–** endurance performance test in which the participant is instructed to cover as much distance as possible within a given time frame (e.g., 30 min).

Insulin Signaling – a term to describe the process in which insulin binds to specific receptors on skeletal muscle to ultimately stimulate a translocation of GLUT4 to the cell membrane for blood glucose disposal (Chang, Chiang, & Saltiel, 2004).

Assumptions

Assumptions for this study include the following:

1) Participants recorded and replicated their food intake 3 days prior to each exercise trial.
2) Participants were in a fasted state for at least 10 hr prior to each exercise trial.
3) Any planned physical activity performed by the participants 3 days prior to the exercise testing were recorded and replicated prior to each trial.
4) Participants abstained from any alcohol or tobacco use 48 hr prior to each exercise trial.
5) Participants abstained from any caffeine intake the day of testing.

Limitations

1) Cycle Ergometer (Velotron) – participants were not able to use their own bikes for this research study. This set-up may alter cycling mechanics; however, participants used the same cycle ergometer for both exercise trials.

Delimitations

1.) Participants for this study were limited to only those males and females that have previous cycling experience and have a VO_2max ≥ 45 ml/kg/min for males and ≥ 40 ml/kg/min for females.
2.) Participants had previous cycling/triathlon experience (competitive experience in race duration > 60 min).
3.) Participants for this study were recruited in the Dallas-Fort Worth area.

Significance of the Study

In 2015, 432,447 individuals within the United States purchased an annual USA Triathlon membership (USA Triathlon, 2015). Also in 2015, 67,329 individuals in the United States purchased a USA Cycling membership (USA Cycling, 2015). In 2016, 507,600 individuals completed a 26.2-mile marathon and 1.9 million individuals completed a 13.1 half marathon (Running USA, 2016). Endurance sports are not just centered on elite level athletes. All levels of fitness and ages can participate in endurance sports such as triathlons, cycling events, and marathons. Depending on the intensity and duration of the aforementioned events, each discipline will require a fueling strategy before and during the event. When these events are performed for prolonged durations (> 60 min) at moderate-to-hard exercise intensity, carbohydrates are the primary fuel source to produce energy. The primary source of carbohydrate oxidation will be from skeletal

muscle glycogen. Therefore, it is important to fully saturate the skeletal muscle glycogen stores before endurance events and potentiate a glycogen sparing effect with the use of exogenous carbohydrates. Due to the popularity of endurance racing and the carbohydrate demand during such events, it seems pertinent to seek effective methods of supplementation to attenuate muscle glycogen during exercise and increase exogenous carbohydrate utilization. Whey protein may be a practical supplement to provide to athlete prior to an endurance event to invoke such responses.

This study examined if 0.7 g/kg/LBM of whey protein administered as a preload to a glucose bolus of 0.9 g/kg/LBM influences cycling performance and the metabolic profile during a 60 min cycling performance trial. This study added quality content to the scientific literature by examining if whey protein was a viable ergogenic aid for endurance athletes.

CHAPTER II

REVIEW OF LITERATURE

Substrate Utilization During Aerobic Exercise

During prolonged moderate to high intensity aerobic exercise (> 65% VO_2max), carbohydrates are the primary substrate utilized to fuel muscle contractions. As exercise intensity progresses from low to high intensity, there is a gradual increase in relative carbohydrate oxidation with a decrease in relative fat oxidation. The substrate shift during exercise is known as the *crossover concept* (see Figure 1; Brooks & Mercier, 1994).

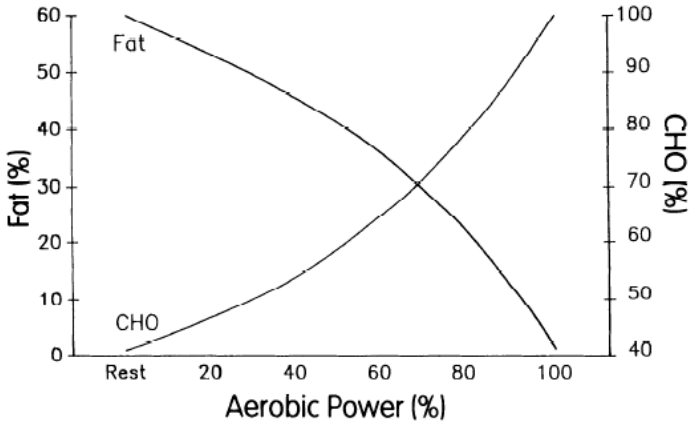


Figure 1. Crossover Concept (Brooks & Mercier, 1994).

Carbohydrates becomes the predominant substrate oxidized during moderate-to-high intensity exercise due to the following physiological responses to exercise; an increased rate of adenosine triphosphate (ATP) synthesis, increase in catecholamine

secretion through the stimulation of the sympathetic nervous system (SNS), activation of type II muscle fibers, and accumulation of calcium (Ca^{+2}) ions and adenosine monophosphate (AMP; Brooks & Mercier, 1994). These are the primary mechanisms promoting a substrate shift during exercise.

Glycogen Metabolism During Exercise

As exercise intensity increases, the primary substrate utilized to fuel muscle contraction is skeletal muscle glycogen (van Loon et al., 2001). Glycogen, a branched-chain polysaccharide, is the storage form of glucose and is primarily located in the liver (~100 g) and skeletal muscle tissue (~350-700 g; Ferrier, 2014; Knuiman, Hopman, & Mensink, 2015). Liver glycogen is primarily catabolized to maintain blood glucose levels during a fasted state or periods of hypoglycemia, while store glycogen within skeletal muscle is broken down and utilized to produce energy for localized muscular contractions (Jensen, Rustad, Kolnes, & Lai, 2011; Knuiman et al, 2015).

During exercise, there is an increase in SNS activity. Increased SNS activity promotes the secretion of catecholamines, epinephrine (EPI) and norepinephrine (NE). EPI is primarily secreted from the adrenal medulla and NE is predominantly released from sympathetic nerve endings (de Diego, Gandía, & García, 2008). Low-intensity exercise (< 50% VO_2max) has minimal effect on the secretion of catecholamines. However, as intensity progresses to moderate to high-intensity exercise, blood levels of EPI and NE rise drastically (Brooks et al., 2005). The primary catecholamine associated with carbohydrate oxidation during aerobic exercise is EPI. EPI binds to β-adrenergic receptors located on the skeletal muscle and liver. The binding of EPI stimulates cyclic

adenosine monophosphate (cAMP) to activate *protein kinase A* (PKA). PKA phosphorylates *glycogen phosphorylase kinase A*. Once phosphorylated, *glycogen phosphorylase kinase A* proceeds to phosphorylate *glycogen phosphorylase A*. *Glycogen phosphorylase A* is the active enzyme to initiate glycogen breakdown within the skeletal muscle and liver (Ferrier, 2014). The rise in EPI secretion is a key stimulus involved in skeletal muscle carbohydrate oxidation during moderate to high intensity exercise. NE also plays a role in maintaining blood glucose homeostasis during exercise. NE can bind to β-adrenergic receptors located on the liver to aid in hepatic glycogenolysis (Brooks et al., 2005).

Glycogen breakdown signaling is also induced by muscular contractions. The increase in muscular recruitment promotes the release of Ca^{2+} from the sarcoplasmic reticulum and an increase in AMP through the breakdown and utilization of ATP. Circulating Ca^{2+} binds to calmodulin (CaM) and can activate *glycogen phosphorylase kinase B*. This promotes the phosphorylation of *glycogen phosphorylase kinase A*, the same step involved when EPI binds to the β-adrenergic receptors. Under rapid ATP degradation, the increase in AMP concentrations can activate *glycogen phosphorylase B* leading to the phosphorylation of *glycogen phosphorylase A* (Ferrier, 2014). A schematic drawing of the influence of EPI, Ca^{2+}, and AMP on glycogen degradation signaling is provided in Figure 2.

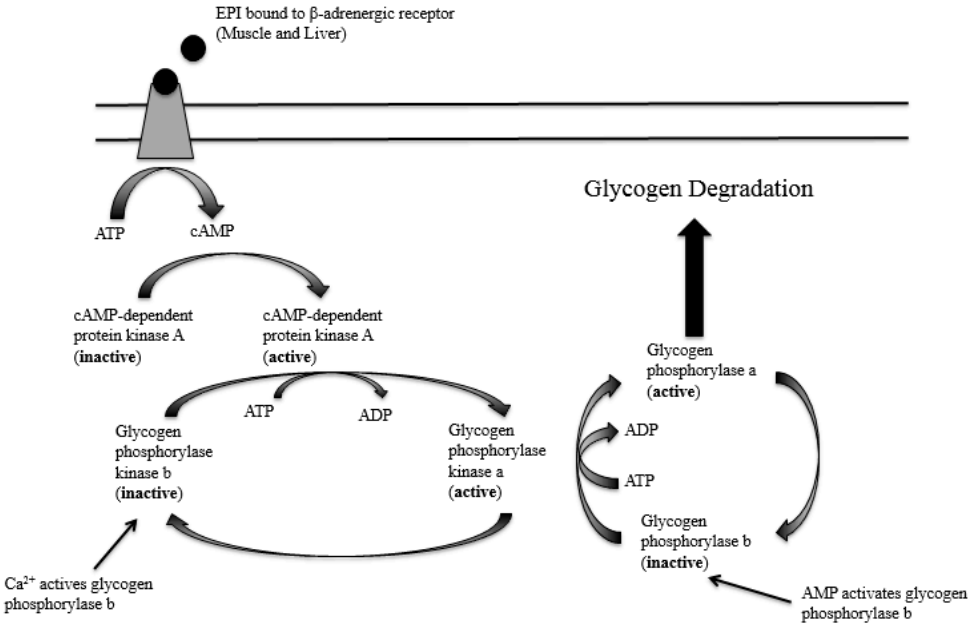


Figure 2. Stimulation of Glycogen Degradation (Ferrier, 2014).

The secretion of EPI, the release of Ca^{2+}, and the accumulation of AMP are stimulated in an intensity dependent manner (Baker & Buchan, 2017; Greiwe, Hickner, Hansen, Racette, Chen, & Holloszy 1985; Zouhal, Jacob, Delamarche, & Grats-Delamarche, 2008). The increase in exercise intensity also stimulates a greater relative contribution of type II muscle fibers. Type II muscle fibers have increased glycolytic enzyme activity, leading to an increase reliance on carbohydrates to sustain muscle contraction (Zierath & Hawley, 2004).

Lipid Metabolism During Exercise

Lipid oxidation is also a primary method to synthesize ATP during aerobic exercise. Lipids are heavily relied upon during low to moderate aerobic exercise intensities (< 65% VO_2max) (Brooks et al., 2005; Brooks & Mercier, 1984; Goodwin, 2010). Lipids make up 90% of a triacylglycerol (TAG) molecule, while the remaining

10% consists of phospholipids, cholesterol (esters) and non-esterified fatty acids (NEFA; Ferrier, 2014). Adipose tissue has α-adrenergic and β-adrenergic receptors located on the outer membrane (Febbraio, Lambert, Starkie, Proietto, & Hargreaves, 1985). The secretion of EPI and NE bind to the β-adrenergic receptors to promote fatty acid mobilization. Catecholamines stimulate lipoprotein lipase (LPL) located in adipose tissue. LPL promotes the breakdown of TAG into free fatty acids and glycerol (Mead, Irvine, & Ramji, 2002). The unbound FFA is also referred to as non-esterified fatty acids and is utilized by the skeletal muscle tissue to produce energy. NEFA are mobilized to the skeletal muscle tissues for oxidation and glycerol is taken up by liver and can be converted into glucose via gluconeogenesis (Brooks et al., 2005; Ferrier, 2014). However, during moderate to high intensity aerobic exercise, the stimulation of lipolysis from catecholamine secretion is inhibited due to increase demand of ATP and increased Ca^{2+} and AMP accumulation. Another factor promoting a decrease in lipid oxidation stems from blood flow redistribution during intense exercise. Blood flow to the adipose tissue is required for FFA mobilization to the skeletal muscle. However, during intense exercise, blood flow is shunted from the adipose tissue and directed towards the metabolically demanding skeletal muscle, impairing FFA mobilization (Goodwin, 2010; Ranallo & Rhodes, 1998). For these reasons, adequate glycogen stores to maintain exercise performance and intensity are key.

Substrate Demand During Endurance Performance

Depending on distance and intensity of the endurance performance bout, carbohydrate oxidation can be heavily relied upon to sustain exercise intensity. During a sprint triathlon (750-m swim, 20-km bike, and 5-km run), 19 male recreational competitive triathletes recorded an average heart rate response of 169.2 ± 22.2 bpm swimming, 163.5 ± 12.1 bpm cycling and 176.2 ± 13.1 running (Pinillos, 2016). The average age of male triathletes was 33 years old. Using the equation, "220-age" to estimate age predicated heart rate max, this group of triathletes would have a HR max of 187 bpm (Fox, Naughton, & Haskell, 1971). Therefore, during each discipline of the triathlon, the athletes were working at 90.5% (swimming), 87.4% (cycling), and 94.2% (running) of their age predicted heart rate max (Fox et al., 1971). During a half marathon, 21 males in-between the ages of 21-49 years reported an average heart rate response of 171.1 ± 7.7 bpm, which calculated out to be 92.4 ± 4.2% of estimated heart rate max. One of the stages during the Tour de France, cyclists compete in an individual time trial (TT) as a test of cycling performance. Eighteen cyclists, mean age of 28.82 years, on Team ONCE (Spain) completed the TT in 52.2 ± 29.8 min, with an average HR response of 165.5 ± 11.68 bpm (Fernández-García, Perez-Landaluce, Rodriguez-Alonso, & Terrados, 2000). Given the age of these cyclists, the TT was completed at 86.6% of age predicated HR max. The high-intensity effort of the athletes in the aforementioned studies demonstrates an increased reliance upon carbohydrates to sustain exercise intensity. The duration for each of these examples of exercise intensity during an

endurance event was less than 90 min, stressing the importance of proper fueling prior to the event.

Even during ultra-endurance events, carbohydrates (CHO) play a critical role in maintaining exercise intensity. Maunder, Kilding, and Plews (2018) analyzed the substrate utilization during an Ironman triathlon (3.9-km swim, 180-km cycle, and 42.2-km run) in elite, top-amateur, and lower-amateur triathletes. Using a theoretical model to estimate substrate utilization during the event, the authors concluded the elite triathletes expended between 2.05-3.49 g/CHO/min and a total expenditure of 985-1675 g CHO. The top-amateur triathletes expended between 1.44-2.88 g/CHO/min with a total expenditure of 788-1573 g CHO, and lastly, the lower-amateur triathletes expended between 1.12-2.56 g/CHO/min and a total expenditure of 875-1999 g CHO. Stored glycogen content in the skeletal muscle alone is not sufficient to provide fuel for ultra-endurance event. Athletes involved in these events may implement strategies to induce a glycogen sparing effect early on in the race and stimulate an increase in exogenous carbohydrate oxidation to aid in maintaining exercise intensity and delaying the onset of fatigue through glycogen depletion.

Acute Carbohydrate Ingestion on Endurance Performance

The scientific literature provides strong evidence that carbohydrate consumption prior to and during an endurance event can improve performance (Vandenbogaerde & Hopkins, 2011). Early studies conducted by Coyle et al. (1983) examined the effects of carbohydrate consumption during prolonged aerobic exercise lasting 2-3 hours. Ten trained cyclists completed two exercise session on a cycle ergometer at 74% VO_2max

until exhaustion. During one of the trials, the cyclists were given 1.0 g carbohydrate/kg body weight following the first 20 min of exercise, and then were provided 0.25 g carbohydrate/kg body weight after 60, 90, and 120 min. During the other exercise trial, the cyclists were given a placebo solution (saccharine and xanthan gum) to match taste and consistency of the carbohydrate solution. The time to exhaustion improved during the carbohydrate trial when compared to the placebo trial (157 ± 5 min vs. 134 ± 6 min, $p <$ .01). Sherman et al. (1991) also reported an improvement in time-trial performance following the consumption of two different doses of carbohydrates.

Participants for this study were required to cycle at 70% VO_2max for 90 min then performed a time trial to complete the work required for 45-min bout at 70% VO_2max. Prior to the exercise testing participants consumed 1.1 g/CHO/kg or 2.2 g/carbohydrate/kg in the form of glucose and maltodextrin. The two doses of carbohydrates were compared to a placebo trial. Following the completion of all three trials, both carbohydrate trials significantly improved time trial performance when compared to the placebo trial by 12.5% and power output was 13.1% higher. Carbohydrate consumption also has been reported to improve power output (Watts) during a 1 hr simulated cycling time trial (El-Sayed, Balmer, & Rattu, 1996). Eight male cyclists performed two separate 1-hr time trials at a self-selected pace. Prior to the exercise trials, the cyclists were given either a placebo or a solution with 8% carbohydrate. With the carbohydrate solution, a significant increase in mean power output of 3% (277 ± 3 vs. 269 ± 3 W) was reported.

Numerous studies have reported similar results to the aforementioned studies. Temesi, Johnson, Raymond, Burdon, and O'Connor (2011) conducted a well-researched systematic review with meta-analysis examining the influence of acute carbohydrate consumption on overall endurance performance. The studies that were included in this analysis administered between 30-80 g/CHO/hr during aerobic exercise ≥ 1 hr. The performance trials consisted of time trials or time to exhaustion protocols. Prior carbohydrate consumptions reported an overall mean improvement in time trial performance by 7.5% and improved time to exhaustion performance by 2.0%.

Carbohydrate consumption 30 min prior to an endurance bout of exercise has also been shown to improve exercise performance. Goodpaster et al. (1996) provided 1 g of amylopectin starch per kg of body mass, 1 g of glucose per kg of body mass or an artificially sweetened placebo to 10 trained males prior to a bout of exercise on separate occasions. The carbohydrate was consumed 30 min prior to a cycling performance trial. The cycling trial consisted of cycling for 90 min at 66% VO_2max followed by a 30 min TT. Results indicated the glucose and amylopectin allowed the cyclists to generate 7.7% and 5.8% more power respectively, compared to the placebo trial. In a review by Hawley and Burke (1997) exploring the effect of carbohydrate timing and aerobic exercise performance, a 7-20% increase in cycling performance was reported when carbohydrates were consumed 30-60 min prior to an endurance bout of exercise. The bulk of literature advocates acute carbohydrate consumption prior to a bout of aerobic exercise compared to placebo elicits an improvement in aerobic performance. The improvement in performance is noted by the ability of exogenous carbohydrates to spare muscle glycogen

and increase carbohydrate oxidation. Currently, there is controversy as to whether adding whey protein to a carbohydrate solution can further augment exogenous carbohydrate oxidation and spare muscle glycogen.

Whey Protein

The composition of milk protein can be divided into two different proteins, casein and whey (Adams & Broughton, 2016; Stevenson & Allerton, 2017). Whey protein accounts for 20% of the total protein in milk, while casein accounts for the remaining 80% (Stevenson & Allerton, 2017). During the cheese manufacturing process, the casein protein is strained and separated into curds, and the remaining whey protein remains in a liquid state (Adams & Broughton, 2016; Krissansen, 2007). Whey protein is categorized as a complete source of protein because it contains all nine essential amino acids (Adams & Broughton, 2016). The nine essential amino acids consist of leucine, isoleucine, valine, threonine, lysine, methionine, histidine, phenylalanine, and tryptophan (Bukhari et al., 2015). Whey protein is particular high in the branched chain amino acids, leucine, isoleucine, and valine (Layman, 2003). Table 1 outlines the peptide composition of whey protein. Whey protein has been utilized in a variety of research studies aiming to explore its influence on health and performance outcomes (Jäger et al., 2017; Krissansen, 2007; Stevenson & Allerton, 2017). One performance measure of interest is the potential ergogenic benefits of co-ingesting whey protein with carbohydrates during an acute bout of aerobic performance.

Table 1

Composition of Whey Protein

Compound	Amount
β-lactoglobulin	45-57%
α-lactalbumin	15-25%
Immunoglobulin	10-15%
Glycomacropeptide	10-15%
Bovine serum albumin	10%
Lactoferrin	~1%
Lactoperoxidase	<1%
EAA	34.1%
NEAA	34.9%

(Adams & Broughton, 2016; Bendtsen, Lorenzen, Bendsen, Rasmussen, & Astrup, 2013; Gorissen et al, 2018; Madureira, Tavares, Gomes, Pintado, & Malcata, 2010)

Metabolic Response to Whey Protein

One of the primary metabolic responses induced by whey protein is the increase in insulin secretion (Adams & Broughton, 2016; Jakubowicz & Froy, 2013). Insulin is a peptide hormone that is secreted from the β-cells of the islet of Langerhans in the pancreas (Wilcox, 2005). Insulin is the primary hormone responsible for fuel utilization and mobilization. A primary function of insulin is to regulate blood glucose uptake into the skeletal muscle, liver, and adipose tissue. Potent stimulators of insulin secretion are carbohydrates and specific amino acids. In healthy individuals, the majority of insulin secretion following a bolus of carbohydrates stems from the release of incretins hormones in the gut. The two known incretin hormones are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These peptides are labeled incretins due to the **in**testinal se**cret**ion of **in**sulin (Zunz & La Barre, 1929). GIP is a hormone secreted from the K cells located in the small intestine and acts on the pancreas

to secrete insulin following the consumption of carbohydrates, specific amino acids, and mixed meals (Seino, Fukushima, & Yabe., 2010). GIP has also been shown to stimulate glucagon secretion from the pancreas and decrease gastric acid production in the GI tract (Seino et al., 2010). GIP (along with GLP-1) has been reported to be a mediator of appetite and satiety (Seino et al., 2010). In a similar fashion, GLP-1 is produced from proglucagon but is secreted from L-cells within the lower small intestine and the colon (Seino et al., 2010). GLP-1 also acts to decrease glucagon secretion and delay gastric emptying within the GI tract (Seino et al., 2010). GLP-1 contributes to the delay in gastric emptying, contributing to an increase in satiety (Seino et al., 2010). Both GIP and GLP-1 are degraded by the enzyme dipeptidyl peptidase-4 (DPP-4). Due to GIP and GLP-1 rapid proteolytic degradation, the half-life is 5 and 2 min, respectively (Seino et al., 2010). It has been shown that ~40% of GIP remains active compared to only 20% for GLP-1 following incretin infusions (Baggio & Drucker, 2007).

