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Nutritional Ergogenic Aids: The Influences of Carbohydrate-Protein Supplementation During Endurance Exercise

Adriana Marina Coletta
University of Tennessee - Knoxville, acoletta@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Adriana Marina Coletta entitled "Nutritional Ergogenic Aids: The Influences of Carbohydrate-Protein Supplementation During Endurance Exercise." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutritional Sciences.

Hollie A. Raynor, Major Professor

We have read this thesis and recommend its acceptance:

Dixie L. Thompson, Michael Zemel

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Nutritional Ergogenic Aids: The Influences of Carbohydrate-Protein Supplementation During Endurance Exercise

A Thesis Presented for
The Master of Science
Degree
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Adriana Marina Coletta
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ABSTRACT

Mixed results, in terms of performance benefits, have been found when comparing carbohydrate (CHO) and carbohydrate-protein (CHO-P) supplementation during endurance exercise. Thus this study assessed performance from three different supplements (CHO-P, CHO, double carbohydrate [CHO-CHO]) as compared to a placebo (PLA) during a time trial (TT) run. Twelve male recreational runners (age = 32.4 ± 9.5 yrs; body mass index [BMI] = 22.7 ± 1.5 kg/m^2; VO_2max = 59.68 ± 7.53 mL/kg/min; 100% white) individually completed four, 12-mile TT runs, 7-10 days apart, at about 75% of their race pace. Dietary and physical activity consistency within the 24-hour time period prior to each run was controlled via individualized diet and activity prescriptions based on the diet consumed and the physical activity engaged in prior to TT 1. Throughout each TT run, participants consumed a 600 mL load of one of the four aforementioned supplements. Supplement order was counterbalanced with a latin-square design. Endurance performance was measured by time to complete the 12-mile run, and time to complete the last 1.2 miles of the run, where participants were instructed to run at maximal effort, 100% race pace. A main effect of time occurred during the TTs for perceived exertion (RPE) and heart rate (HR). RPE (Borg 10-point scale) significantly increased from the mid-point of the TT to completion of the run (4.7 ± 0.7, 9.7 ± 0.9, $p < 0.001$); HR significantly increased from the start of the run to the start of the maximal effort, and was significantly higher at completion of the effort (84.4 ± 14.5 bpm, 166.0 ± 8.3 bpm, 178.8 ± 7.4 bpm, $p < 0.001$). No significant difference was found in overall time to complete the 12-mile run or maximal effort between the supplements (PLA = 88.6 ± 11.6 min, CHO = 89.1 ± 11.3 min, CHO-P = 89.1 ± 11.8 min, CHO-CHO = 89.6 ± 11.9 min; PLA = 8.3 ± 1.2 min, CHO = 8.2 ± 1.2 min, CHO-P = 8.2 ± 1.2 min, CHO-CHO = 8.4 ± 1.5 min). These findings suggest that type of supplementation

(CHO, CHO-CHO, CHO-P) consumed during an endurance exercise bout has no influence on enhancing endurance performance in male recreational runners during TT runs less than 100 minutes in length.

INTRODUCTION

Ergogenic aids are external influences that can positively influence physical and mental performance. Carbohydrate (CHO) supplements, such as Gatorade®, and carbohydrate-protein (CHO-P) supplements, such as Accelerade®, are considered nutritional ergogenic aids. Use of these supplements during training and/or competition, in addition to an optimal diet and strict training regimen, may provide the athlete with a competitive edge above his/her competition.

Traditionally CHO supplementation is utilized during endurance exercise bouts, while CHO-P supplementation is used post-exercise for muscle recovery. Previous research has demonstrated consistently that supplementation with CHO, in comparison to water alone, during an endurance exercise bout lasting greater than one-hour at moderate-vigorous intensity benefits performance. Recently, research has begun to assess CHO-P supplementation during the endurance exercise bout in order to determine if performance is enhanced over that of CHO supplementation.

Mixed results, in terms of performance benefits, have been found when comparing CHO and CHO-P supplementation during endurance exercise. This may be attributed to the varying study designs and protocols that have been used when comparing CHO-P and CHO supplementation during endurance exercise. These variances include: type of methodology (time to fatigue or time trial [TT] method), tested supplement composition (isocarbohydrate and isocaloric), inclusion of a placebo, control for order effects, and glycogen status of participants at the start of the exercise bout. Thus, more research is needed addressing the identified gaps in the literature.

