THE EFFECT OF CARBOHYDRATE VERSUS CARBOHYDRATE:PROTEIN SUPPLEMENTATION DURING PROLONGED, AEROBIC ACTIVITY FOR ENDURANCE CYCLISTS

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ABSTRACT OF THESIS

Introduction: Cycling is becoming a more popular sport for novice and experienced athletes. Although it is understood that nutrition plays a vital role, it is not clear which nutrients allow athletes to cycle at optimal performance.

Objective: The purpose of the present study was to investigate and compare the effects of a carbohydrate (CHO) only supplement and a 4:1 or 7:1 carbohydrate-protein (CHO-PRO) supplement on long distance cyclists during prolonged activity.

Subjects and Protocol: Twenty-eight male and female endurance cyclists volunteered to participate in up to three cycling tests at an aerobic intensity varying between 50-70% VO2 max for 3 to 4 hours. Participants chose either treatment 1 (CHO only) or treatment 2 (CHO-PRO) for tests 1 and 2. For the third test, participants chose either treatment 1 or 2 based on their preference. Participants were allowed to use their own nutritional supplements as long as it met the criteria. Supplement intake was the responsibility of each participant and based on specific direction from their personal coach.

Methods: Pre- and post-test information, including diet, exercise, health, and experiment questions, was collected by questionnaire to determine if treatments affected performance or if other variables existed which could affect the overall effects of the treatments. Body weight was measured before and after each event. Performance was measured by average watts and average heart rate.

Results: Performance as measured by watts was not significantly different between events ($p=0.144$); however, heart rate was significantly different between events ($p=0.026$) and thus each event was analyzed separately. Males who consumed the CHO:PRO supplement performed better than those on the CHO only supplement for the first event, which trended towards significance ($p=0.059$). Performance based on watts was not significantly different between supplements for the other two events ($p=0.878$ and $p=0.255$, respectively). There was no difference in performance as measured by heart rate based on supplement ($p=0.677$; $p=0.802$; $p=0.250$, respectively). Overall, 79% (n=15) of the athletes that completed the longest test (event three) and had a choice between CHO and CHO:PRO, chose to use the CHO:PRO treatment, while 21% (n=4) chose the CHO treatment.

Conclusions: There was no difference in performance, measured in watts and heart rate, when subjects used carbohydrate or carbohydrate and protein supplements during the endurance events. However, when able to opt for a carbohydrate only or carbohydrate plus protein supplement, the majority of cyclists chose to use the supplement with additional protein.

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CHAPTER 1: INTRODUCTION

Cycling has become more popular throughout the world with 1.2 billion total cycling outings completed in the United States by adults in 2010 (2011 Outdoor Foundation). Whether a person is cycling recreationally or competitively, it is vital that they consume the necessary amount of energy to fuel their body for longer distance rides because without that energy, their body may fatigue and they will not be able to sustain the distance. Endurance cyclists completing more than one hour of physical exercise must consume energy to maintain their level of performance during the activity. Although carbohydrates are known to help cyclists perform better during prolonged activity, it is unclear whether other nutrients could help them achieve optimal performance.

The addition of protein supplementation in combination with carbohydrates has been tested more recently; however, results vary based on the type of cycling effort and distance. It is beneficial for long distance cyclists to know how many grams of carbohydrates they need to maximize their aerobic performance, which in turn allows them to finish the distance they are attempting to complete. It would also be beneficial for cyclists to know if a certain amount of protein could enhance their performance because this could allow them to complete a competition at a higher level, at a faster speed, and with less fatigue. This research was designed to compare the effects of a carbohydrate supplement (CHO) with and without protein (CHO:PRO) on long distance cycling performance.

Hypothesis

The proposed problem leads to the hypothesis that a nutritional supplement containing protein and carbohydrate will more positively affect athletic performance for long distance cyclists during prolonged activity compared to carbohydrate alone.

Subproblems

Subproblems for the experiment include the following: (1) what the effect of CHO alone is with no added protein, (2) what the best source of CHO for optimal performance is, (3) which type of protein (i.e. whey, casein, isolate, concentrate) will better optimize performance, and (4) what amount of protein, if any, is needed to see significant improvement in performance in long distance events. In addition, other subproblems include the question of whether the level of training (i.e. recreational versus well-trained versus elite athlete) impact protein needs for long distance cycling.

Limitations

Limitations for the proposed research are due to the parameters of the experimental design. The limitations that were associated with the subjects include the following: (1) subjects were required to recall data and feelings after each test, (2) subjects did not have a set diet to follow prior to testing, (3) subjects were not matched, and (4) subjects consumed different products from one another that could include other performance enhancing substances (i.e. caffeine). In addition to subject limitations, there were expense and location constraints. Performance was only measured by watts and heart rate because of the limitations of space and lab facilities. Subjects self-reported heart rate data from their personal heart rate monitors which could vary in accuracy based on the monitor brand. Also, Compu-Trainers for each bike will be the only objective resource for measuring and determining performance.

Delimitations

Delimitations exist for this research study. Subjects consumed a set amount of carbohydrates for treatment one and a set amount of carbohydrates to protein for treatment two.

Subjects were already enrolled in the Over-Distance Program at Peak Performance Professionals and agreed to participant in this study because it coincides with the program dates. In addition, subjects were considered "well-trained" because they are enrolled in a program that requires them to cycle for over three hours each time. All subjects completed a VO2 submaximal test before the program begins. All subjects were training in the months leading up to each test date and between test dates to keep up their fitness to reach training goals. The final noted delimitation is that data collection will be based on subject recollection, questionnaires, and the bike Compu-trainers to measure performance.

Assumptions

It is assumed that the subjects answered all questionnaires truthfully and with as much detail as possible. Also, subjects consumed their standard pre-race, 3-day diet prior to each test date, and that subjects weighed in before and after each test. It is also assumed that subjects consumed the correct amount of CHO or CHO:PRO assigned to them during testing and informed the researcher if more supplementation was used during testing due to fatigue or other GI distress. It is assumed that subjects hydrated properly before and during testing, subjects maintained in an aerobic zone during the entire test, and subjected reported accurate heart rate data after each test using the subjective form administered to them. Given the noted assumptions, the researcher will try to ensure that all assumptions are taken into consideration.

List of Definitions

Aerobic State: With oxygen; low intensity; the athlete remains in a state that allows muscles to function with oxygen

Anaerobic State: Without oxygen; high intensity; the athlete performs at maximal effort and muscles function without the presence of oxygen

Carbohydrate Loading: Strategy to increase the amount of fuel stored in muscles to improve performance

Compu-Trainer: The bike unit designed to simulate race courses and conditions for athletes

Endurance: The ability of an athlete to remain active for a long period of time; used in aerobic and anaerobic exercise. Endurance varies according to the type of exertion – minutes for high intensity anaerobic activity, hours or days for low intensity aerobic activity

Ergogenic Aide: Any external influence that can be used to enhance performance in high intensity activities (i.e. Caffeine)

Heart Rate: Number of heart beats per unit of time (bpm); varies based on the bodies need to absorb oxygen and excrete carbon dioxide

Heart Rate Monitor: A devise used by athletes to track average heart rate for activity and also heart rates for each completed lap

Ironman Competition: An event completed by athletes; it is comprised of a consecutive 2.4 mile swim, a 112 mile bike, and a 26.2 mile run

RPE: Rate of perceived exertion; this is an athlete's conclusion as to how hard they are working; typically a scale from one to ten

VO2 sub max: Maximum capacity of an athlete's body to transport and use oxygen during activity; reflects the physical fitness of that athlete (V=volume, O=oxygen)

Watts: Measures the rate of energy transferred as athlete cycles; it is the amount of effort required to push a cyclist and their bike through the air against friction

CHAPTER 2: REVIEW OF LITERATURE

Introduction

Carbohydrates serve several important functions both in sports performance and overall health. In terms of athletic competition, carbohydrates are vital for optimal performance and act as the primary and preferred fuel of all muscle movement since they yield the most efficient production of ATP (Skolnik & Chernus, 2010). Previous studies investigating the effects of carbohydrate consumption have found that carbohydrate loading is beneficial for endurance cyclists (Mayo Clinic, 2010). In addition, carbohydrate intake during exercise has been repeatedly shown to improve performance in all types of exercise. Carbohydrates spare muscle glycogen because the muscles take up glucose from the blood to be utilized for aerobic glycolysis instead of using stored energy (Skolnik & Chernus, 2010). Some investigators hypothesize that carbohydrate intake alone during an event of activity will not maximize a cyclists performance; thus, research is underway to find the most effective nutritional approach to improve overall performance during activity.

Recent studies have investigated the effects of added protein to carbohydrate supplements during physical activity. Both Ivy, Res, Sprague, & Widsor (2003) and Saunders, Kane, & Todd (2004) reported that aerobic capacity and performance was significantly improved after the consumption of a carbohydrate-protein beverage compared to carbohydrate alone. However, when supplements are matched for calories, the carbohydrate-protein supplement has been shown to not affect aerobic capacity or performance compared to carbohydrate alone (Martinez-Lagunas et al., 2010). Further research is warranted to clarify any potential benefits of additional protein to carbohydrate supplementation during physical activity.

Supplemental protein during endurance activity may aid in muscle repair, adaptation, and prevention of muscle damage. Endurance training involves long periods of physical effort which causes soft-tissue damage and muscle breakdown (Skolnik & Chernus, 2010). Recent studies have found a decrease in blood markers of muscle tissue breakdown indicating that the protein was useful in preventing muscle damage (Skolnik & Chernus, 2010). Regardless of these findings, there is no consensus among researchers as to the optimal amount of protein to include in a nutritional regimen during competition in order to see benefits affecting muscle repair, damage, and adaptation.

Research on this topic is difficult because nutritional needs during endurance activity likely differs between individuals, and there are so many other variable that affect performance. Variables that affect performance include the level of intensity of activity, amount of aerobic versus anaerobic glycolysis that occurs, type of event, distance and length of time of activity; all in addition to the nutritional regimen (quantity and content) prior to and during the event. The ideal macronutrient consumption during an event to optimize performance will continue to be debated until more detailed research emerges. The purpose of this literature review is to critically analyze the evidence on nutritional supplementation during aerobic activity. Background related to carbohydrate and protein metabolism will be discussed first.

Background

Carbohydrate Metabolism

Endurance exercise training is characterized by an increase in an individual's oxidative capacity, which occurs through building mitochondria size and density and with the accumulation of myosin fibers (Breen et al., 2011). During activity, the demand for ATP increases, thus more glucose is needed for energy production. Carbohydrate is the preferred fuel source during exercise since it yields the most ATP in the greatest quantity and at the greatest efficiency. The carbohydrate that is consumed is digested into glucose, which is absorbed into the blood stream. The pancreas is then stimulated to secrete insulin, which allows muscle cells to take up glucose from the blood stream. Once inside the muscle cells, glucose may be converted into glycogen, or may be burned for energy via glycolysis. In the process of glycolysis, under aerobic conditions (such as endurance exercise), a single molecule of glucose is converted into two 3-carbon molecules, which then enter the TCA cycle. Reducing equivalents produced during the TCA cycle drive the electron transport chain, which using the electrochemical gradient across cell membranes, drives the synthesis of ATP, with oxygen as the final acceptor of the electrons. Under aerobic conditions, for each molecule of glucose, 36 molecules of ATP are formed. Muscle cells need a continuous source of ATP to drive the work of the tissue. In contrast, during anaerobic glycolysis, oxygen is not available at the rate needed as the final electron acceptor, and thus alternate electron acceptors are utilized. During anaerobic glycolysis, ATP production per molecule of glucose is much less efficient.

The advantages of carbohydrate supplementation during aerobic exercise have been extensively studied to evaluate their effect on performance. Aerobic activity is performed at a lower intensity and also referred to as endurance type exercise. Anaerobic activity is performed at a higher intensity and is much more difficult to maintain for long periods of time due to the inability to maintain efficient ATP production at the rate it is needed. Typically, investigators have seen improved aerobic performance in cyclists consuming carbohydrate supplements versus only water or a placebo during prolonged activity lasting over two hours, the point at which muscle glycogen may be depleted (Saunders et al., 2004). This review focuses on supplementation during exercise, although nutrition before and after endurance activity also plays a vital role for cyclists.

During endurance exercise, the most effective and most commonly used strategy by cyclists is to consume carbohydrate-rich beverages or foods during competition (Ivy et al., 2010). Often, the main goal for athletes is to optimize performance. Maximal muscle and liver glycogen stores, which are critical for optimum performance as an energy source during prolonged aerobic exercise, are achieved by consuming carbohydrate-rich beverages and foods, but the amount and timing can vary from athlete to athlete. If muscle glycogen stores are depleted, exercise must be stopped or intensity is significantly reduced due to lack of readily accessible, efficiently available energy (Ivy et al., 2003). Carbohydrate supplementation during the activity can limit the depletion of muscle glycogen during aerobic exercise. In addition, physical performance during exercise of aerobic to anaerobic intensity is dictated by the ability to maintain required rates of carbohydrate oxidation (Toone et al., 2010). Performance can be greatly improved by maximizing the availability of carbohydrate before exercise, as well as during exercise.

Protein Metabolism

During prolonged endurance exercise, muscles absorb amino acids from the bloodstream to be used within the muscle tissue. When carbohydrate stores are low in the muscles, tissue will burn amino acids for energy (Skolnik & Chernus, 2010). Within muscle tissue, amino acids are catabolized into the amino group and the carbon skeleton, which may be burned for energy via glycolysis if needed. However, amino acids are a much less efficient energy source than carbohydrate. Furthermore, amino acids are needed more to maintain lean body mass and thus ideally are only used for ATP production when glucose is not readily available. When adequate carbohydrate is present, there is less demand for protein to act as a fuel source, which spares the amino acids for other use. Consumption of a carbohydrate-protein beverage has been associated with reduction of markers for plasma creatine kinase (Saunders et al., 2007; Romano-Ely et al., 2006) and muscle soreness (Romano-Ely et al., 2006) when compared to carbohydrate alone beverages.

Although many studies suggest that adding protein to carbohydrate supplementation will produce ergogenic effects, some researchers have found protein consumed during endurance activity to not affect performance. Vegge, Ronnestad, & Ellefsen (2012) noted that different sources of protein in supplements such as whey and casein provide different amino acid profiles which can cause differences in nutrient absorption and metabolic responses. Whey protein causes a different absorption process to occur and is absorbed at a different rate than casein protein, which affects whole body protein metabolism. Thus, amino acid composition of the supplements may have an impact on the ergogenic effects of carbohydrate and protein supplementation. Certainly there is great debate on supplementation during endurance exercise and it is apparent that more research geared towards coingestion of protein with carbohydrate supplementation is necessary to find consistent results exemplifying optimal performance during prolonged activity for endurance athletes.

Protein ingestion before endurance exercise may augment net muscle protein accumulation during post-exercise recovery (Beelen et al., 2010). Protein consumption prior to and during resistance type exercise also stimulates muscle protein synthesis, which may improve muscle adaptive responses to exercise training (Beelen et al., 2010). There is little research investigating the effects of protein ingestion on endurance exercise, however.

Evidence suggests that carbohydrate and protein consumption during endurance exercise is associated with improved muscle recovery, accelerated glycogen replenishment, and reduced post-exercise muscle damage (Saunders et al., 2007). More evidence is needed to determine the metabolic effects of combined protein and carbohydrate supplementation, in comparison with carbohydrate alone, during endurance events, as well as the affects of muscle adaptation and damage.

Macronutrient Supplementation during Endurance Exercise

Carbohydrate supplementation is required during endurance exercise lasting over 2 hours in order to maintain efficient ATP production and maintain performance. The ability of other macronutrients, combined with carbohydrate or alone, to improve performance is still debated between investigators (Cathcart et al., 2011). While endogenous triglycerides are an energy source during endurance activity, supplements rarely contain lipids because of the difficulty of fat digestion and likelihood of discomfort during activity. It is possible that a carbohydrate-protein supplement during activity may be more effective than a carbohydrate supplement alone (Ivy et al., 2003). Carbohydrate supplementation limits the need to access and use muscle glycogen. In addition, several publications reported enhanced glycogen resynthesis post-exercise when protein was ingested with carbohydrate after completion of activity (Ivy et al., 2003). The mechanism of this benefit is thought to be an amino acid stimulated, augmented insulin response, which is unlikely to have additional physiological benefits over carbohydrate ingestion alone during exercise (Cathcart et al., 2011). Regardless of this unlikelihood, results have suggested improvements in time to exhaustion at fixed work rates (Saunders et al., 2004) as well as variable exercise intensities (Ivy et al., 2003) in subjects consuming supplements with carbohydrate and protein that were matched for carbohydrate content, but not calorie content, during exercise. Not all studies have been able to find clear benefits of combined carbohydrate and protein supplementation in comparison to carbohydrate alone when matched for carbohydrate amounts.

It remains unclear as to whether adding calories through the addition of protein or replacing a portion of the carbohydrate calories with protein has any ergogenic benefits on endurance exercise (Cathcart et al., 2011).

Current Recommendations for Nutritional Supplementation during Exercise

A variety of agencies and institutions, such as SCAN (Sports, Cardiovascular, and Wellness Nutrition), American College of Sports Medicine, and the AND (Academy of Nutrition and Dietetics), offer evidence-based nutritional recommendations to athletes regarding the amounts of carbohydrate to consume to optimize performance, improve metabolic processes, and potentially see positive ergogenic effects. The Academy of Nutrition and Dietetics recommends that endurance athletes consume 10-13 grams CHO/kg of body weight during endurance events; whereas SCAN recommends endurance athletes consume up to 90 grams CHO/hour. Recently, as researchers continue to investigate nutritional profiles that optimize performance, other nutritional components, such as protein, are being scrutinized and new suggestions are being developed.