Assumptions lead researchers to speculate that GIP may be less vulnerable to degradation by DDP-4 (Baggio & Drucker, 2007). GLP-1 and GIP bind to specific receptors (GLP-1R and GIPR) expressed on the pancreatic β-cells to further augment insulin secretion (Seino et al., 2010). The insulin secretion from the incretin effect may account for 50-70% of total insulin secretion following glucose consumption in healthy individuals (Baggio & Drucker, 2007). Following the ingestion of a mixed meal or carbohydrates, insulin is secreted in a biphasic pattern into the blood stream via the portal veins (Rorsman et al., 2000). The initial phase of insulin secretion is generally rapid, while the second phase is less concentrated and more gradual (Wilcox, 2005). The

majority of glucose disposal occurs within the skeletal muscle and hepatic tissue to be utilized as energy or stored as glycogen. Skeletal muscle is the primary disposal tissue for glucose at rest, accounting for ~70% of total glucose uptake following the consumption of carbohydrates or a mixed meal (Abdul-Ghani & DeFronzo, 2010; DeFronzo et al., 1981; Thiebaud et al., 1982).

In skeletal muscle, insulin binds to specific insulin receptors located on the cell membrane. The binding-interaction promotes a downstream signal to stimulate the translocation of glucose transporter protein 4 (GLUT4) to the cell membrane (Carnagarin et al., 2015; Karim, Adams, & Lalor, 2012). Insulin binds to an insulin receptor composed of two extracellular α subunits on the cell membrane and two intermembrane β subunits (Chang et al., 2004). The binding of insulin promotes the increase of tyrosine kinase activity from the β subunits (Chang et al., 2004). This induces the phosphorylation of intracellular tyrosine residues such as insulin-receptor substrate 1 (IRS-1; Chang et al, 2004). The phosphorylation of IRS-1 binds with p85 subunit of phosphatidylinositol 3-kinase (PI3K) to activate p110 (Chang et al., 2004; Henriksen, 2002). The activation of p110 produces phosphatidylinositol 3,4,5-trisphophate (PIP_3) (Chang et al., 2004). PIP3 stimulates phosphoinositide-dependent kinases (PDK) and proceeds to phosphorylate AKt (protein kinase B) and PKCλ (see Figure 3; Chang et al., 2004; Henriksen, 2002). AKt directly phosphorylates AS160 (TBC1D4) and TBC1D1 (Sakamoto & Holman, 2008). AS160 (TBC1D4) promote the activity of guanosine-5`-triphosphate (GTP) to guanosine diphosphate (GDP) located on the vesicles containing GLUT4 proteins. GDP is depicted as an inhibitor of GLUT4 protein translocation to the cell membrane (Klip,

Sun, Chiu, & Foley, 2014). The phosphorylation of AS160/TBC1D1 from AKt (PKB) stimulates a less active form of AS160/TBC1D4. The inactive form promotes GDP to GTP utilizing Rab-GAP (GTPase-activing protein) (Rab-GDP to Rab-GTP) (Sakamoto & Holman, 2008). Rab-GTP promotes the activation of GLUT4 translocation to the plasma membrane (see Figure 3).

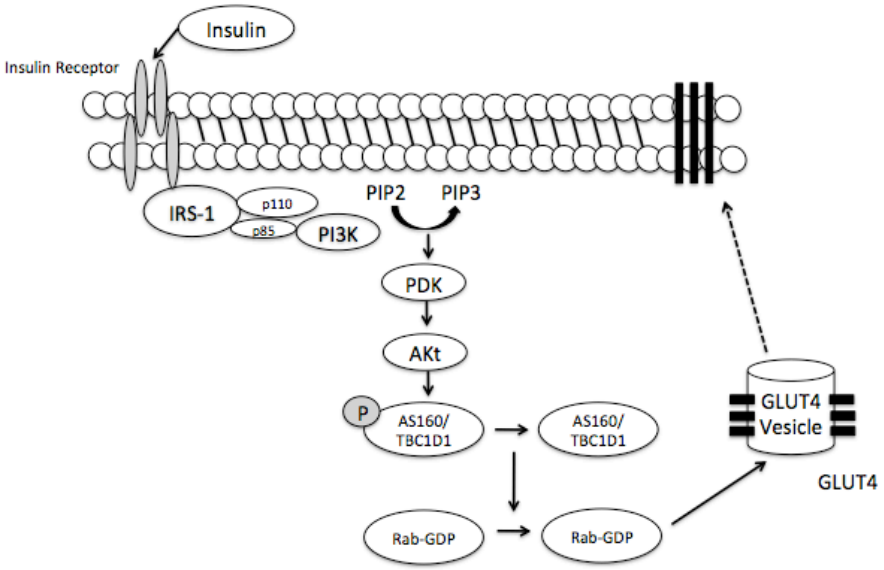


Figure 3. Regulated insulin-signaling pathway (Sakamoto & Holman, 2008; Chang et al, 2004).

GLUT4 proteins transport glucose from the blood stream into the skeletal muscle to be stored as glycogen or utilized for energy production (Wilcox, 2005).

The increase in insulin can also influence the degree of glycogenolysis within the skeletal muscle tissue. In the skeletal muscle and liver, insulin can phosphorylate *protein phosphatase-1* and stimulate *glycogen phosphorylase B*, the inactive form of *glycogen phosphorylase A* (see Figure 4). Insulin can also stimulate *protein phosphatease-1*, and promoting the activation of glycogen *phosphorylase kinase B*, the inactive form of

glycogen *phosphorylase kinase A* (see Figure 4; Ferrier, 2014). These actions inhibit muscle glycogen breakdown.

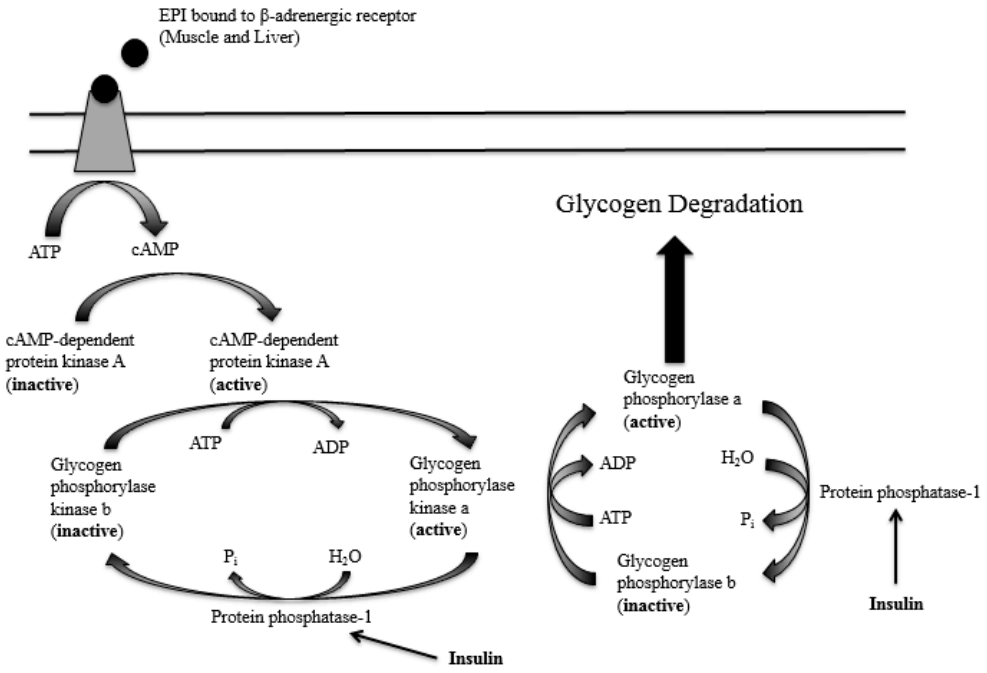


Figure 4. Influence of insulin on glycogen degradation (Ferrier, 2014).

Significant insulin secretion also inhibits lipolysis. Lipolysis is initiated by the enzyme adipose triglyceride lipase (ATGL). The interaction of AGTL with a TAG yields diacylglycerol to interact with hormone-sensitive lipase (HSL). Increase insulin secretion inactivates HSL in the adipose tissue therefore impairing the ability of diacylglycerol breakdown. Insulin also subdues ATGL activity in the adipose tissue (Ferrier, 2014).

Whey protein promotes a significant increase in insulin secretion and therefore stimulates glucose uptake into the skeletal muscle and liver. Due to the secretion of insulin, whey protein also stimulates glucagon to counter-regulate the potential decline in

blood glucose concentration. Glucagon is secreted from the α cells of the pancreas. Low blood glucose levels and whey protein initiate the release of glucagon. The primary function of glucagon is to increase hepatic glycogenolysis and gluconeogenesis to maintain blood glucose concentrations (Ferrier, 2014).

However, it appears the glucagon secretion following the consumption of whey protein does not invoke a hypoglycemic response but rather maintains or improves postprandial glycemia at rest. Whey protein ingested as a preload or co-ingested with a bolus of carbohydrates or mixed meal has consistently been reported to improve blood glucose peak and area under the curve (AUC) in healthy individuals. Twelve, healthy males and females consumed 18 g of whey protein co-ingested with 25 g of glucose in a fasted state to assess the glycemic response following consumption (Nilsson et al., 2007). Following the consumption of the beverage, overall glucose AUC was significantly lower when whey protein was incorporated into the beverage (whey + glucose = 45 ± 10.8 mmol • min/L; glucose = 103.4 ± 21.0 mmol • min/L). The decrease in glucose AUC is due to the 60% increase in serum insulin AUC when whey protein was consumed with the carbohydrate (17.0 ± 2.0 vs. 10.6 ± 1.3 nmol •min/L).

Whey protein also stimulated an 80% increase in GIP AUC and a 22% increase in GLP-1 AUC when compared to the intake of carbohydrate only (Nilsson et al., 2007). This response was consistent with previous work by Nilsson, Stenberg, Frid, Holst, and Bjork (2004) examining the influence of various types of protein on glycemia and insulinemia in healthy subjects. Implementing a similar dosing strategy, whey protein significantly decreased glucose AUC (57%), and increased insulin AUC (90%) and GIP

AUC (54%) compared to the consumption of carbohydrates alone. Shelley, Desbrow, Grant, Anoopkumar-Dukie, and Leveritt (2013) reported similar results when administering 20 g of whey protein with 50 g of glucose after an overnight fast. The co-ingestion of 20 g of whey protein in healthy males and females produced a reduction in glucose AUC by 47%. Whey protein also increased insulin AUC by 58.9% compared to the consumption of glucose only. Similar responses were reported when healthy males consumed either a carbohydrate dose equivalent to 0.2 g/kg or a carbohydrate dose of 0.2 k/kg co-ingested with 0.2 g/kg of whey protein hydrolysate.

The addition of whey protein to a bolus of carbohydrates significantly decreased glucose AUC and increased insulin AUC (exact values were not reported; Claessens, Saris, & van Baak, 2009). Zafar, Wasilen, AlRaefaei, Alrashidi, and AlMahmoud (2013) delivered 25 g of whey protein with 50 g of glucose in healthy young females. Glucose AUC concentrations were reduced 38% compared to 50 g of glucose. Whey protein consumed by healthy individuals, in a fasted, rested state has yielded positive results for overall glycemic control. However, it is noteworthy to examine the metabolic responses when whey protein is given before or during exercise.

Whey Protein and Endurance Performance

The notion of incorporating whey protein with carbohydrates during endurance exercise is controversial. The current body of literature provides a discrepancy in the data, leading to an indecisive conclusion. Whey protein co-ingested with carbohydrates has been speculated to improve aerobic performance by stimulating an increase in insulin secretion leading to an increase in exogenous carbohydrate utilization, invoking a muscle

glycogen sparing effect, regulate glycemic control, decrease the onset of central fatigue, and improve fluid availability (Betts & Stevenson, 2011; Ivy et al., 2003; Saunders et al., 2007).

Ivy et al. (2003) stimulated an ample amount of researchers to examine the influence of protein co-ingested with carbohydrates during endurance performance. Ivy et al. (2003) hypothesized the addition of (whey) protein to a carbohydrate solution during prolonged endurance performance contributed to prolonging exercise intensity due to proteins insulinotropic effect, increasing exogenous carbohydrate utilization, leading to the sparing of muscle glycogen. Nine-trained male cyclists completed three different exercise testing trials. Each participant was required to complete an interval based fatiguing protocol on a cycle ergometer, followed by cycling at 85% VO_2max until volitional fatigue. During the exercise performance, the participants consumed 1 of the 3 test solutions; (1) 200 ml of a placebo supplement, (2) 200 ml solution with 7.75% carbohydrate (per 100 ml) supplement, or (3) 200 ml solution with 7.75% carbohydrate / 1.94% protein (per 100 ml) supplement. The test solutions was provided to the participants every 20 min during the testing until the final segment at 85% VO_2max. Outcomes from this study reported a 36% improvement in time to exhaustion when comparing the carbohydrate-protein supplement to the carbohydrate only supplement. Other noteworthy outcomes from this study include no significant differences in respiratory exchange ratio (RER) between the carbohydrate and carbohydrate-protein supplement. In addition, there were no significant differences in glucose, insulin, or FFA levels during the testing period. Saunders, Luden, and Herrick (2007) also aimed to

examine the performance outcomes when ingesting a protein-carbohydrate gel compared to a carbohydrate-gel. Thirteen recreational cyclists (men = 8, women = 5) performed two separate time to exhaustion tests at 75% of VO_2peak. During each test, the participants consumed 1 of 2 the interventions, (1) carbohydrate only gel consisting of 0.15 g CHO/kg or (2) carbohydrate + protein gel consisting of 0.15 g CHO/kg + 0.038 g PRO/kg. The gels were consumed every 15 min throughout the testing period. The carbohydrate + protein gel invoked a 13% improvement in time to exhaustion when compared to the carbohydrate only gel (116.6 ± 28.5 min vs. 102.8 ± 25 min).

Similar to the findings of Ivy et al., (2003), there was no change in blood glucose values between the two trials. Romano-Ely, Todd, Saunders, and Laurent (2006) examined the influenced of a carbohydrate-protein-antioxidant beverage on overall time to exhaustion while cycling at 70% and 80% VO_2max. Fourteen male cyclists consumed the beverages every 15 min during their time trial. The carbohydrate-protein-antioxidant beverage contained 26 g of carbohydrate, 6.5 g protein, 200% recommended dietary allowance of vitamin E and 200% for vitamin C per serving. The carbohydrate only solution provided the participants with 35 g of carbohydrates per serving. Although the findings were not statistically significant, the carbohydrate-protein-antioxidant beverage improved cycling time to exhaustion by 3 min at 70% VO_2max and by 2 min at 80% VO_2max. It is important to note that the previous studies administered a carbohydrate dose that is considered less than optimal (< 60 g/CHO/hr; McLellan et al., 2014).

When optimal carbohydrate delivery is administered during an endurance performance measure, the addition of protein appears to have little effect. Twelve trained

male cyclists performed two separate exercise trials consisting of 120 min of cycling at ~55% VO_2max followed by a 60-min time trial. During the exercise trials, the participants were required to consume a carbohydrate beverage (65 g/hr) or a carbohydrate + protein beverage (65 g/hr + 19 g/hr) every 15 min during the testing. The results indicated no significant differences in time trial performance between both trials (carbohydrate beverage time trial 60:13 ± 1:33 min; carbohydrate + protein beverage time trial 60:51 ± 2:40 min; Breen et al., 2010). Van Essen and Gibala (2006) examined the influence of a 2% protein co-ingested with a 6% carbohydrate solution on cycling performance during an 80-km time trial. Ten male trained cyclist completed three separate 80-km time trial bouts in which they consumed either a 6% carbohydrate solution (60 g/CHO/hr), a 6% carbohydrate solution with the addition of 2% protein (60 g/CHO/hr, 20 g/protein/hr) or a placebo. The solutions were consumed every 15 min during the time trial. Results inferred no significant difference in time trial performance between the carbohydrate and carbohydrate-protein solution (135 ± 9 min vs. 135 ± 9 min). Both trials improved time trial performance by 4.4% when compared to the placebo trial. There was also no significant difference in insulin, glucose, or FFA concentrations between the carbohydrate and carbohydrate-protein solution during the cycling trial. Authors concluded when carbohydrate delivery is optimal (> 60 g/hr), the addition of protein offers no additional performance benefit (Van Essen & Gibala, 2006).

Often times, multi-sport endurance athletes will complete multiple training sessions in one day. The addition of whey protein following a training session, which proceeds the next training session, has been shown to increase exogenous carbohydrate

utilization and induce a muscle glycogen sparing effect (Betts, Williams, Boobis, & Tsintzas, 2008). Six males performed two exercise trials on separated days consisting of a 90-min treadmill run at 70% VO_2max followed by a 4-hour recovery period, followed by a 60-min treadmill run at 70% VO_2max. Following the completion of the first exercise bout, individuals received either the carbohydrate solution (sucrose) consisting of 0.8 g/kg/hr or the carbohydrate-protein solution consisting of 0.8 g/CHO/kg/hr and 0.3 g/pro/kg/hr. The solutions were provided in 30-min intervals throughout the 4-hour recovery period. During the recovery period, the carbohydrate-protein solution significantly increased serum insulin concentrations compared to the carbohydrate only solution. The protein-carbohydrate solution also induced a greater rate of carbohydrate oxidation during the second bout of exercise when compared to the carbohydrate only trial (48.4 ± 2.2 vs. 41.7 ± 2.6 mg/kg/min). Fat oxidation rates also decrease with the carbohydrate-protein solution when compared to the carbohydrate only solution (4.71 ± 0.80 vs. 2.56 0.35 mg/kg/min). Figure 5, published by Betts et al. (2008), represents the overall substrate utilization during the second bout of exercise. Figure 5 represents the brief overview of the substrate utilization while implementing whey protein with carbohydrate consumption.

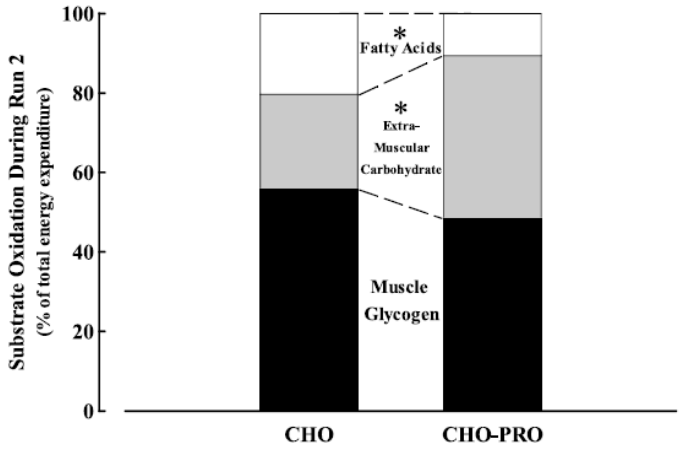


Figure 5. Substrate Oxidation during Run 2. (Betts et al., 2008).

However, there is a paucity of data addressing the influence of a whey protein preload prior to carbohydrate consumption during an aerobic bout of exercise. Morufuji et al. (2011) examined the influence of a whey protein preload prior to an acute bout of aerobic exercise in rodents. Male Sprague-Dawley rats where divided into three groups, the first group (n = 7) received 1.0 ml/100 g of body weight solution with only water, the second group (n = 7) received 1.0 ml/100 g body weight solution with 30% glucose and the last group (n = 7) received 1.0 ml/100 g body weight solution with 30% glucose plus 10% whey protein hydroslate. All groups were given their test solution 10 min prior to a glycogen-depleting bout of exercise consisting of swimming for 60 min with an external load equivalent to 3% of their body weight. Although this was not a test for performance, the rats that consumed the glucose plus whey protein hydroslate solution significantly attenuated skeletal muscle glycogen content compared to the glucose only and the water only interventions. An increase in skeletal muscle glycogen content following exercise may have been due to the significant increase in insulin concentration in the glucose plus

protein group when compared to the other test groups (glucose + protein = 1.92 ± 0.39 ng/ml; to glucose = 0.75 ± 0.33 ng/ml; water = 0.75 ± 0.17 ng/ml). The increase in insulin concentration may have increased exogenous carbohydrate oxidation, therefore promoting a glycogen sparing effect. To the author's knowledge, this study is the first to implement whey protein with carbohydrates 10 min prior to an aerobic bout of exercise. This type of research design has yet to be explored in a human population. The proposed study aims to examine the influence of whey protein consumed 20 min prior to carbohydrate consumption on cycling performance and metabolic responses.

CHAPTER III

RESEARCH METHODS

Experimental Overview

This single blind, crossover design study recruited recreational active cyclists or triathletes to test the influence of a whey protein preload prior to carbohydrate consumption during a cycling performance test. The participants performed two experimental trials on separate days. Participants consumed either a placebo preload (water plus a zero calorie lemonade sweetener) or a whey protein preload (0.7 g/kg/LBM) 20 min prior to the consumption of a carbohydrate load (0.9 g/kg/LBM). Following the consumption of the beverage, participants performed a cycling performance test consisting of cycling at 90% of their lactate threshold for 30 min, followed by a 30 min time trial. Throughout the experimental trials, venous blood was collected to analyze plasma concentrations of glucose, insulin, glucagon, and lactate. The aim of this study was to examine the performance and metabolic outcomes of a whey protein preload prior to a cycling performance test.

Participants

This study included males and females between the ages of 18 and 44 years of age who were considered recreationally active cyclists or triathletes. Recreationally active was defined based on the participants VO_2max collected during the preliminary data collection trial and current cycling training regimen. Males must record a VO_2max $\geq$ 45

ml/kg/min and females must record a VO_2max ≥ 40 ml/kg/min. These minimum threshold values are consistent with recreational trained cyclist and triathletes, although significantly lower than elite-level cyclist and triathletes (Saunders et al., 2007). Participants were currently cycling at least 2 hr per week. Participants had previous race experience in which the athlete has cycled for at least 60 minutes continuously within the last 12 months. This criterion was chosen because the cycling performance test lasted approximately 60 min.

Information regarding training/racing status was obtained during the preliminary visit. All females were tested during the mid-follicular phase of their menstrual cycle. Specific exclusion criteria for participation include: (1) any current nicotine use or have quit within the last 6 months, (2) females that are or plan on becoming pregnant during the testing period, (3) current or history of any known cardiopulmonary, cardiovascular, or metabolic disease, (4) current or previous musculoskeletal injuries that may impair cycling performance or limit hip or knee range of motion, (5) irregular menstrual cycle within the last 3 months, (6) currently taking any form of contraceptives (e.g., oral, injections, patches, intravaginal), (7) hormonal substance or medication use within the last year that may influence exercise performance (e.g., anabolic steroids, growth hormone, statins, or glucocorticoids), and (8) greater than moderate alcohol consumption as defined by the U.S. Department of Health and Human Services (> 1 drink per day for women and > 2 drinks per day for men; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015).