The aim of the presented study was to determine whether potential performance benefits from ingesting a CHO-P supplement during endurance exercise, as assessed using the TT

method, was attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to the tested CHO supplements. This study assessed performance from three different supplements (CHO-P, CHO, double carbohydrate [CHO-CHO]) as compared to a placebo (PLA) during a TT run; the CHO and CHO-P supplements were iscarbohydrate and the CHO-P and CHO-CHO supplements were isocaloric. Twelve male recreational runners (age = 32.4 ± 9.5 yrs; body mass index [BMI] = 22.7 ± 1.5 kg/m^2; VO_2max = 59.68 ± 7.53 mL/kg/min; 100% white) individually completed four, 12-mile TT runs, 7-10 days apart, at about 75% of their race pace. Dietary and physical activity consistency within the 24-hour time period prior to each run was controlled via individualized diet and activity prescriptions based off of the diet consumed and the physical activity engaged in prior to TT 1. Throughout each TT run, participants consumed a 600 mL load of one of the four aforementioned supplements. Supplement order was counterbalanced with a latin-square design. Endurance performance was measured by time to complete the 12-mile run, and time to complete the last 1.2 miles of the run, where participants were instructed to run at maximal effort, 100% race pace.

Overall, a main effect of time occurred during the TTs for perceived exertion (RPE) and heart rate (HR). RPE (Borg 10-point scale) significantly increased from the mid-point of the TT to completion of the run (4.7 ± 0.7, 9.7 ± 0.9, $p < 0.001$); HR significantly increased from the start of the run to the start of the maximal effort, and was significantly higher at completion of the effort (84.4 ± 14.5 bpm, 166.0 ± 8.3 bpm, 178.8 ± 7.4 bpm, $p < 0.001$). No significant difference was found in overall time to complete the 12-mile run or maximal effort between the supplements (PLA = 88.6 ± 11.6 min, CHO = 89.1 ± 11.3 min, CHO-P = 89.1 ± 11.8 min, CHO-CHO = 89.6 ± 11.9 min; PLA = 8.3 ± 1.2 min, CHO = 8.2 ± 1.2 min, CHO-P = 8.2 ± 1.2 min, CHO-CHO = 8.4 ± 1.5 min). These findings suggest that type of supplementation (CHO, CHO-

CHO, CHO-P) consumed during an endurance exercise bout has no influence on enhancing endurance performance in male recreational runners during TT runs less than 100 minutes in length.

CHAPTER I

LITERATURE REVIEW

ABSTRACT

Traditionally CHO supplementation is utilized during endurance exercise bouts, while CHO-P supplementation is used post-exercise for muscle recovery. Previous research has demonstrated consistently that supplementation with CHO, as opposed to water alone, during an endurance exercise bout lasting greater then one-hour at moderate-vigorous intensity benefits performance. The physiological mechanisms associated with these resultant benefits have also been confirmed. Recently, research has begun to assess CHO-P supplementation during the endurance exercise bout in order to determine if use of these supplements enhance endurance performance over that of the traditional CHO supplementation. The physiological mechanisms associated with CHO-P supplementation during endurance exercise have yet to be confirmed. Additionally, mixed results have been found in regards to performance benefits associated with CHO-P supplementation during endurance exercise. These mixed results may be attributed to the varying study designs and protocols that have been used when comparing CHO-P and CHO supplementation during endurance exercise. These variances include: type of methodology (time to fatigue or time trial method), tested supplement composition (isocarbohydrate and isocaloric), inclusion of a placebo, control for order effects, and glycogen status of participants at the start of the exercise bout. Thus, more research is needed addressing the identified gaps in the literature and so the aim of the presented study was to determine whether potential performance benefits from ingesting a CHO-P supplement during endurance exercise, as assessed using the TT method, is attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to the tested CHO supplements.

BACKGROUND & SIGNIFICANCE

Ergogenic aids are external influences that can positively influence physical and mental performance. There are several types of ergogenic aids such as physiological, psychological, biomechanical, pharmacological and nutritional aids.[1] Nutritional ergogenic aids are food products and supplements that enhance physical performance.[1] Supplement use during training and/or competition, in addition to an optimal diet and strict training regimen, may provide the athlete with a competitive edge above his/her competition.