Evidence on Nutrition Supplementation during Endurance Cycling

There are various studies explaining the effects and overall benefits of carbohydrate consumption during aerobic activity, but few have determined the exact amount necessary per hour or which macronutrients are needed to maximize performance. Some studies are finding that increased carbohydrate intake during endurance activity can improve performance, but the effect of the timing of carbohydrate intake on performance is still unknown. Regardless of the current findings, endurance cyclists commonly consume carbohydrate-rich beverages and foods during the event. The following is a critical analysis of research on the effect of supplementation of carbohydrate alone during endurance activity on performance.

The Effect of Carbohydrate Supplementation on Performance

The study “Bananas as an Energy Source during exercise: A Metabolomics Approach” compared the effects of ingesting banana versus a 6% carbohydrate drink during a cycling event on performance, post-exercise inflammation, oxidative stress, and immune function (Nieman et al., 2012). This randomized-controlled crossover study included 14 male cyclists experienced in time trials and road racing. They ranged from age 18 to 45 years old and had to maintain their current weight for the duration of the study and avoid any vitamin and mineral supplementation. Baseline testing included a maximal power test, oxygen consumption, ventilation, and heart rate assessments during a graded exercise test. Demographic and training histories were gathered by questionnaire and athletes met with a dietitian. Subjects were instructed to follow a moderate carbohydrate diet for three days before each time trial. They received a food list of appropriate foods to eat during this time and also recorded their food intake over the course of three days. One week later, subjects completed a 75-km time trial during which they were randomized to either consume 0.2 g/kg body weight of banana or 6% carbohydrate solution every 15 minutes. They were crossed over for the second 75-km time trial three weeks later. Blood counts, plasma cytokines, oxidative stress, and antioxidant capacity were evaluated.

There were no significant differences for the athletes in energy, macronutrient intake, potassium consumption, vitamin C, and dietary fiber prior to the time trials. Mean power, heart rate, rate of perceived exertion, and total time did not differ for subjects between the two treatments. Subjects who consumed banana reported feeling more full and bloated during the trial; however, subjects lost 0.4 kg more body weight during the banana trial. Overall, the data indicated that ingestion of banana or a 6% carbohydrate solution during a 75-km time trial resulted in similar performance. Nieman et al. (2012) concluded that cyclists ingesting banana or carbohydrate solution at a rate of 0.2 g/kg carbohydrate per 15 minutes are capable of completing the 75-km time trial with no differences in performance measures.

Although strengths and weaknesses were not addressed by the authors, they did exist. Strengths of the study included the use of well trained, experienced road racers who were baseline tested before the first time trial and each received diet instruction that resulted in similar diet histories during the three days pre-testing. Subjects completed each time trial in a controlled environment and were randomized to avoid potential biases. Time trials were divided by three weeks allowing each athlete to recover from the previous activity. Weaknesses included the small number of subjects used for the experiment as well as the food diary kept by each subject. Another possible weakness could be the frequency of carbohydrate consumption. Banana or the carbohydrate solution was consumed every 15 minutes during the event which may not be ideal for every cyclist. This timeframe may have also contributed to the bloating some cyclists experienced. Certainly, this study focused on the effects of only carbohydrates on experienced cyclists during prolonged activity, but did not address the ideal amount of carbohydrate. Many professional cyclists want to know an exact amount of carbohydrate needed per hour of aerobic activity and also the type of carbohydrate to consume in order to improve performance.

Burke, Claassen, Hawley, & Noakes (1998) studied the effects of pre-exercise meals on metabolism and performance. The purpose of this study was to examine if the glycemic index (GI) of pre-exercise carbohydrate (CHO) intake has an impact on exercise metabolism and performance when larger amounts of CHO are consumed during exercise. Six trained male cyclists participated in this study. Each subject cycled during three randomized trials with each trial separated by seven days. Before each trial, one of the test meals was consumed (two hours before cycling 70% VO2 max). Test meals included the following: a high glycemic (HGI) CHO rich meal of instant mashed potato (GI=87); a low glycemic meal (LGI) CHO rich meal of lightly cooked pasta (GI=37); or a control meal (Con) of low-energy jelly. Subjects had to consume the meal within 15 minutes and then rest for two hours. Fifteen minutes before exercise, the subjects consumed a glucose solution and continued to drink the solution every 20 minutes (a total of 24ml/kg each trial) during the steady-state ride. On completion of the endurance ride, subjects were given one minute to rest before they started a timed and more anaerobic performance ride.

Results of the study showed a significant interaction between diet and time ($p < 0.05$). Serum glucose concentrations were significantly increased above fasting values for the HGI and LGI trials (7.9 ± 0.6 and 6.6 ± 0.5 mmol/L) compared to control (4.4 ± 0.3 mmol/L). In addition, there was a significant rise in serum insulin after the HGI meal (74.1 ± 10.1 uU/ml) and persisted longer than the LGI trial (43.9 ± 8.9 uU/ml). Free fatty acid (FFA) concentrations in the control trial were significantly greater than the HGI trial. There was no significant interaction of diet and time for Respiratory Exchange Ratio (RER), total CHO oxidation, and oxidation of the ingested glucose drink. However, after 20 minutes of exercise, oxidation of the CHO drink was significantly lower in the LGI trial than in control. The amount of CHO that was oxidized during the 120 minutes of steady state exercise was, on average, 380g and did not differ among trials. Similarly, Rate of Perceived Exertion (RPE) did not differ among trials. Performance during the time trial after the 120 minutes of steady-state exercise did not significantly differ among trials. Time to complete the 300 kJ of cycling was 947 ± 23, 953 ± 36, and 970 ± 26 seconds for HGI, LGI, and control trials, respectively.

The most valuable finding from the present study was when large amounts of CHO, approximately 170 grams, were ingested during prolonged cycling, there were minimal differences in the metabolic and performance between the pre-event meals. This finding is valuable because performance was affected by carbohydrate consumption during activity more than what was consumed before as a pre-exercise meal. The ingestion of a HGI CHO-rich meal produced a greater glycemic and insulinemic response compared to the LGI CHO-rich meal during exercise. Carbohydrate consumption throughout exercise minimized any potential differences in either circulating blood metabolites or substrate oxidation during the trials, hence, nutrients consumed during activity appears to be more significant than no nutrient consumption at all. Finally, all subjects reported that for competition events, they would choose to eat the pre-exercise meal that was most familiar to them, suggesting that cyclists prefer to eat what they know has been effective to improve their performance when cycling. Based on this, when endurance athletes consume the recommended amounts of CHO during exercise, they may choose their pre-exercise meal in accordance with their personal preferences and previous experiences.

Strengths and limitations existed for the present study. One strength was that athletes were blinded to the treatment groups; however, this was also a limitation because it appeared the cyclists knew which treatment they were on and which they would normally choose to eat before a competition. Testing the effect of the pre-exercise meal on performance is a strength of this study as it is not considered in other research studies. This too can act as a limitation because the pre-exercise meal may not be what is affecting cycling performance during activity but rather what the cyclist is consuming during activity. There is a possibility that the pre-exercise meal aids performance during activity but further research is necessary with the exclusion of supplementation during activity to isolate the pre-exercise meal.

These studies indicate that carbohydrate prior to and during endurance exercise is helpful to achieve optimal performance. The exact form of the supplement, the quantity and timing, and whether additional macronutrients such as protein may be beneficial, is still unclear.

Studies Suggesting a Potential Benefit of Supplemental Protein on Performance

Rebecca J. Toone and James A. Betts conducted a randomized double blinded study in 2010 that tested the effects of nutrient ingestion on cycling time-trial performance. The study, “Isocaloric Carbohydrate Versus Carbohydrate-Protein Ingestion and Cycling Time-Trial Performance,” was designed to compare the effects of energy-matched carbohydrate (CHO) and carbohydrate-protein (CHO-PRO) supplements on cycling time-trial performance. They hypothesized that replacing a portion of carbohydrate in a supplement with protein would not significantly improve physical performance when the supplement was ingested during a competitive race simulation before a cycling time-trial.

Participants included twelve highly trained male cyclists and triathletes (2.9 ± 2.1 years of competitive time-trial experience). All participants were similar in age, BMI, VO2 max, and had completed a similar amount of training hours per week. The study was designed to have participants perform two main trials in a randomized, counterbalanced order separated by 5-10 days. The supplements were given in a double-blind manner, such that an individual not involved in the study provided the supplements in unmarked containers. Each 62 minute trial included a ten minute warm up followed by a 45 minute variable intensity protocol and a 6 km time trial. Fifteen minutes before the start of the warm up and every fifteen minutes throughout the protocol (but not during the 6 km trial), participants ingested either a solution containing CHO alone or a matched solution for available energy containing a 3:1 mixture of CHO-PRO.

The solution profiles for CHO and CHO-PRO were matched for taste, consistency, and odor to ensure successful double blinding. The total amount of CHO (sucrose) ingested was 95

± 7 grams in CHO trials and 72 ± 5 grams in the CHO-PRO trials. This equals an ingestion rate of approximately 1.4 and 1.1 grams/minute, respectively. Furthermore, equal energy content was achieved between trials by including 22 ± 2 grams, or 2.2%, of whey protein isolate in the CHO-PRO solution. Carbohydrate and CHO-PRO solutions were provided in equal volumes for each treatment.

Results of the study indicated that mean time-trial performance was longer when the CHO-PRO supplement had been ingested relative to the energy-matched CHO supplement ($p=0.048$). Order of supplementation had no effect on performance. Blood glucose was regularly tested during each trial and results showed that blood glucose concentrations were lower ($p=0.02$) in CHO-PRO trials than the CHO trials. Similarly, peak glucose concentrations in each participant were lower ($p=0.04$) in CHO-PRO trials than in CHO trials. In addition, blood lactate was regularly tested during the protocol and concentrations were no different between the CHO and CHO-PRO treatments at any point. When the actual time-trial occurred, there was a substantial increase in blood lactate concentrations across both treatments compared to baseline ($p < .001$).

Mean rates of whole-body lipid oxidation for the entire cycling protocol were 0.05 ± 0.03 grams/minute for the CHO treatment and 0.04 ± 0.02 grams/minute for the CHO-PRO treatment. Carbohydrate oxidation increased significantly with the onset of exercise ($p < .001$) with rates averaging 3.38 ± 0.13 grams/minute during the entire protocol for the CHO treatment and 3.43 ± 0.17 grams/minute with the CHO-PRO treatment. In addition, Rate of Perceived Exertion (RPE) was significantly higher during the CHO-PRO trials than the CHO trials ($p=0.01$), but with no apparent interaction of treatment and time. Finally, mean heart rate during the protocol was not different between CHO and CHO-PRO groups, averaging 151 ±9 beats/minute for both treatments.

The main finding of this research study was that ingesting carbohydrate alone in sufficient quantities during a competitive race simulation, results in a significantly enhanced time-trial performance relative to the energy matched mixture of carbohydrate and protein. The researchers noted that the overall difference in time-trial performance between each treatment was only ~1%; however, that equates to approximately 60 m of distance, which is considered worthwhile in a competitive environment. Furthermore, no metabolic effects were apparent between treatments that could have affected performance outcomes. The researchers stated that one major limitation of the study was, in fact, a lack of further metabolic analysis. The mechanism as to why CHO is more effective than CHO-PRO remains unclear and further research is necessary.

Using supplemental carbohydrate during prolonged exercise at a variable intensity has been found to spare muscle glycogen and increase aerobic endurance. Ivy et al. (2003) compared the effects of a carbohydrate and a carbohydrate-protein supplement on aerobic endurance performance.

Nine trained, male cyclists participated in a double blind, randomized crossover design. Subjects had a mean age of 27.3 ± 1.3 years, weighed 69.6 ± 2.5 k, and had a VO2 max of 61.3 ± 2.4 ml/kg/min. On three separate occasions, the subjects cycled to fatigue, defined when the subject could no longer maintain the required exercise intensity for fifteen seconds continuously, or dropped below the required exercise intensity for the third time.

During each cycling test, subjects were blindly administered a 200ml supplement of either a placebo, a 7.75% carbohydrate supplement, or a 7.75% carbohydrate / 1.94% protein supplement. All supplements had the same amount of vitamins and electrolytes. Each supplement was provided immediately before the start of the exercise and every 20 minutes thereafter until the exercise intensity was increased to 85% VO2max; supplements were then stopped. Cycling tests started with a 30 minute warm up at 45% VO2max and was followed by cycling six times for eight minutes at 75% VO2max, alternated with cycling six times for eight minutes at 45% VO2max. Next, subjects cycled for three minutes at 75% VO2max and for three minutes at 45% VO2max. This was repeated nine times until the last piece of the test when subjects cycled at 85% VO2max until fatigue. Each cycle test was separated by a seven day period.

Overall, supplementation significantly improved performance compared to the placebo supplement. The carbohydrate treatment resulted in a significant increase in time to exhaustion when compared to the placebo. In addition, the carbohydrate-protein treatment resulted in a significant increase in time to exhaustion when compared to the carbohydrate treatment. There was a 55% increase in performance based on watts when comparing carbohydrate with placebo and 36% increase when comparing carbohydrate-protein with carbohydrate. Furthermore, carbohydrate oxidation was significantly reduced (at 174 minutes; placebo = 1.79 ± 0.9 g/min, carbohydrate = 2.48 ± 0.15 g/min, carbohydrate-protein = 2.31 ± 0.13 g/min), and fat oxidation was significantly increased during the placebo treatment (174 minutes; 0.76 ± 0.04 g/min) compared to the carbohydrate (174 minutes; 0.56 ± 0.04 g/min) and carbohydrate-protein (174 minutes; 0.55 ± 0.03 g/min) treatments. There were no significant differences in respiratory exchange, carbohydrate oxidation, or fat oxidation between the carbohydrate and carbohydrate-protein treatments. Results also indicated that blood glucose levels were significantly higher during the carbohydrate and carbohydrate-protein treatments compared to the placebo treatment.

Mean blood glucose was not different between the carbohydrate and carbohydrate-protein groups. Blood lactate was not significantly different between treatments until fatigue. At this time, blood lactate averaged 4.95 ± 1.1 mmol/L for the placebo treatment, 5.67 ± 0.9 mmol/L for the carbohydrate treatment, and 6.22 ± 0.8 mmol/L for the carbohydrate-protein treatment.

The results of this research study suggested that while carbohydrate supplementation improved endurance performance, there were additional improvements in performance when protein was combined with carbohydrate as an energy source during prolonged aerobic activity. Both carbohydrate and carbohydrate-protein supplementation increased the plasma insulin concentration during exercise above that produced by the placebo. Moreover, researchers found that after 180 minutes of variable intensity exercise, the subjects were only capable of cycling approximately 13 minutes at 85% VO2max with the placebo treatment. The subjects were able to cycle for approximately 20 and 27 minutes at 85% VO2max for the carbohydrate and carbohydrate-protein treatments, respectively. Overall, the authors concluded that providing a carbohydrate supplement during endurance exercise would improve overall performance, and that the addition of protein to a carbohydrate supplement would provide an additional ergogenic effect. However, more research is needed to draw definitive conclusions.

Romano-Ely, Todd, Saunders, & Laurent (2006) in a randomized crossover trial tested the effects of a carbohydrate-protein-antioxidant drink on cycling performance and muscle damage in elite athletes. They compared the effects of two commercially available beverages, matched for total calories. The products were a carbohydrate (CHO) beverage (Gatorade) and a carbohydrate-protein beverage (Accelerade and Endurox) fortified with vitamins C and E (CHOPA). Researchers posed two questions: (1) Does CHOPA alter time to fatigue during prolonged bouts of cycling compared with an isocaloric CHO beverage, and (2) Does CHOPA alter biomarkers of post-exercise muscle damage and muscle soreness compared with an CHO beverage.

Fourteen male volunteers completed this study, achieving a statistical power of 0.08. Subjects were included if they trained at least 4 days/week and demonstrated a VO2max above 45 ml*kg-1*min-1. The testing protocol followed the same protocol used by Saunders et al (2004), using three phases to gather data and draw conclusions. Phase one consisted of subject data collection and the final two phases were performance rides. The first performance ride was at 70% VO2max and the second performance ride was at 80% VO2max. Each ride was separated by a 22 to 24 hour rest period. After a one to two week wash out period, phase three began. It was identical to phase two, but the beverages were alternated. Again, beverages were randomized such that half the subjects consumed CHO and half consumed CHOPA during both trials.

The purpose of the first performance test was to induce glycogen depletion that would reduce performance for the second ride. The purpose of the second performance ride was to provide a measure of performance in a state of partial glycogen depletion. Thus, researchers hypothesized that differences in performance between beverages would be greater in the second ride. The beverages contained equal fluid volumes and equal calories. During the performance rides, subjects consumed the treatment beverage at a rate of 2 ml/kg body mass every 15 minutes. The CHOPA beverage contained 20% fewer carbohydrate calories than the CHO beverage. The CHOPA beverage contained 7.5% carbohydrate and 1.8% protein, and the CHO beverage contained 9.8% carbohydrate. The flavor of both treatments was matched to ensure blinding of subjects.

Results of this study indicated that there were no significant differences in VO2 max values between treatment trials at either 70% or 80% intensity. Diet records reflected no differences in total calories, carbohydrate content, or protein content for the 24 hour period prior to the trial and each ride within each phase. For both treatments, time to fatigue was shorter in trials at 80% VO2max compared to the trials at 70% max. However, time to fatigue was not significantly different between trials supplemented with a CHO beverage compared to a CHOPA beverage at 70% intensity (CHO: 95.8 ± 29.7 min, CHOPA: 98.1 ± 28.7 min; $p = 0.77$), 80% intensity (CHO: 42.3 ± 18.6 min, CHOPA: 42.9 ±21.8 min; $p = 0.91$), or as a total over the 2 day period (CHO: 138.1 ± 39.3 min, CHOPA: 140.9 ± 43.7 min; $p = 0.77$). Creatine Phosphokinase (CPK) values were assessed to determine muscle damage and values were significantly elevated ($p < 0.05$) over baseline values during the CHO trial (202.6 ± 120 vs. 582.0 ± 474.9 U*L-1), but not the CHOPA trial (187.5 ± 119.0 vs. 272.9 ± 169.4 U*L-1), indicating greater muscle damage. Soreness was subjectively rated and researchers found peak muscle soreness significantly higher in the CHO trial (median = 3.0, range = 1.0-5.0) than the CHOPA trial (median = 1.0, range = 0.0-2.0) at 24 hours.