Study Design Summary

Each participant performed two different randomized experimental trials and one preliminary visit. The order of the trials for the participants was counterbalanced to negate the influence of any order effects. The trials consisted of two conditions: a placebo trial (**placebo –** 350 ml of deionized water mixed with a zero calorie lemonade flavoring [1 serving] was given to each participant 20 min prior to a carbohydrate load of 0.9 g/kg/LBM) and a whey protein preload trial (**whey** – 0.7 g/kg/LBM of whey protein isolate mixed with 350 ml of deionized water and a zero calorie lemonade flavoring [1 serving] was given to each participant 20 min prior to the glucose load of 0.9 g/kg/LBM). The glucose load was consumed 10 min prior to the cycling performance test (CPT). The CPT consisted of cycling on an electrical controlled cycle ergometer for 30 min at 90% lactate threshold followed by a 30-min time-trial (see Figure 6). A baseline blood sample was taken immediately before the preload (whey or placebo) at -30 min. Blood draws occurred immediately before the 0.9 g/kg/LBM of glucose is consumed and throughout the exercise trial. Each trial was separated by at least one week, but no more than two weeks in-between each trial. Each trial took ~90 min. Total time commitment for each participant for the entire study was ~4-5 hr.

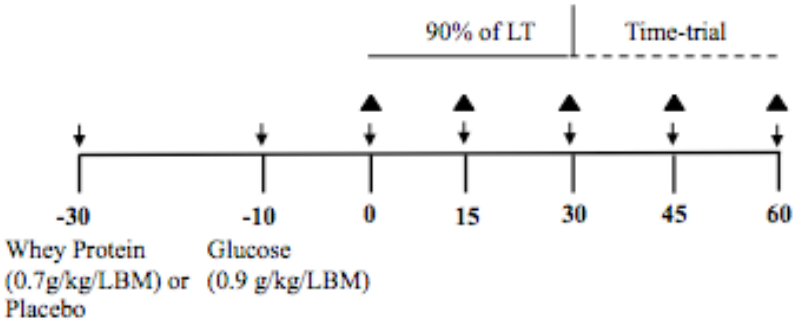


Figure 6. Schematic timeline of testing procedures. ▲= collection of expired gas, ↓= blood sample and VAS.

Preliminary Lab Visit

Participants visited the Exercise Physiology Laboratories located in Pioneer Hall for all testing trials. Each participant was asked to abstain from any food or drink, except water, at least 2-3 hr prior to the preliminary trial. During the preliminary visit, each participant was provided informed consent; filled out a medical history questionnaire and the Physical Activity Readiness Questionnaire Plus (PAR-Q Plus). A physical activity questionnaire was provided to each participant. This questionnaire obtained participant information about exercise frequency, intensity, duration, and mode. Participants were informed to not consume any commercially available supplements within their daily routine prior to and throughout the duration of the study. Participants were also informed to temporarily stop taking any other commercially available supplements or over the counter medications because it may interfere with substrate utilization during exercise (e.g., stimulants, pseudoephedrine). Participants were given detailed description on how to record food logs and activity logs 3 days prior to testing. Each participant was asked to

replicate the meals consumed three days prior to both experimental trials. Participants were required to replicate the same amount of physical activity performed 3 days prior to both experimental trials. Participants were asked to abstain from alcohol or nicotine use 48 hr prior to the interventions. During this visit, each participant was educated on how to adjust the resistance applied to the electronically braked cycle ergometer (Velotron; RacerMate, Seattle, WA).

Participant Blinding

During the preliminary visit, participants were told they were testing a new commercially available supplement to assess the ergogenic effects on cycling performance. The aim was to mask the flavor (by using a zero calorie lemonade sweetener, aspartame) between both preloads (whey and placebo) to eliminate the outcome expectancy effect of the preload. The outcome expectancy effect leads participants to achieve a particular outcome (Tippens et al., 2014). Therefore, it was important participants did not know when the whey protein was consumed. Upon completion of the exercise trial, participants were debriefed on the supplemental interventions and results.

Body Composition

Basic anthropometric data was measured during this trial. Height (cm) was measured using a stadiometer and weight (kg) was measured on a standardized digital scale. Lean body mass (kg), fat mass (kg) and bone mineral density (g/cm^2) was assessed utilizing a dual-energy x-ray absorptiometry (DXA). The DXA procedure occurred in Woodcock Hall in the Institute for Women's Health in rooms 013 and 010. During this

session, the participants were encouraged to ask any question they may have regarding the research procedures.

Test of Aerobic Capacity

Participants performed a lactate threshold (LT) test on an electronically controlled cycle ergometer. This test provided the work rate (W) at lactate threshold and the participants VO_2max (ml/kg/min). A VO_2max was achieved if the participant reached a plateau in VO_2 with an increase in workload. If a plateau in oxygen uptake was not achieved, secondary criteria was measured to determine if the individuals reached a VO_2max; (1) heart rate values during the test is within 10 bpm of age-predicted HR_{max} (HR_{max} was determined by using 220-age); (2) RER $\geq$ 1.10; and (3) blood lactate concentration > 8.0 mmol (blood lactate was measured from the capillary blood using a spring-loaded lancet 2 min following the termination of the test; Howley, Bassett, & Welch, 1995). Prior to performing the test, participants were fit with a heart rate monitor strapped around the level of the xyphoid process. The heart rate signal was transmitted to a digital watch and was monitored by the researcher.

The participants were allowed to adjust the seat height and handle bars to accommodate their cycling fit. The seat height and handle bar length was recorded for the subsequent exercise trials. The participants were fit to an indirect calorimetry (TrueOne 2400; ParvoMedics, Sandy, UT) to collect expired gasses (VO_2 and VCO_2) every minute of the test. The participants began the LT protocol at a power output of 50 W and the workload increased by 30 W every 4 min until volitional fatigue or if cycling cadence fell below 70 rpm (Urhausen, Coen, Weiler, & Kinderman, 1993). At the end of each stage, a

capillary blood lactate sample was collected. Lactate threshold was determined by using the maximal distance (Dmax) method developed by Cheng et al. (1992). The Dmax method involves calculating the VO_2 value that measure the maximal distance from a 3rd order polynomial line and the perpendicular distance from the two end points of VO_2. Participants then performed a 5-min cool-down at a self-selected intensity.

Experimental Trials

Approximately or at least 1 week after the LT test, participants were asked to come back to the Exercise Physiology Lab to complete the first of two experimental trials. Upon entering the lab, participants were instructed to sit upright on a chair and rest for 5 min. An indwelling Teflon catheter was placed in an antecubital or forearm vein following the resting period. The first baseline blood draw (-30 min) was collected to measure plasma glucose, insulin, glucagon, and non-esterified fatty acids (NEFA) concentrations. A baseline capillary blood lactate sample was also obtained. After the baseline blood draw (-30 min), participants consumed 1 of the 2 preloads (whey protein or placebo).

Whey + Carbohydrate

Following the baseline blood draw, participants consumed 0.7 g/kg/LBM of whey protein isolate, mixed with 350 ml of water and a zero calorie lemonade sweetener. Participants rested for 20 min and a second blood draw was collected. Participants then consumed 0.9 g/kg/LBM of glucose (Thermo Scientific, Middletown, VA) 10 min prior to the exercise bout. The exercise bout began 30 min following the consumption of the whey protein.

Placebo + Carbohydrate

Following the baseline blood draw, participants consumed 350 ml of water mixed with a zero calorie lemonade sweetener. Participants rested for 20 min and a second blood draw was collected. Participants then consumed 0.9 g/kg/LBM of glucose (Thermo Scientific, Middletown, VA) 10 min prior to the exercise bout. The exercise bout began 30 min following the consumption of the placebo.

Cycling Performance Test (CPT)

The exercise trial consisted of cycling for 30 min at 90% LT followed by a 30-min time trial. Participants were allowed to freely adjust the resistance added to the cycle ergometer throughout the 30-min time trial. During this trial, the total distance covered was hidden from the participants until the completion of both experimental trials. Throughout the CPT, power output (Watts) and cycling cadence (rpm) were measured and recorded.

Expired Gas Analysis

Throughout the CPT, expired pulmonary gasses (VO_2 and VCO_2) were collected every 15 min. VO_2 and VCO_2 were collected to determine the respiratory exchange ratio. VO_2 was analyzed to determine the average percent of LT at which the participants are working during the CPT.

Cardiorespiratory Responses

During both exercise trials, participants were fit with a Polar Heart Rate (Polar USA, Kempele, Finland strap (same process as preliminary visit) to measure and record heart rate every 15 min during the testing period.

Perceptual Measures

During both CPT, rating of perceived exertion (RPE) was measured using the Borg Rating of Perceived Exertion Scale every 10 min (Borg, 1998). The researcher asked the participant during exercise to rate their level of effort at a given moment. The participant replied by selecting a number on a scale of 6-20 indicating level of exertion. The number 6 indicates no exertion at all, while the number 20 indicates maximal exertion. Participants were also asked to rate leg pain every 10 min throughout the CPT using a visual analog scale (VAS). Every 10 min during the CPT, the researcher displayed a horizontal visual scale ranging from 0-10 to assess exertional leg pain. The value 0 indicates no pain, and 10 indicates the worst possible pain (Erdek & Pronovost, 2004).

Blood Sampling and Analysis

Venous Blood Collection

All blood samples were taken in the morning (starting between 0700 and 0800 hr.) in a fasted state (8-12 hr.). Time slots for each participant were kept consistent between both trials. Each blood sample was collected into BD Vacutainer K2 EDTA 4 ml tubes. A total of 7 blood samples (-30, -10, 0, 15, 30, 45, and 60 min) were taken for each trial. Each tube was prepped with aprotinin (Milliplex®, 2019 Merck, Darmstadt, Germany) for the measurement of glucagon. A total of 14 blood draws were taken for each participant, resulting in a total of 56 ml of blood collected for both trials. For reference, the average amount of blood donated in a single session according to the American Red Cross is between 450 to 500 ml (American Red Cross, 2018).

Each sample was immediately inverted slowly to mix the blood and inhibitor cocktail. The sample was centrifuged at 3000 RPM for 10 min at 4 °C. Plasma samples were aliquoted into cryotubes for immediate measurement of plasma glucose (mg/dl) and lactate (mmol/L) using an YSI 2900 Bioanalyzer (YSI Life Science, OH, USA). The remaining samples were kept frozen at -80 °C for further analysis. Insulin, c-peptide, and glucagon were analyzed with the Luminex MagPix® system using the Human Metabolic Hormone Magnetic Bead Panel kit (EMD Millipore Corporation, Billerica, MA). The analysis procedure followed the manufacture guidelines for preparation (Milliplex®). This procedure required 25 µl of each sample in duplicate neatly dispersed into each well. Non-esterified fatty acids (NEFA) concentration was measured using the process described by Wako Diagnostics (Wako Diagnostics, Richmond, VA).

Capillary Blood Sampling

Capillary blood was collected and measured throughout the lactate threshold test for concentration of blood lactate (mmol) using a calibrated, portable blood lactate analyzer (Lactate Scout +, EKF Diagnostic, Germany). A spring-loaded lancet was applied to the participant's fingertip to obtain a small droplet (~25 μl) of blood for analysis. Blood lactate samples were obtained at -30, -10, 0, 15, 30, 45, and 60 min.

Statistical Analysis

Performance results were predicated on total distance completed during the 30 min of exercise. Results from the time trial were analyzed using paired-samples t-test. Glucose, insulin, glucagon, NEFA and lactate time point data were analyzed using a two-way repeated measure ANOVA (condition x time). The level of significance was set at p

≤ .05 for all analyses. When applicable, a Bonferonni *post hoc* comparison analysis was implemented. All data was analyzed using Statistical Package for the Social Science (Version 21, IBM Corporation, Armonk, NY, USA).

CHAPTER IV

PRESENTATION OF FINDINGS

Brief Summary

A total of 10 participants completed this study. Results revealed there was no significant difference in time trial performance (km covered) between the whey protein trial and placebo trial. During the whey protein trial a significant spike in plasma insulin concentration was found at the beginning (timepoint 0) of the cycling performance trial. The whey protein trial also stimulated a significant increase in plasma glucagon concentrations during the entirety of the cycling performance trial when compared to the placebo trial. In addition, there were no significant differences in substrate utilization markers (e.g., respiratory exchange ratio and non-esterified fatty acids) between experimental trials.

Participants

A total of 20 participants were recruited for this study. One participant was excluded following the preliminary trial due to a low aerobic fitness score from the lactate threshold test. Four participants were removed from the study due to lack of communication with the primary investigator after the preliminary trial. Three participants were removed from the study due to scheduling conflicts following the preliminary trial. Twelve participants completed one of the experimental trials; however, one participant was excluded due to the time in-between experimental trials (> 2 weeks)

and one due to lack of motivation to complete the second trial. Therefore, a total of 10 (males; $n = 9$, female: $n = 1$) participants completed the study (see Table 2). All participants verbally stated they replicated the 3-day activity log prior to each of the experimental trials. Although each participant verbally stated they replicated their 3-day dietary log prior to the experimental trials, only six participants turned back in the hard copy. Therefore, 3-day dietary data only represents six participants (see Table 3). All nutritional data was analyzed using MyFitnessPal. The average amount of whey protein isolate consumed by the participants for the whey protein trial was 41.9 ± 6.7 g/lean body mass and the average amount of glucose consumed by the participants for both experimental trials was 53.9 ± 8.6 g/lean body mass.

Table 2

Participant Characteristics

Variable	**Data**
Age	32.2 ± 8.7
Height (cm)	175 ± 13.5
Weight (kg)	81.4 ± 11.5
Body fat (%)	23.7 ± 5.1
Lean body mass (kg)	61.2 ± 9.6
VO_2max (ml/kg/min)	45.7 ± 5.9
Lactate Threshold (W)	203 ± 37.6

Note. Data is presented as means ± standard deviation. Centimeters (cm); kilograms (kg); millimeters per kilogram per min (ml/kg/min); watts (W); grams (g).

Table 3.

3-Day Dietary Data

Macronutrient	Grams
Protein	111.4 ± 37.7
Carbohydrate	319.6 ± 89.4
Fat	78 ± 16.4

Note. Data is presented as means ± standard deviation.

Performance Outcomes

There was no significant difference for distance (km) covered following the whey protein (WP) time trial (16.8 ± 0.34 km) and placebo (PL) time trial (17 ± 0.4 km) (p = .346; see Figure 7). There was also no significant difference between the average absolute power output between both experimental trials (WP = 202.63 ± 16.03 W vs. PL = 213.17 ± 16.03 W; p = .519).

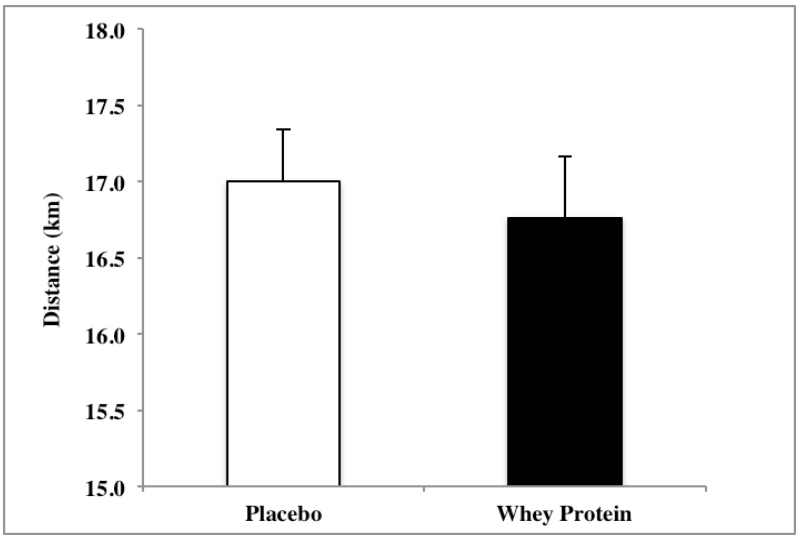


Figure 7. Distance covered during both of the experimental trials. All data is presented as means ± SEM. km = kilometers.

Metabolic Outcomes

Following the statistical analysis of insulin (time x trial), one extreme outlier was detected. Consequently, plasma samples from one participant were omitted from data analysis. Therefore a total of 9 participants' plasma samples were analyzed to measure insulin, glucose, glucagon, c-peptide, and NEFA.

Glucose

There was a significant crossover interaction effect (trial) for plasma glucose (mg/dl) between the two trials ($p = 0.009$). However, the consumption of the two different preloads did not produce any significant simple effects for any timepoint during the CPT. Timepoint comparison of plasma glucose data is displayed in Figure 8.

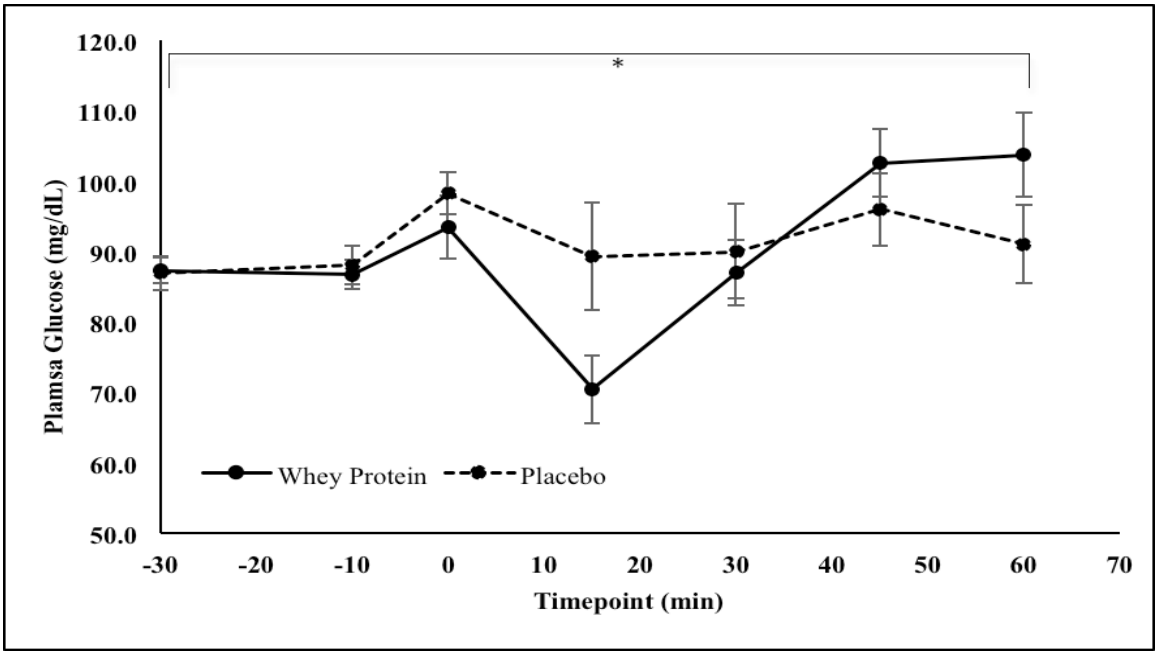


Figure 8. Comparison of plasma glucose concentrations between the whey protein and the placebo trial. All data is reported as mean ± SEM. * Significant interaction effect.

Insulin

There was a significant interaction effect for plasma insulin concentration (time x trial; $p = .007$) between the two experimental trials. The whey protein preload produced a significant increase ($p = .047$) in plasma insulin at timepoint 0 (222.88 ± 45.1 pg/ml) when compared to the consumption of the placebo (85.95 ± 45.1 pg/ml). Although there were no other significant time point differences, the whey protein preload produced a non-significant elevated response in plasma insulin. Timepoint comparison of plasma insulin data is displayed in Figure 9.

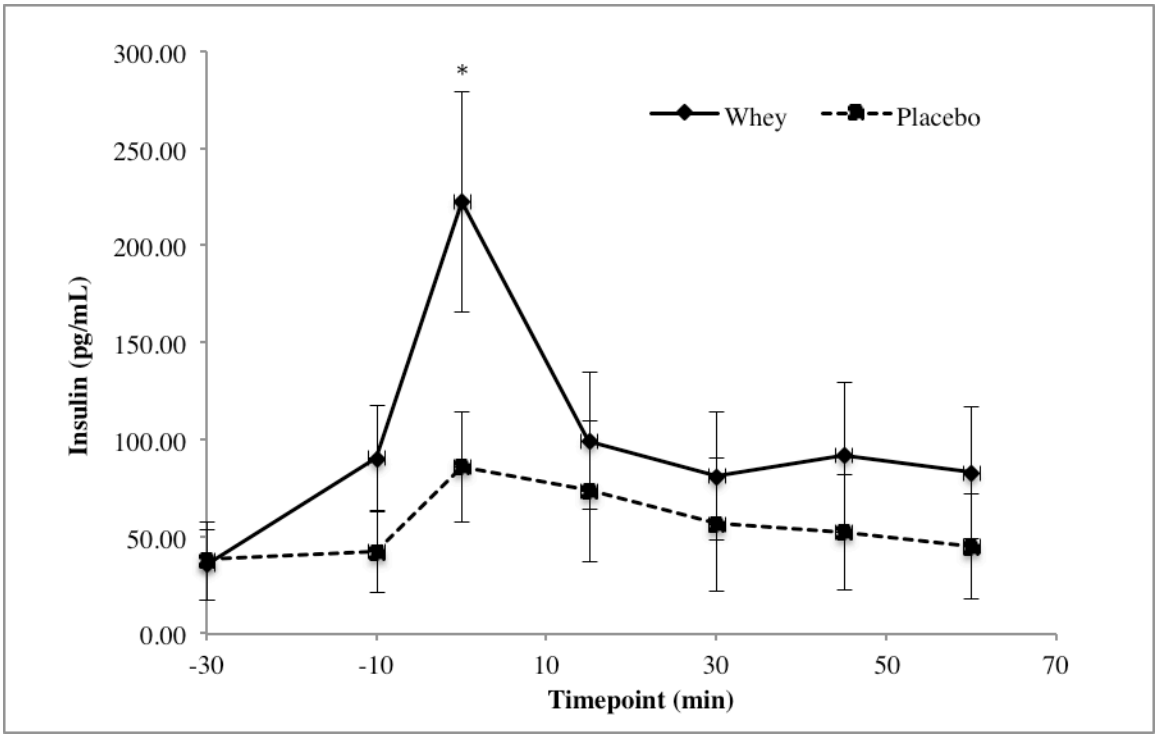


Figure 9. Comparison of plasma insulin concentrations between the whey protein and the placebo trial. All data is reported as mean ± SEM. * significantly different from the placebo timepoint.

C-Peptide

There was no significant interaction for plasma c-peptide (time x trial; $p = .112$) between the two experimental trials. Timepoint comparison of plasma c-peptide data is displayed in Figure 10.