Substrate Utilization During Exercise

Food serves as the body's most essential fuel source. A diet that includes optimal levels of macro and micronutrients is important in preventing disease, sustaining overall health, and providing energy for activity. For the athlete, an optimal diet is an integral component of maximizing performance.[2] Athletes frame their diets based on their training and competing schedule to have optimal energy availability and utilization during events.[2]

Substrate utilization during endurance exercise has been investigated from the late 1960s onward.[3] According to McArdle and colleagues,[4] the body uses three different energy processes as it shifts from rest to steady-state, aerobic, exercise, which is defined as an activity lasting greater than 2-3 minutes.[2] These three energy processes occur in an overlapping manner.[6] Adenosine Tri-Phosphate (ATP) is the primary source of energy needed by the muscle cells for muscle contraction and resulting power output regardless of substrate utilization, aerobic/anaerobic conditions, or exercise intensity.[5] At the start of exercise (~0-3 seconds), the phosphagen system supplies an immediate fuel source to the working muscle cells via the substrates phosphocreatine (PCr) and ATP. In coordination with stored ATP in the muscle cell,

PCr, a high-energy bond, splits, providing energy to the cell.[6] The phosphagen system satisfies the body's initial energy demand as it shifts from a state of rest to activity, thus this is the first energy system used.

The second energy process is derived from the anaerobic breakdown of muscle glycogen resulting in a bi-product of pyruvic acid and eventually lactic acid, as some of this pyruvic acid converts to lactic acid.[6] These two processes comprise the anaerobic energy system, and can regenerate ATP at high rates.[6] The anaerobic system usually occurs within the first few minutes of exercise and is capable of generating energy, ATP, at the rates necessary to shift the body from a state of rest to exercise. It is important to recognize that the limitation of the anaerobic system is not attributed to a lack of oxygen; while oxygen is still present in small amounts within this system, the anaeorobic system is limited by energy demand placed on the body as it proceeds into continuous, steady-state, aerobic exercise. Additionally, the amount of energy that can be released at one time within the anaerobic system cannot satisfy the energy demands of the working muscles. The reduction in stored PCr, accumulation of lactic acid from anaerobic glycolysis, and resultant decrease of pH levels in the cell can cause a vast reduction in power output and cessation of exercise.[6] A vast reduction in power output is due to a decrease in performance capacity of the muscle, otherwise commonly referred to as muscle fatigue.[6] Cessation of exercise is a result of either a decreased rate of ATP utilization by the working muscles due to muscle fatigue, or a reduced rate of ATP re-synthesis.[6] While total depletion of ATP in the working muscle does not occur, it is possible to experience total depletion of PCr.[6]

The third energy process, or the aerobic system, occurs when exercise is performed for greater than 2 minutes, typically under constant conditions where VO_2 (oxygen uptake) remains stable and steady state exercise is achieved. At this time, the oxygen needs and energy demand of

the working muscle cells are met and the body is no longer in a state of oxygen deficit. After a few minutes of activity, oxygen becomes present in adequate amounts, and aerobic or oxidative metabolism begins.[6] Aerobic metabolism becomes the major source of ATP production for the working muscle cells during continuous exercise. Under aerobic conditions, glycolysis, ß-oxidation, the Krebs cycle, and the electron transport chain utilize carbohydrates (CHOs), lipids and proteins for energy. The intensity of the endurance exercise will determine which primary substrate, CHO or lipid, is utilized more abundantly to produce energy.[7] The concept of energy flux in relation to exercise intensity, also known as the crossover concept, serves as a major factor in indicating which substrate will be utilized during endurance exercise.[3] For example at low-intensity endurance exercise, ≤ 40% VO_2max, lipids serve as the preferred energy source, while CHO is utilized at a lesser extent; at higher intensities, > ~60% VO_2max, CHO via muscle glycogen then liver glycogen (as muscle glycogen stores begin to deplete) is preferred over lipids. It is understood that protein only contributes about 5-10% of the body's total energy demands, regardless of exercise intensity.[8]