The present research study determined that time to fatigue was not different between CHO and CHOPA treatments. The CHOPA treatment contained the same number of total calories and 25% fewer carbohydrate calories than the CHO treatment. Under these conditions, performance time during the CHOPA trial was similar to the CHO trial. Thus, when matched for total calories, carbohydrate-protein beverages are equally effective as CHO beverages in providing performance benefits, less muscle damage, and less muscle soreness during exercise. Investigators note that future studies comparing carbohydrate-protein and carbohydrate beverages are necessary to continue finding significant results as to whether performance improvements exist based on the type of nutrients consumed. Overall, data from the present study added to the growing body of evidence that protein added to carbohydrate supplements during and after exhaustive exercise does not improve performance but may ease muscle damage.

One strength of this study is the type of beverage. The type of beverages used was matched for total calories, eliminating energy content as a variable. Moreover, the experimental design was a limiting factor because it was designed to mimic day to day training and dietary practices that are common among competitive cyclists. Within subject variations of training and diet led to variable data. Subjects were relied on to keep training and diet normal prior to the each test. It was stated in this study that records were reported reliable and sensitive enough to see changes within subjects. Also, during the experiment, subjects cycled to exhaustion on two separate days with rest periods between. Experimenting on the same day with rest periods between may have different effects on cyclists. Another limitation is using CPK as a marker of muscle damage. CPK can be released from other sources such as monocytes in addition to the muscles which may cause skewed results. Finally, researchers used two beverages as treatments, but for more valid comparisons, a third beverage would be recommended such that a CHOPA, isocaloric CHO, and isocarbohydrate-CHO beverage are used during experimentation. This design could clarify if the benefits of carbohydrate-protein beverages are due to additional calories or due to the addition of protein.

Cathcart, Muragatroyd, McNab, Whyte, & Easton (2011) assessed the effects of combined CHO-PRO supplementation on physiological responses during eight days, or stages, of cycling competition in a hot environment. Researchers hypothesized that CHO-PRO supplementation would prevent body mass loss, enhance thermoregulation, and improve competitive exercise performance. Twenty-eight trained mountain bikers (4 female, 24 male) participated and each was a competitor in the 2007 TransAlp Challenge mountain bike race. Five of the subjects withdrew from the competition and were therefore excluded from the study.

Subjects were matched by predicted VO2max and then randomly assigned to one of the two treatment groups; either placebo (PLA) or CHO-PRO. The four female subjects were divided equally between PLA and CHO-PRO groups. Participants were provided with solid and liquid supplements on the morning of each stage. Both solid and liquid supplements were provided to permit ad libitum consumption during every stage. Cyclists' may have preferences to use solids or liquids while competing and researchers did not want to limit participants. The PLA liquid supplement contained 76g CHO, 460mg sodium, and 122mg potassium. The PLA solid supplement contained 37g CHO, 3g whey PRO, 5g fat, and 55mg sodium. The CHO-PRO liquid treatment contained 72g CHO, 18g whey PRO, 648mg sodium, and 170mg potassium. Solid CHO-PRO supplements contained 24g CHO, 13g whey PRO, 4g fat, and 100mg sodium. Subjects and researchers were blinded to the supplements and could consume as much supplement as they wanted but had to avoid any other food or beverage offered on the bike course.

The competition was a multi-day event during which participants were required to cross the Central Alps in eight consecutive days, or stages. Each stage consisted of one cross country marathon. First, there were no significant differences in physical characteristics, including, age ($p=0.82$), height ($p=0.18$), pre-race body mass ($p=0.24$), or predicted VO2max ($p=0.95$) between treatment groups. There were no significant differences between groups in the mean rate at which liquid ($p=0.11$) and solid ($p=0.14$) supplements were ingested during the race. The mean rates of CHO ($p=0.24$) and fat ($p=0.16$) intake during the race were not significantly different between the two groups. Subjects in the CHO-PRO group ingested PRO at a significantly greater rate than in the PLA group ($p < 0.01$, 95% CI 11.94-18.13 g/h) and also had a significantly higher energy intake ($p=0.03$, 95% CI 7.77-131.96 kcal/h). There were no significant differences in mean daily caloric ($p=0.82$), CHO ($p=0.34$), PRO ($p=0.52$), or fat ($p=0.23$) intakes between the two groups.

There was, however, a significant decline in body mass from pre- to post- exercise on all race stages ($p < 0.01$) with no differences between groups. The mean change in body mass from pre-to post-exercise for all stages (kg and % of pre-exercise mass) were -1.3 ± 0.1 kg and -1.8 ± 0.2% for PLA, and -1.4 ± 0.1 kg and -2.0 ± 0.2% for CHO-PRO. Each subject in both groups had a normal range of serum CK. It did increase significantly from baseline levels for both groups and remained significantly elevated, but still within normal range, until the end of the race. Palpated muscle soreness was evaluated subjectively. Results indicated no significant differences between treatment groups ($p=0.44$). Muscle soreness increased significantly from baseline values to the end of race stage one ($p < 0.01$) and remained elevated until the end of the race for all groups.

Blood glucose results were determined in addition to urine osmolality and tympanic membrane temperature. There was a significant increase in blood glucose pre- to post-exercise on both stage 1 and 8 ($p < 0.01$), for both groups. There was a significant main effect of supplementation on urine osmolality ($p=0.04$) but time ($p=0.96$) or race stage ($p=0.09$) did not have a significant main effect. There were no significant interactions with the exception of race stage x group on urine osmolality ($p=0.04$). Urine osmolality was significantly higher post-exercise in CHO-PRO compared to PLA on races stages 4 ($p=0.03$), 5 ($p < 0.01$), 6 ($p=0.01$), 7 ($p=0.04$), and 8 ($p=0.03$). The urine osmolality results suggested that subjects in the PLA group did excrete a greater proportion of the ingested fuel. Race stage ($p < 0.01$) and supplementation (p=0.01) had a significant main effect on rise in temperature from pre- to post-exercise and was significantly higher in PLA compared to CHO-PRO on race stages 5 (p=0.01), 6 (p=0.03), and 7 (p=0.01), suggesting that the addition of protein to the carbohydrate supplement could have an effect on thermal regulation.

There was a significant main effect of race stage on heart rate and exercise performance measured by time (p=0.04, p=0.02, respectively) but supplementation did not have a significant effect on heart rate or performance (p=0.16, p=0.24, respectively). Mean HR was significantly higher on race stage 1 compared to all other stages for both groups (PLA; 154 ± 3 bpm, CHO-PRO; 149 ± 4 bpm). Mean peak HR was significantly higher as well (PLA; 181 ± 1 bpm, CHO-PRO; 174 ± 1 bpm). The total time it took to complete the eight stages was significantly faster in the CHO-PRO group compared to the PLA group (2,277 ± 127 vs. 2,592 ± 68 minutes; p=0.02), suggesting that added protein to carbohydrate supplement affects overall time to completion and essentially performance. There was no significant differences in completion of stage 1 between groups (p=0.09), but all other individual stages were completed by CHO-PRO subjects faster than the PLA subjects ($p < 0.05$ for all comparisons between stages by group).

Overall results indicated that the CHO-PRO treatment group maintained their body mass during the competition better than PLA, had a rise in temperature more so than PLA, and completed the race 12% faster than those in the PLA treatment group. Not all of the variables were controlled for, so this difference in groups could have been due to the increased calories during exercise from the additional protein in the CHO-PRO group. Although results from this study suggest that the CHO-PRO was more helpful than the PLA supplement for performance, the benefits of the use of protein are still unclear. PRO consumption during exercise did not have any additional benefit on muscle damage compared to PLA. Many studies have reported reductions in CK and muscle soreness with the addition of PRO and the present study is a direct contrast to those investigations. The reason as to why CHO-PRO supplementation did not affect muscle damage is unclear.

Limitations of this study include baseline performance and physiological responses to exercise not being measured due to the competition restraints. Also, a randomized-crossover design would have strengthened the results by helping to control for potential confounding variables between individuals. This nutritional intervention was not in a controlled environment; however, the research setting of a real competition lends itself to meaningful insight as to whether or not an ergogenic effect is possible during actual races. Finally, combined CHO-PRO supplementation during a prolonged mountain bike race, in a hot environment, was effective in preventing body mass loss, reducing tympanic membrane temperature, and improving exercise performance. The exact mechanism remains unknown, but these effects might be mediated by a PRO-induced increase in substrate availability and/or improvements in thermoregulatory function. Further research is necessary to validate the present study results and determine the mechanism for improved performance.

McCleave et al. (2011) investigated whether the addition of moderate levels of protein (1.2%) to a low carbohydrate (3%) mixture (CHO+PRO) would improve time to exhaustion (TTE) compared to a traditional 6% carbohydrate (CHO) supplement in well-trained female athletes exercising at, or slightly below, threshold. The study followed a randomized, double-blind, repeated measures design. Two experimental trials were completed with 7 days separating the two trials. In each trial, fourteen female cyclists cycled at varying intensities between 45% and 75% VO2max for varying intervals followed by a ride to exhaustion.

Supplements were consumed in the same dose for each subject. Doses of 275ml were consumed immediately and every 20 minutes of the ride. Subjects were asked to consume only as much as they felt comfortable as they reached exhaustion. The CHO+PRO supplement contained a mixture of dextrose, maltodextrin, and fructose, with a whey protein isolate. The CHO treatment was only comprised of dextrose. CHO+PRO contained 50% the CHO content compared to the CHO treatment, as well as 33% less calories. Both treatments contained equal electrolyte concentrations, but they were not matched for calories.

The first 30 minutes of cycling was performed at 45% VO2max, which was monitored during the experiment by investigators, followed by six intervals of 8 minute durations. Intervals were then reduced to three minutes, and at three hours of total cycling, subjects began the performance ride to exhaustion. Exhaustion was determined as the point in which subjects could no longer maintain a cadence of 60 rpm.

Performance measures showed that TTE was significantly greater ($p < 0.05$) for the CHO+PRO (49.94 ± 7.01 minutes), with a 15.2% increase in performance compared to the CHO treatment (42.36 ± 6.21 minutes). Subjects performed the exhaustion ride at an average of 75.06% VO2max. Intensities for individual subjects ranged from 7.25% below threshold to 5.1% above threshold. In addition, blood and plasma analyses showed no significant differences in pre-exercise plasma glucose levels between groups. Plasma glucose concentration increased significantly from pre-exercise to the end of exercise for the CHO treatment. Mean blood glucose over the course of the event for the CHO group (4.47 ± 0.12 mmol/L-1) was significantly greater compared to the CHO+PRO group (4.07 ± 0.12 mmol/L-1). No significant differences between supplements were found in average blood lactate concentrations or average plasma myoglobin concentration. Additionally, there were no significant treatment differences in either CHO or fat oxidation rates. During exercise, average heart rate was significantly lower during the CHO+PRO (130.17 ± 3.13 b/min-1) treatment compared to the CHO (132.80 ± 2.92 b/min-1) treatment, suggesting a greater efficiency of the heart during the carbohydrate-protein trial. There were no significant differences for RPE for either treatment.

The primary finding of this study was that CHO+PRO enhanced TTE more than CHO alone when exercising at an intensity slightly below threshold. Improvement in TTE occurred regardless of the fact that CHO+PRO contained 50% fewer CHO content and 33% less calories. These results were similar to the previous findings that the addition of PRO to a CHO supplement enhanced endurance performance, measured in watts, compared to the traditional 6% CHO supplement. It is likely that the improved performance seen with the CHO+PRO treatment was a result of the additional PRO and use of a mixture of CHO sources because, as seen in this experiment, the improvement in performance occurred despite calorie differences than a standard 6% supplement solution.

Although the results seem positive, limitations exist for the present study. The solutions were not matched for total calories which can in turn cause some subjects to perform better than others. In addition, there was a mixture of carbohydrate sources used for the CHO+PRO, not a single source. This can also impact performance because oxidation rates can be higher compared to single source solutions. Performance was defined as the point at which subjects could no longer maintain a cadence above 60 rpm during the experiment. Although this is a good method to derive results, it also acts as a limitation because this type of experiment is not representative of realistic cycling events. Moreover, subjects completed the performance ride at an average of 75.06% VO2max, which is 1.5% lower than the average ventilatory threshold (VT). Measuring performance for each individual is a clear strength of this study because individual differences could be measured and accounted for. These strengths and limitations attribute to the necessary need for continued future research.

To continue, “The effect of a low carbohydrate beverage with added protein on cycling endurance performance in trained athletes” (Ferguson-Stegall et al., 2010) was developed to assess the effect of a supplement containing a mixture of carbohydrates compared with a supplement containing lower total carbohydrate content and a moderate amount of protein (3.0% CHO, 1.2% PRO) on total TTE. Also, researchers wanted to determine if there was a difference in muscle damage between the two supplements. The study followed a double-blind, randomized experimental design. Fifteen trained endurance athletes were recruited to participate (8 males, 7 females). They were required to ride at varying intensities alternating between 45% and 70% VO2max for three hours, and then increase intensity between 74% and 85% VO2max until exhaustion. Two trials, separated by seven days, occurred in which subjects consumed a 6% carbohydrate beverage (CHO), containing dextrose, or a 3% CHO/1.2% protein (MCP) beverage, containing dextrose, maltodextrin, and fructose, during exercise. Beverages were consumed in doses of 275ml every 20 minutes of exercise.

The cycling protocol consisted of 30 minutes at low intensity (45% VO2max), followed by 1.5 hours with alternating intensities every 8 minutes between 45% and 70% VO2max. From hours two to three, the intensity continued to alternate between the same intensities but at three minute intervals. After three hours of total riding, intensity increased up to 85% VO2max until exhaustion. Time of exhaustion was determined when subjects could no longer maintain a cadence of 60 rpm. Ventilatory threshold was calculated from the VO2max testing the week before the experiment began. This percentage then told researchers if subjects were cycling at or below VT. Researchers essentially sought to determine if VT contributed to an increase in TTE.

Although it did not reach statistical significance, TTE results indicated that for the combined group (n=15) TTE was greater in the MCP group than in the CHO group, by 19.3% ($p=0.064$). However, for subjects cycling at or below VT (n=8), TTE in the MCP group was significantly greater than CHO (45.64 ± 7.38 minutes vs. 35.47 ± 5.94 minutes; $p=0.006$).

Additionally, there were no significant differences in plasma insulin levels by treatment, but plasma glucose was significantly lower when subjects consumed the MCP supplement compared to the CHO supplement. No significant differences between treatments existed for lactate concentration or plasma myoglobin levels ($p=0.189$). No significant differences were seen for carbohydrate or fat oxidation, RPE, or heart rate between treatment groups.

The researchers suggested that the use of three different carbohydrate sources in the MCP beverage may have led to optimal use of carbohydrate transporters within the body due to the rate of absorption increasing beyond that of the CHO treatment. This would also suggest that the varying carbohydrate sources lead to optimal performance rather than the addition of protein to the supplement solution. Using three different carbohydrate sources is a limitation in this study because too many variables exist between treatment groups making it difficult to determine what is actually affecting overall performance. In the end, researchers stated that compared to a traditional 6% carbohydrate supplement a mixture of carbohydrate and protein may improve aerobic endurance at exercise intensities near threshold.

Strengths of the present study include using VT rather than percent of VO2max to determine performance outcomes for each subject. Also, the use of three different MCP beverages may have optimized carbohydrate absorption rather than a single source carbohydrate. Additionally, researchers used muscle myoglobin as the muscle damage marker which could have showed hours post-exercise rather than immediately. Regardless of the strengths and limitations, the present study appears to have valid results, but again, more research is needed to establish actual, concrete conclusions.

The addition of protein to a carbohydrate supplement appears to have more positive than negative effects on performance. Studies by Toone et al. (2010), Ivy et al. (2003), and McCleave et al. (2011) indicated that protein added to a carbohydrate supplement during exercise resulted in improvements in performance and time to exhaustion or completion. Romano-Ely at al. (2006) demonstrated that protein had no effect on cyclists or performance when measuring time to fatigue. More research is necessary to isolate the variables causing these differences and to determine the protein requirements needed for consumption during exercise for endurance cyclists.

Studies Suggesting a Potential Benefit of Supplemental Protein on Muscle Damage

Improved endurance performance in athletes who consume a carbohydrate beverage while exercising versus only water has been consistently documented by researchers. It is believed that performance improves because carbohydrate beverages increase blood glucose levels, thus increasing available energy to fuel the work, and decreasing reliance on glycogen stores. There is also evidence that the addition of protein to a carbohydrate supplement may have positive effects on muscle damage and adaptation. Although the addition of protein suggests positive effects on performance, there are other potential benefits to this type of supplementation such as decreased muscle damage and enhanced adaptation. While the studies in the previous section focused on performance as an outcome, the following studies also examined muscle damage as an outcome of additional protein to carbohydrate supplementation.

Saunders et al. (2004) developed a randomized controlled crossover study, “Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage” to determine whether endurance cycling performance and post-exercise muscle damage were altered well-trained cyclists when consuming a carbohydrate and protein (CHO+P) beverage. It was designed to address if cycling time to fatigue improved when subjects consumed a CHO+P beverage versus an isocarbohydrate (CHO), but not isocaloric, beverage.