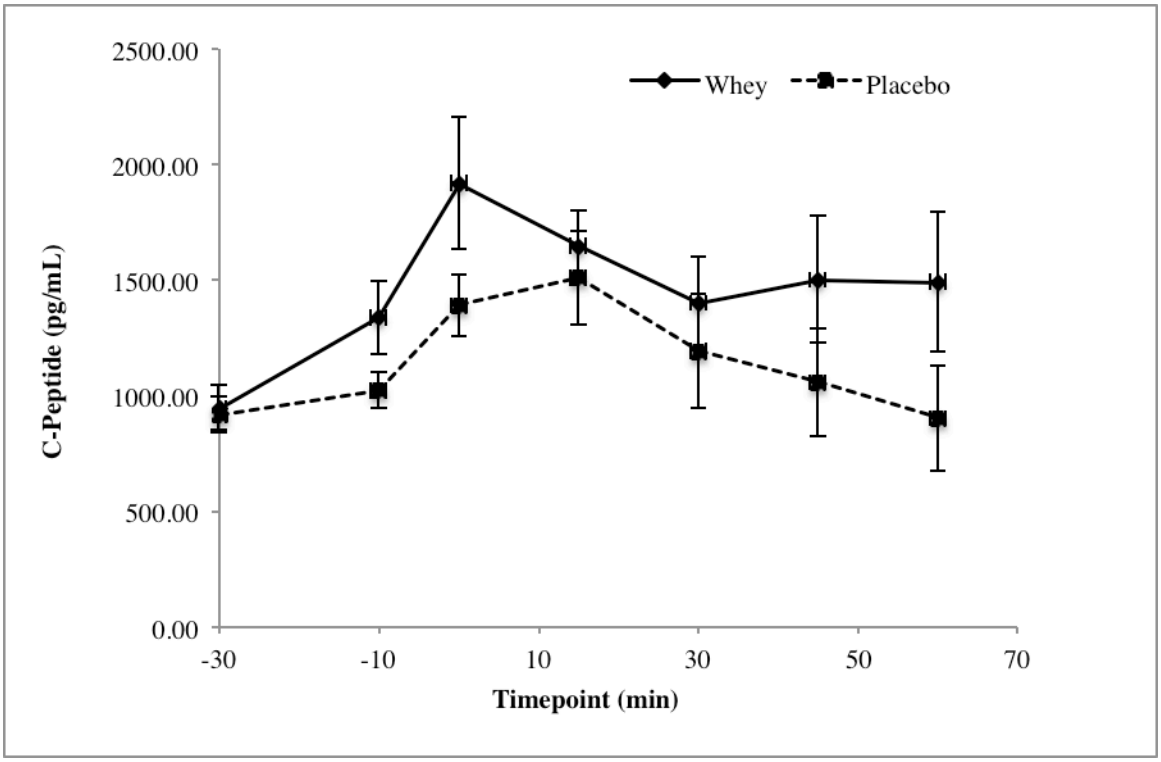


Figure 10. Comparison of plasma c-peptide concentrations between the whey protein and the placebo trial. All data is reported as mean ± SEM.

Glucagon

There was a significant interaction (time x trial, $p > .001$) between the two experimental trials (see Figure 11). Bonferonni post-hoc analysis reported a significant differences ($p = .015$) between timepoint -10 of the WP trial (178.85 ± 20.1 pg/ml) and the PL trial (100.84 ± 20.1 pg/ml). Timepoint 0 of the WP trial (219.21 ± 24.6 pg/ml) was

significantly different (p = .004) than the PL trial (102.51 ± 24.6 pg/ml). Timepoint 15 of the WP trial (371.32 ± 33.3 pg/ml) was significantly different (p < .001) then the PL trial (109.17 ± 33.3 pg/ml). Timepoint 30 of the WP trial (348.78 ± 29.1 pg/ml) was significantly different (p < .001) then the PL trial (117.82 ± 29.1 pg/ml). Timepoint 45 of the WP trial (281.77 ± 20.2 pg/ml) was also significantly different (p < .001) than the PL trial (116.41 ± 20.2 pg/ml). Lastly, timepoint 60 of the WP trial (264.31 ± 21.2 pg/ml) was also significantly different (p < .001) than the PL trial (123.1 ± 21.2 pg/ml).

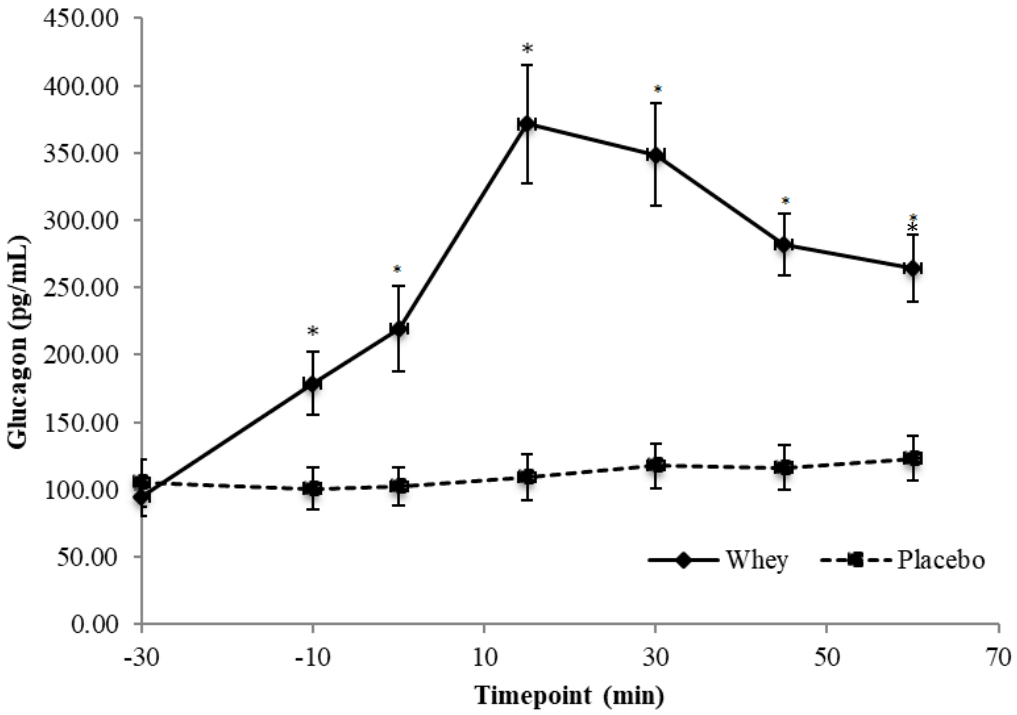


Figure 11. Comparison of plasma glucagon concentrations between the whey protein and the placebo trial. All data is reported as mean ± SEM. * = significantly different from PL trial.

Non-Esterified Fatty Acids

There were no significant main effects (time x trial, $p = .978$) for plasma NEFA concentrations between the two experimental trials (see Figure 12).

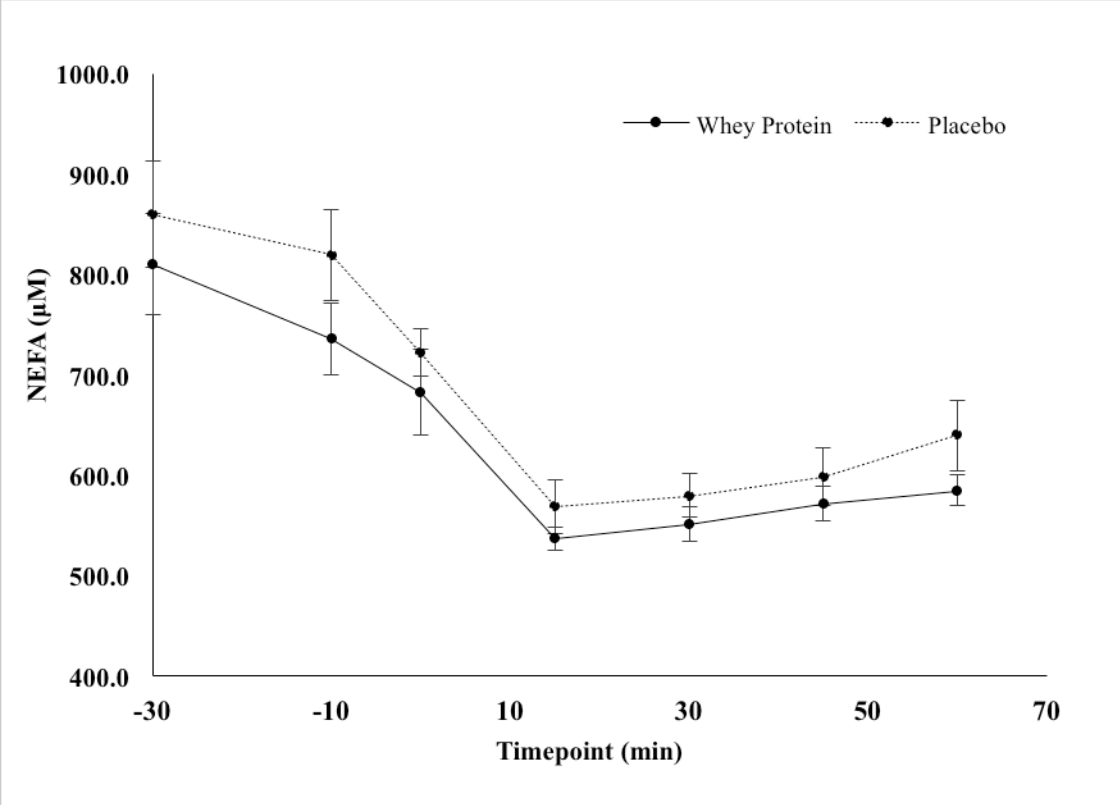


Figure 12. Comparison of plasma NEFA concentrations between the whey protein and the placebo trial. All data is reported as mean ± SEM.

Physiological, Perceptual, and Cardiorespiratory Outcomes

There were no significant main effects reported for VO_2 (ml/kg/min; $p = .330$), RER ($p = .648$), plasma lactate (mmol/L; $p = .077$), HR ($p = .298$), RPE ($p = .674$), or leg pain ($p = .741$) between the two experimental trials. Timepoint data for the aforementioned variables are listed in Table 4 and Table 5.

Table 4

Physiological Responses During Exercise

Variable	15	30	45	60
VO_2 (ml/kg/min)				
Whey	31.28 ± 1.6	31.1 ± 1.5	33.06 ± 1.6	35.43 ± 1.7
Placebo	31.08 ± 1.4	30.48 ± 1.6	34.77 ± 1.8	36.39 ± 1.8
RER				
Whey	0.89 ± .02	0.89 ± .02	0.92 ± .01	0.95 ± .02
Placebo	0.90 ± .01	0.89 ± .01	0.92 ± .01	0.94 ± .02
Lactate				
Whey	2.15 ± .22	2.02 ± .18	3.53 ± .47	4.24 ± .64
Placebo	2.11 ± .29	1.96 ± .29	3.75 ± .52	4.27 ± .61

Note. All data is reported as means ± standard error. RER = respiratory exchange ratio.

Table 5

Cardiorespiratory and Perceptual

Variable	10	20	30	40	50	60
Heart Rate						
Whey	138.8 ± 3.7	143.4 ± 3.3	144.8 ± 3.6	154.6 ± 3.8	156.2 ± 4.2	157.9 ± 3.5
Placebo	136.9 ± 4.6	137.8 ± 3.9	139.4 ± 4.9	154.9 ± 3.6	154.7 ± 3.1	159 ± 2.9
RPE						
Whey	11.4 ± 0.4	12.3 ± 0.4	13.4 ± 0.5	14.8 ± 0.5	15.6 ± 0.5	17 ± 0.5
Placebo	11.25 ± 0.6	11.95 ± 0.4	12.75 ± 0.5	14.7 ± 0.4	15.75 ± 0.3	17.1 ± 0.4
Leg Pain						
Whey	3.55 ± 0.7	4.35 ± 0.5	5.1 ± 0.5	6.35 ± 0.5	6.95 ± 0.5	7.8 ± 0.5
Placebo	3.65 ± 0.7	4.25 ± 0.6	4.75 ± 0.5	6.25 ± 0.5	7.15 ± 0.3	8 ± 0.3

Note. All data is reported as means ± standard error. RPE = rating of perceived exertion.

CHAPTER V

DISCUSSION AND SUMMARY

To the author's knowledge, this is the first study to examine the influence of a whey protein preload prior to CHO consumption on aerobic cycling performance and metabolic outcomes. The primary purpose of this study was to examine the influence of a whey protein preload prior to CHO consumption compared to the consumption of CHO alone on time trial (TT) performance, glycemic responses (e.g., glucose, insulin, & glucagon), substrate utilization, cardiovascular, and perceptual outcomes during a bout of aerobic cycling in recreational trained cyclists and triathletes.

The primary findings from this study were the following: (1) there was no significant difference in time trial performance between the whey protein trial (WP) and the placebo trial (PL); (2) there was a significant increase in plasma insulin concentrations during the whey protein at the beginning of the cycling performance trial (CPT: timepoint 0); (3) there was a significant crossover interaction effect for plasma glucose; and (4) there were no significant differences in substrate utilization or mobilization (e.g., RER and NEFA) between the two trials.

Cycling Performance

This study showed that a whey protein preload prior to a bolus of CHO did not improve 30 min TT performance when compared to a placebo preload (WP = 16.8 ± 0.34 km and PL = 17 ± 0.4 km) in recreationally trained triathletes and cyclists. Additionally,

there was no significant difference between average absolute power outputs during the TT (WP = 202.63 ± 16.03 W vs. PL = 213.17 ± 16.03 W). Previous literature examining the influence of whey protein co-ingested with CHO during cycling has reported inconsistent findings (Breen et al., 2010; Highton, Twist, Lamb, & Nicholas, 2013; Ivy et al., 2003; Saunders et al., 2007; Van Essen & Gibala, 2006). Ivy et al. (2003) reported a 36% improvement in time to exhaustion when whey protein was co-ingested with a CHO (1.94% protein + 7.75% CHO beverage) compared to the consumption of CHO alone (7.75% CHO beverage). Saunders et al. (2007) also reported a 13% improvement in time to exhaustion when whey protein was co-ingested with CHO (0.038 g protein + 0.15 g CHO kg/BW beverage) compared to the consumption of CHO only (0.15 g CHO kg/BW beverage). These results lead other researchers to challenge these findings. Van Essen and Gibala (2006) stated the CHO intake of the participants in the aforementioned studies was not an optimal dose for the exercise duration and intensity (Ivy et al., [2003] ~47 g/CHO/hr and Saunders et al., [2007] ~37 g/CHO/hr). Van Essen and Gibala (2006) hypothesized the whey protein consumed during the bout of cycling simply provided extra calories, therefore providing more energy during the time to exhaustion test. The optimal dosing of CHO intake during exercise is advocated to be ~ 60-70 g/CHO/ hr (Jeukendrup, 2004). Van Essen and Gibala (2006) provided a solution of 2% protein and 6% CHO, the equivalent of 20 g of whey protein per hour and 60 g of CHO per hour during an 80-km TT. When compared to the consumption of only 60 g of CHO per hour, there was no significant difference between the two trials (Whey protein + CHO = 135 ± 2 min vs. CHO = 135 ± 2 min). Breen et al. (2010) also reported no significant

differences in late stage time trial performance when participants were provided 65 g/CHO/hr or 65 g/CHO/hr co-ingested with 19 g/PRO/hr (CHO only = 247 ± 11 W and whey protein co-ingested with CHO = 247 ± 13 W).

The average CHO intake prior to the CPT in the current study was 53.9 ± 8.6 g, which is consider a less than the optimal dose of CHO intake for 60 min of cycling at a moderate to vigorous exercise intensity (Jeukendrup, 2004). However, we did not want to provide participants with an absolute dose of CHO, therefore, CHO intake was based off lean body mass. The average whey protein intake was 41.9 ± 6.7 g, which is equivalent to 167.6 calories. Therefore, with the addition of an extra 167.6 calories provided from the whey protein, one may speculate the extra calories would produce an increase in TT performance based on previous research. However, this was not true for the current study. Results from this study showed no significant differences between the two experimental trials. Even though the whey protein provided additional calories, it seemed the average CHO intake was sufficient to provide enough energy for the 60 min of cycling. Although it was not significantly different, the average absolute power output during the placebo trial was 5.3% higher than the whey protein trial (WP = 202.63 ± 9.58W vs. PL = 212.17 ± 12.68W). If a cyclist/triathlete can improve absolute power out by 10 W but exclude whey protein prior to exercise, these results may provide practical application.

Insulin Response

One of the central metabolic responses of the whey protein preload was a hyperinsulinemic response at the beginning of the CPT (see Figure 9). At timepoint 0, WP produced a 61.43% higher plasma insulin concentration when compared to PL.

Although the proceeding timepoints failed to reach significance, it is noteworthy to mention plasma insulin concentrations remained elevated in WP by 25.7% (timepoint 15), 30.35% (timepoint 30), 43.16% (timepoint 45), and 46.01% (timepoint 60) during the CPT when compared to PL. A theoretical mechanism cited by previous researchers hypothesized the insulinogenic response from whey protein may potentiate exogenous CHO utilization and invoke a muscle glycogen sparing effect and thus, improve late stage time trial performance (Ivy et al., 2003; Saunders, 2007). Previous literature has examined the insulin response from whey protein during cycling, however, the dosing of whey protein was too small to further stimulate an increase in plasma insulin during exercise when compared to the consumption of CHO alone (Highton et al., 2013;Van Essen & Gibala, 2006).

Stimulating an insulinogenic response from whey protein before or during exercise has yet to be examined. Previous literature has shown the consumption of a whey protein (18-50 g) preload or co-ingested with a CHO source (e.g., glucose) at rest stimulates a hyperinsulinemic response, delays gastric emptying and improves blood glucose values over time (Adams & Broughton, 2016; Nilsson et al., 2007; Petersen et al., 2009; Stanstrup, Schou, Holmer-Jensen, Hermansen, & Dragsted, 2014; Stevenson & Allerton, 2017). Nilsson et al. (2007) reported a 60% increase in serum insulin $AUC_{0\text{-}120}$ when 18g of whey protein were consumed with 25 g of glucose compared to the consumption of glucose alone. Roberts et al., (2012) also reported ~59% increase in insulin $AUC_{0\text{-}120}$ when 20 g of whey protein was co-ingested with 50 g of glucose compared to the consumption of only 50 g of glucose. Similar to the current findings,

both of these studies also reported a peak insulin response 30 min following the consumption of the whey protein. However, the aforementioned studies examined the metabolic response of whey protein at rest, this study observed the response during exercise.

The hyperinsulinemic response at the beginning of the CPT (timepoint 0), in addition to the potential to delay gastric emptying, was theorized to stabilize blood glucose levels over time. This mechanism has been presented in previous literature at rest and post-exercise (Kaastra et al., 2006; Roberts et al., 2013; van Loon et al., 2000a; van Loon et al., 2000b;). However, the hyperinsulinemic response at the beginning of the CPT (see Figure 9) promoted an abrupt decrease in blood glucose levels 15 min into the CPT (see Figure 8), invoking a physiologically response referred to as rebound hypoglycemia (Jentjens & Jeukendrup, 2002; Jeukendrup & Killer, 2003).

Rebound Hypoglycemia

The consumption of whey protein as a preload to a bolus of CHO invoked a physiological response called *rebound hypoglycemia* (Jentjens & Jeukendrup, 2002; Jeukendrup & Killer, 2003). Rebound hypoglycemia can simply be defined as the sudden decline in blood glucose levels below baseline with a subsequent return back to baseline values or above (Kondo, Tanisawa, Suzuki, Terada, & Higuchi, 2019; Short, Sheffield-Moore, & Costill, 1997). Previous literature has shown that pre-exercise CHO consumption 15-30 min prior to a bout of exercise may cause a significant decline in blood glucose levels (≤ 70 mg/dl); however, this is dose dependent (Jeukendrup & Killer, 2010; Kondo, et al., 2019; Short et al., 1997). Although this value does not represent

clinical hypoglycemic (< 40 mg/dl), it simply refers to drop in blood glucose levels at the beginning of exercise, generally below baseline (Short et al., 1997). The consumption of a high glycemic substance (e.g., dextrose) 15-30 minutes before a bout of exercise can cause a rapid increase in blood glucose. An increase in blood glucose levels stimulates the β-cells of the pancreas to secrete insulin (Rorsman et al., 2000).

Insulin concentrations following a high glycemic substance (e.g., glucose) have been shown to peak around 30 minutes following consumption (Bloomer, Peel, Moran, & MacDonnchadh, 2016). Short et al. (1997) examined the influence of varying doses of pre-exercise CHO intake on the glycemic kinetics during exercise. Participants were provided 22 g, 45 g, or 75 g of a maltodextrin/dextrose or a placebo beverage 30-60 min prior to cycling at 66% VO_2max for 120 min. Authors noted all doses promoted a significant decline in blood glucose values 15 min into the exercise bout when compared to the placebo trial, with 75 g stimulating the greatest decline. There was also a dose-dependent response in serum insulin concentrations, with 75 g of the maltodextrin/dextrose beverage eliciting the highest insulin concentration. Kondo et al. (2019) also reported a hypoglycemic effect when participants consumed 150 g of glucose 30 min prior to cycling for 60 min at 75% VO_2max. Participants experience a sudden decline in blood glucose levels 15 min into the exercise bout (peak nadir = 127.8 mg/dl to 88.2 mg/dl). There was also a 14.0-fold increase in serum insulin concentration 15 min before the sudden decline in blood glucose values. In the current experiment, the PL did not stimulate a sudden decline in blood glucose values below baseline during the CPT.

Average resting blood glucose concentrations were 87.0 mg/dl during the PL trial. The average intake of glucose 10 min prior to beginning the CPT was ~54 g. Contrary to previous findings, participants blood glucose values remained stable at 15 (89.3 mg/dl) and 30 min (90 mg/dl) into the CPT, despite a 56% and 49% increase in plasma insulin concentrations above baseline (37.85 pg/ml) at timepoint 0 (85.95 pg/ml) and 15 (73.77 pg/ml), respectively. However, WP did stimulate a rebound hypoglycemic effect during the CPT. A driving impetus to consume whey protein prior to exercise was to mitigate the possibility of experiencing a sudden decline in blood glucose levels to due its ability to delay gastric emptying and stabilize blood glucose levels at rest (Kaastra et al., 2006; Shelley et al., 2013; van Loon et al., 2000a; van Loon et al., 2000b;). Although, gastric emptying rate was not measured during the current study, it appeared to not play a role in aiding in glycemic regulation due to the rebound hypoglycemic effect witnessed. At the beginning of the CPT, plasma insulin concentration was 61.43% higher (p = .047) during WP (222.88 pg/dl) than PL (85.95 pg/dl). The hyperinsulinemic response at timepoint 0 during WP also promoted a 27% drop in blood glucose values below baseline 15 min into the CPT. In addition to insulin's role in glucose uptake, the addition of skeletal muscle contractions (e.g., cycling) can promote a synergistic effect on blood glucose levels. It has been well established that skeletal muscle contractions also aid in the uptake and disposal of blood glucose levels through GLUT4 phosphorylation and translocation to the cell membrane surface (Richter & Hargreaves, 2014). However, the homeostatic mechanism to regulate the drop in blood glucose levels induces a secretion of counter-regulatory hormones to rebalance glycemic homeostasis.