One molecule of triaclyglycerol (lipid) yields about 460 molecules of ATP,[4] whereas one glucose molecule produces only 32 ATP.[9] The glycerol molecule formed during lipid oxidation travels to the liver to serve as a precursor for the synthesis of glucose.[4] This process occurs at a slower and less efficient rate in comparison to CHO oxidation, which is why glycerol is not administered exogenously as a supplement during high-intensity endurance exercise.[4] During endurance exercise bouts at lower intensity levels, energy demands are not as high in comparison to higher intensity activities; therefore, lipids are the primary substrate for low-intensity endurance exercise bouts. Since CHO oxidation occurs at a much quicker and more efficient rate in comparison to lipids, CHOs are considered the most important substrate for moderate- to high-

intensity endurance exercise.[7] Thus, ingestion of CHO during exercise will increase the amount of exogenous CHO available for oxidation and decrease the contribution of CHO from liver glycogen.[7] Since liver and muscle glycogen stores are limited, oral CHO supplementation during endurance exercise at moderate- to high-intensity should positively influence athletic performance.[7]

CHO-Based Sport Drinks

CHO-based sports drinks contain 6-8 grams of carbohydrate per 100 milliliter of fluid; this is commonly referred to as a CHO concentration of 6-8%. The carbohydrates can be in the form of sucrose, glucose and polymers/maltodextrins, all of which are equally effective in blood glucose maintenance and CHO oxidation in improving performance.[7] According to the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada, the benefits of carbohydrate consumption from sport drinks, such as Gatorade® or Powerade®, during endurance exercise lasting greater than one hour helps sustain performance, fluid and electrolyte balance.[2] Consistently research has shown that in comparison to water, CHO sport drinks containing a 6-8% CHO concentration along with the addition of ~20-30 meq/L sodium and chloride (chloride as the anion) and ~2-5 meq/L of potassium benefit athletic performance during prolonged exercise, $\geq$ 1 hour, at moderate- to high-intensities of about 70% VO_2max.[10] Athletes using CHO supplementation during endurance exercise have demonstrated the ability to work at higher intensities for a longer period of time in comparison to drinking water alone.[10]

There are several proposed mechanisms in which CHO sport drinks may enhance endurance performance. These mechanisms include: maintaining consistent blood glucose and levels of CHO oxidation, preventing hypoglycemia, sparing endogenous glycogen, and

synthesizing glycogen during low-intensity exercise.[11] During endurance exercise of moderate- to high-intensities, CHO is the main substrate utilized. As muscle glycogen stores begin to diminish, after about 15-20 minutes, supplementing with CHO maintains blood glucose concentrations and helps maintain and/or prevent both muscle and liver glycogen stores from being utilized (depending on glycogen status prior to starting the exercise bout). Therefore, CHO supplementation during moderate- to high-intensity endurance exercise is ideal to ensure maintaining performance levels throughout the exercise bout.

In addition to maintaining a constant, easily accessible source for energy, CHO beverages serve to rehydrate endurance athletes after long (≥ 1 hour), strenuous bouts of exercise. These drinks also contain sodium, chloride, and potassium, three minerals excreted in sweat. The logical benefits and minimal risk of including sodium in these sport drinks include stimulating thirst, promoting fluid consumption, and delaying dehydration.[12] This warrants recommendation for inclusion of sodium in sport drinks. In addition, inclusion of chloride is attributed to its role as an anion to sodium, and potassium has been noted to help replace electrolyte losses.[13]

Carbohydrate-Protein (CHO-P) Based Sport Drinks

Recently, several studies have investigated the addition of protein to CHO sport drinks and its influence on endurance performance.[14-27] Typically protein is ingested after the exercise bout to enhance and expedite muscle recovery.[22] It is generally accepted that protein only contributes a small amount of energy to endurance exercise in comparison to carbohydrates and lipids; ingestion of protein in addition to CHO during endurance exercise may increase protein oxidation and alter substrate utilization, sparing circulating blood glucose and minimizing glycogen depletion in both the working muscle and liver.[8] This consequently minimizes muscle

and liver glycogen depletion and will maintain circulating blood glucose concentrations resulting in a faster and more efficient source of energy leading to greater endurance performance.