Fifteen male volunteer cyclists were recruited to achieve a power of 0.80. Testing procedures included three phases: the first phase was physical fitness assessment testing; the second and third phases were cycling tests. During the performance test, each subject performed two bouts of cycling ergometry to fatigue, with a 12 to 15 hour rest period between rides. In the first ride, subjects were required to ride at 75% VO2 max at a self-selected cadence >50 RPM until they were unable to maintain that minimum cadence for 30 seconds. In the second ride and under the same conditions as the first with the exception that glycogen stores were partially depleted, subjects rode at 85% VO2max. Metabolic measures, heart rate, and rate of perceived exertion (RPE) were determined. The phase two treatment protocol included nutrition supplements and consisted of a 127 ml CHO+P drink or CHO drink every 15 minutes of exercise at 1.8 ml/kg. Subjects also consumed 10 ml/kg of the supplement within 30 minutes after the exercise bouts. Half the subjects consumed the CHO+P beverage and half consumed the CHO beverage. The CHO+P beverage was a 4:1 carbohydrate/protein ratio, such that the beverage had a carbohydrate content of 26 grams of carbohydrate (7.3%) and 6.5 grams of whey protein (1.8%). The CHO beverage was identically matched in carbohydrate content but lacked protein calories. After phase two was complete, the subjects returned after a 7 to 14 day washout period for phase three and repeated the two performance rides. The only difference was that treatments were reversed for subjects.

The results of the first performance ride indicated that time to exhaustion at 75% of

VO2max was 29% longer ($p < 0.05$) during the CHO+P trial than during the CHO trial. In addition, during the second performance ride, cycling at 85% VO2max, subjects rode 40% longer ($p < 0.05$) when consuming CHO+P beverage than when consuming CHO beverage. Post-exercise muscle damage was indirectly assessed using plasma CPK levels. Pre-exercise CPK levels were not significantly different between trials; however, post-exercise CPK levels (12 to 15 hours after the first performance ride) were significantly lower ($p < 0.05$) after the CHO+P trial than the CHO trial, indicating less muscle damage. There were no significant differences during performance rides for serum glucose or lactate, ventilation, VO2, RPE, and heart rate between treatment groups. Overall, results suggested that the addition of protein to a carbohydrate beverage results in significant improvements in cycling time to fatigue and reductions in post-exercise muscle damage. Researchers noted that further research is necessary to determine whether these effects were the result of higher total caloric content in the CHO+P beverage or due to protein-mediated mechanisms.

As many of the presented studies have already suggested, carbohydrate beverages consumed during exercise improve endurance performance, and the addition of protein may offer even more benefits. It remains unclear as to whether these effects are related to total energy intake or specific effect of protein. Valentine, Saunders, Todd, & Laurent (2008) developed a study, "Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption," to examine the effects of a carbohydrate-protein (CHO+Pro) beverage provided during exercise on time to exhaustion and markers of muscle disruption after exercise. They specifically wanted to compare a CHO+Pro beverage with a placebo (CHO beverage) and determine which treatment could equal or exceed maximal exogenous CHO oxidation levels. The researchers also wanted to see if a CHO+Pro beverage attenuated plasma CK, serum myoglobin (Mb), muscle soreness, and improved muscle function compared to the placebo treatment.

Participants included 12 male cyclists that performed at least four days of aerobic training per week and had a VO2 peak above 45 ml/kg/min. Each cyclist performed a prolonged cycling test to exhaustion at 75% VO2max, and also had to cycle at a cadence greater than 50 rpm. Time to exhaustion was recorded at the time point when participants were unable to maintain the minimum cadence for 20 seconds. Participants received 1.8 ml/kg of beverage every 15 minutes for the duration of the ride (1L/hour). One of the four treatment beverages was consumed in each experimental ride: (1) a CHO+Pro beverage (77.5 g CHO/hr, 19.4 g Pro/hr), (2a) a CHO beverage matched for carbohydrate calories (CHO 77.5 g CHO/hr), (2b) a second CHO beverage matched for total calories (CHO+CHO; 96.9 g CHO/hr), and (3) a water placebo (0.0 g CHO or Pro). Carbohydrate content of the beverages was sucrose and maltodextrin. The protein source was a whey-protein concentrate.

After completion of the cycling bout, each participant completed a muscle-function test 22 to 24 hours post cycling. The test consisted of a standard warm up rep at approximately 50% of 1RM, following the maximal number of repetitions to fatigue at 70% of 1RM on a leg-extension machine. In addition, a subjective rating of muscle soreness was determined at baseline and 22 to 24 hours after each cycling test. Following the cycle test and muscle-function test, participants returned after a 5 to 10 day washout period and repeated the same protocols. The only difference was the type of beverage that was administered. This was repeated until all participants completed trials with all four beverages. The beverages were administered to participants using a double-blind protocol.

Researchers found that participants rode significantly longer ($p < 0.05$) in the CHO+Pro and CHO+CHO treatments than the placebo (18% and 13%, respectively). The difference in time to exhaustion between the CHO treatment and placebo trial (9.7%) was not significant. Also, differences in time to exhaustion between the CHO+Pro versus CHO and CHO+CHO treatments were not statistically significant (7.4% and 4.0%, respectively). Results for metabolic measures during exercise testing suggested various indications. First, blood glucose levels did not change during exercise in the placebo group, but it did increase significantly compared to all other groups ($p < 0.05$; CHO, 102.3 ± 11.1 mg/dl; CHO+CHO, 107.7 ± 10.6 mg/dl; CHO+Pro, 103.9 ± 8.9 mgl/dl). Furthermore, blood lactate levels decreased from the onset of exercise to the end of exercise in all trials and were not significantly different between treatments. Moreover, RER decreased from the onset of the test to the last common time point achieved for all four trials but that decrease was not significant. VO2 and heart rate increased slightly during all trials compared to baseline, but only the significant increase in heart rate compared to baseline was in the CHO+CHO trial. RPE increased significantly from baseline during all trials with CHO+Pro being significantly lower than the CHO trial (CHO+Pro; 15.0 ± 1.8, CHO; 16.0 ± 1.4). Serum insulin levels were maintained in all trials except the placebo treatment, resulting in significantly lower insulin at the end of exercise than the other treatments (placebo, 7.7 ± 7.7 µU/ml, CHO, 14.6 ± 14.5 µU/ml; CHO+CHO, 17.9 ± 14.4 µU/ml; CHO+Pro, 20.1 ± 16.8 µU/ml). Post exercise CK concentrations increased significantly ($p < 0.05$) from pre-exercise values for placebo (91%), CHO (119%), and CHO+CHO (116%), but not for the CHO+Pro (-8%). Similarly, Mb concentrations increased significantly after exercise in the placebo (392%), CHO (163%), and CHO+CHO (174%) trials, but not after the CHO+Pro trial (37%). More leg-extension repetitions were completed after the CHO+Pro trial than the other three treatments, and in addition, more repetitions were completed in the CHO trial than the placebo trial. Post exercise muscle soreness was not significantly different between any treatments.

Time to exhaustion was used in the present study as a way to measure performance and endurance. The present study does support that protein added to a carbohydrate beverage improved performance compared to placebo beverages. Moreover, carbohydrate-protein beverages have previously been reported to reduce indices of post exercise muscle disruption when compared to CHO beverages matched for carbohydrate content or total calories (Ivy et al., 2003; Saunders et al., 2004, 2007). Results from the present study also suggest carbohydrate ingestion alone might have a positive effect on post exercise muscle recovery and soreness. Overall, the subjects taking the CHO+Pro beverage had the longest time to exhaustion, although it was not significantly longer than either CHO beverage. It is possible that improvements in performance with additional protein are related to caloric differences between treatments, as they were not matched for total calories.

Strengths and limitations exist for the present study. Acting as a strength and limitation, the beverages used for this comparison were matched for total carbohydrate content but not total calories. This design acts as a strength because the additional protein content is the only energy difference between the CHO+P and CHO beverages. And difference in performance or recovery can be attributed to something other than the carbohydrate content. This is also a limitation because increased availability of total calories from the CHO+P beverage may contribute to differences between trials. Certainly more research needs to be conducted in order to determine the best possible beverage solution that could have positive effects on performance outcomes.

The effect of protein ingestion on muscle damage was further studied by Saunders, Luden, & Herrick (2007) in the randomized crossover study, “Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents post-exercise muscle damage." It was designed to address if endurance performance improved when subjects consume an oral carbohydrate+protein (CHO+P) gel versus a CHO only gel. Also, investigators wanted to determine if consumption of a CHO+P gel reduced muscle damage compared to a CHO energy gel, and if the benefits of a CHO+P gel was consistent between men and women. The overall purpose was to see if benefits existed for CHO+P gels versus beverages.

Thirteen recreationally competitive cyclists (8 males, 5 females) volunteered to participant in the study. Two experimental rides with blinded treatments were conducted with a 7 to 14 day washout period. Trials were prolonged bouts of cycling at a self-selected cadence of >50 rpm at 75% VO2max. Time to exhaustion was used to compare endurance performance between gel treatments. The alternate gel treatment was given for the second cycling trial. Gels were matched for total carbohydrate content, and as a result, the CHO+P gel contained 25% more calories than the CHO alone gel. Gel feedings were administered every 15 minutes after the onset of exercise. They consisted of 0.146 g CHO/kg body weight such that the concentration of CHO relative to the fluid was 7.3% by volume.

Results indicated that subjects performed 13% longer ($p < 0.05$) in the CHO+P trial (116.6 ± 28.5 minutes) than in the CHO trial (102.8 ± 25.0 minutes). Responses between treatments for each gender were similar with no significant treatment x gender interactions ($p=0.980$). Similarly, no differences between CHO and CHO+P trials were observed for the following variables: heart rate, RER, blood lactate, blood glucose, and RPE. Post-exercise muscle damage was assessed indirectly by analysis of plasma CK concentrations. There was a significant main effect for time on plasma CK concentration ($p < 0.05$), but not treatment x time interaction for CK ($p=0.294$). CK did significantly increase from pre-exercise to post-exercise in the CHO trial (183 ± 116 and 267 ± 214 U/L-1), but not in the CHO+P trial (180 ± 133 and 222

± 141 U/L-1). There were no significant treatment x gender (p=0.916) or time x treatment x gender (p=0.751) interactions in CK responses.

The primary finding of the current study was that cyclists performed 13% longer at 75% VO2max when receiving a CHO+P gel versus a CHO gel. Additionally, the relative improvements in performance in the CHO+P trial (14.6 and 12.8%) were similar between genders. Researchers found that the CHO+P gel was effective at preventing muscle damage due to the lack of elevations that were seen in post-exercise CK levels following the CHO trial. Low CK levels could be related to the subjects recruited for the study. One theory is that the relative muscular effort of this particular group of subjects was lower than other study subjects. Also, these subjects may have been more highly trained than previous studies. Regardless, it seems unlikely that small differences in training status between groups could explain the differences in post-exercise CK levels between various studies.

Beelen et al. (2011) assessed the impact of protein coingestion with carbohydrate during endurance exercise on whole body protein balance and skeletal muscle protein synthesis rates. The study “Impact of protein coingestion on muscle protein synthesis during continuous endurance type exercise” was developed to test the hypothesis that protein coingestion would not augment muscle protein synthesis rates during more continuous endurance type exercise. Muscle protein synthesis rates were assessed in 12 male cyclists during two hours of moderate intensity cycling while receiving carbohydrate with and without additional protein.

The study followed a double-blind, randomized crossover design where each subject participated in two treatments separated by one week. Cycling consisted of two hours at 55% of the subjects’ individual VO2max. Subjects were required to ingest either carbohydrate (CHO) or carbohydrate and protein (CHO+PRO) during exercise. Subjects consumed the test drink every

15 minutes of exercise. The CHO beverage treatment was a dose of 1.0 g/kg/hr, 50% glucose and 50% maltodextrin solution. The CHO+PRO beverage treatment was an isocaloric dose of 0.8 g/kg/hr carbohydrate plus 0.2 g/kg/hr protein hydrolysate, made from casein protein.

After the experimental tests were completed, results indicated no significant differences between treatments for plasma glucose concentrations; however, there were significant differences between treatments over time ($p < 0.05$) for plasma insulin concentrations. Total plasma insulin responses were greater in CHO+PRO compared with CHO (9.1 ± 1.8 and 18.1 ± 4.1 mU/l-1/2hr for CHO and CHO+PRO). Plasma amino acid concentrations were significantly lower during exercise in the CHO+PRO treatment compared to the CHO treatment ($p < 0.01$). Similarly, whole body protein synthesis (46.2 ± 1.3 vs. 56.5 ± 1.8) and oxidation rates (6.0 ± 0.4 vs. 10.1 ± 1.2) were higher in the CHO+PRO treatment compared to the CHO treatment ($p < 0.01$). Protein breakdown did not differ between treatment groups ($p=0.06$, respectively).

The current study illustrated that muscle protein synthesis rates were greater during continuous endurance type exercise while subjects ingested carbohydrate or carbohydrate plus added protein compared to basal fasting conditions. Protein coingestion during exercise improved whole body protein synthesis and net protein balance but did not further augment mixed muscle protein synthesis rates during exercise. Additionally, muscle protein rates were stimulated during two hours of continuous endurance type exercise compared with basal fasting muscle protein synthesis rates (0.064 ± 0.006 vs. 0.036 ± 0.004%/hr respectively, $p < 0.01$). These results differ from previous studies in that few studies have examined the impact of endurance type exercise on whole body protein synthesis rates. Most of these studies have reported a decrease or no effect of exercise on muscle protein synthesis rates, but this particular study speculated that greater exercise intensity (~60% VO2max compared to other studies at

~50% VO2max) is responsible for the differences in rates. Muscle protein synthesis does not typically occur in the absence of insulin, the impact of increasing insulin concentrations on muscle protein turnover is thought to be related to the decrease in muscle protein breakdown rather than stimulation of protein synthesis. Overall, this is the first study to suggest that muscle protein synthesis is stimulated during endurance exercise when carbohydrate or carbohydrate plus protein are ingested. Protein coingestion does not further augment muscle protein synthesis during endurance activity. Further research is necessary to determine the metabolic pathways leading to increased muscle protein synthesis.

Breen et al. (2011) conducted a similar study to test the effects of protein coingestion on protein synthesis. The primary aim of "The influence of carbohydrate-protein coingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis" was to investigate the impact of ingesting added protein to a carbohydrate supplement on protein synthesis responses following prolonged endurance exercise. A secondary aim of the study was to determine the potential intracellular signaling mechanisms which regulate the protein synthesis response following post-endurance exercise protein ingestion.

Ten trained male cyclists were recruited to participate in this study. The design was formatted similar to previous studies with two trials conducted in a double-blind, randomized crossover fashion. Subjects consumed a carbohydrate (CHO) or carbohydrate-protein (C+P) beverage during both trial rides. During each trial, subjects cycled for 90 minutes at high intensity (75% VO2max) at a self-selected cadence > 60 rpm before immediately consuming one of the treatments. They then consumed that treatment 30 minutes following exercise. Subjects consumed the alternate treatment on the second trial date which was separated by two weeks. Mitochondrial and myofibrillar muscle protein synthesis (MPS) were measured by combining isotopic tracer infusion and muscle biopsy techniques. In addition, VO2, RER, heart rate, and RPE were recorded over 25-30 minutes, 55-60 minutes, and 85-90 minutes of exercise. Treatment beverages were randomized and matched for color and taste, but eight out of the ten subjects were able to correctly identify the order of treatments. The CHO beverage consisted of 25.2g CHO and the C+P beverage consisted of 25.4g CHO plus 10.2g whey protein isolate.

Results indicated that there were no between-trial differences in heart rate, cadence, VO2, and RER during the trial rides. Approximately 30 minutes after consuming the first treatment beverage, plasma glucose concentration increased by 32% for CHO treatment and 20% for the C+P treatment, but this was not significant. Serum insulin increased more for C+P (285 ± 32%) compared to the CHO treatment (60 ±8%; $p < 0.001$). Plasma lactate concentration also increased for the CHO trial (150 ± 16%) and the C+P trial (175 ± 19%) compared to resting values. No differences existed for plasma lactate concentrations between treatment groups, however. Plasma urea did not change for the CHO trial compared to resting values. From 15 minutes to 4 hours post-exercise, plasma urea concentration did significantly increase for C+P ($p < 0.05$). Myofibrillar protein synthesis rates were ~35% higher for C+P compared to CHO ($p=0.025$). Rates of mitochondrial and myofibrillar protein synthesis were 30.2 ± 0.9% lower in the C+P group when an unadjusted plasma precursor was used to calculate protein phosphorylation, and this remained significant between groups ($p=0.03$).

This study expands on previous study results showing that added protein to carbohydrate supplementation stimulates an increase in myofibrillar MPS compared to CHO alone. The rates of mitochondrial MPS did not increase with consumption of C+P compared to CHO. The authors theorized that this was due to enhanced mRNA translation evidenced by differences in the phosphorylation intermediates with C+P versus CHO alone. The lack of protein synthesis response could be related to the pattern of mitochondrial protein response to exercise. The authors attempted to rationalize the current study results related to mitochondrial protein synthesis, but in turn, the main result they were able to find was that when protein is coingested with carbohydrate after cycling exercise, only myofibrillar protein synthesis increases. This may then enhance the synthesis of proteins associated with fatigue or may serve to counteract a fasted-state rise in myofibrillar protein breakdown during and immediately following endurance exercise. Thus, post-endurance exercise protein consumption may have significant implications for the adaptive response to endurance exercise and the recovery of muscle function. Additionally, the current study results may be dependent on the subject training status, intensity, and duration of exercise, as well as timing and quantity of nutrition strategies.

The effect of carbohydrate with additional protein supplementation on performance has been studied in great detail; however, further research is necessary in order to make specific conclusions regarding athletic intake and performance. Not only does the coingestion of protein and carbohydrate during prolonged aerobic activity augment performance changes, it is also possible that protein may enhance muscle repair and adaptation with the prevention of muscle damage. This area of research is currently limited and unclear. Finally, protein coingestion with carbohydrate has been suggested in previous studies to improve performance, but some research suggests that there is a neutral effect during prolonged exercise. More well conducted studies are needed to elucidate the effect of protein consumption during exercise on performance and muscle recovery.