Counter-Regulatory Hormones

In order to inhibit a severe drop in blood glucose levels due to the synergist effect of hyperinsulinemia from whey protein combined with skeletal muscle contractions, the hormone glucagon is secreted from the α-cells of the pancreas (Ferrier, 2014). Glucagon stimulates glycogenolysis and gluconeogenesis in the liver in response to a drop in blood glucose levels (Brooks et al., 2005). Previous literature has shown a significant glucagon response to the consumption of whey protein (Claessens et al., 2008). Claessens et al. (2008) provided 0.6 g/kg of whey protein to non-obese subjects at rest. Plasma glucagon concentrations peaked 30 min (~175 pg/ml) after the consumption of the whey protein. In the current study, participants consumed 0.7 g/kg/LBM of whey protein prior to the CPT. Similar to Claessens et al. (2008), plasma glucagon concentrations were 219 pg/ml 30 min following the consumption of the whey protein, but PL only achieved a plasma glucagon concentration of 102.5 pg/ml. However, in the current study, plasma glucagon concentrations peaked 15 min into the CPT (371.32 pg/ml), 45 min after the consumption of the whey protein (see Figure 11). Glucagon concentrations peaked 45 min after the consumption of the whey protein due to the hyperinsulinemic response at the beginning of the CPT, followed by the sudden drop in blood glucose levels at timepoint 15.

In order to regulate blood glucose levels, glucagon stimulates hepatic glycogenolysis to restore blood glucose homeostasis (Taborsky, 2010). Following peak glucagon concentration during WP, blood glucose values at the 30 min time point were back to baseline values (87 mg/dl). Although speculative, other counter-regulatory hormones, such as epinephrine, may have been involved in decreasing plasma insulin

concentration and thus, further regulate blood glucose levels (Brooks et al., 2005; Gelfand, Matthews, Bier, & Sherwin, 1984; Sprague & Arbeláez, 2011). Previous literature has shown that aerobic exercise > 60% of VO_2max stimulates a large increase in plasma epinephrine levels (Brooks et al., 2005; Hackney, 2006). Individuals in this study were cycling at 90% of LT for 30 min, followed by a 30 min TT. Norepinephrine secretion has also been purported to inhibit glucose-dependent insulin secretion (Ahrén, 2000; Thorens, 2014). At rest, norepinephrine targets the β-cell 2α-adrenergic receptor and prevents the secretion of insulin (Rosengren et al., 2010). The exercise intensity in the current study should have been sufficient to drive an increase in epinephrine and norepinephrine levels during exercise, aiding in the decrease in plasma insulin concentration.

Substrate Utilization

One of the theoretical purposes of consuming whey protein as a preload prior to aerobic exercise was to increase exogenous CHO utilization during exercise (Ivy et al., 2003). Increasing exogenous CHO utilization during exercise allows the athlete to maintain moderate-vigorous exercise intensity and possibly promote a glycogen sparing effect, which may prove to be critical during the late stages of an endurance race. This study hypothesized respiratory exchange ratio (RER) would increase and NEFA concentration would decrease during WP compared to PL, indicating an increase in CHO oxidation. However, there were no significant differences between RER or NEFA concentrations for any of the timepoints when comparing WP to PL.

During submaximal aerobic exercise ~50-60% VO_{2max}, there is an increase in NEFA concentration, promoting an increase in fat availability for oxidation (Bassami, Ahmadizad, Doran, & MacLaren, 2007; van Hall, 2015). Maximal fat oxidation rates have been shown to be around ~50% VO_{2max} (Randell et al., 2017). During this study, participants were above the maximal fat oxidation intensity; therefore, it was expected to observe a decrease in NEFA concentrations below baseline levels. Due to the insulinogenic effect of whey protein, it was theorized there would a decline in NEFA concentration during WP compared to PL. Stimulating an increase in plasma insulin concentrations stimulates lipoprotein lipase located in adipose tissue to promote fat storage and inhibits degradation (Brooks et al., 2005). Insulin also inhibits hormone sensitive lipase located with the skeletal muscle tissues that inhibits fat oxidation (Brooks et al., 2005). However, it appeared the hyperinsulinemic effect of whey protein did not influence fat metabolism during the CPT due to the negligible difference in RER and NEFA concentration when compared to PL.

The lack of NEFA concentrations between the two trials may have been due to the exercise intensity of the exercise bout, however, this is only speculation. Both trials produced similar exercise intensities, which is the one of the overall primary drivers of influencing a change in substrate utilization (Brooks et al., 2005).

The significant increase in glucagon has also been shown to influence substrate metabolism (Brooks et al., 2005; Goodwin, 2010). One of the primary effects of increase glucagon concentrations is the inhibition of fatty acid synthesis by phosphorylating ACC (Ferrier, 2014). Glucagon also inhibits the glycolytic metabolism by inhibiting PFK-1 in

the liver (Ferrier, 2014). Although WP elicited a significant increase in plasma glucagon during the WP compared to the PL, there were no differences in RER or NEFA concentrations compared to PL.

Finishing Summary

The consumption of a whey protein preload prior to CHO consumption resulted in no significant difference in TT performance when compared to the consumption of CHO alone. Contrary to previous findings, the PL trial did not induce a rebound hypoglycemic effect, however, the consumption of the whey protein preload did. The WP trial triggered a hyperinsulinemic response with a drastic rise in plasma glucagon concentration during the CPT.

Limitations

The involvement of a diverse group of endurance athletes in a research study may bring up the possibility of specific limitations to the research design. One limitation of the current study is the use of an electronically controlled cycle ergometer to test cycling performance. This may have presented the athletes with two issues: (1) the use of different cycle ergometer and fit and (2) having to cycle indoors for the study without any external stimulus (e.g., music or television). This may interfere with ecological validity; however, this method insures consistency among the participants. All participants were also required to come into the laboratory in a fasted state and begin a bout of cycling in the morning (e.g., between 7:00-9:00 A.M.). The manipulation of pre-exercise nutrients and timing of exercise may have influenced the athlete's perceived readiness before the

exercise bout began. Although one female was included in this study, statistical analysis was performed with just all males to observe if the one female stimulated a change in the results. However, the statistical analysis was the same with the female removed. Another possible limitation was the potential fluctuation in nutrient intake between the two trials for 4 of the 10 participants. Only 6 participants turned in and replicated their 3-day food log diary, while 4 of the participants failed to turn back in their dietary analysis. There was a potential for inadequate pre-exercise fueling between the experimental trials. Lastly, the current study did have a small sample size and therefore was underpowered. For example, the effect size for distance covered during the 30-min time trial was d = 0.166 (small effect).

Future Studies

1) Examining the influence of varying doses of a whey protein preload prior to CHO consumption of overall cycling performance. The current study only examined one dose of whey protein. The dosing in the current study has been shown to elicit a significant insulin response at rest (Nilsson et al, 2007; Akhavan et al., 2014). Future studies can begin to examine the influence of varying doses.

2) To further explore the mechanisms as to why the insulinogenic response from the whey protein drastically declines during exercise. Future studies can examine the concentration of the incretin hormones (GLP-1 and GIP) prior to and during exercise. Whey protein has been shown to stimulate an increase in the concentration of incretin hormones and further augment insulin secretion (Baggio & Drucker, 2007; Jakubowicz & Froy, 2013). The concentrations of incretin hormones during exercise may help further

understand the insulin decline. Measuring the catecholamines, norepinephrine, and epinephrine may also help explain the mechanistic decline in plasma insulin concentration during exercise.

3) Casein protein has been shown to have a slow absorption rate and aid in the delay of gastric emptying (Dangin et al., 2001). Due to its potential to further delay gastric emptying, it may be pertinent to compare the performance and metabolic outcomes of whey protein and casein protein as a preload to aerobic cycling performance.

REFERENCES

Abdul-Ghani, M. A., & DeFronzo, R. A. (2010). Pathogenesis of insulin resistance in skeletal muscle. *Journal of Biomedical Biotechnology, 476279*, 1-19. doi: 10.1155/2010/476279.

Adams, R. L., & Broughton, K. S. (2016). Insulinotropic effects of whey: Mechanisms of action, recent clinical trials, and clinical applications. *Annals of Nutrition and Metabolism, 69*(1), 56-63. doi:10.1159/000448665

Ahrén, B. (2000). Autonomic regulation of islet hormone secretion- Implications for health and disease. *Diabetologia, 43*, 393-410.

Akhavan, T., Luhovyy, B. L., Brown, P. H., Cho, C. E., & Anderson, G. H. (2014). Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin response in young adults. *American Journal of Clinical Nutrition*, 91, 966-975.

Alghannam, A. F., Jedrzejewski, D., Tweddle, M. G., Gribble, H., Bilzon, J., Thompson, D., Tsintzas, K., & Betts, J. A. (2016). Impact of muscle glycogen availability on the capacity for repeated exercise in man. *Medicine Science Sports & Exercise, 48*(1), 123-131.

American Red Cross. (2018). Blood needs and blood supply. https://www.redcrossblood.org/donate-blood/how-to-donate/how-blood-donations-help/blood-needs-blood-supply.html. September, 21, 2019.

Baggio, L. L., & Drucker, D. L. (2007). Biology of incretins. *Gastroenterology*, *132*, 2131-2157.

Baker, J. S., & Buchan, D. (2017). Metabolic stress and high intensity exercise. *Physical Medicine & Rehabilitation Research, 2*(1), 2-2.

Bassami, M., Ahmadizad, S., Doran, D., & MacLaren, D. P. (2007). Effects of exercise intensity and duration of fat metabolism in trained and untrained males. *European Journal of Applied Physiology, 101*(4), 525-532.

Bendtsen, L. Q., Lorenzen, J. K., Bendsen, N. T., Rasmussen, C., & Astrup, A. (2013). Effect of dairy proteins on appetite, energy expenditure, body weight, and composition: a review of the evidence from controlled clinical trials. *Advanced Nutrition*, 4, 418-438.

Betts, J. A., & Stevenson, E. (2011). Should protein included in CHO-based sports supplements. *Medicine & Science in Sports & Exercise*, *43*(7), 1244-1250. doi: 10.1249/MSS.0b013e31821b0dcc

Betts, J. A., Williams, C., Boobis, L., & Tsintzas, K. (2008). Increased carbohydrate oxidation after ingesting carbohydrate with added protein. *Medicine & Science in Sports & Exercise, 40*(5), 903-912.

Bloomer, R. J., Peel, S. A., Moran, R. G., & MacDonnchadh, J. J. (2016). Blood glucose and insulin response to artificially- and sugar-sweetened sodas in healthy men. *Integrated Food in Nutrition & Metabolism, 3*(1), 268-272.

Borg, G. (1998). *Borg's perceived exertion and pain scale*. Champaign, IL: Human Kinetics.

Breen, L., Tipton, K. D., & Jeukendrup, A. E. (2010). No effect of carbohydrate-protein on cycling performance and indices of recovery. *Medicine and Science in Sports and Exercise, 42*(6), 1140. doi:10.1249/MSS.0b013e3181c91f1a

Brooks, G. A., Fahey, T. D., & Baldwin, K. M. (2005). *Exercise Physiology: Human bioenergetics and its application. Fourth Edition*. New York, NY: McGraw-Hill.

Brooks, G. A., & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: The "crossover" concept. *Journal of Applied Physiology, 76*(6), 2253-2261. doi:10.1152/jappl.1994.76.6.2253

Bukhari, S. S. I., Phillips, B. E., Wilkinson, D. J., Limb, M. C., Rankin, D., Mitchell, W. K., Kobayashi, H., Greenhaff, P. L., Smith, K., & Atherton, P. J. (2015). Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. *American Journal of Physiology & Endocrinology Metabolism*, 308, E1056-E1065.

Carnagarin, R., Dharmarajan, A. M., & Dass, C. R. (2015). Molecular aspects of glucose homeostasis in skeletal muscle - A focus on the molecular mechanisms of insulin resistance. *Molecular and Cellular Endocrinology, 417*, 52-62.

Chang, L., Chiang, S. H., & Saltiel, A. R. (2004). Insulin signaling and the regulation of glucose transport. *Molecular Medicine*, *10*(7-12): 65-71.

Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A., & Hesselink, M. (1992). A new approach for determination of ventilatory and lactate threshold. *International Journal of Sports Medicine, 13*(7), 518-522.

Claessens, M., Saris, W. H., & van Baak M. A. (2008). Glucagon and insulin responses after ingestion of different amounts of intact and hydrolysed proteins. *British Journal of Nutrition, 100*(1), 61-69.

Coyle, E. F., Hagberg, J. M., Hurley, B. F., Martin, W. H., Ehsani, A. A., & Holloszy, J. O. (1983). Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *Journal of Applied Physiology, 55*(1), 230-235. doi:10.1152/jappl.1983.55.1.230

Dangin, M., Boirie, Y., Garcia-Rodenas, C., Gachon, P., Fauquant, J., Callier, P., & Beaufrere, B. (2001). The digestion rate of protein is an independent regulating factor of postprandial protein retention. *American Journal of Physiology-Endocrinology And Metabolism, 280*(2), E340-E348.

de Diego, A. M. G, Gandia, L., & Garcia, A. G. (2008). A physiological view of the central and peripheral mechanisms that regulate the release of catecholamines at the adrenal medulla. *Acta Physiologica, 192*(2), 287-301. doi:10.1111/j.1748-1716.2007.01807.

DeFronzo, R. A., Jacot, E., Je'quier, E., Maeder, E., Wahren, J., & Felber, J. P. (1981). The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catherization. *Diabetes, 30*, 1000-10007.

El-Sayed, M. S., Balmer, J., & Rattu, A. J. (1996). Carbohydrate ingestion improves endurance performance during a 1 h simulated cycling time trial. *Journal of Sports Sciences, 15*(2), 223.

Erdek, M. A., & Pronovost, P. J. (2004). Improving assessment and treatment of pain in the critically ill. *International Journal for Quality in Health Care: Journal of the International Society for Quality in Health Care, 16*(1), 59-64. doi:10.1093/intqhc/mzh010

Febbraio, M. A., Lambert, D. L., Starkie, R. L., Proietto, J., & Hargreaves, M. (1985). Effect of epinephrine on muscle glycogenolysis during exercise in trained men. *Journal of Applied Physiology, 84*(2), 465-470.

Fernández-García, B., Perez-Landaluce, J., Rodriguez-Alonso, M., & Terrados, N. (2000). Intensity of exercise during road race pro-cycling competition. *Medicine & Science in Sports & Exercise, 32*(5), 1002-1006.

Ferrier, D. R. (2014). *Biochemistry & nbsp;* (6th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Fox, S. M., Naughton, J. P., & Haskell, W. L. (1971). Physical activity and the prevention of coronary heart disease. *Annuals of Clinical Research, 3*(6), 404-432.

Gangurde, H. (2011). Whey Protein. *Scholars Research Journal, 1*(2), 69.

Gelfand, R. A., Matthews, D. E., Bier, D. M., & Sherwin, R. S. (1984). Role of counterregulatory hormones in the catabolic response to stress. *Journal of Clinical Investigation, 74*, 2238-2248.

Goodpaster, B. H., Costill, D. L., Fink, W. J., Trappe, T. A., Jozsi, A. C., Starling, R. D., & Trappe, S. W. (1996). The effects of pre-exercise starch ingestion on endurance performance. *International Journal of Sports Medicine, 17*, 366-372.

Goodwin, M. L. (2010). Blood glucose regulation during prolonged, submaximal, continuous exercise: A guide for clinicians. *Journal of Diabetes Science and Technology, 4*(3), 694.

Gorissen, S. H. M., Crombag, J. J. R., Senden, J. M. G., Waterval, W. A. H., Bierau, J., Verdijk, L. B., & van Loon, L. J. C. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids, 50*, 1685-1695.

Greiwe, J. S., Hickner, R. C., Hansen, P. A., Racette, S. B., Chen, M. M., & Holloszy, J. O. (1985). Effects of endurance exercise training on muscle glycogen accumulation in humans. *Journal of Applied Physiology, 87*(1), 222-226.

Hackney, A. C. (2006). Stress and the neuroendocrine system: The role of exercise as a stressor and modifier of stress. *Expert Review of Endocrinology & Metabolism, 1*(6), 783-792.

Hansen, M. Bangsbo, J., Jensen, J., Bibby, B. M., & Madsen, K. (2015). Effect of whey protein hydrolysate on performance and recovery of top-class orienteering runners. *International Journal of Sport Nutrition & Exercise Metabolism, 25*, 97-109.

Hargreaves, M., & Briggs, C. A. (1985). Effect of carbohydrate ingestion on exercise metabolism. *Journal of Applied Physiology, 65*(4), 1553-1555.

Hawley, J. A., & Burke, L. M. (1997). Effect of meal frequency and timing on physical performance. *British Journal of Nutrition, 77*(S1), S103. doi:10.1079/BJN19970107

Hearris, M. A., Hammond, K. M., Fell, J. M., & Morton, J. P. (2018). Regulation of muscle glycogen metabolism during exercise: Implications for endurance performance and training adaptations. *Nutrients, 10*(3), 298. doi:10.3390/nu10030298

Henriksen, E. J. (2002). Exercise effects of muscle insulin signaling and action invited review: Effect of acute exercise and exercise training on insulin resistance. *Journal of Applied Physiology*, *93*, 788-796.

Highton, J., Twist, C., Lamb, K., & Nicholas, C. (2013). Carbohydrate-protein coingestion improves multiple-sprint running performance. *Journal of Sports Science*, *31*(4), 361-369.

Huang, S., & Czech, M. P. (2007). The GLUT4 glucose transporter. *Cell Metabolism*, *5*(4), 237-252.

Howley, E. T., Bassett, J. R., & Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Medicine & Science in Sports & Exercise*, *27*(9), 1292-1301.

Ivy, J. L., Res, P. T., Sprague, R. C., & Widzer, M. O. (2003). Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. *International Journal of Sport Nutrition and Exercise Metabolism, 13*(3), 382-395. doi:10.1123/ijsnem.13.3.382

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J.,Wells, S. D., Skwiat, T. M., & Wilborn C. D. (2017). International society of sports nutrition position stand: Protein and exercise. *Journal of the International Society of Sports Nutrition, 14*, 1-25. doi:10.1186/s12970-017-0177-8

Jakubowicz, D., & Froy, O. (2013). Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and type 2 diabetes. *Journal of Nutrient Biochemistry*, *24*, 1-5.

Jensen, J., Rustad, P. I., Kolnes, A. J., & Lai, Y. (2011). The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. *Frontiers in Physiology, 2*, 112. doi:10.3389/fphys.2011.00112

Jentjens, R. L., & Jeukendrup, A. E. (2002). Prevalence of hypoglycemia following pre-exercise carbohydrate ingestion is not accompanied by higher insulin sensitivity. *International Journal of Sport Nutrition, Exercise. & Metabolism, 12*(4), 398-413.

Jeukendrup, A. E. (2004). Carbohydrate intake during exercise and performance. *Nutrition, 20*(7), 669-677. doi:10.1016/j.nut.2004.04.017

Jeukendrup, A. E. (2008). Carbohydrate feeding during exercise. *European Journal of Sport Science, 8*(2), 77-86. doi:10.1080/17461390801918971

Jeukendrup, A. E., & Killer, S. C. (2003). The myths surrounding pre-exercise carbohydrate feeding. *Annuals of Nutrient Metabolism, 57*(2 Suppl), 18-25.

Joyner, M. J., & Coyle, E. F. (2008). Endurance exercise performance: The physiology of champions. *The Journal of Physiology, 586*(1), 35-44. doi:10.1113/jphysiol.2007.143834

Kaastra, B., Manders, R. J. F., van Breda, E., Kies, A., Jeukendrup, A. E., Keizer, H. A., Kuipers, H., & van Loon, L. J. C. (2006). Effects of increasing insulin secretion on acute postexercise blood glucose disposal. *Medicine & Science in Sports & Exercise, 38*(2), 268-275.

Karim, S., Adams, D. H., & Lalor, P. F. (2012). Hepatic expression and cellular distribution of the glucose transporter family. *World Journal Gastroenterology, 18*(46), 6771-6781.

Klip, A., Sun, Y., Chiu, T. T., & Foley, K. P. (2014). Signal transduction meets vesicle traffic: The software and hardware of GLUT4 translocation. *American Journal of Physiology-Cell Physiology,* 306(10): C879-C886.

Knuiman, P., Hopman, M. T., & Mensink, M. (2015). Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. *Nutrition & Metabolism (Lond), 12*(59).

Kondo, S., Tanisawa, K., Suzuki, K., Terada, S., & Higuchi, M. (2019). Preexercise carbohydrate ingestion and transient hypoglycemia: Fasting versus feeding. *Med Sci Sports Exerc, 51*(1), 168-173.

Krissansen, G. W. (2007). Emerging health properties of whey protein and their clinical implications. *Journal of the American College of Nutrition*, 26(6), 713S-723S.

Layman, D. K. (2003). The role of leucine in weight loss diets and glucose homeostasis. *Journal of Nutrition*, 133(1), 26.

Ma, J., Stevens, J. E., & Cukier, K. (2009). Effects of protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care, 32*, 1600-1602.

Madureira, A. R., Tavares, T., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2010). Invited review: physiological properties of bioactive peptides obtained from whey proteins. *Journal of Dairy Science*, 93, 437-455.

Maunder, E., Kilding, A., & Plews, D. (2018). Substrate metabolism during ironman triathlon: Different horses on the same courses. *Sports Medicine, 48*(10), 2219-2226. doi:10.1007/s40279-018-0938-9

McLellan, T., Pasiakos, S., & Lieberman, H. (2014). Effects of protein in combination with carbohydrate supplements on acute or repeat endurance exercise performance: A systematic review. *Sports Medicine, 44*(4), 535-550. doi:10.1007/s40279-013-0133-y

Mead, J., Irvine, S., & Ramji, D. (2002). Lipoprotein lipase: Structure, function, regulation, and role in disease. *Journal of Molecular Medicine, 80*(12), 753-769. doi:10.1007/s00109-002-0384-9

Morifuji, M., Kanda, A., Koga, J., Kawanaka, K., & Higuchi, M. (2011). Preexercise ingestion of carbohydrates plus whey protein hydrolysates attenuates skeletal muscle glycogen depletion during exercise in rats. *Nutrition, 27*(7-8), 833-837.