Another benefit of the addition of protein to CHO based sport drinks is that in the intestines, amino acids contain multiple transport pathways that stimulate fluid and electrolyte absorption independent from mechanisms associated with glucose absorption.[8] This may result in faster fluid/fuel transport across the endothelial cells of the intestines.[8] Along with this benefit, if branched chain amino acids are used as the protein source as opposed to whey protein, these amino acids can be oxidized and used as an additional fuel source in the skeletal muscles during exercise.[24] Theoretically, the addition of protein to a CHO supplement would then yield greater endurance performance. Other proposed physiological mechanisms by which CHO-P drinks may enhance performance include: reduction of central fatigue by inducing the ratio of free tryptophan to branch chain amino acids, and production of a favorable influence on muscle energy metabolism such that the rate of glycogen breakdown decreases, TCA cycle intermediates found in muscle pools increases, and PCr use decreases.[8,28]

Typically, a liquid form of CHO-P supplementation is ingested after exercise in order to promote muscle recovery and glycogen re-synthesis.[8] Research has been conducted on the influences of CHO-P supplementation in relation to muscle recovery as early as 1992.[29] However, research on CHO-P supplementation during exercise, similar to studies done with CHO supplementation, did not start until much later, 2003.[15]

Research Investigating Possible Mechanisms for CHO-P Supplementation's Impact on Endurance Performance

In 1992, Zawadzki and colleagues investigated the influences of the CHO-P complex on muscle glycogen storage after exercise.[29] The idea of the CHO-P complex in reference to glycogen storage from this study coincides with the proposed mechanism made by Saunders more than ten years later that ingestion of a CHO-P supplement during endurance exercise may spare blood glucose and/or muscle glycogen stores.[8] In this randomized, partially counterbalanced study, nine competitive male cyclists participated in three endurance exercise bouts each separated by seven days. Investigators compared three types of supplements: a CHO supplement consisting of 112.0 grams of a dextrose-maltodextrin mixture, a protein (PRO) only supplement consisting of 40.7 grams of a milk and whey protein isolate mixture, and a CHO-P supplement consisting of both 112.0 grams of the CHO supplement and 40.7 grams of the PRO supplement.[29] Thus the tested supplements were matched in CHO content but not caloric content.

During each exercise bout participants were instructed to complete a two-hour ride to exhaustion (fatigue) on a cycle ergometer at a set work rate. The set work rate varied between 60-75% VO_2max depending on individual fitness level and was determined during preliminary testing. Supplementation was administered immediately after exercise and two hours post exercise. The purpose of the two-hour time frame for the exercise bout was to elicit blood glucose and muscle glycogen depletion. A 12-hour fast prior to the start of each trial was also done to facilitate glycogen storage depletion.[29] Dietary and physical activity control within the 24-hours prior to each exercise bout was not reported. Tissue collections and blood samples were taken both before and immediately after exercise in order to assess muscle glycogen levels. In comparison to both the CHO and PRO supplements, it was found and concluded that

administration of CHO-P supplementation following exercise enhanced post-exercise muscle glycogen stores due to a carbohydrate and protein interaction on insulin secretion.

Similarly, Koopman and colleagues assessed whole body protein and addition of protein to CHO supplements during ultra endurance exercise in order to determine if protein balance was improved.[16] In this randomized, cross-over design, eight competitive, endurance-trained athletes participated in two separate exercise trials lasting six hours.[16] Within each trial either a CHO or CHO-P supplement was administered. Both supplements were matched in CHO concentration such that the CHO supplement contained a mixture of 80 grams/liter (g/L) maltodextrin and 25 g/L glucose and the CHO-P supplement consisted of the same CHO content with the addition of 30 g/L of rice hydrolysate. Throughout each trial participants were instructed to cycle at 50% VO_2max while completing 2.5 hours of cycling on a cycle ergometer, followed by one hour of running on a treadmill, and then another 2.5 hours on the cycle ergometer. Supplementation was administered at 30-minute intervals in 4 ml/kg body weight servings during the trials. Within the week before all trials, CHO loading was prohibited. In addition, a 2-day diet record was collected prior to the first trial and participants were then instructed to consume the same exact diet 48 hours before their second trial. In opposition of this dietary control, participants entered all trials in a state of glycogen depletion such that they were required to begin each trial after an overnight fast; hours of fast was not reported. Participants were also instructed to refrain from heavy activity three days prior to each exercise bout. The method to check for compliance of dietary and physical activity controls was not reported.