Studies Suggesting no Benefit of Supplemental Protein on Performance

Vegge et al. (2012) studied the effects of carbohydrate and protein on cycling performance following a randomized, double-blind crossover design that tested the effects of carbohydrate with the addition of marine protein on cyclists during activity. The authors made three hypotheses: (1) there would be no ergogenic effect with consumption of a carbohydrate plus protein beverage on performance after a 5 minute power test following a 120 minute steady aerobic ride compared to consumption of just carbohydrate; (2) adding codfish-based hydrolyzed protein to the carbohydrate plus protein beverage would improve performance compared to just the carbohydrate plus protein beverage and also the carbohydrate only beverage; (3) the ergogenic effect from the codfish plus carbohydrate plus protein beverage would correlate with cycling performance level. Subjects included 12 well-trained, male cyclists ages 19-27 years old. They were instructed to avoid intense exercise 48 hours before each test, restricted from eating 90 minutes before each test, and were also restricted from consuming caffeine four hours before each test. Cycling was completed at the same time on testing days and beverages were administered at the same time during each test. All treatment beverages contained the same amount of carbohydrate but varied in protein. The carbohydrate only beverage contained 8.3% maltodextrin (60 grams/hour). The carbohydrate plus protein beverage contained 8.3% maltodextrin and 2.1% intact whey protein. The carbohydrate plus protein plus codfish contained 0.4% codfish, 1.7% intact whey protein, and 8.3% maltodextrin. All beverages were matched for total calories. There was no control without protein in this study.

Results of the study illustrated no differences on performance between any of the beverages during a 5 minute power test following 120 minutes of steady cycling. With the ingestion of the added codfish during the 5 minute power test in comparison to ingestion of only carbohydrate, the subjects with a lower performance level compared to more experienced cyclists showed better performance in watts/kg. The authors concluded that adding hydrolyzed protein to a carbohydrate solution provided an ergogenic effect on mean power performance in athletes with a lower performance level compared to experienced athletes.

Strengths of the present study included the study design, use of a controlled environment for testing, and beverage mixtures using equal parts carbohydrate and protein. The test protocol is also a strength because cyclists were restricted from certain ergogenic aids such as caffeine prior to testing. Limitations included the small sample size, lack of training and diet history, and the use of food logs before each test rather than a set diet regimen. It is possible that some subjects ate better or worse than others in the days leading up to the test which can affect the overall results. Another potential weakness could be the percentage of protein included in each solution. Perhaps a higher percentage of whey protein to codfish or vice versa would have lead to different, and even significant results. The overall results support the hypothesis that consumption of carbohydrate and unprocessed protein does not affect cyclist performance during a 5 minute power test following 120 minutes of steady cycling. However, the data presented in the study still indicate that further research is necessary, and that potential performance effects of adding protein to a carbohydrate supplement are still unknown for long distance cyclists. The author noted that future research should focus on longer lasting submaximal tests as well as muscular exertion by measuring glycogen content in the body. Certainly this study contributes to and acts as a starting point for future research of added protein to carbohydrate solutions consumed during exercise.

Rauch, Hawley, Woodey, Noakes, & Dennis (1999) developed a study to investigate the effects of ingesting a commercial sports bar containing fat, carbohydrate, and protein on exercise performance compared to a isocaloric amount of carbohydrate. Their study, “Effects of ingesting a sports bar versus glucose polymer on substrate utilization and ultra-endurance performance” tested macronutrient contribution on performance in low-intensity, aerobic activity lasting longer than three hours.

Six competitive, endurance cyclists were chosen as subjects for the study. They had a mean age of 31 ± 3 years, a mean body mass of 77 ± 1 kg, a mean VO2 max of 69 ± 2 mo*kg-1*min, and a mean power output (PPO) of 414 ± 18 watts. Subjects completed two randomized experimental trials which were separated by 7 days. During the first trial, they ingested 1.5 energy bars plus 700 ml of water every hour. During the second experimental trial, subjects ingested 700 ml of a 100 g/L glucose polymer solution every hour. Energy consumption for both rides was similar with each bar containing CHO (19 grams, primarily from corn syrup with trace amounts of honey and sugar), fat (7 grams), protein (14 grams), sodium (200 mg), potassium (50 mg), dietary fiber (<1 gram), and trace amounts of vitamins A, B, C, and E. Each trial lasted 330 minutes and was performed at 55% VO2 max followed by a performance ride. Subjects were only allowed to consume the bar or CHO solution during the 330 minute period and nothing during the time trial. During the last 30 minutes of the entire trial, subjects were allowed to ingest half of what they had been ingesting during each previous hour.

Results of the study indicated that after completing a 330 minute cycling test, an average power output of 203 ± 8 watts for all groups was achieved. This corresponds directly to the 55% VO2max intensity cyclists were asked to ride. RER values fell from 0.89 ± 0.01 and 0.86 ± 0.01 after 60 minutes of exercise to 0.85 ± 0.01 and 0.79 ± 0.01 at the end of the ride for the CHO and bar trials. In addition, heart rate rose progressively during both rides, but was not different between to the two trials. Subjective ratings of perceived exertion were also not significantly different over the duration of the ride. Ratings of perceived exertion did, however, significantly increase from 10.3 ± 0.7 and 10.3 ± 0.6 respectively after 60 minutes to 14.3 ± 0.7 and 13.3 ± 0.6

at the end of the 330 minute ride with the ingestion of CHO and the bar ($p < 0.05$).

Results of the time trial were divided between those subjects that were able to finish the time trial (n=4) and those that were not capable (n=2). At the end of the time trial, VO2 was significantly higher than that of the two subjects that failed to complete the time trial when ingesting the bar (4.17 ± 0.36 L/min vs. 2.47 ± 0.441; $p <0.05$), but similar to those subjects who completed the time trial when ingesting the bar (4.09 ± 0.34). RER values were significantly higher during the time trial, when subjects ingested CHO, compared to those of the two fatigued subjects. RER was also significantly higher at the end of the time trial for those that did not fatigue ($p < 0.05$). Further, the heart rate at the end of the time trial was significantly lower than those subjects that did not fatigue (176 ± 9 bpm) when ingesting the bar.

Additionally, the total amount of CHO oxidized during the 300 minute period in the CHO trial was significantly greater than during the bar trial (741 ± 38 grams vs. 573 ± 48 grams; $p < 0.05$). The rates of CHO oxidation were significantly lower at the end of the 300 minute period when subjects ingested the bar compared to CHO (1.2 ± 0.15 vs. 2.04 ± 0.14 grams*min-1; $p < 0.05$). Total fat oxidation was significantly higher in the bar trial compared to the CHO trial (280 ± 24 vs. 203 ± 25 grams; $p < 0.05$). With the ingestion of the bar, the rate of fat oxidation increased significantly over time from 0.59 ± 0.05 g*min-1 after 5 minutes to 1.09 ± 0.08 g*min-1 at the end of the 300 minute ride ($p < 0.05$). The percentage contribution towards total energy production from CHO oxidation during the time trial after ingestion of the CHO was significantly higher than after the bar (82 ± 3% vs. 59 ± 4%; $p < 0.05$). Conversely, fat oxidation accounted for only 18 ± 3% of the energy after CHO ingestion compared to 41 ± 4% after the bar. Changes in performance during the time trial after CHO and bar ingestion were associated with the drop in the rate of CHO oxidation (R=0.81, p=0.05).

There are clearly many significant findings from this research study. The first important finding was that when subjects ingested a sports bar containing a mixture of CHO, fat, and protein during 330 minutes of submaximal exercise, their performance during an immediate time trial was impaired, compared to when they ingested CHO throughout a prolonged ride. The authors assumed that almost all of the ingested CHO was essentially oxidized and the minimum contribution of endogenous CHO was approximately 460 grams for both trials. Therefore, the sports bar did not have a noticeable glycogen sparing effect on subjects. The subjects that did not complete the time trial complained of leg fatigue, which further suggests that muscle glycogen was depleted. A second conclusion from the study is that ingesting less CHO promotes the utilization of fat and alternately, the ingestion of fat in the bar may have increased fat oxidation for subjects. Researchers presume that the ingestion of a sports bar containing 7 grams of fat elevates the rates of fat oxidation during prolonged, submaximal exercise when compared to CHO ingestion alone.

Martinez-Lagunas, Ding, Bernard, Wang, & Ivy (2010) developed a study to investigate the aerobic capacity of an isocaloric carbohydrate (CHO) plus protein (PRO) drink compared to a low-calorie CHO plus PRO drink and a traditional 6% CHO sports beverage. The purpose of this study was to investigate the ability of PRO to reduce the need for CHO in sports beverages without reducing the efficacy of the drink. The hypothesizes were the following: (a) aerobic capacity will be enhanced with CHO and CHO plus PRO supplementation compared to a non-caloric placebo beverage, and (b) aerobic capacity would not be compromised, but possibly improved, when reducing CHO content of a sports drink from 6% to 4.5% or 3% when combined with PRO.

Twelve trained cyclists (5 women, 7 men) were recruited for this study. Aerobic capacity was measured by time to fatigue while cycling. Consumption of fuel was either a traditional 6% sports beverage (CHO), a 4.5% carbohydrate plus 1.15% protein beverage (CHO/PRO H), a 3% carbohydrate plus 0.75% protein beverage (CHO/PRO L), or a flavored water placebo (PLA). Four randomized and counterbalanced experimental trials occurred; thus, the study design followed a double-blind, placebo-controlled, and within-subject repeated measures experimental design.

Each experimental trial was divided by seven days and all four trials were completed by each subject. The protein used in the experiment consisted of whey protein and the carbohydrate source consisted of dextrose. Cycling trials began with a 24 minute warm up at 55% VO2max and was followed by twelve, eight minute intervals alternating between 55% and 75% VO2max. This was then followed by ten shorter intervals of three minutes each alternating between the same intensities. After this sequence, subjects cycled at 80% VO2max until fatigue. Subjects were required to maintain a pedal cadence of 70 to 100 rpm and when a cadence of 60 rpm occurred twice, they were asked to stop cycling. This was considered the point of fatigue.

As expected by the investigators, the average time to fatigue during exercise at 80% VO2max after 150 minutes of varying intensity cycling was significantly longer ($p < 0.05$) during the CHO, the CHO/PRO H, and CHO/PRO L trials compared to the PLA trial. The time to fatigue was 83% longer during the CHO trial, 107.5% during the CHO/PRO H trial, and 96.6% during the CHO/PRO L trial compared to the PLA trial. Comparing the CHO to CHO/PRO H trial, 9 out of 12 cyclists performed better on the CHO/PRO trial, but this was not significant ($p=0.073$) in terms of riding for a longer period of time. In addition, heart rate gradually increased during the ride, but no significant treatment or treatment by time differences in heart rate were found for any of the trial treatments. RPE also increased gradually during the course of the ride. Average RPE was significantly lower during the CHO (12.8 ± 0.4), CHO/PRO H (12.3 ± 0.4), and CHO/PRO L (12.6 ± 0.4) trials compared to the PLA trial (13.1 ± 0.4). The average Respiratory exchange ratio, RER, during the CHO (0.899 ± 0.005), the CHO/PRO H (0.893 ± 0.006), and the CHO/PRO L (0.888 ± 0.008) trials was significantly higher ($p < 0.05$) than during the PLA trial (0.872 ± 0.007). Average RER was not significantly different between CHO, CHO/PRO H, and CHO/PRO L trials. No significant treatment or treatment by time differences in energy expenditure was found for the four experimental trial beverages. Average CHO oxidation during the CHO (2.23 ± 0.17 g/min-1), CHO/PRO H (2.14 ± 0.15 g/min-1), and CHO/PRO L (2.11 ± 0.17 g/min-1) trials was significantly higher ($p < 0.05$) than during the PLA trial (1.90 ±0.15 g/min-1). In general, CHO oxidation was significantly higher during the CHO, CHO/PRO H, and CHO/PRO L trials compared to the PLA trial from 40 to 152 minutes of exercise. Similarly, average fat oxidation during the CHO (0.41 ± 0.04 g/min-1), CHO/PRO H (0.44 ± 0.05 g/min-1), and CHO/PRO L (0.46 ± 0.05 g/min-1) was significantly lower than during the PLA trial (0.53 ± 0.05 g/min-1). Significant differences were found in fat oxidation from 40 to 135 minutes of exercise.

Metabolic analysis showed average blood glucose was significantly higher ($p < 0.05$) during the CHO (4.11 ± 0.11 mmol/L-1), CHO/PRO H (4.10 ± 0.11 mmol/L-1), and CHO/PRO L (3.94 ± 0.10 mmol/L-1) trials compared to the PLA trial (3.61 ± 0.10 mmol/L-1). At fatigue, blood glucose was significantly higher during the CHO/PRO H trial compared with the CHO, CHO/PRO L, and PLA trials. Average plasma insulin was significantly higher for all trials except the PLA trial. Additionally, average plasma insulin during the CHO/PRO H trial was significantly higher than during the CHO/PRO L trial. Blood lactate concentration rose and fell during the all exercise trials in relation to exercise intensity, but was significantly higher (p <

0.05) at fatigue compared to at rest in all four trials.

Study results indicated that CHO, CHO/PRO H, and CHO/PRO L trials had longer times to fatigue than the PLA trial. CHO and CHO plus PRO supplementation during exercise enhanced aerobic capacity and performance compared to just water or a placebo beverage. Conversely, time to fatigue was not significantly different between the CHO, CHO/PRO H, and CHO/PRO L treatments. This supports the hypothesis that aerobic capacity is not compromised when the amount of CHO is reduced from 6% to 4.5% or 3% if replaced with a small amount of PRO. Study findings did not support the second hypothesis because aerobic capacity did not improve after ingestion of the CHO/PRO H or CHO/PRO L treatments beyond that produced by CHO alone.

The author notes one existing limitation of the current research study. The research design was developed with the absence of a low CHO-only trial. Only a performance reduction with a low CHO supplement can validate the necessity for adding protein to a lower CHO treatment. In summary, the CHO/PRO H supplement was not found to be more beneficial to improving aerobic capacity than a traditional 6% isocaloric CHO supplement. It was found, though, that with the addition of protein, the carbohydrate and caloric content of a sports beverage could be substantially reduced without loss of efficacy. Further investigation is needed regarding the specific physiological mechanisms in which added protein maintains the efficacy of a sports beverage containing less CHO.

Interest in the role of protein coingestion with carbohydrate has led to the findings of increased endurance performance, but Rowlands & Wadsworth (2011) discovered other results in the study “No effect of protein coingestion on exogenous glucose oxidation during exercise.” Eight male cyclists and triathletes completed this double-blind, randomized, crossover study. It was designed to compare the effects of added protein to a glucose solution on exogenous glucose and endogenous carbohydrate and fat oxidation rates. Additionally, plasma substrate, gut comfort, and perceived exertion were also investigated. Each subject completed four tests. Test one was an incremental test to volitional exhaustion and tests 2, 3, and 4 were 150 minute steady-state rides ingesting the experimental solutions. Subjects cycled at 50% VO2max for 150 minutes. Experimental solutions consisted of protein plus glucose (protein-glucose), glucose only (glucose), and noncaloric placebo (water). Every 15 minutes, cyclists ingested 150ml of a test solution for a total ingestion of 1.5L solution. The protein-glucose solution contained the same ingredients as the glucose plus 2% (3g) milk protein concentrate.

Results of the study indicated that the addition of protein had minimal effect on the exogenous glucose rate but led to a small increase in endogenous carbohydrate oxidation rate relative to the glucose and water solutions. Protein coingestion resulted in a small reduction in fat oxidation relative to water, but the difference compared to glucose was minimal. Consumption of the glucose solution led to a small increase in total carbohydrate oxidation, coupled with small decreases in endogenous carbohydrate and fat oxidation rates compared to water. Moreover, plasma glucose measures resulted in moderate to large increases compared to water. Increased plasma glucose concentration was observed in the protein-glucose solution (9.2% ± 3.4%) and glucose solution (15.9% ± 3.6%). No differences in lactate concentrations existed. The addition of protein had trivial effects on overall RPE and leg muscle soreness for all subjects.

Analysis of the current study results indicated that the addition of 2% protein offered no clear benefit to endurance activity. There was also no clear effect on gut comfort or physical exertion compared to a solution with only glucose. The minimal effect of protein coingestion on the exogenous glucose oxidation rate suggests that it is unlikely to be the mechanism for improved endurance exercise. Regardless, protein did show an effect on metabolism through the lowering of plasma glucose concentration. Protein has been shown to have blood glucose moderating and insulin enhancing effects when combined with carbohydrate during exercise; however, the lower blood glucose concentrations suggest either slower absorption of glucose from the gut or faster absorption of glucose from the plasma into tissues. Because plasma insulin was not measured, the researchers can only speculate about glucose absorption. Overall, protein coingestion with glucose during exercise had a neutral effect on exogenous carbohydrate oxidation, gut comfort, and RPE. Data concludes that protein coingestion will not negatively affect gut comfort, RPE, or reduce the rate of carbohydrate delivery to muscles during prolonged endurance exercise.

Conclusion

A wide array of research is being conducted to test the effects of protein coingestion with carbohydrate on performance measures and muscle damage. Various investigations suggest that carbohydrate alone maximizes athletic performance; whereas, other investigators are suggesting that in order to optimize performance, protein coingestion may be beneficial during exercise. The varying results and suggestions from recent studies indicate the necessity for further research to clarify the optimal nutritional supplementation regimen during endurance activity in order to optimize performance.

CHAPTER 3: METHODS

The purpose of this research study was to determine the effects of a carbohydrate versus a carbohydrate-protein supplement on performance by long distance cyclists. In order to do this, a controlled trial was conducted in which cyclists were asked to ride three hours or more for each of three events and consume either a carbohydrate or carbohydrate plus protein supplement during the exercise. Approval was received for this study from Mount Mary University's Institutional Review Board.