Nilsson, M., Holst, J. J., & Björck, M. E. (2007). Metabolic effects of amino acid mixtures and whey protein in healthy subjects: Studies using glucose-equivalent drinks. *American Journal Clinical Nutrition*, 85, 996-1004.

Nilsson, M., Stenberg, M., Frid, A. H., Holst, J. J., & Bjorck, I. M. (2004). Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: The role of plasma amino acids and incretins. *American Journal of Clinical Nutrition, 80*(5), 1246-1253.

Oosthuyse, T., Carstens, M., & Millen, A. (2015). Whey or casein hydrolysate with carbohydrate for metabolism and performance in cycling. *International Journal of Sports Medicine, 36*(8), 636-646. doi:10.1055/s-0034-1398647

Ormsbee, M. J., Bach, C. W., & Baur, D. A. (2014). Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance. *Nutrients*, *6*(5), 1782-1808.

Pfeiffer, B., Stellingwerff, T., Zaltas, E., & Jeukendrup, A. E. (2010a). Oxidation of solid versus liquid CHO sources during exercise. *Medicine & Science in Sports & Exercise, 42*(11), 2030-2037.

Pfeiffer, B., Stellingwerff, T., Zaltas, E., & Jeukendrup, A. E., (2010b). CHO oxidation from a CHO gel compared with a drink during exercise. *Medicine & Science in Sports & Exercise, 42*(11), 2038-2045.

Petersen, B. L., Ward, L. S., Bastian, E. D., Jenkins, A. L., Campbell, J., & Vuksan, V. (2009). A whey protein supplementation decreases post-prandial glycemia. *Nutrition Journal*, 8(47), 1-5.

Pinillos, F. G., Camara-Perez, J. C., Hermoso, V. M., & Roman, P. A. L. (2016). A HIIT-based running plan improves athletic performance by improving muscle power. *Journal of Strength and Conditioning Research, 31*(1).

Ranallo, R. F., & Rhodes, E. C. (1998). Lipid metabolism during exercise. *Sports Medicine, 26*(1), 29-42.

Randell, R. K., Rollo, I., Roberts, T. J., Dalrymple, K. J., Jeukendrup, A. E., & Carter, J. M. Maximal fat oxidation rates in an athletic population. (2017). *Medicine & Science in Sports & Exercise, 49*(1), 133-140.

Rayner, C. K., Ma, J., Jones, K. L., Clifton, P. M., & Horowitz, M. (2014). Protein "pre-loads" in type 2 diabetes: What do we know and what do we need to find out? *Diabetologia, 57,* 2603-2604.

Richter, E. A., & Hargreaves, M. (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews, 93*(3), 993-1017. doi:10.1152/physrev.00038.2012

Romano-Ely, B. C., Todd, M. K., Saunders, M. J., & Laurent, T. S. (2006). Effect of an isocaloric carbohydrate-protein-antioxidant drink on cycling performance. *Medicine and Science in Sports and Exercise, 38*(9), 1608-1616. doi:10.1249/01.mss.0000229458.11452.e9

Rorsman, P., Eliasson, L., Renstrom, E., Gromada, J., Barg, S., & Gopel, S. (2000). The cell physiology of biphasic insulin secretion. *News Physiological Science, 15*, 72-77.

Rosengren, A. H., Jokubka, R., Tojjar, D., Granhall, C., Hansson, O., Li, D. Q., Nagaraj, V.(2010). Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science, 327*(5962), 217-220.

Running USA. (2016). 2016 Annual Marathon Report. Advanced Solutions International, Inc. "Industry News." *Home*, www.runningusa.org/RUSA.

Sakamoto, K., & Holman, G. D. (2008). Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *American Journal of Physiology. Endocrinology, & Metabolism*, *295*, E29-E37.

Saunders, M. J. (2007). Coingestion of carbohydrate-protein during endurance exercise: Influence on performance and recovery. *International Journal of Sport Nutrition & Exercise Metabolism, 17*, S87-S103.

Saunders, M. J., Luden, N. D., & Herrick, J. E. (2007). Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. *Journal of Strength and Conditioning Research, 21*(3), 678-684.

Seino, Y., Fukushima, M., & Yabe, D. (2010). GIP and GLP-1, the two incretin hormones: Similarities and differences. *Journal of Diabetes Investigation*, 1(1/2), 8-23.

Shelley, R. M., Desbrow, B., Grant, G., Anoopkumar-Dukie, S., & Leveritt, M. (2013). Glycemic response to carbohydrate and the effects of exercise and protein. *Nutrition, 29*(6), 881-885. doi:10.1016/j.nut.2012.12.022

Sherman, W. M., Peden, M. C., & Wright, D. A. (1991). Carbohydrate feedings 1 h before exercise improves cycling performance. *The American Journal of Clinical Nutrition, 54*(5), 866-870. doi:10.1093/ajcn/54.5.866

Short, K. R., Sheffield-Moore, M., & Costill, D. L. (1997). Glycemic and insulinemic responses to multiple preexercise carbohydrate feedings. *International Journal of Sports Nutrition, 7*(2), 128-137.

Spiller, G. A., Jensen, C. D., Pattison, T. S., Chuck, C. S., Whittam, J. H., & Scala, J. (1987). Effect of protein dose on serum glucose and insulin response to sugar. *American Journal of Clinical Nutrition, 46*(3), 474-480.

Sprague, J. E., & Arbeláez, A. M. (2011). Glucose counterregulatory responses to hypoglycemia. *Pediatric Endocrinology Review, 9*(1), 463-475.

Stanstrup, J., Schou, S. S., Holmer-Jensen, J., Hermansen, K., & Dragsted, L. O. (2014). Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites compared to casein, gluten, and fish protein. *Journal of Proteome Ressearch, 13*(5), 2396-2408.

Stevenson, E. J., & Allerton, D. M. (2017). The role of whey protein in postprandial glycaemic control. *Proceedings of the Nutrition Society*, *77*(1), 42-51. doi: 10. 1017/S0029665117002002

Taborsky, G. J. (2010). The physiology of glucagon. *Journal of Diabetes Science & Technology, 4*(6), 1338-1344.

Temesi, J., Johnson, N. A., Raymond, J., Burdon, C. A., & O'Connor, H. T. (2011). Carbohydrate ingestion during endurance exercise improves performance in adults. *The Journal of Nutrition, 141*(5), 890-897. doi:10.3945/jn.110.137075

Thiebaud, D., Jacot, E., DeFronzo, R. A., Maeder, E., Jequier, E., & Felber, J. P. (1982). The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes, 31*(11), 957-963.

Thorens, B. (2014). Neural regulation of pancreatic islet cell mass and function. *Diabetes, Obesity, and Metabolism, 16*(Suppl. 1), 87-95.

Tippens, K. M., Purnell, J. Q., Gregory, W. L., Connelly, E., Hanes, D., Oken, B., & Calabrese, C. (2014). Expectancy, self-efficacy, and placebo effect of a sham supplement for weight loss in obese subjects. *Jounral of Evidence Based Complementary Alternative Medicine, 19*(3), 181-188. doi:10.1177/2156587214528513

Tsintzas, K., & Williams, C. (1998). Human muscle glycogen metabolism during exercise. Effect of carbohydrate supplementation. *Sports Medicine, 25*(1), 7-23.

Urhausen, A., Coen, B., Weiler, B., & Kindermann, W. (1993). Individual anaerobic threshold and maximum lactate steady state. *International Journal of Sports Medicine, 14*(3), 134-139.

USA Cycling. (2015). USA cycling 2015 annual report. Available at http:/www.usacycling.org/about-us/fiscal-information.

USA Triathlon. (2015). USA triathlon 2015 membership report. Available at http:/www.teamusa.org/usa-triathlon/about/multisport/demographics.

U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 dietary guidelines for Americans. Retrieved from http://health.gov/dietaryguidelines/2015/guidelines/.

Van Essen, M., & Gibala, M. J. (2006). Failure of protein to improve time trial performance when added to sports drink. *Medicine & Science in Sports & Exercise, 38*(8), 1476-1483.

Vandenbogaerde, T. J., & Hopkins, W. G. (2011). Effects of acute carbohydrate supplementation on endurance performance: a meta-analysis. *Sports Medicine, 41*(9), 773-792.

van Hall, Gerrit. (2015). The physiological regulation of skeletal muscle fatty acid supply and oxidation during moderately-intensity exercise. *Sports Medicine, 45*(Suppl 1), S23-S32.

van Loon, L. J., Kruijshoop, M., Verhagen, H., Saris, W. H. M., & Wagenmakers, A. J. M. (2000a).Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *Journal of Nutrition., 130*, 2508-2513.

van Loon, L. J., Saris, W. H. M., Kruikshoop, M., & Wagenmakers, A. J. M. (2000b). Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *American Journal of Clinical Nutrition, 72*, 106-111.

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H., & Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilization n humans. *Journal of Physiology, 536*(Pt 1), 295-304.

Wilcox, G. (2005). Insulin and insulin resistance. *Clinical Biochemistry Review*, 26, 19-39.

Zafar, T. A., Wasilen, C., AlRaefaei, A., Alrashidi, N., & AlMahmoud, E. (2013). Whey protein sweetened beverages reduce glycemic and appetite response and food intake in young females. *Nutrition Research, 33*(4), 303-310.

Zierath, J. R. & Hawley, J. A. (2004). Skeletal muscle fiber type: Influence on contractile and metabolic properties. *PLoS Biology, 2*(10), e348. doi:10.1371/journal.pbio.0020348

Zouhal, H., Jacob, C., Delamarche, P., & Grats-Delamarche, A. (2008). Catecholamines and the effects of exercise, training and gender. *Sports Medicine*, 38(5), 401-423.

Zunz, E., & La Barre, J. (1929). Contributions a l'étude des variations physiologiques de la sécrétion interne du pancréas relations entre les sécrétions externe et interne du pancreas. *Acta International Physiology & Biochemistry, 31*, 20-44.

APPENDIX A

Institutional Review Board Approval Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378
email: IRB@twu.edu
https://www.twu.edu/institutional-review-board-irb/

DATE: December 13, 2018

TO: Mr. Chris Irvine
Kinesiology

FROM: Institutional Review Board (IRB) - Denton

Re: Approval for The Influence of a Supplemental Preload Prior to Carbohydrate Consumption on Cycling Performance (Protocol #: 20317)

The above referenced study was reviewed at a fully convened meeting of the Denton IRB (operating under FWA00000178). The study was approved on 12/13/2018. This approval is valid for one year and expires on 12/13/2019. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. George King, Kinesiology
Dr. Vic Ben-Ezra, Kinesiology
Graduate School

APPENDIX B

Participant Recruitment Information

Texas Woman's University Kinesiology Department

Research Participants Needed!!!

Examining A New Supplement To Potentially Improve Cycling Performance!

Male and female participants are needed to examine the influence of a supplement preload on cycling performance. If you are:

- Male or female
- 18-44 years old
- Recreationally active cyclists or triathlete

CONTACT US!

Benefits of Participation Include:

- Test of Maximal Oxygen Consumption (VO_2max)
- Determination of Lactate Threshold
- Analysis of Substrate Utilization During Cycling
- Metabolic Profile During Cycling

If interested, please contact:
Chris Irvine
cirvine@twu.edu
(817)-505-7966

Participation is voluntary and may be discontinued at any time.
All email conversations are confidential and kept between the addressee and research team member. There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions

APPENDIX C

Training History

Training Questionnaire

How long have you been exercising? _____

How many days a week do you exercise? _____

Average duration of workout session _____

How many days a week do you participate in the following?

Swim _____

Bike _____

Run _____

Weights _____

Please list the endurance events you have participated in (e.g., Olympic triathlon)

_____
_____
_____
_____
_____
_____

APPENDIX D

Informed Consent

Texas Woman's University

Informed Consent to Participate in a Research Project

TITLE OF THE STUDY: The Influence of a Supplemental Preload Prior to Carbohydrate Consumption on Cycling Performance

PRINCIPAL INVESTIGATOR: Chris Irvine cirvine@twu.edu (817) 505-7966
ADVISOR: Vic Ben-Ezra, Ph.D. VBenEzra@twu.edu (940) 898-2597

WHO IS DOING THE STUDY? Department of Kinesiology, Texas Woman's University

PURPOSE: You are being invited to participate in a study for my dissertation that is examining the influence of a new sport supplement that may potentially improve cycling performance. Cycling performance will be measured by a 30 min time trial. The secondary purpose of this study will be to investigate the metabolic responses invoked by the consumption of a supplemental preload during a performance cycling trial. Outcomes from this study may provide further insight for utilizing this supplement to improve aerobic performance and the metabolic profile during prolonged, submaximal cycling in trained individuals.

WHERE IS THIS STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?
The study will take place on the Texas Woman's University Denton campus in the Department of Kinesiology (Exercise Physiology laboratory, room 116) located in Pioneer Hall and at the Institute of Women's Health located in Woodcock Hall (rooms 013 and 010). The entire study should last a total of ~5 hours.

PROCEDURES

You are being asked to participate in this study which is designed to examine the influence of a sport supplement preload on cycling performance. Exclusion criteria for participation include any current tobacco use or have quit within the last 6 months. Current or previous (in the last 6 months) musculoskeletal injuries that may impair cycling performance or limit hip or knee range of motion. If you are or plan on becoming pregnant during the testing period will also be excluded from participation in this study. Lastly, if you have any allergies to whey or dairy products, you will be excluded from this study.

If you qualify to participate, you will be asked to come to the lab on **3 different occasions** with 1 week in-between each visit. One visit will last approximately 1 hour and 30 minutes and the other visits approximately 1 hour and 45 minutes each, for a total of ~5 hours of your time. We will ask that you do not eat or drink anything (besides water) for at least 2-3 hours before each session. During the first visit, you will perform a lactate threshold test of the cycling ergometer. This test will last approximately 12-21 min. During your next two session, you will be asked to complete a 60 min cycling performance test. Each of trials are explained in more detail below:

Initial: _____

Preliminary Data Collection

You will be invited to come to the Exercise Biochemistry Laboratory located in Pioneer Hall prior to the testing trials. You will need to abstain from any food or drink, except water, at least 2-3 hr prior to the preliminary trial. During the preliminary visit you will be given an informed consent and fill out a medical history questionnaire. An exercise questionnaire will also be provided to you and will ask you about your exercise frequency, intensity, duration, and mode. You will also be informed to temporarily stop taking any other commercially available supplements because it may interfere with outcome variables that are important for this study. You will also be given detailed description on how to record food logs and activity logs 3 days prior to testing. You will be asked to replicate the meals consumed and exercise completed 3 days before each of the trials. You will need to abstain from alcohol use 48 hr prior to the interventions. After you have signed the informed consent, you will proceed to perform the lactate threshold test.

Health History & Physical Activity Questionnaire

To assess your general health and physical activity status, you will be asked to complete a short questionnaire. When answering questions, please be as honest and accurate as possible. This form should take approximately 10 minutes to complete.

Body Composition

Basic anthropometric data will also be measured during this trial. Height (cm) will be measured using a stadiometer, weight (kg) will be measured on a standardized digital scale, and waist circumference (cm) will be measured using a measuring tape wrapped around your body, approximately one inch above the belly button. Lean body mass (kg), fat mass (kg) and bone mineral density (g/cm2) will be assessed utilizing a dual-energy x-ray absorptiometry (DXA). The DXA procedure will occur in Woodcock Hall in the Institute for Women's Health in rooms 013 and 010. During this session, you are encouraged to ask any question they may have regarding the research procedures.

Lactate Threshold Test

You will perform a lactate threshold (LT) test on an electronically controlled cycle ergometer. This test will provide the work rate (W) at lactate threshold and your VO_2max (ml/kg/min). Prior to performing the test, you will be fit with a heart rate monitor strapped just below your chest line. The heart rate signal will be transmitted to a digital watch and will be monitored by the researcher. You will be allowed to adjust the seat height and handle bars to accommodate your cycling fit. You will be fit to an indirect calorimetry (TrueOne 2400; ParvoMedics, Sandy, UT) to collect expired gasses (VO_2 and VCO_2) every minute of the test. You will begin the LT protocol at a power output of 50 W and the workload will increase by 30 W every 4 min until volitional fatigue or if cycling cadence falls below 70 rpm. At the end of each stages, a capillary blood lactate sample will be collected.

Capillary Blood Sampling

Capillary blood will be collected and measured during the lactate threshold testing protocol using a calibrated, portable blood lactate analyzer (Lactate Scout +, EKF Diagnostic, Germany). A spring-loaded lancet will be applied to your fingertip to obtain a small droplet (~25 µL) of blood for analysis.

Initial: _____

Metabolic Measures

During both exercise session investigators will be utilizing a metabolic cart to analyze several respiratory variables from your expired breath. A metabolic cart is a device that continually measures the amount of oxygen consumed and carbon dioxide produced during rest or at exercise. You will breathe in regular room air through a snorkel-like mouthpiece during this test, and a computer will analyze the air that you breathe out.

Heart Rate

Throughout each test your heart rate will be examined utilizing a Polar heart rate monitor. This consist of wearing a small elastic, flexible belt around your chest to hold the heart rate monitor in place. The signals are picked up by a wrist watch receiver in view of the investigators. The results will be shown in real-time to the investigators.

Rating of Perceived Exertion

During each exercise stage, you will be asked to rate how difficult you are exerting yourself using a rating of perceived exertion scale. This scale ranges from 6 to 20; 6 indicating very, very, light exertion and 20 indicating very, very hard exertion.

Exercise Trials

Upon entering the lab, you will be instructed to sit upright on a chair and rest for 5 min. An indwelling Teflon catheter will be placed in your arm following the resting period. The first baseline blood draw (-30 min) will be collected to measure plasma glucose, insulin, glucagon, lactate and non-esterified fatty acids (NEFA) concentrations. After the baseline blood draw (-30 min), you will consume 1 of the 2 preloads.

Endurance Protocol

The exercise trial will consist of exercising for 30 min at 90% LT followed by 30 min time trial. You will be allowed to freely adjust the resistance added to the cycle ergometer throughout the 30 min time trial. During this trial, the total distance covered will be hidden from you until the completion of both experimental trials. Throughout the exercise trials, power out (Watts) and cycling cadence (rpm) will be measured and recorded. During both trials, you will be allowed to consume water.

Preloads

Prior to the endurance protocol, you will consume a preload supplement solution of 350 mL (which is equivalent to ~1.5 cups of fluid). One of the two solutions will contain a new supplement that may improve cycling performance. Twenty minutes following the preload, you will consume 0.8 g/CHO/kg.

Perceptual Measures

During both exercise trials, rating of perceived exertion (RPE) will be measured using the Borg Rating of Perceived Exertion Scale every 10 min. The researcher will ask you to rate your level of effort at a given moment. You will reply by selecting a number on a scale of 6-20 indicating level of exertion. The number 6 indicates no exertion at all, while the number 20 indicates maximal exertion. Following the testing trials, you will be asked to rate their level of gastrointestinal stress.

Initial: _____

Visual Analog Scale

You will be asked to rate feelings of overall leg pain during the exercise trials using a visual analog scale.

BENEFITS OF PARTICIPATION

You will be given information about your own aerobic fitness capacity (lactate threshold and VO2max), cycling performance, and body composition. If interested, you will be given an explanation of your own personal results. You may request to receive a copy of the abstract of the completed study.

POTENTIAL RISKS OF PARTICIPATION

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Cardiac Event: Exercise rarely causes any problems in normal participants, but in individuals with known or hidden heart disease, exercise may cause chest pain, dizziness, or bouts of irregular heart rhythms. Exercise will be stopped immediately if there are any signs of excessive strain. With any type of exercise testing or training, there is the possibility of injury or discomfort. During intense training and/or testing, the risk of having a heart attack or even dying goes up slightly; however, the risk in a patient with no history of heart disease is low, and your overall risk of heart attack or death over the time of exercise training will actually decrease. Exercise may also occasionally be accompanied by abnormal blood pressure, nausea, fainting, muscle soreness, joint and bone injury, and in rare instances, heart attack, stroke, or death. Every precaution will be taken to minimize these risks by closely monitoring your vital signs (heart rate and blood pressure) throughout exercise, and all technicians are certified in cardiopulmonary resuscitation and automated external defibrillator techniques. Automated external defibrillator techniques involve implementing the proper placement of the pads on the individual and following the procedures instructed by the machine if a cardiac event were to occur. A telephone is also present in the Exercise Physiology lab in the event that emergency treatment is needed.

Small risk if infection: There is also a small risk of the catheter perforating (going through) the vein or not being inserted into a blood vessel. Also, you may experience discomfort, bleeding, and/or bruising. On a rare occasion, they may feel dizzy or faint. The likelihood of these complications is very remote (about 1 in 10,000) when this procedure is carried out by trained personnel and proper equipment is used, as it will be in this case. Universal precautions will be used during all blood draw procedures. Sites for blood draws will be cleaned with alcohol immediately prior to each venipuncture. Each new needle that is opened will be disposed of in biohazard boxes immediately after use. A registered nurse or a trained phlebotomist will obtain these blood samples.

Shortness of Breath, lightheadedness, nausea: High-intensity exercise training has been associated with shortness of breath, fatigue, light-headedness, and in some cases nausea. In order to minimize these effects, you will do a 5-minute warm-up and be instructed through an active recovery during each exercise session. Active recovery has been associated with a reduction in the afore-

Initial: _____

mentioned risks. Furthermore, each exercise session will be supervised by the principle investigator and both heart rate and rating of perceived exertion will be recorded for each session. At any point during the study, you are allowed to stop testing.

Muscle soreness/joint soreness/muscle fatigue: You may also experience local muscle and/or joint soreness following exercise. To reduce (but not completely avoid) the effect of muscle/joint soreness, you will be allowed a time for a warm-up and cool-down, and will be advised about stretching exercises.

Emotional discomfort: During the collection of personal information (i.e., menstrual and menopausal history, dietary information) you may feel emotionally uneasy. To minimize emotional discomfort with the collection of this information, you will have the option to share this information with a research team member of the same gender.

Embarrassment: During measurement of height and weight you may feel embarrassed. To minimize this embarrassment you will have the option to have measurements taken by a research team member of the same gender. Additionally to ensure privacy, procedures will be performed in a small private room located in the exercise physiology lab (PH 116) and not shared with anyone except the principal investigators and faculty advisor.

Loss of Confidentiality: There is potential for a loss of confidentiality during communication through email, downloading, other Internet transactions, or data stored offline. To minimize this risk, all collected data will be stored in a password-protected computer and locked filing cabinet in the principal investigator's office. Further, you will be coded with a combination of letters/numbers that cannot identify you (e.g. W-001, W - 002). Confidentiality will be protected to the extent that is allowed by law.