Each participant's whole body protein was measured via protein tracers at rest, during 30-minute intervals throughout the exercise bout, and during recovery. Koopman and her team of investigators found that ingestion of the CHO-P supplement resulted in greater net protein

balance at rest, exercise, and recovery in comparison to the CHO supplement.[16] These results suggest that during ultra-endurance exercise CHO-P supplementation results in an increase in protein and CHO oxidation, a decrease in free fatty acid oxidation, and a consequential sparing of muscle glycogen stores to a large extent. This provides further evidence towards the use of CHO-P supplements to improve endurance performance.

Along with the aforementioned research, Miller and her team of investigators assessed the metabolic responses associated with ingestion of CHO-P drinks in comparison to CHO drinks during endurance exercise.[18] In this randomized, partially counterbalanced study, nine male competitive endurance athletes participated in three endurance exercise bouts each separated by about 7-10 days. Within each trial, one of three supplements was tested. Per 200 mL serving, the CHO supplement contained 45 grams of dextrose, the CHO-P supplement, which was skim milk, contained 17 grams of protein and 27 grams of CHO, and the artificially sweetened placebo contained no calories. Both the CHO and CHO-P supplement were matched for caloric content.

For each trial, participants were instructed to run for two hours on a treadmill at 65% VO_2max. Supplementation was administered at the start of the exercise bout and at 20, 40, 60 and 80 minutes during the bout. In addition, prior to the start of the trials a 3-day diet record was collected and analyzed in order to determine typical energy and macronutrient intake of all participants; however participants entered all trials in a state of glycogen depletion such that they were required to begin each trial after an overnight fast; hours of fast was not reported. Additionally, vigorous exercise was restricted before all trials. Blood samples were taken to analyze plasma glucose, lactate, free fatty acid (FFA), and amino acid (AA) concentrations before and after exercise. Overall, an increase in glucose and lactate concentrations between pre-

and post-exercise for each supplement was found. FFA levels were significantly higher in the placebo trial than either the CHO or CHO-P trial. AA concentration increased relative to pre-exercise levels for the CHO-P trials and decreased in the CHO and placebo trials. According to Miller and colleagues, these findings demonstrate the body's high priority of blood glucose maintenance during exercise.[18] Furthermore, CHO and CHO-P drinks inhibited the typical resultant increase in free fatty acids due to exercise and promoted use of CHO during exercise.[18]

Although the presented research does not directly measure endurance performance in relation to CHO-P supplementation during exercise, these findings provide rationale and indications for further investigation on the benefits of CHO-P supplementation during endurance exercise.

Research Investigating CHO-P Supplementation and Endurance Performance

Within the research comparing CHO-P versus CHO supplementation during endurance exercise and the resultant influences on performance, methodologies for assessing endurance performance has varied. Most commonly cycling, via a cycle ergometer, has been the endurance exercise used to assess athletic performance. Cycling, on a cycle ergometer, enables the participant to be under close surveillance and allows for a wide array of performance manipulations and measures. Thus the use of cycle ergometer as the endurance activity has provided a high degree of experimental control, helping to eliminate potential confounding variables (i.e.- varying temperature and humidity levels, inconsistent work rate), allowing research in this area to contain a high degree of internal validity. Additionally, either competitive or recreational cyclists have been used as the participants in these studies. Competitive cyclists may be defined as those who have had years of experience competing in the sport and have

competed at the national and/or international level; recreational cyclists may be defined as those who cycle most days of the week and have experience in competing at the local level. Within these studies, either the time to fatigue methodology or the time trial (TT) method was used to measure endurance performance (Appendix A, Table 1).

Time to Fatigue Methodology

To assess endurance performance using the time to fatigue method, research protocols comprise of instructing participants to either cycle at a consistent intensity level, ≥ 65% VO_2max, until complete fatigue, or cycle at varying intensity levels and at the final level continue until fatigue. Within the varying intensity protocol, exercise intensity either increases or alternates between several levels of intensity in intervals, usually ≥ 5 minutes in length; the number of intervals included is subject to trial protocol. At the final level of intensity, after the given time frame has expired, participants are instructed to cycle until fatigue. Within both protocols, fatigue is defined as the inability to continue a specific work rate for a given period of time, typically 15 seconds; however, the given period of time is not standard and has varied among studies (for example, 20 seconds versus 15). Time until fatigue is how endurance performance is measured; a longer time to fatigue indicates greater endurance performance.