Twenty-eight endurance cyclists (11 females, 17 males) participated in this research study. Participants were clients at Peak Performance Professionals (P3), which is a training facility for cycling and triathlon athletes. Subjects were participating in the P3 Over-Distance Training Series, an endurance training program with rides up to four hours.

Study Protocol

The study consisted of three endurance cycling events with nutrition supplementation during the events. Events were scheduled for January 19 and 20, February 16 and 17, and March 23 and 24, 2013. Participants could ride on either Saturday or Sunday depending on their personal schedules. The length of events one, two, and three were 3 hours, 3.5 hours, and 4 hours, respectively. The length of the events differed because they were part of the pre-established Over Distance Training Series that the clients had enrolled in already. For all three cycling events, participants were asked to cycle between 50-70% of their VO2max, which was determined in the fall and winter before the program began.

Data Collection

Data was collected prior to each of the cycling events, during the cycling events, and after completion of each cycling event in order to assess performance and confounding variables. Prior to the start of the study, each participant signed a study consent form and completed VO2max testing. VO2max data indicates fitness levels based on oxygen consumption and gives a basis for exercise intensity. Demographic data was collected via the 'Pre-Study Questionnaire' (Appendix A) and adapted by the researcher from the questionnaire administered at P3. Other relevant information, including current physical activity, physical limitations, and recent nutritional habits were collected via the 'Athlete Health Questionnaire' (Appendix B) prior to the start of the study. This questionnaire was comprised of 3 open-ended and 15 yes or no questions. Subjects also completed a 3-day food record prior to the start of the study. They were to log the three days leading up to the first event and were asked to record all food and beverage items consumed with portions stated if possible.

Prior to and after each cycling event, participants were weighed without clothing. In order to assess performance, heart rate and watts during the cycling event was monitored by each athlete as well as by a trained researcher. The overall average watts for each subject for each event were obtained from a Compu-Trainer, which is a computer software program connected to a cyclist's bike. A Compu-Trainer simulates realistic courses and conditions such as wind and hills. Average heart rate was monitored by individual heart rate monitors worn and monitored by each athlete. Heart rate was reported by those participants that wore a heart rate monitor.

Following completion of each event, subjects were weighed again. Subjects also completed the 'Post-Event Questionnaire' (Appendix C), designed by the researcher, within 2 hours of finishing the event in order to assess hydration, GI distress, and physical and mental feelings during the event. It also assessed any significant changes in lifestyle, diet, or sleep patterns in the three days leading up to each event. The questionnaire included 15 open ended questions with scaled responses.

Nutritional Supplements

Two categories of supplements were tested in this study. The first contained only carbohydrate as the macronutrient, and the second contained both carbohydrate and protein at various ratios (4:1-7:1). A list of commercially available supplements that met the pre-established criteria was provided to the cyclists from which to choose (Table 1). Commercially available supplements were used instead of making our own supplement solutions in order to adhere to client preferences, avoid potential GI distress from unknown ingredients that participants may not tolerate, and to have more applicable findings.

For the first event, subjects chose whether they were going to consume a carbohydrate or a carbohydrate plus protein supplement (described in detail below). For the second event, subjects had to consume the alternate supplement (the one they did not consume for the first event). For the third and longest trial, participants could choose either supplement based on their preference and prior experience during the first two tests.

The amount of supplement that each participant consumed was dependent on his or her pre-determined needs based on prior VO2max testing at Peak Performance Professionals. Carbohydrate consumption varied from 45 grams to 85 grams per hour depending on the individual athlete.

Data Analysis

Data was analyzed using SPSS Statistics, version 21.0.0. One-way ANOVA and independent t-tests were used to determine whether there were differences in performance indicators among the three cycling events. Independent t-tests were used to calculate differences in performance by supplement use, and also to determine whether gender and hydration status were confounding variables.

Table 1. Treatment Options for Participants
Participants were able to choose other products as long as they fit the same nutrient profile. They recorded their 'assumed intake' before the event and their 'actual intake' after the event.

Treatment 1: CHO					
Manufacturer	**Product Name**	**Serving Size**	**Calories**	**CHO (grams)**	**PRO (grams)**
Hammer	Gel	1 pouch	90	21	0
	Heed	1 scoop	100	26	0
Clif Bar	Gel	1 pouch	110	22	0
	Blocs	3 pieces	100	24	0
Gu	Gel	1 pouch	100	25	0
	Chomps	4 pieces	90	23	0
Gatorade	Chews	6 pieces	100	24	0
	Powder	1 tbsp	50	14	0
Power Bar	Gel	1 pouch	110	27	0
	Perform Drink	1 scoop	70	17	0

Treatment 2: CHO-PRO					
Manufacturer	**Product Name**	**Serving Size**	**Calories**	**CHO (grams)**	**PRO (grams)**
Accelerade (4:1)	Sports Drink	1 scoop	120	21	5
	Hydro	1 scoop	60	10	2.5
	Accel Gel	1 pouch	100	20	5
Hammer (7:1)	Perpetuem	2 scoops	270	54	7
	Perpetuem Solids	3 tablets	100	20	3
	Sustained Energy	3 scoops	320	68	10

CHAPTER 4: RESULTS

A total of 28 participants (males = 17, females = 11) participated in the study, although not every participant completed all three events. Average age, height, and weight are shown by gender (Table 2). Subjects were categorized into triathletes and cyclists, those that trained with a heart rate monitor and that did not, and also by the average amount of sleep per night. Of the 28 participants, 71% were triathletes that were capable of cycling for many hours at a time. Subjects were all capable of completing a four hour endurance ride but varied in level of fitness. There were only 3 participants that did not train with a heart rate monitor (HRM) (11%), thus, heart rate data could not be collected for those individuals. Based on the pre-test questionnaire, 61% of participants averaged over 7 hours of sleep per night, meeting the general recommendation stated by the Mayo Clinic in 2012.

Table 2. Subject Information

Total Subjects	**n=28**	**%n**
Triathletes	20	71%
Cyclists	8	29%
Trains w/ HRM	25	89%
Does not Train w/ HRM	3	11%
<7 Hours of Sleep / Night	11	39%
>7 Hours of Sleep / Night	17	61%

Dietary habits and perceived level of fitness was evaluated on the pre-test questionnaire. Dietary habits were evaluated with a rating scale and based on the participants' opinion. The majority of participants perceived their dietary habits as adequate or needing work (n=13 and n=12, respectively; Table 3). Perceived level of fitness was also evaluated using a rating scale from one to five, one was being unfit, and five being elite level. The majority of participates determined they were a level 4 (61%), with the next most common rating at a level 3 (32%) (Table 4). Also, subjects were questioned on variables that could affect performance during a competition, and in training. Subjects were asked what the most difficult factor is for them while competing: nutrition, hydration, distance, controlling heart rate, starting out too fast, or other (Table 5). For this question, some athletes chose more than one answer.

Table 3. Personal Dietary Habits Evaluation

Rating Scale	Participants	
Excellent	2	7%
Adequate	13	46%
Needs Work	12	43%
Horrible	1	4%

Table 4. Personal Level of Fitness Evaluation

Rating Scale	Participants	
5 (elite level)	1	4%
4	17	61%
3	9	32%
2	1	4%
1 (unfit level)	0	0%

Table 5. Personal Competition Performance Evaluation

Difficulty	Participants	
Nutrition	7	21%
Hydration	7	21%
Distance	1	3%
Controlling Heart Rate	7	21%
Starting too Fast	7	21%
Other or NA	4	12%

Table 6. Subject Anthropometrics and Supplement Use

	Total Subjects	Males	Females
	n=28	n=17	n=11
Average Age (years)	44.54 ± 9.1	42.35 ± 9.7	47.91 ± 7.3
Average Height (inches)	68.29 ± 3.6	70.18 ± 3.0	65.36 ± 2.0
Weight (lbs)	169.82 ± 34.0	184.76 ± 25.5	146.73 ± 33.5
SUBJECTS	**Event 1**	**Event 2**	**Event 3**
Total Subjects	18	20	19
Subjects that chose CHO	12	7	4
% Subjects that chose CHO	66%	35%	22%
Subjects that chose CHO/PRO	6	13	15
% Subjects that chose CHO/PRO	33%	65%	79%

Supplement Use

Grams of carbohydrate and grams of protein consumed during the events were recorded by each subject and calculated by the researcher in order to confirm whether they met their estimated nutritional needs. Supplement use during the events was recorded differently by each subject and resources were limited to determine their estimated dietary needs. In effect, estimated dietary needs for each athlete was not determined nor taken into consideration when measuring performance outcomes.

Post Event Evaluation

After completing each test, subjects completed a post-test questionnaire (Figure 1) to evaluate their performance during the test. Nine questions from the post-test questionnaire were used to evaluate each subject's opinion regarding the treatment and experiment.

Figure 1. Post-Event Questionnaire

Use the following scale to answer questions 1, 2, and 3:
Extremely Difficult 1 2 3 4 5 Extremely Easy

1. **After the 1st hour of cycling, how did you feel about the workload? Did it seem easy or difficult?**
2. **After the 2nd hour of cycling, how did you feel about the workload? Did it seem easy or difficult?**
3. **After the 3rd hour of cycling, how did you feel about the workload? Did it seem easy or difficult?**
4. **At any point during the event, did you suffer from GI distress (i.e. tightness, cramping, bloating)?**
5. **Do you think you hydrated (water and sports drinks) enough during the entire ride?**
6. **Did you feel fatigued at any point during the ride (i.e. too tired to want to continue riding)?**
7. **Using the scale below, in the three days leading up to the event, did your diet remain optimal for training (i.e. typical training food regiment as recorded in the 3-day food log)?**
   Poor training diet 1 2 3 4 5 Optimal training diet
8. **Using the scale below, in the three days leading up to the event, did your training and exercise remain normal (i.e. typical before a longer event)?**
   Unusual training week 1 2 3 4 5 Typical training week
9. **Using the scale below, were there any irregularities in your life (i.e. wedding, games, sickness, etc.) that occurred?**
   Not a typical week 1 2 3 4 5 Everything was normal

Of the 18 subjects who participated in the first experiment, 16 of those answered the first two questions of the post-test. Most of the subjects felt the workload was a 3 (n=8) and 4 (n=7), with only 1 subject (6.3%) rating the workload a 2. During the second hour of cycling, 68.7% of subjects rated the workload a 3 (n=11) and 25% of subjects rated it a 4 (n=4). Again, 6.3% rated the workload a 2 (n=1). Fifteen subjects answered the third post-test question; 53% rated the workload a 3 (n=8), 13% rated it a 4 (n=2), and 6.3% rated it a 2 (n=1). The percentage of subjects finding the first event extremely easy from the first hour to the second hour decreased from 43.7% to 25% and finally to 13% for the third hour. During the first test experiment, 6.3% of subjects (n=1) had GI issues related to the treatment or cycling event. There were 28.6% of subjects (n=4) that did not feel hydrated enough during the event, and 14.3% (n=2) that had feelings of fatigue during the ride. In addition, 20% (n=3) of subjects felt they did not eat well leading up the event; whereas, 6.6% (n=1) felt they had an optimal diet leading up to the experiment. The majority of subjects (53%, n=8) were indifferent and diet was seemingly normal for eating patterns. Exercise completed the week of the experiment was also evaluated in the post-test questionnaire. There were 12.5% (n=2) of subjects that thought their training week was unusual with majority of subjects (50%, n=8) finding it be a typical exercise week. Finally, subjects were asked about any irregularities in their life leading up to the event. The majority of subjects seemed to have a less typical week than usual with 83.3% of subjects (n=10) rating it a 2. The other 16.7% of subjects (n=2) rated question nine a 3.

The results of the post-event questionnaire following the second event are as follows. Of the 19 subjects that answered the post-test questionnaire, 45% of them (n=9) rated the workload of the first hour a 3. While 45% of subjects (n=9) rated the workload a 4, closer to extremely easy. One subject rated the first hour a 2, but none rated it a 1, or felt it was extremely difficult. For the second hour of work, 30% of subjects (n=6) rated the workload a 4, which dropped from the first hour. Half of the subjects (50%, n=10) rated the second hour of work a 3, with ~~now~~ 15% of subjects (n=3) rating the workload a 2. For the third hour of cycling, 15% of subjects (n=3) rated the workload a 4, and 55% (n=11) rated it a 3. While 30% (n=6) rated the workload a 2. Thus, during the second experiment trial, participant performance decreased as overall time increased based on post-test questionnaire feedback from subjects. Additionally, 20% of subjects (n=4) experienced GI issues during the event, and 5% (n=1) did not feel hydrated enough. There were 39% (n=7) that felt fatigued during the event, which was nearly tripled from the first trial. When asked about their diet leading up to the event, 35% of subjects (n=7) felt it was normal and 40% (n=8) rated it a 4 or a 5. There were 25% of subjects (n=5) that did not feel it was a typical week of eating. When asked about their training week, 45% of subjects (n=9) rated it a 5, a typical training week, with 10% of subjects (n=2) rating it a 1, not typical. Of the subjects that answered the final question regarding irregularities in their life, 67% (n=10) rated it a 2 and 33% (n=5) rated it a 3.

For the third and final test, when rating the workload for the first hour, 47.6% of subjects (n=10) rated it a 3. Nine subjects, or 42.9%, rated it a 4, with one subject rating it a 5 and one subject rating it a 2. For the second hour of work, there were no subjects that rated the workload a 5, extremely easy. There were 61.9% of subjects (n=13) that rated it a 3, an increase from the first hour of work. There were only 23.8% of subjects (n=5) that now rated workload of the second hour a 4, and 19% (n=4) that rated it a 2. For the final hour of work, over half of the subjects (55%, n=11) rated the workload a 3, with 20% (n=4) rating it a 2. As concluded from the first two trials, this also indicates that as time increases, overall performance decreases as evidenced by subject feedback on workload from the post-test questionnaire. Moreover, 9.5% (n=2) suffered from GI distress related to treatment during the event, and 5.3% (n=1) did not feel hydrated enough while cycling. There were 36.8% of subjects (n=7) that felt fatigued during the event. When asked to evaluate their diet leading up to the event, 35% of subjects (n=7) and 10% of subjects (n=2) rated their diet a 4 or a 5. There were 55% of subjects (n=11) that rated their diet a 2 or a 3 which would indicate a suboptimal diet regimen leading up the event. Many subjects had a normal training week with 55% of subjects (n=11) rating it a 4 or a 5, and 45% of subjects (n=9) rating their training week a 1, 2, or 3. Majority of subjects felt there were irregularities in their lives leading up to the event with 86.7% (n=13) rating that a 2, and 13.3% (n=2) rating it a 3. No subjects felt it was a normal week.

The Post-Event Questionnaire results were also analyzed by supplement use. For the first event, the subjects that felt fatigued during the ride were both following the carbohydrate treatment (n=2), while those on the carbohydrate plus protein treatment did not feel fatigued. The one subject that suffered GI distress during the ride was on the carbohydrate only treatment. Only one subject on the CHO:PRO treatment found the ride to be difficult (rating of a 2) by the second hour; whereas, five subjects on the CHO:PRO treatment found that by the third hour the workload was becoming more difficult.

For the second event lasting 3.5 hours, the three subjects felt fatigued during the duration of the ride were following the CHO:PRO treatment and the four subjects who felt fatigued during the ride following the carbohydrate only treatment. Of the four subjects that suffered from GI distress during the second event, two were following the carbohydrate plus protein treatment and two were following the carbohydrate only treatment. For the third hour of cycling, there were five subjects consuming the carbohydrate plus protein treatment that found the workload to be difficult with only one subject on the carbohydrate only treatment finding it difficult. This could indicate that as cycling time increases, there is no benefit to supplemental protein in terms of perceived difficulty and that carbohydrate becomes more valuable to reduce the perceived workload.

For the third event, five subjects on the CHO:PRO treatment felt fatigued whereas only two on the carbohydrate treatment felt fatigued cycling. The two subjects that suffered from GI distress were following the carbohydrate plus protein treatment. All subjects that found the workload to be difficult by the final hour of cycling were all on the carbohydrate plus protein treatment, suggesting that protein does not ease perceived difficulty. After reviewing the differences in post-event questionnaire results compared to the treatment subjects were using, it appears that those on carbohydrate supplement alone were able to perform better overall during the course of the endurance event.

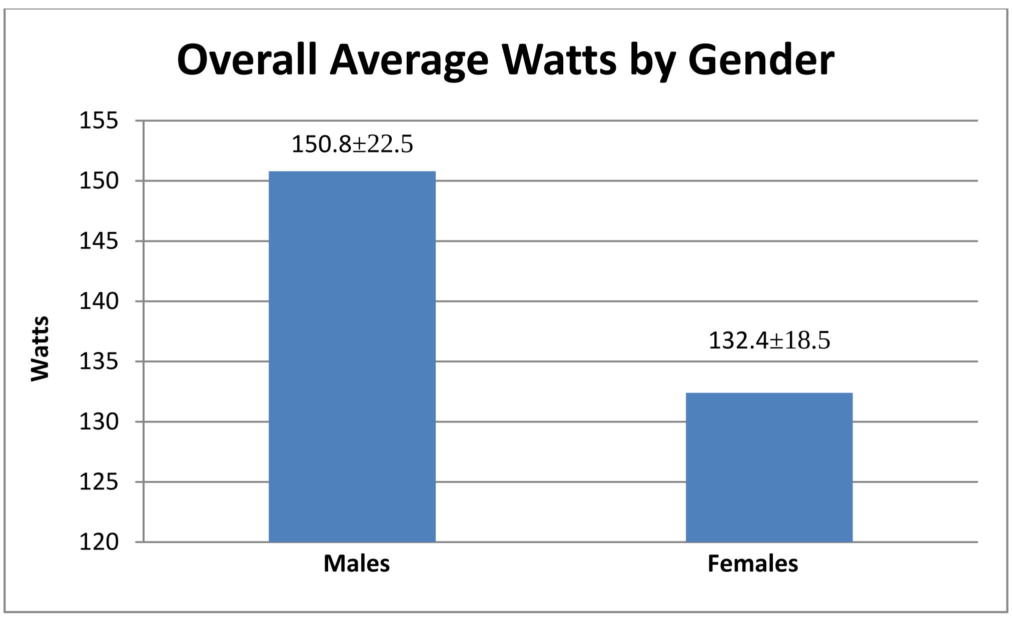
Performance

Because the events differed in length of time, and to determine whether the three events could be analyzed together, a one-way ANOVA was conducted to determine whether there was a difference in performance by event. Watts was not significantly different between events ($p=0.144$); however, heart rate was significantly different between events ($p=0.026$). Subjects performed differently depending on which event it was, regardless of which treatment they were following.