Loss of Time: You will be allocating time to be available to participate in this research study. To minimize unwanted loss of your time during the study, schedules will be made and given to both the research team and to you (the participant). These schedules will inform both parties of the day, and time of day that you are scheduled to be in the lab. These schedules will also outline what you will be doing during your time in the lab. This will allow the research team to plan in advance to ensure that everything is performed and completed in the available time frame.

***Bruising*:** The risk of bruising resulting from catheter blood draws is minimal due to this procedure being performed by trained personnel. Universal precautions will be used during all blood draw procedures. To minimize bruising, pressure will be applied to the site for approximately five minutes after each blood draw.

***Discomfort with blood draws*:** The risks of collecting a blood sample include the possibility of requiring more than one attempt to obtain the blood sample, local discomfort (pinch when the needle enters your skin), minor bruising or bleeding at the site (10%), possible temporary lightheadedness, infection (<0.01%), or development of a blood clot (<0.01%). A trained and experienced individual (a registered nurse or a trained phlebotomist) will obtain these blood samples.

***Gastro-Intestinal Distress (Nausea)*:** To minimize the possibility of GI distress with administration independently and combined forms of the supplement, you will have

Initial: _____

immediate access to restroom facilities, additional water will be available and research will be stopped immediately if any signs or issues of nausea or gastrointestinal distress are witnessed by the research team. You will be notified of taste and possible GI distress during consent when administering oral drinks.

***Radiation Exposure from DXA Scan*:** The risks associated with a DXA scan include exposure to small amounts of radiation. DXA scanning utilizes radiation to obtain an image of your body. Everyone receives a small amount of unavoidable radiation from the environment each year. Some of this radiation comes from space and some from naturally-occurring forms of radioactive water and minerals. The DXA scan technique gives your body the equivalent of about 4 extra days' worth of this natural radiation. The dose to patient from DXA is considered small (0.08–4.6 μSv (Njeh, 1999) The radiation dose we have discussed is what will be received from this study only and does not include any exposure you may have received or will receive from other tests. If you are pregnant or trying to get pregnant, they will not be included in this study. It is possible that having several of these tests may add to possible risk of injury or disease. To minimize this risk only 1 scan will take place at the preliminary visit for this research project.

Food Allergy: If an allergic reaction does occur following the consumption of whey protein, the research study will be terminated immediately and EMS will be contacted by one the research members.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

CAN MY TAKING PART IN THIS STUDY END EARLY?

As mentioned, we are aware that this study requires a modest time commitment from you as a volunteer. In the event that something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed, simply to check and make sure that everything is OK. Should our testing reveal information that suggests you need to be referred for medical care, we will refer you to your primary care physician.

RIGHT TO ASK QUESTIONS AND/OR WITHDRAW FROM THE STUDY

If you have questions or concerns at any time during the course of this investigation or after you complete the study, you may contact Chris Irvine at (817) 505-7966. Chris Irvine's office is located in room 215 Pioneer Hall.

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

PARTICIPATION

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to

Initial: _____

which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly signed this consent form.

PRIVACY OF RECORDS

Only you, Chris Irvine, and members of the research team will have access to your results. All data collected in this study will kept in a locked file cabinet on the TWU campus and will be coded by subject number rather than by name. The results of the research will be published; however, no publication will contain information which will allow you to be identified.

AUTHORIZATION

Signature of Study Participant: _____

Printed Name of Study Participant:_____Date: _____

Investigator's Signature: _____

Printed Name of Investigator:_____ Date: _____

APPENDIX E

Health History Questionnaire

Wellness & Sport Evaluation Program
Health Questionnaire

Name _____ **Date** ____/____/20_____
(Last) (First) (Middle)

		Circle One	Notes
(1)	**Have you been under the care of a physician during the past 2 years?**	**Yes No**	
(2)	**Are you allergic to penicillin, any drugs, medicine, or latex?**	**Yes No**	
(3)	**Have you ever had excessive bleeding which required special treatment?**	**Yes No**	
(4)	**Women: Is there a chance you might be pregnant?**	**Yes No**	
(5)	**Women: Are you taking any birth control medication?**	**Yes No**	
(6)	**Have you had adverse reaction to local anesthetics?**	**Yes No**	

(7) Do you use recreational drugs?_____ If yes, what?_____
(8) Do you use tobacco? _____ If so, what form? _____
(9) Date of last medical exam _____
(10) Circle Yes to any of the following which you have had or have at present. Circle No to those that you have not had.

Yes	**No**	**High Blood Pressure**	**Yes**	**No**	**Unexplained Shortness of Breath**
Yes	**No**	**High Blood Cholesterol**	**Yes**	**No**	**Chronic Cough or Bronchitis**
Yes	**No**	**Chest Pain or Pressure (Angina)**	**Yes**	**No**	**Tuberculosis (TB)**
Yes	**No**	**Heart Disease or Attack**	**Yes**	**No**	**Emphysema**
Yes	**No**	**Heart Pacemaker**	**Yes**	**No**	**Asthma**
Yes	**No**	**Heart Failure**	**Yes**	**No**	**Hay Fever**
Yes	**No**	**Heart Surgery**	**Yes**	**No**	**Allergies or Hives**
Yes	**No**	**Fainting or Lightheadedness**	**Yes**	**No**	**Sinus Trouble**
Yes	**No**	**Artificial Heart Valve**	**Yes**	**No**	**Cancer**
Yes	**No**	**Congenital Heart Lesions**	**Yes**	**No**	**Leukemia or Lymphoma**
Yes	**No**	**Mitral Valve Prolapse**	**Yes**	**No**	**Radiation or Chemotherapy**
Yes	**No**	**Stroke**	**Yes**	**No**	**Anemia**
Yes	**No**	**Transient Ischemic Attack**	**Yes**	**No**	**Bruise Easily**
Yes	**No**	**Lupus**	**Yes**	**No**	**Bleeding Disorders**
Yes	**No**	**Rheumatic Fever**	**Yes**	**No**	**Sickle Cell Disease**
Yes	**No**	**Scarlet Fever**	**Yes**	**No**	**Alcoholism**
Yes	**No**	**Chronic Fatigue**	**Yes**	**No**	**Drug Addiction**
Yes	**No**	**Artificial Joints**	**Yes**	**No**	**Blood Transfusion**
Yes	**No**	**Kidney Dialysis**	**Yes**	**No**	**Liver Disease**
Yes	**No**	**Kidney Disease**	**Yes**	**No**	**Yellow Jaundice**
Yes	**No**	**Eating Disorder**	**Yes**	**No**	**Hepatitis**
Yes	**No**	**Rheumatoid Arthritis**	**Yes**	**No**	**AIDS / HIV Infection**
Yes	**No**	**Arthritis**	**Yes**	**No**	**Cold Sores / Fever Blisters**
Yes	**No**	**Chronic Head, Neck, or Back Pain**	**Yes**	**No**	**Psychiatric Treatment**
Yes	**No**	**Diabetes Requiring Insulin**	**Yes**	**No**	**Depression / Bipolar**
Yes	**No**	**Diabetes Not Requiring Insulin**	**Yes**	**No**	**Nervousness / Anxiety**
Yes	**No**	**Hypoglycemia**	**Yes**	**No**	**Dizzy Spells**
Yes	**No**	**Hyperthyroidism (High)**	**Yes**	**No**	**Epilepsy or Seizures**
Yes	**No**	**Hypothyroidism (Low)**	**Yes**	**No**	**Condition Requiring Cortisone Medicine**
Yes	**No**	**Ulcers**	**Yes**	**No**	**Glaucoma**
Yes	**No**	**Pulmonary Disease**	**Yes**	**No**	**Spine or Hip Fractures**

List all prescription medications that you are currently taking.

Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____

Please list all non-prescription medication or vitamins or nutritional supplements you are currently taking.

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

List all surgical procedures that you have had in the past.

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

List all hospitalizations of 24 hours or more for any reason.

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Other Health Information

Please use this space to record any other personal health information that was not listed above.

_____

_____

"I Attest To The Fact That The Information Given Above Is Correct And I Consent To Receive Clinical Services."

_____

(Parent or Guardian must sign for patient under age 18.)

This section for office use only:

Comments:

_____

_____

_____

_____

_____

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it ***does not limit your current ability*** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

☑ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

🛑 **If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.**

⚠ **Delay becoming more active if:**

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2017 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2)3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation: background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

01-01-2017

APPENDIX F

Participant Take Home Logs

3-Day Exercise Log

Day 1

Mode	Intensity	Duration	RPE

Day 2

Mode	Intensity	Duration	RPE

Day 3

Mode	Intensity	Duration	RPE

Day 1: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening Snacks**	**Serving Size**
Number of hours of sleep last night					

Day 2: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening Snacks**	**Serving Size**
Number of hours of sleep last night					

Day 3: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening Snacks**	**Serving Size**
Number of hours of sleep last night					

APPENDIX G

Data Collection Sheets

ID: _____ DATE & TIME: _____

TRIAL: _____ 90% LT _____

HEIGHT (IN): _____ WEIGHT (KG): _____

TIME	BEVERAGE	BLOOD DRAW	EXPIRED GASES
-30			
-10			
0			
15			
30			
45			
60			

TIME	RPE	VAS	HR
0			
10			
20			
30			
40			
50			
60			

	Dosing
Whey	
Glucose	

LACTATE THRESHOLD

ID: _____ DATE & TIME: _____

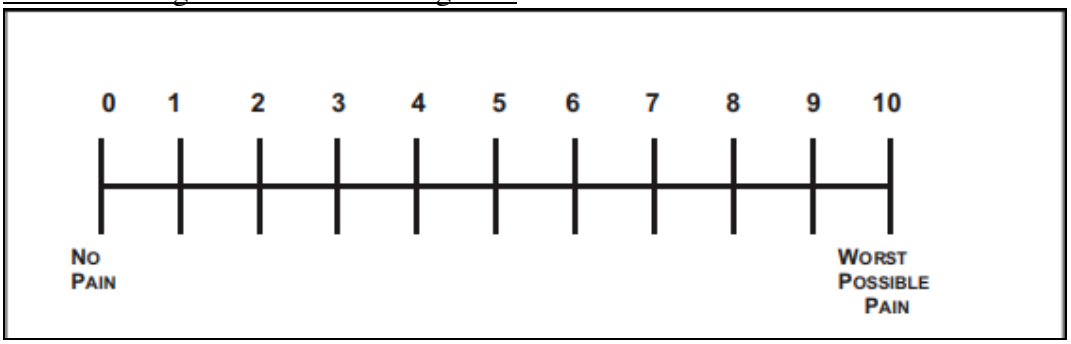
HEIGHT (CM): _____ WEIGHT (KG): _____

AGE: _____

Time	Workload (watts)	HR (bpm)	RPE	BL_A (mmol)
0-4	50			
4-8	80			
8-12	110			
12-16	140			
16-20	170			
20-24	200			
24-28	230			
28-32	260			
32-36	290			
36-40	320			
40-44	350			
44-48	380			
48-52	410			
52-56	440			
56-60	470			

NOTES:_____
_____
_____
_____
_____

Visual Analog Scale to Assess Leg Pain



Borg Rating of Perceived Exertion

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

APPENDIX H

Statistical Outputs

Between-Subjects Factors

		Value Label	N
Trial	1.00	whey protein	9
	2.00	placebo	9

Descriptive Statistics

Trial		Mean	Std. Deviation	N
Insulin_M30	whey protein	35.8011	54.71281	9
	placebo	37.8500	60.65197	9
	Total	36.8256	56.04416	18
Insulin_M10	whey protein	90.4122	82.40382	9
	placebo	42.1856	63.64232	9
	Total	66.2989	75.61202	18
Insulin_0	whey protein	222.8756	171.00908	9
	placebo	85.9533	85.72518	9
	Total	154.4144	148.93898	18
Insulin_15	whey protein	99.2822	106.62560	9
	placebo	73.7667	109.36102	9
	Total	86.5244	105.59649	18
Insulin_30	whey protein	81.0800	99.99746	9
	placebo	56.4722	103.66068	9
	Total	68.7761	99.61254	18
Insulin_45	whey protein	91.8922	113.94437	9
	placebo	52.2300	89.33017	9
	Total	72.0611	101.39744	18
Insulin_60	whey protein	82.9978	101.99151	9
	placebo	44.8111	81.83212	9
	Total	63.9044	91.82852	18
Glucose_M30	whey protein	87.3889	6.00238	9
	placebo	86.9667	7.79455	9
	Total	87.1778	6.75222	18
Glucose_M10	whey protein	86.8056	7.07754	9
	placebo	88.1000	9.34692	9

	Total	87.4528	8.07025	18
Glucose_0	whey protein	93.5222	15.02812	9
	placebo	98.2889	9.81917	9
	Total	95.9056	12.55653	18
Glucose_15	whey protein	70.5167	14.43472	9
	placebo	89.3000	22.88384	9
	Total	79.9083	20.92551	18
Glucose_30	whey protein	86.9500	14.12095	9
	placebo	90.0778	22.65666	9
	Total	88.5139	18.38450	18
Glucose_45	whey protein	102.6778	15.51570	9
	placebo	95.9111	17.27357	9
	Total	99.2944	16.30400	18
Glucose_60	whey protein	103.7778	18.81401	9
	placebo	90.9778	18.72410	9
	Total	97.3778	19.36302	18
Cpep_M30	whey protein	947.6133	295.04087	9
	placebo	916.6422	235.92735	9
	Total	932.1278	259.63811	18
Cpep_M10	whey protein	1338.6556	475.76524	9
	placebo	1022.3344	236.98642	9
	Total	1180.4950	399.29252	18
Cpep_0	whey protein	1918.7567	859.67737	9
	placebo	1390.1389	402.10585	9
	Total	1654.4478	705.58027	18
Cpep_15	whey protein	1647.3333	459.30818	9
	placebo	1510.2667	609.26513	9
	Total	1578.8000	528.14251	18
Cpep_30	whey protein	1402.6267	601.52243	9
	placebo	1192.3144	738.10879	9
	Total	1297.4706	662.08722	18
Cpep_45	whey protein	1502.4133	821.41796	9
	placebo	1056.8022	698.35070	9
	Total	1279.6078	774.32821	18

Cpep_60	whey protein	1491.6644	907.67968	9
	placebo	901.4678	683.25608	9
	Total	1196.5661	836.42322	18
Glucagon_M30	whey protein	94.4767	42.46387	9
	placebo	105.0200	52.85706	9
	Total	99.7483	46.82673	18
Glucagon_M10	whey protein	178.8544	71.30021	9
	placebo	100.8467	47.19052	9
	Total	139.8506	71.07112	18
Glucagon_0	whey protein	219.2122	95.65508	9
	placebo	102.5067	42.18524	9
	Total	160.8594	93.53413	18
Glucagon_15	whey protein	371.3244	131.67865	9
	placebo	109.1700	52.09294	9
	Total	240.2472	166.21846	18
Glucagon_30	whey protein	348.7822	113.43855	9
	placebo	117.8167	49.63883	9
	Total	233.2994	146.06836	18
Glucagon_45	whey protein	281.7722	69.47725	9
	placebo	116.4067	50.19405	9
	Total	199.0894	103.42039	18
Glucagon_60	whey protein	264.3067	75.09519	9
	placebo	123.1444	50.05421	9
	Total	193.7256	95.43343	18
NEFA_M30	whey protein	810.0000	168.49613	9
	placebo	859.6222	163.76510	9
	Total	834.8111	163.19607	18
NEFA_M10	whey protein	735.2000	112.94135	9
	placebo	818.8778	150.62832	9
	Total	777.0389	136.13703	18
NEFA_0	whey protein	681.8556	134.36678	9
	placebo	721.8556	77.64442	9
	Total	701.8556	108.42854	18
NEFA_15	whey protein	535.9333	38.65310	9

	placebo	567.7889	89.97932	9
	Total	551.8611	69.14997	18
NEFA_30	whey protein	550.7333	59.21013	9
	placebo	578.8889	73.35038	9
	Total	564.8111	66.26873	18
NEFA_45	whey protein	570.7444	55.14214	9
	placebo	597.7889	86.69792	9
	Total	584.2667	71.84492	18
NEFA_60	whey protein	584.0667	53.37921	9
	placebo	639.6333	116.62494	9
	Total	611.8500	92.51397	18

Multivariate Tests[a]

Effect			Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Between Subjects	Intercept	Pillai's Trace	0.997	837.044[b]	5.000	12.000	0.000	0.997
		Wilks' Lambda	0.003	837.044[b]	5.000	12.000	0.000	0.997
		Hotelling's Trace	348.768	837.044[b]	5.000	12.000	0.000	0.997
		Roy's Largest Root	348.768	837.044[b]	5.000	12.000	0.000	0.997
	Trial	Pillai's Trace	0.740	6.829[b]	5.000	12.000	0.003	0.740
		Wilks' Lambda	0.260	6.829[b]	5.000	12.000	0.003	0.740
		Hotelling's Trace	2.845	6.829[b]	5.000	12.000	0.003	0.740
		Roy's Largest Root	2.845	6.829[b]	5.000	12.000	0.003	0.740

Within Subjects Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Time	Pillai's Trace	1.834	9.272	30.000	480.000	0.000	0.367
	Wilks' Lambda	0.048	13.962	30.000	370.000	0.000	0.454
	Hotelling's Trace	5.857	17.650	30.000	452.000	0.000	0.539
	Roy's Largest Root	3.484	55.750[c]	6.000	96.000	0.000	0.777
Time * Trial	Pillai's Trace	1.144	4.749	30.000	480.000	0.000	0.229
	Wilks' Lambda	0.216	5.764	30.000	370.000	0.000	0.264
	Hotelling's Trace	2.165	6.525	30.000	452.000	0.000	0.302
	Roy's Largest Root	1.284	20.551[c]	6.000	96.000	0.000	0.562

Univariate Tests

Time * Trial	Insulin	Sphericity Assumed	51938.550	6	8656.425	4.936	0.000	0.236
		Greenhouse-Geisser	51938.550	2.541	20443.729	4.936	0.007	0.236
		Huynh-Feldt	51938.550	3.249	15985.869	4.936	0.003	0.236
		Lower-bound	51938.550	1.000	51938.550	4.936	0.041	0.236
	glucose	Sphericity Assumed	2644.625	6	440.771	4.593	0.000	0.223
		Greenhouse-Geisser	2644.625	2.693	982.106	4.593	0.009	0.223
		Huynh-Feldt	2644.625	3.492	757.307	4.593	0.004	0.223
		Lower-bound	2644.625	1.000	2644.625	4.593	0.048	0.223
	cpeptide	Sphericity Assumed	1175854.120	6	195975.687	2.214	0.048	0.122
		Greenhouse-Geisser	1175854.120	2.467	476635.007	2.214	0.112	0.122
		Huynh-Feldt	1175854.120	3.134	375251.326	2.214	0.095	0.122

		Lower-bound	1175854.120	1.000	1175854.120	2.214	0.156	0.122
	glucagon	Sphericity Assumed	228996.371	6	38166.062	17.789	0.000	0.526
		Greenhouse-Geisser	228996.371	2.750	83284.343	17.789	0.000	0.526
		Huynh-Feldt	228996.371	3.584	63890.335	17.789	0.000	0.526
		Lower-bound	228996.371	1.000	228996.371	17.789	0.001	0.526
	nefa	Sphericity Assumed	10947.519	6	1824.587	0.194	0.978	0.012
		Greenhouse-Geisser	10947.519	2.764	3961.269	0.194	0.886	0.012
		Huynh-Feldt	10947.519	3.607	3034.952	0.194	0.927	0.012
		Lower-bound	10947.519	1.000	10947.519	0.194	0.666	0.012

Tests of Between-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Trial	Glucose_M30	0.802	1	0.802	0.017	0.899	0.001
	Glucose_M10	7.540	1	7.540	0.110	0.745	0.007
	Glucose_0	102.245	1	102.245	0.635	0.437	0.038
	Glucose_15	1587.661	1	1587.661	4.338	0.054	0.213
	Glucose_30	44.023	1	44.023	0.124	0.730	0.008
	Glucose_45	206.045	1	206.045	0.764	0.395	0.046
	Glucose_60	737.280	1	737.280	2.093	0.167	0.116

Source	Dependent Variable	Type III Sum of	df	Mean Square	F	Sig.	Partial Eta

		Squares					Squared
Trial	Insulin_M30	18.891	1	18.891	0.006	0.941	0
	Insulin_M10	10466.151	1	10466.151	1.931	0.184	0.108
	Insulin_0	84364.627	1	84364.627	4.611	0.047	0.224
	Insulin_15	2929.696	1	2929.696	0.251	0.623	0.015
	Insulin_30	2724.942	1	2724.942	0.263	0.615	0.016
	Insulin_45	7078.913	1	7078.913	0.675	0.423	0.041
	Insulin_60	6561.997	1	6561.997	0.768	0.394	0.046

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Trial	Glucagon_M30	500.228	1	500.228	0.218	0.647	0.013
	Glucagon_M10	27383.46	1	27383.46	7.491	0.015	0.319
	Glucagon_0	61290.84	1	61290.84	11.216	0.004	0.412
	Glucagon_15	309262.287	1	309262.287	30.845	0	0.658
	Glucagon_30	240052.895	1	240052.895	31.313	0	0.662
	Glucagon_45	123055.951	1	123055.951	33.5	0	0.677
	Glucagon_60	89670.478	1	89670.478	22.019	0	0.579

Descriptive Statistics

Trial		Mean	Std. Deviation	N
Lactate_15	whey protein	2.1520	0.72558	10
	placebo	2.1130	0.90391	10
	Total	2.1325	0.79800	20
Lactate_30	whey protein	2.0190	0.59635	10
	placebo	1.9560	0.92391	10
	Total	1.9875	0.75752	20
Lactate_45	whey protein	3.5280	1.57273	10
	placebo	3.7510	1.63804	10
	Total	3.6395	1.56707	20
Lactate_60	whey protein	4.2370	2.12036	10
	placebo	4.2680	1.92648	10
	Total	4.2525	1.97177	20
VO2_15	whey protein	31.2750	5.02069	10
	placebo	31.0800	4.50846	10
	Total	31.1775	4.64527	20
VO2_30	whey protein	31.0950	4.71643	10
	placebo	30.4750	5.13589	10
	Total	30.7850	4.80964	20
VO2_45	whey protein	33.0600	5.18790	10
	placebo	34.7700	5.82963	10
	Total	33.9150	5.44209	20
VO2_60	whey protein	35.4260	5.23055	10
	placebo	36.3900	5.60366	10
	Total	35.9080	5.29887	20
RER_15	whey protein	0.8920	0.05224	10
	placebo	0.9000	0.04595	10
	Total	0.8960	0.04806	20
RER_30	whey protein	0.8850	0.04972	10
	placebo	0.8870	0.04218	10
	Total	0.8860	0.04489	20
RER_45	whey protein	0.9200	0.03197	10
	placebo	0.9210	0.04677	10