In order to observe differences in performance by supplement for all subjects (both males and females) combined, independent t-tests were also conducted with the treatment as the independent variable and performance (watts) as the dependant variable. Performance by watts for each supplement group was not significantly different in any of the 3 events ($p=0.092$, $p=0.232$, $p=0.862$, respectively). The first event suggested a trend towards significance. Subjects using the CHO treatment had an average watts of 141.2 ± 18.5 and the subjects using the CHO:PRO treatment had an average watts of 139.2 ± 30.4. Interestingly enough, significance declined as events became longer suggesting that as time increases for each event, the type of supplement becomes less influential. Other possible variables, such as caffeine use or injuries, could have impacted participant performance. Furthermore, when evaluating performance based on heart rate by supplement use, heart rate was not significantly different based on supplement (p=0.143, p=0.330, p=0.205 for the three trials respectively).

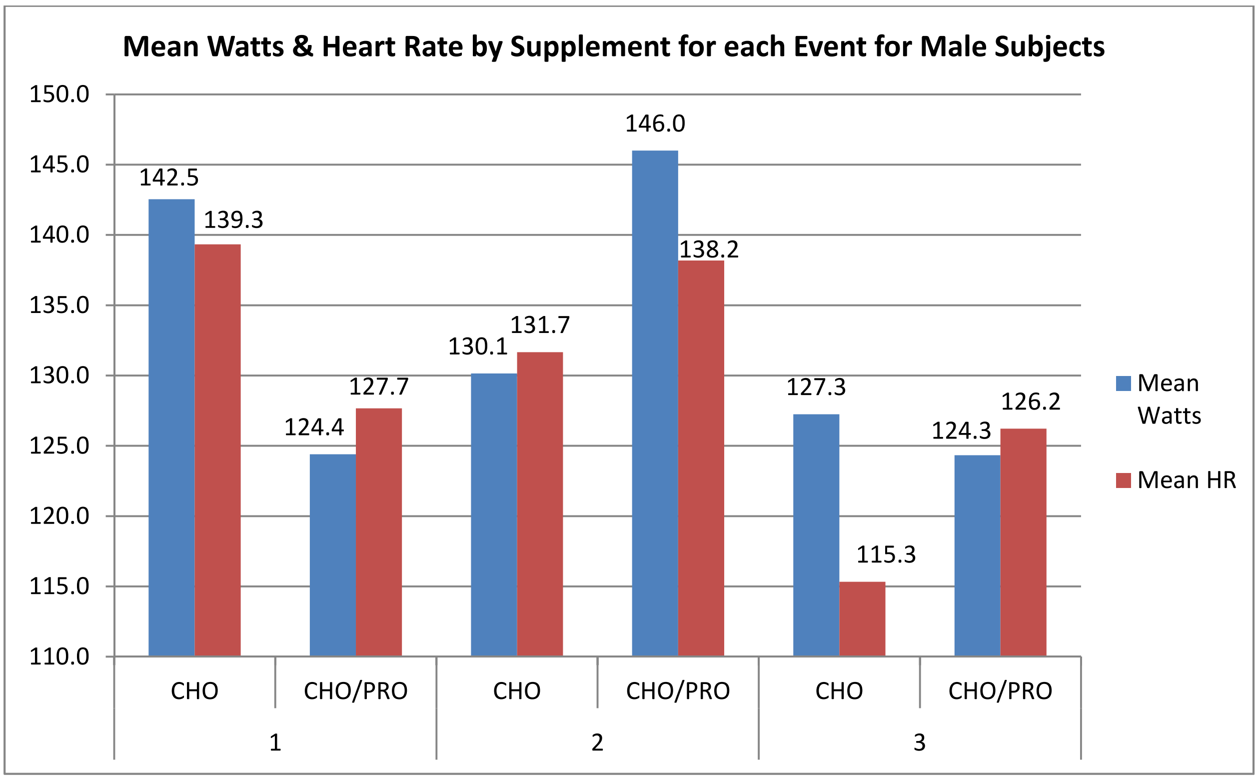
Gender was investigated as a potential confounding variable on performance, to determine whether males and females could be analyzed together. There was a significant difference in performance based on gender (Figure 2). Males had a higher overall average watts (150.8 ± 22.5) compared to females (132.4 ± 18.5) and males had significantly higher wattage compared to females for each of the three events (p=0.002, p=0.000, p=0.010 for the three events respectively). Thus, males and females were separately evaluated for performance based on supplement.

Figure 2. Average Watts by Gender for All 3 Events



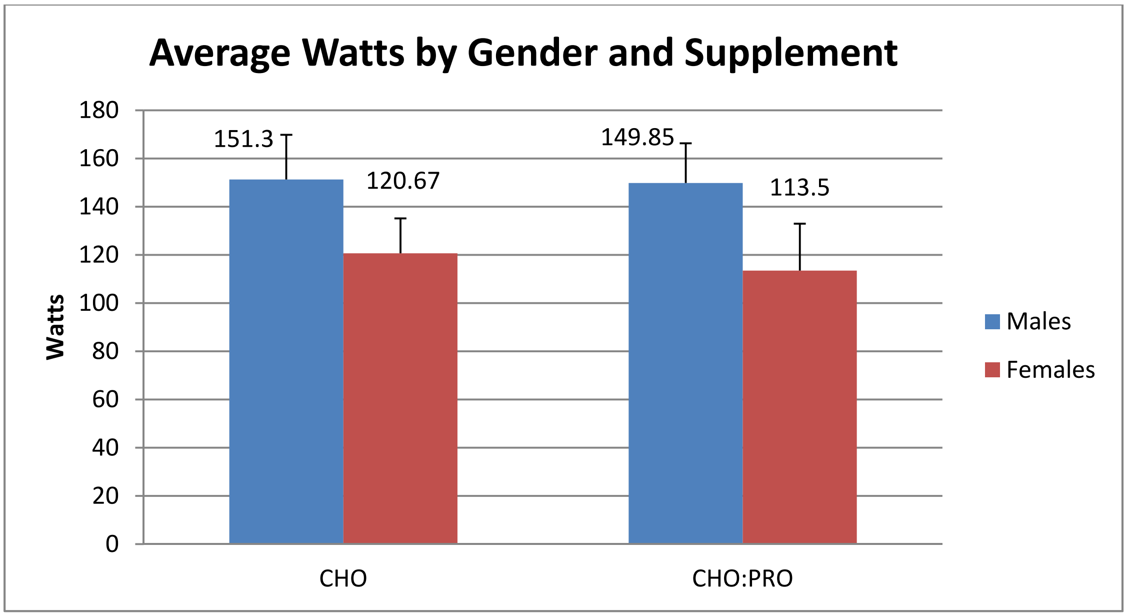
Males who consumed the CHO only supplement performed better than those on the CHO:PRO supplement for the first event, which trended towards significance (p=0.059). Performance based on watts was not significantly different between supplements for the other two events (p=0.878 and p=0.255, respectively), but for event two, male subjects that consumed the CHO:PRO supplement performed better than those on the CHO only supplement. Additionally, there was no difference in performance as measured by heart rate based on supplement (p=0.677; p=0.802; p=0.250, respectively).

Figure 3. Mean Watts & Mean Heart Rate by Supplement for each Event for Males



Because watts did not differ by event, performance (in watts) based on supplement use was compared between individuals (Figure 4). Performance, measured in watts, did not differ between individuals depending on which supplement they consumed. Males averaged 151.3±18.54 and 149.85±16.44 watts for carbohydrate only and carbohydrate plus protein, respectively (p=0.803). Females averaged 120.67±14.5 and 113.5±19.49 watts for carbohydrate only and carbohydrate plus protein, respectively (p=0.167).

Figure 4. Average Watts by Gender and Supplement



Subject Preference

Overall, 79% (n=15) of the athletes that completed the longest test (event three) and had a choice between CHO and CHO:PRO, chose to use the CHO:PRO treatment, while 21% (n=4) chose the CHO treatment (Table 7). Of those 15 subjects, 9 of them had completed a previous event with the CHO treatment. This may be the most telling of all findings such that subjects felt overall improvements in performance and essentially chose to use additional protein with their carbohydrate supplementation for the longest endurance event. Although this is an insightful finding, it should be noted that subjects had the freedom to choose their supplement for each event and were not limited to a certain number of events, as some subjects were only able to come to one or two events. The freedom of treatment supplements and event attendance could be the driving force behind this finding because of subject and treatment variability.

Table 7. Subject Treatment Choice for each Event

Event	CHO	CHO/PRO
1	66% (n=12)	33% (n=6)
2	35% (n=7)	65% (n=13)
3	21% (n=4)	79% (n=16)

CHAPTER 5: DISCUSSION & FUTURE RESEARCH

A total of 28 subjects participated in the three events (males = 17, females = 11). Each participant was able to choose their treatment for each event; either a carbohydrate treatment or a carbohydrate plus protein supplement. Watts and heart rate were used to determine performance outcomes for each participant. If performance measures decrease overtime, then that may indicate possible fatigue, but if subjects are able to maintain or gradually increase wattage while maintaining an aerobic heart rate, then that may indicate an improvement in performance, the ultimate goal for each athlete.

When paired t-tests were used to compare performance by gender, age, weight, fitness level, and average hours of sleep per night, significance was observed for all variables ($p=0.000$). Participants were grouped by gender for each test. A significant p value suggests that the indicated variables affect overall performance. Interestingly enough, when males and females were grouped together, only age and weight were significant.

Results were derived from the post-test questionnaire completed by each participant. It was observed that as time elapsed during the first event, subjects experienced a decline in overall performance. Participants noted that by the end of the event, cycling was not as easy as when they started. This could be related to nutritional status before starting the event, nutrition during the event, hydration, and possibly fitness level. Other variables do exist as well that may not have been included in this experiment. Only one participant noted that they had an optimal diet the week of the event, with most participants finding it typical with nothing unusual to impact this event.

For the second event, participant performance also declined over time. There were five participants that noted their diet was optimal the week before the event, but most participants were indifferent about their diet. It was also a normal training week for nine of the participants. For the final event, overall performance declined as time increased. Diet leading up to the event was noted as optimal for two participants, while 11 participants felt they had a typical training week. Although participant feedback indicates that performance declined over time and that they maintained a suboptimal diet regimen, the results of this study suggest a possible impact from diet and training based on the methods used to record diet and activity. Overall, statistical results indicated that diet score and fitness level based on gender were significant confounding variables. After subjective data was evaluated, statistical analysis was completed, distribution of participant wattage and heart rate were evaluated. Watts is a significant variable for all three events when evaluating performance by gender.

After evaluation of differences in performance by event, it was indicated that watts was not significant, but heart rate was significant when comparing each event and for all subjects combined. To determine further evaluation, participants were divided by gender. Watts was then a significant confounding variable based on event, while heart rate was not. This potentially suggests that watts are a better indicator of overall performance than heart rate. Based on gender, males held a much higher wattage than females which could suggest males have the potential for less fatigue and better performance outcomes. For events one and two, males also held a higher heart rate than females, but this could simply be athletic gender differences being observed. Males did not perform as well as females for the third, and longest, event, only holding a mean heart rate of 122 bpm. This drop in mean heart rate could be related to the mean drop in watts for this particular event.

Differences in performance by supplement were observed as well for each event. It was determined through statistical analysis that the treatment was not significant when compared to performance measured in both watts and heart rate for the combined group. When females were excluded from the statistical analysis, performance measured by watts or heart rate was not affected by treatment except for the first event when considering watts. Although treatment was not a confounding variable for overall performance, trends were observed from the group statistics. Majority of participants had the option to use either treatment, but (80%) chose to use the CHO:PRO treatment for the final event. Event one and two were split with 66% and 35% using the CHO treatment and 33% and 65% using the CHO:PRO treatment. For the final event, watts were similar for each treatment group (CHO=127, CHO:PRO=124). This suggests that regardless of the type of nutrient, some form of supplementation is necessary. Additionally, when evaluating performance based on heart rate by supplement use, heart rate was higher for the CHO:PRO group during events two and three but not event one. This could be because only three people were using the CHO:PRO treatment for event one which is a small sample size. Overall, many confounding variables exist when evaluating performance measures. Statistical analysis revealed that based on gender for each event, significance is not seen when comparing performance to supplement use. The original hypothesis that protein combined with CHO will significantly affect athletic performance during prolonged activity appears to be false according to the present study results – treatment did not significantly affect overall performance.

Perplexing Findings

Based on the current research analysis and results, there appears to be perplexing findings. As mentioned in the results, performance is not affect by treatment when separated by CHO and CHO:PRO treatments. It would seem likely that the shortest event would be less significant that the final, longest event, but that was not found in the analysis. Treatment simply did not prove significant in any event. This finding was not expected when formulating the hypothesis and based on other current research studies. It was also interesting to find that less sleep per night on average did not affect overall performance, nor did perceived fitness level when analyzed by a complete sample group. Gender played a role in this experiment, as significance was found only when the sample group was divided into males and females. Regardless of gender, however, it was interesting to find that age (uncontrollable) and weight (controllable) have a significant impact on performance during aerobic activity such that as an athlete ages, they are able to perform better or worse. This finding could lead to future research testing the effects on performance.

Finally, the most perplexing finding was that heart rate acts as a performance indicator more than watts when considering treatment. Both heart rate and watts seem to play a role in overall performance but under different circumstances and under different conditions. It could be predicted that over the course of an event, as time increases, heart rate becomes a better performance indicator than watts, or vise versa. The findings of this experiment lead to additional questions and hypotheses about the research topic and other possible confounding variables.

It is not certain why the majority of athletes chose the CHO:PRO treatment versus CHO alone, but what is suggested from the statistical analysis is that some form of supplementation is necessary to improve overall performance. Moreover, heart rate affects performance for the combined group of males and females, but when divided by gender, heart rate does not significantly affect performance for either males or females. Watts, however, becomes a significant variable for each gender. Differences in performance by treatment were not significant, but trends were evaluated and results indicate other variables exist, including fitness level, hydration, nutrition that day, nutrition that week, and other indicators such as ambient temperature, body temperature, hormones, illness, etc. (described in greater detail below).

Comparison to Current Research

Testing the effects of protein coingestion with carbohydrate on performance measures is currently being conducted in various research studies. Certain investigations suggest that carbohydrate alone optimizes athletic performance. Other investigators suggest that performance can only be optimized with the addition of protein during exercise. The conflicting findings from recent studies indicate that all confounding variables could potentially affect performance during prolonged endurance activity and that each must be extensively researched.

Compared to other studies related to the current research, similarities exist. Various research studies have measured performance based on watts and heart rate for cycling time trials. A diet recall appears to be a standard as well as providing subjects with a 4:1 or 7:1 ratio of carbohydrates to protein supplementation. Also similar to the present study is that wash out periods are used. For this experiment, athletes had about one month between events which would be enough time to recover, refuel, rehydrate, and ensure the next trial is successful.

Differences to other research studies include study design and access to equipment. The subjects and researchers in other studies are blinded to the treatment, and testing occurs with various types of equipment including those for lactic threshold, sub max or anaerobic threshold, blood chemistry levels, and muscle damage indices. In order to have a larger sample size, subjects were allowed to pick their supplements for each treatment and the researcher was present at each event; blinding would have been a challenge. Also, there was no access to laboratory equipment and no money budgeted for this experiment; hence, only the Compu-Trainers at Peak Performance Professionals could be used for the events. Additionally, differences are observed in the sample size and gender of athletes. Many other research studies are conducted with less than ten subjects and all of the same gender and athletic capabilities. The current sample size was a variety of athletes with many different athletic capabilities. Subjects underwent a sub max test prior to the event, but typically one is also conducted at least one week before the experiment begins for other studies. Compliance from participants and access to equipment would have been a challenge and costly for the researcher. Many other studies match energy for carbohydrates and protein versus allowing differences in calories. Athletes in the current study were not matched for energy, but were able to choose their supplements for treatment based on advice from their coach and also what they prefer. If the choice did not exist, sample size would have been significantly compromised. Finally, other current studies have subjects complete an aerobic cycling test followed by a time to exhaustion trial. The present study only considered the effects on performance for the aerobic portion, not the anaerobic time trial. The protocol of this experiment was to simply find relationships in performance and aerobic training. Although there are similarities to other studies that measured aerobic performance as a segment of their research, it is too difficult to compare results due to the unique study design of the current research experiment. Other studies have indicated relationships between supplement use and performance, but that relationship was not fully observed in the current study and could have been related to the overwhelming presence of confounding variables.

Strengths

There are various strengths to this research study with the first being the study design. Set up as an experimental, crossover design, subjects were used as their own controls and each event was a single experiment test. This allows for more accurate results and better comparisons between subjects. Each subject trains regularly at Peak Performance Professionals under the guidance of Bob Hanisch, MA, CDE, CSCS. They have all completed sub max testing and are aware of the appropriate zones in which to train. Subject sample size was larger for this experiment than other current experiments recently conducted. Subjects were able to perform each event on a Compu-Trainer with data being stored for each subject within the system database. Additionally, the experimental cycling room maintained a temperature of 55 to 58 degrees during all three events. The course was exactly the same for the second and third trials, following the Ironman WI course. Subjects had access to water during the course of all three events and were not limited to a particular amount of fluids. They also had a choice of which products to use for the treatment as long as the treatment protocol was followed for each. These strengths in subject and study design allowed for a smoother experiment and also better compliance from each participant. Finally, the same post-test questionnaire was completed by each participant after each event. Participants were able to see the questions in advance and had the opportunity to determine answers during each event. Although many strengths exist for this experiment, there are also several limitations that suggest further future research.

Confounding Variables: Hydration & Diet

Hydration seemed to play a role in each event as majority of participants lost over one pound each time. Hydration status for each cyclist was determined by difference in pre- and post-event weights. Weight loss of less than one pound was considered ‘good’ hydration and weight loss over one pound was considered ‘poor’. Athletes that gained weight were excluded from this analysis. There were two subjects excluded from test two and three subjects excluded from test three. Results for the first event indicated that out of 18 total participants, four lost less than one pound and 14 lost over one pound. Subjects that were identified to be in good hydration status had better performance than subjects that were identified to be in poor hydration status, based on performance measured in watts ($p=0.000$). For the second trial, five subjects had ‘good’ and 12 subjects had ‘poor’ hydration status, and performance did not differ between the two, although there was a trend towards improved performance for those in good hydration status ($p=0.119$). For the final event, six had ‘good’ and ten had ‘poor’ hydration status. Performance did not differ by hydration status in event three ($p=0.627$). These findings suggest that fluid status was a confounding variable for individual cyclists and also indicates that majority of cyclists were not adequately hydrated during the events. More analysis and research needs to be conducted in order to determine performance results based on hydration.

In addition to hydration status, diet was also considered and paired t-tests were conducted to determine if perceived quality of diet was related to performance. As previously stated, athletes were instructed to record a three day diet recall before the first event and also for each event day. Diet records were reviewed for any red flags that may have impacted performance, but essentially, they were not detailed enough to derive useful information for analysis. As a result, for this particular analysis, the athletes’ perceived diet scores were used. The subjects that were poorly scored for diet were not excluded from the analysis because 20 to 40% of subjects fell within these categories. Diet score did not significantly affect performance when measured in watts for each event (p=0.235, p=0.742, p=0.014, respectively).

Limitations & Challenges

Acting as a strength of the study, the sample size is also a limitation of the current research. The sample size is considerably large, not typical of this type of research; yet, it is also quite small to determine significant results and conclusions. The sample size is also a mix of males and females with more males participating. This can skew the results if gender is not considered. Subjects were also different for each event and able to choose the supplement they were taking for each event. They were able to choose if they were participating in one, two, or three events. This can skew the results as each subject can be affected differently by the length of time of each event and also the supplement chosen. Subjects had the freedom to do one treatment for all three events if that was most comfortable to them. Additionally, the actual fitness level of each participant is also a limiting factor because performance would be measured differently for less or more fit athletes. Because each subject is acting as their own control, fitness level may not play a significant role in performance for this experiment.

Moreover, the course followed during the first event did not match the course for the second and third events due to athlete preference. According to participants, it was considered an easier course with few challenging hills. This acts as a limitation because it can impact the overall performance of each participant, suggesting significant results on one course but not the other. Additionally, subjects were required to record a three day diet intake, record intake before and during each event, and record final watts and heart rate. They were also required to complete a pre-test and post-test questionnaire for each event. The researcher can only assume that each subject recorded accurate, honest, and reliable information. Subjects were asked to complete nude weigh in's before and after each event. It can only be assumed that each subject was nude and recorded their weight accurately. The scale used for weigh in's was a household scale that Peak Performance Professionals uses regularly for their athletes. This is a potential limitation in that the scale could have been incorrect for any given event. It was a challenge to get compliance from participants to do their own weigh in's so the research had to continuously call athletes over to the scale to ensure weigh in's were completed. For two of the three events, at least one participant did not weigh in; thus, that piece of data could not be used in the analysis.

Acting as a confounding variable, hydration also was a limitation of the study. Participant hydration during all three events was not regulated – they could consume as much or as little fluids as they wanted. Many subjects noted how many ounces of fluid they consumed, but overall, majority of subjects were skeptical as to an accurate amount. This could negatively impact the overall performance results such that hydration could impact performance more than the type of supplement.

Finally, a possible limitation could be the experiment environment which was a controlled setting. Prior research designs have been conducted in more controlled environments with subjects attached to machines; however, there are also experiments conducted during competition in a real-life setting. It has not been determined which environment creates more accurate findings, but it would be expected that each is a limiting factor to the experiment.

Future Research

With limited resources to complete the present research, definite future research is needed to determine more conclusive results. It was unexpected to find no significant results from the treatment on performance, but that could be because too many variables still existed. A more controlled environment with more experimental laboratory analysis may help to isolate the variables from one another. Future research would benefit not only the participants in the current study, but any athlete of any fitness level hoping to performance well in their sport. Optimizing performance is essential for any athlete, but the evidence still does not prove what variable affects performance more. The current study observed treatment, time, fitness level, diet, sleep, age, weight, and hydration as possible variables on performance. The main hypothesis was to find significance in the treatment, which was not observed. Clearly, more research needs to be conducted in order to determine which confounding variables are most effective on performance. Isolating each of these and also other variables is crucial to the outcome for performance. Athletic studies though are challenging given the possible sample size, environmental control, and current chemical state of each person at that time. Only speculation and assumptions from current research testing carbohydrate plus protein supplementation can be used to advise athletes at this point until more detailed and specific research is developed.

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Appendix A. Pre-Study Questionnaire

Name: ______________________________Date of Birth:_____________Age Today: ________

Address:____

City:____________________State:_______Zip:________

Phone 1:________________(Circle: Home / Work / Cell) Phone 2:________________

Email: ________________________Preferred Contact Time: Circle: AM NOON PM

Height:____________Weight: ____________

Married: Yes / No Children: Yes / No

Occupation:__________________________Normal Hours of Work:______________________

TRAINING INFORMATION

Focused Sport:______________________________Training for:________________________

I train with a heart rate monitor: Yes / No Brand/Model:________________________

What is your current resting heart rate:_______________________

What is your perceived level of fitness (1 low – 5 high): 1 2 3 4 5

Current Anaerobic Threshold Data:

Bike:_____________________________________

Run:_____________________________________

Swim:____________________________________

Are you fitted on your bike: Yes / No / Unsure

Do you keep a training log: Y / N

Do you have any injuries or recurring injuries:____
____

Best competition times for preferred races: Please list event distance, time, and date)

	Event	Time	Date
Cycling Race:			
Running Race:			
Swimming Race:			
Half Ironman:			
Ironman:			
Other:			

My dietary habits are: Excellent Adequate Needs Work Horrible

Do you take any form of supplements: Y / N
If yes, what do you take? ____
____

Is there anything special or unusually to know about your dietary habits? _____________________
____

Please describe your current nutritional habits. When do you typically eat?
Breakfast:____
Lunch:____
Dinner:____
Snacking:____

When was your last physical exam:____________________Are you cleared to train? Y / N

What is your longest training week (hours/week):_______________Shortest:__________________

Do you take rest days during the week: Y / N If so, how many:___________

How many days during the week do you strength train or cross train:___________

How many hours of sleep do you average per night:_______

Do you have difficulties in sleeping? Y / N
Do you feel exhausted during the day? Y / N

What time do you usually go to bed:_____________What time do you usually wake up:________

What is most difficult for you when completing competitions? Circle.

Nutrition Distance Controlling Heart Rate Going out too Fast
Hydration Other:_____________________________________

List up to three goals that you hope to accomplish during the over-distance program?

1. ____
2. ____
3. ____

Additional Comments/Concerns:____

____

Appendix B. Athlete Health Questionnaire

1. Do you have a personal history of heart disease?	YES	NO
2. Have you ever experienced pain or discomfort in your chest that has not been formally diagnosed?	YES	NO
3. Have you ever experienced a rapid fluttering or throbbing of the heart?	YES	NO
4. Do you have a known heart murmur?	YES	NO
5. Do you have any shortness of breath?	YES	NO
6. Do you have difficulty breathing while standing or have sudden breathing problems at night?	YES	NO
7. Have you had problems with dizziness or fainting?	YES	NO
8. Do you have any form of swelling in the ankles?	YES	NO
9. Have you experienced severe leg pain while walking?	YES	NO
10. Do you have any history of metabolic diseases (thyroid, liver, renal)?	YES	NO
11. Do you have diabetes?	YES	NO
12. Have you been told you have high blood pressure? Current blood pressure? _____/______	YES	NO
13. Do you have high cholesterol (over 240mg/dl)?	YES	NO
14. Are you a cigarette smoker?	YES	NO
15. Would you describe your lifestyle as sedentary?	YES	NO

16. List any surgeries in the last 2 years or any surgeries that have resulted in complications that limit your activity: ____
____

17. Has your physician limited your physical activity in any way?_____________________
____

18. Current Medications:____
____

Appendix C. Post-Event Questionnaire

After completion of each test, please answer the following questions. Some questions may not pertain to this event date. Ideally and if possible, please immediately complete or return data to the researcher within 2-3 days after the event. This data will help to eliminate any confounding variables unrelated to each treatment.

After the first hour of cycling, how did you feel about the workload? Did it seem easy or difficult?

EXTREMELY DIFFICULT 1 2 3 4 5 VERY EASY

Other comments:____
____

After the second hour of cycling, how did you feel about the workload? Did it seem easy or difficult?

EXTREMELY DIFFICULT 1 2 3 4 5 VERY EASY

Other comments:____
____

After the third hour of cycling, how did you feel about the workload? Did it seem easy or difficult?

EXTREMELY DIFFICULT 1 2 3 4 5 VERY EASY

Other comments:____
____

At any point during the event, did you suffer from GI distress (i.e. tightness, cramping, bloating)? YES NO

If yes, how many times did you experience GI discomfort? 1 2 3 4 5+

If yes, which hour of cycling did you have distress? Hr 1 Hr2 Hr3 Hr4

If you had to guess what caused GI distress, which of the following would best describe the cause (please circle):

Today's Breakfast Under Hydration Over Hydration Low Electrolytes

Nutrition Supplement Other

Other comments regarding GI distress:____

____

Do you think you hydrated (water and sports drinks) enough during the entire ride?

YES NO

About how much water/sports drink did you drink during each hour (please circle)?

NOTE: standard water bottle is 24oz

Hour 1: less than 12oz 12oz 16oz 24oz 32oz more than 32oz

Hour2: less than 12oz 12oz 16oz 24oz 32oz more than 32oz

Hour3: less than 12oz 12oz 16oz 24oz 32oz more than 32oz

Other comments regarding hydration:____

____

Did you feel fatigued at any point during the ride (i.e. too tired to want to continue riding)?

YES NO

If so, when did you feel any fatigue or feelings that you did not want to continue cycling (please circle)?

Before starting today Hour 1 Hour 2 Hour 3 After hour 3

If so, did you take additional food or drink to compensate? YES NO

What did you consume and how much?____

Other comments regarding fatigue:____

____

Using the scale below, in the three days leading up to the event, did your diet remain optimal for training (i.e. typical training food regiment as recorded in the 3-day food log)?

Poor training diet 1 2 3 4 5 Optimal training diet

Other comments regarding diet: ____

____

Using the scale below, in the three days leading up to the event, did your training and exercise remain normal (i.e. typical before a longer event)?

Unusual Training Wk 1 2 3 4 5 Typical Training Week

Other comments regarding exercise:____

____

Using the scale below, were there any irregularities in your life (i.e. wedding, sickness, etc.) that occurred?

Not a typical week 1 2 3 4 5 Everything was the same as usual

Other comments regarding irregularities in life:_____________________________________

____

What watts did you average throughout the ride? ___________________

What heart rate zone (or threshold range) did you average throughout the ride?

Less than 50% threshold 50-60% 60-70% 70-80% Over 80% threshold

Heart rate data can help determine performance. If possible, please record below heart rate data collected by your heart rate monitor that would be helpful to the experiment. Try to provide details so the researcher can identify potential results.

Event 1 - *# of subjects that responded for each*					
Rating	**1**	**2**	**3**	**4**	**5**
Question 1	0	1	8	7	0
Question 2	0	1	11	4	0
Question 3	0	5	8	2	0
Question 7	0	3	8	3	1
Question 8	0	1	1	4	8
Question 9	0	10	2	0	0
	YES	**NO**			
Question 4	1	15			
Question 5	10	4			
Question 6	2	12			
Event 2 - *# of subjects that responded for each*					
Rating	**1**	**2**	**3**	**4**	**5**
Question 1	0	1	9	9	1
Question 2	0	3	10	6	1
Question 3	0	6	11	3	0
Question 7	1	4	7	6	2
Question 8	2	4	4	1	9
Question 9	0	10	5	0	0
	YES	**NO**			
Question 4	8	12			
Question 5	19	1			
Question 6	7	11			
Event 3 - *# of subjects that responded for each*					
Rating	**1**	**2**	**3**	**4**	**5**
Question 1	0	1	10	9	1
Question 2	0	3	13	5	0
Question 3	0	4	11	4	0
Question 7	0	5	6	7	2
Question 8	5	3	1	3	8
Question 9	0	13	2	0	0

	YES	NO
Question 4	5	15
Question 5	17	1
Question 6	7	12

Appendix D. Subject Plans for each Event

	TEST 1 (3 hours)		
Subject	**PLAN**	**TOTAL CHO (g)**	**TOTAL PRO (g)**
3	CHO/PRO	135	17.5
13	CHO/PRO	162	21
15	CHO/PRO	448	9
16	CHO/PRO	45	10
22	CHO/PRO	136	20
23	CHO/PRO	97	21

	TEST 1 (3 hours)	
Subject	**PLAN**	**TOTAL CHO (g)**
1	CHO	150
6	CHO	149
8	CHO	98
10	CHO	126
11	CHO	122
12	CHO	98
17	CHO	134
18	CHO	50
19	CHO	144
21	CHO	109
26	CHO	220
28	CHO	148

	TEST 2 (3.5 hours)		
Subject #	**PLAN**	**TOTAL CHO (grams)**	**TOTAL PRO (grams)**
1	CHO/PRO	200	19
3	CHO/PRO	81	10.5
6	CHO/PRO	165	16
7	CHO/PRO	na	na
8	CHO/PRO	189	25
10	CHO/PRO	130	40
11	CHO/PRO	121	28
14	CHO/PRO	196	20
18	CHO/PRO	22	4.5
20	CHO/PRO	60	17
21	CHO/PRO	103	10

	TEST 2 (3.5 hours)	
Subject #	**PLAN**	**TOTAL CHO (grams)**
2	CHO	187
4	CHO	78
12	CHO	99
13	CHO	178
15	CHO	323
22	CHO	125
23	CHO	94

24	CHO/PRO	165	23
26	CHO/PRO	100	16

	TEST 3 (4 hours)				TEST 3 (4 hours)	
Subject #	**PLAN**	**TOTAL CHO (grams)**	**TOTAL PRO (grams)**	**Subject #**	**PLAN**	**TOTAL CHO (grams)**
1	CHO/PRO	220	24	11	CHO	140
2	CHO/PRO	150	5	20	CHO	38
3	CHO/PRO	162	21	24	CHO	126
4	CHO/PRO	82	17	30	CHO	98
6	CHO/PRO	236	35			
7	CHO/PRO	100	10			
8	CHO/PRO	216	28			
13	CHO/PRO	154	14			
15	CHO/PRO	222	14			
22	CHO/PRO	163	29			
26	CHO/PRO	100	16			
28	CHO/PRO	134	21			
31	CHO/PRO	93	10			

Appendix E. Weight Changes during Testing.

Subject #	Test 1			Test 2			Test 3		
	Wgt IN	Wgt OUT	Difference	Wgt IN	Wgt OUT	Difference	Wgt IN	Wgt OUT	Difference
1	158.4	155.6	-2.8	160.4	157.6	-2.8	159	157.7	-1.3
2				117.8	118	0.2	115.6	115.7	0.1
3	173.5	170.7	-2.8	172.7	171.6	-1.1	173.4	170.2	-3.2
4				213.6	208.8	-4.8	215.7	211	-4.7
5									
6	216.1	213.1	-3	214	209.9	-4.1	215.5	212	-3.5
7				157.2	157	-0.2	155.4	155.8	0.4
8			0	210.6	207.8	-2.8	208.2	207.3	-0.9
9									
10	213.4	210.4	-3	202	200.8	-1.2			
11	157.7	156.6	-1.1	159.1	158.6	-0.5	160.4	159.6	-0.8
12	133.7	131.1	-2.6	129	127.6	-1.4			
13	221.5	219.8	-1.7	217.8	217	-0.8	215.2	212.4	-2.8
14				186.2	183	-3.2			
15	202.5	199.4	-3.1	199.3	196.8	-2.5	198	194.2	-3.8
16	129.5	129	-0.5				129.5	129	-0.5
17	185.5	182.1	-3.4						
18	149.1	148	-1.1	149.9	151.1	1.2			
19	162.6	160.8	-1.8				160.6	158.7	-1.9
20				122.8			124.6	124.9	0.3
21	216.4	215.6	-0.8	216.8	215.2	-1.6			
22	124.1	123.5	-0.6	121.6	121.4	-0.2	122.4	123.7	1.3
23	132.3	130.7	-1.6	129.3	132.1	2.8			
24				169	169	0	167.2	166.6	-0.6
25									
26	228	227	-1	224	221.7	-2.3	221	221	0
27	164.4	163.2	-1.2	163	165	2	165	163	-2
28	208.4	211.1	2.7				204	205	1
29							137.8	137.6	-0.2

30							160.1	157.9	-2.2
31							159.8	156.2	-3.6

Key: gray color indicates subject did not participate; blank spaced indicated subject did not weight in/out.