	Total	0.9205	0.03900	20
RER_60	whey protein	0.9540	0.05461	10
	placebo	0.9410	0.06082	10
	Total	0.9475	0.05665	20

Univariate Tests

Source			Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time * Trial	Lactate	Sphericity Assumed	0.252	3	0.084	0.077	0.972	0.004
		Greenhouse-Geisser	0.252	1.129	0.223	0.077	0.815	0.004
		Huynh-Feldt	0.252	1.220	0.207	0.077	0.833	0.004
		Lower-bound	0.252	1.000	0.252	0.077	0.785	0.004
	VO2	Sphericity Assumed	17.059	3	5.686	1.080	0.365	0.057
		Greenhouse-Geisser	17.059	1.331	12.820	1.080	0.330	0.057
		Huynh-Feldt	17.059	1.477	11.554	1.080	0.336	0.057
		Lower-bound	17.059	1.000	17.059	1.080	0.312	0.057
	RER	Sphericity Assumed	0.001	3	0.000	0.553	0.648	0.030
		Greenhouse-Geisser	0.001	1.491	0.001	0.553	0.531	0.030
		Huynh-Feldt	0.001	1.686	0.001	0.553	0.551	0.030
		Lower-bound	0.001	1.000	0.001	0.553	0.467	0.030

Descriptive Statistics

Trial		Mean	Std. Deviation	N
HR_10	whey protein	138.8000	11.57392	10
	placebo	136.9000	14.43337	10
	Total	137.8500	12.77034	20
HR_20	whey protein	143.4000	10.28699	10
	placebo	137.8000	12.34504	10
	Total	140.6000	11.42665	20
HR_30	whey protein	144.8000	11.44844	10
	placebo	139.4000	15.52203	10
	Total	142.1000	13.56039	20
HR_40	whey protein	154.6000	12.13992	10
	placebo	154.9000	11.38664	10
	Total	154.7500	11.45644	20
HR_50	whey protein	156.2000	13.27320	10
	placebo	154.7000	9.70739	10
	Total	155.4500	11.34379	20
HR_60	whey protein	157.9000	11.12005	10
	placebo	159.0000	9.20145	10
	Total	158.4500	9.94974	20
RPE_10	whey protein	11.4000	1.17379	10
	placebo	11.2500	1.78341	10
	Total	11.3250	1.47144	20
RPE_20	whey protein	12.3000	1.15950	10
	placebo	11.9500	1.38343	10
	Total	12.1250	1.25525	20
RPE_30	whey protein	13.4000	1.57762	10
	placebo	12.7500	1.47667	10
	Total	13.0750	1.52415	20
RPE_40	whey protein	14.8000	1.47573	10
	placebo	14.7000	1.33749	10
	Total	14.7500	1.37171	20

RPE_50	whey protein	15.6000	1.42984	10
	placebo	15.7500	1.08653	10
	Total	15.6750	1.23837	20
RPE_60	whey protein	17.0000	1.49071	10
	placebo	17.1000	1.28668	10
	Total	17.0500	1.35627	20
LP_10	whey protein	3.5500	2.11411	10
	placebo	3.6500	2.16089	10
	Total	3.6000	2.08124	20
LP_20	whey protein	4.3500	1.63384	10
	placebo	4.2500	1.96143	10
	Total	4.3000	1.75769	20
LP_30	whey protein	5.1000	1.72884	10
	placebo	4.7500	1.51383	10
	Total	4.9250	1.59171	20
LP_40	whey protein	6.3500	1.66750	10
	placebo	6.2500	1.55009	10
	Total	6.3000	1.56777	20
LP_50	whey protein	6.9500	1.60641	10
	placebo	7.1500	1.05541	10
	Total	7.0500	1.32685	20
LP_60	whey protein	7.8000	1.68655	10
	placebo	8.0000	1.05409	10
	Total	7.9000	1.37267	20

Univariate Tests

Source			Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time * Trial	HR	Sphericity Assumed	197.567	5	39.513	1.216	0.308	0.063
		Greenhouse-Geisser	197.567	1.367	144.532	1.216	0.298	0.063
		Huynh-Feldt	197.567	1.523	129.688	1.216	0.301	0.063
		Lower-bound	197.567	1.000	197.567	1.216	0.285	0.063
	RPe	Sphericity Assumed	2.217	5	0.443	0.517	0.763	0.028
		Greenhouse-Geisser	2.217	3.027	0.732	0.517	0.674	0.028
		Huynh-Feldt	2.217	3.909	0.567	0.517	0.719	0.028
		Lower-bound	2.217	1.000	2.217	0.517	0.481	0.028
	LP	Sphericity Assumed	1.160	5	0.232	0.331	0.893	0.018
		Greenhouse-Geisser	1.160	2.205	0.526	0.331	0.741	0.018
		Huynh-Feldt	1.160	2.666	0.435	0.331	0.780	0.018
		Lower-bound	1.160	1.000	1.160	0.331	0.572	0.018

APPENDIX I

Blood Analysis

Set up for Magpix Analysis – 96 well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 BackG	Standard 4	QC-1 Control	WP 14 0	WP 14 60	P 14 15	WP 16 -30	WP 16 30	P 16 -10	P 16 45		
B	0 BackG	Standard 4	QC-1 Control	WP 14 0	WP 14 60	P 14 15	WP 16 -30	WP 16 30	P 16 -10	P 16 45		
C	Standard 1	Standard 5	QC-2 Control	WP 14 15	P 14 -30	P 14 30	WP 16 -10	WP 16 45	P 16 0	P 16 60		
D	Standard 1	Standard 5	QC-2 Control	WP 14 15	P 14 -30	P 14 30	WP 16 -10	WP 16 45	P 16 0	P 16 60		
E	Standard 2	Standard 6	WP 14 -30	WP 14 30	P 14 -10	P 14 45	WP 16 0	WP 16 60	P 16 15			
F	Standard 2	Standard 6	WP 14 -30	WP 14 30	P 14 -10	P 14 45	WP 16 0	WP 16 60	P 16 15			
G	Standard 3	Standard 7	WP 14 -10	WP 14 45	P 14 0	P 14 60	WP 16 15	P 16 -30	P 16 30			
H	Standard 3	Standard 7	WP 14 -10	WP 14 45	P 14 0	P 14 60	WP 16 15	P 16 -30	P 16 30			

MagPix Analysis Procedure for Insulin, C-Peptide and Glucagon

1. Prep Antibody-Immobilized Beads
    a. Sonicate each vial for 30 seconds and then vortex for 1 min.
    b. Add 150 µL of each anti-body to the Mixing bottle and bring to 3.0 ml with Bead Diluent and vortex.
2. Prep Quality Controls
    a. Add 250 µL of DI water to Q1 and Q2.
3. Prep Wash Buffer
    a. Add 540 ml of DI water to the 60 ml wash buffer.
4. Prep Serum Matrix
    a. Add 1 ml of DI water to Serum Matrix.
5. Add 250 µL of DI water to the Standard.
6. Perform a serial dilution for the 6 standards.

Procedure of Analysis

1. Add 200 µL of Buffer into each well and shake for 10 min, then decant.
2. Add 25 µL of each standard and the controls.
3. Add 25 µL of Buffer to all the wells.
4. Add 25 µL of matrix solution to just the background, standards, and controls.
5. Add 25 µL of samples into the wells.
6. Add 25 µL of the beads to each well.
7. Incubate overnight for 16-18 hours.

8. Remove well contents and wash.
9. Add 50 µL Detection Antibodies per well.
10. Shake for 1 hour.
11. Add 50 µL of Streptavidin-Phycoerythrin to each well.
12. Shake for 30 min.
13. Repeat step 8.
14. Add 100 µL of Sheath fluid per well
15. Read on Luminex.

This procedure was performed for 4 different plates*

Plasma NEFA 96 Well Plate Set-Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 5	WP 01 -10	WP 01 45	P 01 0	P 01 60	WP 02 15	P 02 -30	P 02 30	WP 03 -10	WP 03 45	P 03 0
B	Standard 1	Standard 5	WP 01 -10	WP 01 45	P 01 0	P 01 60	WP 02 15	P 02 -30	P 02 30	WP 03 -10	WP 03 45	P 03 0
C	Standard 2	Standard 6	WP 01 0	WP 01 60	P 01 15	WP 02 -30	WP 02 30	P 02 -10	P 02 45	WP 03 0	WP 03 60	P 03 15
D	Standard 2	Standard 6	WP 01 0	WP 01 60	P 01 15	WP 02 -30	WP 02 30	P 02 -10	P 02 45	WP 03 0	WP 03 60	P 03 15
E	Standard 3	Standard 7	WP 01 15	P 01 -30	P 01 30	WP 02 -10	WP 02 45	P 02 0	P 02 60	WP 03 15	P 03 -30	P 03 30
F	Standard 3	Standard 7	WP 01 15	P 01 -30	P 01 30	WP 02 -10	WP 02 45	P 02 0	P 02 60	WP 03 15	P 03 -30	P 03 30
G	Standard 4	WP 01 -30	WP 01 30	P 01 -10	P 01 45	WP 02 0	WP 02 60	P 02 15	WP 03 -30	WP 03 30	P 03 -10	P 03 45
H	Standard 4	WP 01 -30	WP 01 30	P 01 -10	P 01 45	WP 02 0	WP 02 60	P 02 15	WP 03 -30	WP 03 30	P 03 -10	P 03 45

NEFA Analysis Procedure

1. Perform a serial dilution with FFA standard to produce a total of 7 standard solutions.
2. Add 50ml FFA diluent A to prepare FFA reagent A.
3. Add 5 µl of each sample to the respective wells.
4. Add 45 µl of dilution buffer to all wells (50 µl to standards).
5. Add 100 µl FFA Reagent A to each well and incubate for 10 min.
6. Add 50 µl of FFA Reagent B to each well and incubate for 10 min.
7. Let plate sit for 5 min at room temperature and then run.

APPENDIX J

Raw Data

WHEY PROTEIN TRIAL RAW DATA

Distance covered during the time trial effort.

Participant ID	Distance Covered (km)
WPC_01	15.83
WPC_02	16.74
WPC_03	18.46
WPC_11	17.3
WPC_12	16.35
WPC_14	16.33
WPC_16	17.5
WPC_17	17.59
WPC_19	14.54
WPC_18	16.95

Glucose timepoint data

Glu_-30	Glu_-10	Glu_0	Glu_15	Glu_30	Glu_45	Glu_60
86.7	86.4	91.1	35	67.9	88.2	82.1
99.9	103	116	88.3	115.5	130	129.5
82.0	84.3	85.7	60.6	74.3	90.0	93.1
90.7	83.4	78	70.1	96.8	118	139.5
80.7	79.8	103.5	94.9	90.8	118	106
85.7	88.8	113.5	54.7	80.1	94.9	99.8
82.7	79.9	79.6	66.3	76.7	100.4	97.5
86.5	85.75	85	63.95	91.65	90.8	82.9
86.4	85.4	79.6	55	68.7	85.1	88.1
91.9	90.9	100.5	80.8	88	96.9	97.6

Average watts at 90% LT (1st 30 min) and average watts during the time trial.

Watts_30	Watts_TT
177.2	175.5
153.4	185.9
230.6	262.1
182.5	222.6
156.2	190.8
172.5	189.4
229.5	204.7
195.2	231.1
148.2	156.2
152	208

Heart rate responses during the CPT

HR_10	HR_20	HR_30	HR_40	HR_50	HR_60
134	139	138	140	144	142
161	158	161	177	179	178
136	142	142	155	159	162
145	146	152	163	169	166
136	143	142	164	167	168
139	148	152	158	161	160
143	147	147	148	141	145
140	144	150	155	154	156
140	149	146	151	150	152
114	118	118	135	138	150

RER measurements during the CPT

RER_15	RER_30	RER_45	RER_60
0.91	0.9	0.89	0.91
0.86	0.84	0.89	0.88
0.91	0.87	0.93	0.94
0.81	0.81	0.9	0.95
0.94	0.94	0.96	1
0.9	0.88	0.93	0.96
0.84	0.87	0.87	0.88
0.94	0.91	0.93	0.97
0.97	0.98	0.97	1.05
0.84	0.85	0.93	1

VO_2 measurements during the CPT

VO2_15	VO2_30	VO2_45	VO2_60
30.7	30.1	29.8	31.2
24.4	24.6	26.7	29.1
35.7	35.1	37.9	40.85
36.5	35	41	40.7
29.95	28.7	34.65	35.9
35.2	35.1	36	37.65
34	34.1	29.5	30.5
35.7	36.05	38.75	44.26
28.2	29.3	27.8	31.4
22.4	22.9	28.5	32.7

Rating of perceived exertion during the CPT

RPE_10	RPE_20	RPE_30	RPE_40	RPE_50	RPE_60
12	13	13	14	14	15
11	12	14	16	17	17
13	14	15	17	17	20
12	11	15	15	15	17
11	12	12	14	14	16
11	13	13	14	14	15
9	13	15	16	18	18
13	13	14	16	16	17
11	12	13	14	16	17
11	10	10	12	15	18

Leg Pain scores during the CPT.

LP_10	LP_20	LP_30	LP_40	LP_50	LP_60
6	6	6	7	7	8
1	3	4	6	5	8
7	7	7	9	9	10
5.5	4.5	7	7.5	7.5	8
2	2	3	4	4	4
3	4	5	6	7	8
2	5	7	8	9	9
5	6	6	7	8	8
2	3	3	4	6	6
2	3	3	5	7	9

NEFA concentration prior to and during the CPT

	-30	-10	0	15	30	45	60
WP_02	1090.0	736.7	610.0	530.0	503.3	523.3	550.0
WP_03	616.7	616.7	536.7	463.3	490.0	563.3	576.7
WP_11	896.7	770.0	690.0	576.7	690.0	616.7	630.0
WP_12	703.3	663.3	656.7	550.0	523.3	550.0	590.0
WP_14	910.0	836.7	710.0	550.0	570.0	616.7	663.3
WP_16	790.0	710.0	643.3	496.7	556.7	676.7	650.0
WP_17	950.0	896.7	950.0	556.7	530.0	523.3	543.3
WP_19	770.0	836.7	816.7	583.3	570.0	556.7	543.3
WP_18	563.3	550.0	523.3	516.7	523.3	510.0	510.0

Insulin concentration prior to and during the CPT

WP_02	**91**	**243.89**	**606.92**	**285.93**	**279.26**	**347.1**	**306.48**
WP_03	8.29	9.85	60.07	35.01	8	10.18	6.39
WP_11	7.17	33.19	86.21	21.81	34.28	87	141.62
WP_12	162.5	203.2	364.43	258.82	222	197.63	157.42
WP_14	8.43	25.77	116.48	23.15	8.17	13.74	7.8
WP_16	14.1	91.92	224.28	130.13	49.47	41.14	40.4
WP_17	5.82	38.59	124.62	17.61	18.03	13.39	4.88
WP_18	18.03	109.38	210.02	105.87	84.47	101.83	66.78
WP_19	6.87	57.92	212.85	15.21	26.04	15.02	15.21

C-peptide concentration prior to and during the CPT

	-30	-10	0	15	30	45	60
WP_02	1542	2109	3843	2371	2469	3082	2975
WP_03	691.2	779.65	1289	1505	853.04	700.16	614.87
WP_11	1009	1490	1833	1629	1724	2221	2718
WP_12	793.56	980.85	1611	1349	1048	952.45	985.18
WP_14	811	1116	1603	1422	942.6	1068	958.35
WP_16	1241	1783	2146	2435	2092	2049	2069
WP_17	567.48	696.4	718.81	1057	679	582.11	411.58
WP_18	950.2	1483	1963	1587	1497	1653	1479
WP_19	923.08	1610	2262	1471	1319	1214	1214

Glucagon concentration prior to and during the CPT

	-30	-10	0	15	30	45	60
WP_02	54.35	331.25	444.28	686.93	515.93	418.9	407.64
WP_03	49	61.64	127.91	329.24	306.25	297.43	277.35
WP_11	101.18	202.49	233.9	380.83	357.7	334	311.71
WP_12	143.61	196.6	272.78	364.46	368.46	257.89	223.6
WP_14	155.28	185.01	167.62	301.22	281.75	245.81	224.58
WP_16	78	167.41	181.38	416.73	529.33	281.89	212.99
WP_17	127.3	179.76	183.88	292.31	213.17	231.17	292.17
WP_18	38.23	137.38	217.97	217.61	217.44	171.45	141.84
WP_19	103.34	148.15	143.19	352.59	349.01	297.41	286.88

PLACEBO TRIAL RAW DATA

Distance covered during the time trial effort.

Participant ID	Distance Covered (km)
WPC_01	16.05
WPC_02	15.21
WPC_03	19.23
WPC_11	17.63
WPC_12	16.06
WPC_14	16.64
WPC_16	18.05
WPC_17	17.63
WPC_19	15.66
WPC_18	17.81

Glucose timepoint data

-30	-10	0	15	30	45	60
99.2	106.5	113	144	149	138	132
94	94.4	102	89.4	78.7	86.6	90.3
85.6	81.1	93.3	83.5	90.5	91.1	98.9
75.7	77.4	86.4	81.3	88.7	88.3	78.7
90.5	91.5	93.7	84.9	86	106	95
79.1	81.2	91.7	72.9	80.9	95.4	89.8
79.9	80.2	100	67.4	75	81.1	62.9
86	86.7	90.5	77.3	83	90.8	90.3
92.7	93.9	114	103	78.9	85.9	80.9

Average watts at 90% LT (1st 30 min) and average watts during the TT

Watts_30	Watts_TT
177.2	180.7
152.4	162.8
233.8	290.5
181.5	223.9
155	181.7
178	199
230.4	246.1
196.1	236.2
149.7	171.8
155	230

Heart rate responses during the CPT

HR_10	HR_20	HR_30	HR_40	HR_50	HR_60
143	143	146	150	146	158
155	153	155	168	166	168
135	137	139	164	166	170
147	144	145	166	167	170
138	139	139	150	149	150
126	131	132	147	147	147
130	133	134	144	147	149
133	138	144	151	151	155
155	151	158	171	164	168
107	109	102	138	144	155

RER values during the CPT

RER_15	RER_30	RER_45	RER_60
0.9	0.89	0.89	0.91
0.85	0.83	0.83	0.83
0.91	0.86	0.9	0.88
0.82	0.84	0.93	0.95
0.91	0.9	0.93	0.97
0.91	0.93	0.95	0.98
0.9	0.89	0.91	0.92
0.95	0.92	0.95	0.94
0.98	0.96	1.01	1.05
0.87	0.85	0.91	0.98

VO_2 measurements during the CPT

VO2_15	VO2_30	VO2_45	VO2_60
30.7	30.2	29.8	31.2
26.9	25.4	27.6	26.1
34.5	36.2	45.7	45.2
33.9	31.5	38.8	39.9
30.5	29.8	32.6	34.1
32.3	32.6	32.6	37.6
33.6	33	35.3	37.8
37.4	37.1	42.2	43
29.7	29.5	33.6	34.1
21.3	19.45	29.5	34.9

Rating of perceived exertion during the CPT

RPE_10	RPE_20	RPE_30	RPE_40	RPE_50	RPE_60
12	13	14	16	16	18
12	12	12	15	16	17
13	13	15	17	17	20
12.5	12.5	12.5	15	16.5	17
11	12	13	14	15	16
10	11	11	13	14	15
11	12	13	14	17	17
13	14	14	16	16	17
11	11	13	14	16	17
7	9	10	13	14	17

Leg pain during the CPT

LP_10	LP_20	LP_30	LP_40	LP_50	LP_60
6	7	7	8	8	9
4	3	4	6	7	7
7	7	7	8.5	9	10
5.5	5.5	5.5	8	7	8
3	4	4	5	6.5	7
1	3	3	5	6	8
2	3	4	6	8	9
5	6	6	7	8	8
2	2	4	4	6	7
1	2	3	5	6	7

Plasma NEFA concentrations

	-30	-10	0	15	30	45	60
P_02	990.0	863.3	776.7	530.0	523.3	496.7	530.0
P_03	996.7	803.3	696.7	536.7	570.0	556.7	616.7
P_11	643.3	1170.0	570.0	496.7	503.3		696.7
P_12	883.3	743.3	803.3	763.3	716.7	696.7	863.3
P_14	583.3	610.0	636.7	603.3	650.0	623.3	670.0
P_16	863.3	830.0	783.3	590.0	630.0	756.7	736.7
P_17	950.0	823.3	730.0	456.7	536.7	496.7	483.3
P_19	770.0	776.7	783.3	516.7	503.3	550.0	556.7
P_18	1056.7	750.0	716.7	616.7	576.7	603.3	603.3

Insulin concentrations

P_02	**120.11**	**117.41**	**222.59**	**334.97**	**308.99**	**259.46**	**226.08**
P_03	8	9.69	11.59	17.22	6.15	6.15	6.15
P_11	9.21	7.72	31.42	13.42	7.72	4.39	4.01
P_12	165.58	182.92	205.44	160.46	135.87	141.62	142.58
P_14	3.6	6.23	22.65	4.54	2.35	4.37	1.85
P_16	10.49	13.39	30.97	23.66	13.74	15.02	5.62
P_17	4.12	6.02	7.33	14.46	5.25	5.25	2.24
P_18	9.63	25.5	91.64	68.86	11.4	9.35	7.56
P_19	9.91	10.79	149.95	26.31	16.78	24.46	7.21

C-peptide

P_02	1427	1372	1933	2947	3047	2761	2627
P_03	660.06	705.68	764.32	1072	613.59	459.97	444
P_11	962	980.31	1440	1423	1034	751.01	712.09
P_12	768.04	728.63	913.93	962.86	714.04	545.69	430.11
P_14	743.36	845.11	1086	974.54	715.42	733	517
P_16	1109	1228	1387	1730	1338	1382	1128
P_17	752.2	1271	1709	1283	1146	981.74	719.44
P_18	894.42	1073	1466	1639	911.78	795.81	663.09
P_19	933.7	997.28	1812	1561	1211	1101	872.48

Glucagon

P_02	171.33	127.91	145.57	109.76	111.23	122.27	124.97
P_03	34.57	49	49.74	45.34	63.74	86.71	123.01
P_11	87.2	67.18	93.09	91.13	82.3	86.71	84.75
P_12	178.69	185.81	167.16	194.64	206.66	215.01	228.5
P_14	117.31	96.86	102.76	135.41	147.45	118.39	127.83
P_16	66.04	79.31	71.26	98.65	115.41	72.63	77.33
P_17	146.51	151.63	143.43	149.78	151.17	161.09	153.01
P_18	43.11	46.29	50.9	25.41	45.5	50.19	56.99
P_19	100.42	103.63	98.65	132.41	136.89	134.66	131.91