Saunders and colleagues used this method to test CHO-P supplementation during endurance exercise, as compared to CHO supplementation, and assess any impact on performance.[21] In this double-blinded, randomized, counter-balanced experiment, 15 competitive male cyclists completed two trials including two rides to fatigue per trial. Supplements tested were matched in CHO content but not total energy (caloric content); the CHO-P supplement, provided by the PacificHealth Laboratory, consisted of a 7.3% CHO concentration and 1.8% whey- protein, and the CHO supplement, Gatorade®, contained 7.3% CHO.

Within each trial, the first ride to fatigue was performed at 75% of the individual's VO_2max, while the second ride was completed 12-15 hours after the first ride at an intensity of 85% VO_2max. The purpose of the second ride was to assess performance in a glycogen depleted state. Supplementation was administered in 1.8 mL/kg body weight servings in 15-minute intervals throughout each exercise bout until fatigue. The investigators did not report controlling for diet or exercise prior to the start of the trials. Metabolic measures, heart rate (HR), ratings of perceived exertion (RPE), and blood samples were measured in 30-minute intervals throughout each exercise bout until fatigue. Performance was measured via time to fatigue during the second ride. Overall, no significant differences were found between treatments or exercise intensities in reference to metabolic measures, HR, RPE, blood glucose or blood lactate levels.[21] Significant differences were found between treatments such that time to fatigue was greater in the second ride during the CHO-P trials; thus, it was concluded that CHO-P supplementation during endurance exercise results in greater performance over using a traditional CHO supplement.[21]

Along with this work, Ivy and colleagues also compared CHO-P and CHO supplementation during endurance exercise to assess influences on athletic performance.[15] In this double-blinded, randomized, partially counter-balanced study, nine competitive male cyclists volunteered and participated in three separate endurance exercise trials separated by approximately seven days each. Within each trial one of three supplements was administered, CHO-P, CHO, or placebo (PLA). The CHO-P and CHO supplements were matched for CHO concentration, 7.75%, but not total caloric content; the CHO-P supplement included an addition of 1.94% whey-protein. The PLA was sweetened with a non-caloric sweetener.

Each trial required participants to cycle on a cycle ergometer for 180 minutes in two different stages. The first stage consisted of a 30-minute warm-up at 45% of their individual

VO_2max. The second stage was 150 minutes in length and consisted of six sets of alternating exercise bouts of varying intensity where participants cycled for eight minutes at 75% VO_2max and eight minutes at 45% VO_2max, followed by nine sets of alternating three minute bouts at 75% and then 45% VO_2max. Once a total exercise time of 180 minutes was reached, participants cycled at 85% VO_2max until fatigue. Fatigue was defined as being unable to maintain the 85% work rate for 15 seconds consistently, or dropping below this intensity on the third attempt. Supplementation during the trials was administered in 200 mL servings before the exercise bout and every 20 minutes thereafter for the first 180-minutes of the ride. Once the cyclist began the ride to fatigue at 85% VO_2max, no supplementation was administered.

Participants were asked to eat the same meals for two days prior to each trial; yet no instruction was provided in terms of how to maintain dietary consistency. All athletes entered each trial in a glycogen-depleted state such that they were instructed to fast within the 12-hour time period prior to the start of all trials. According to McArdle and colleagues, even if a diet consisting of adequate amounts of macronutrients is consumed, an eight to twelve hour time period without consuming any food or drink containing calories results in significant depletion of glycogen stores.[4] In addition to dietary control, participants were required to perform a standardized workout (instruction provided) two days prior to each trial.[15] The method to check for compliance for both the dietary and physical activity controls was not reported. Along with dietary and physical activity measures, blood glucose levels, insulin levels and the respiratory quotient (RQ) were measured at the start of exercise, in between each stage, and immediately after exercise. Performance was measured via the time to fatigue during the final intensity level of 85% VO_2max.

Blood glucose was significantly higher in the CHO and CHO-P trials in comparison to the PLA and was not found to be significantly different between CHO and CHO-P trials, indicating that CHO oxidation between trials was the same. In addition, plasma insulin responses were similar and there were no differences in RQ, CHO oxidation or lipid oxidation between the CHO and CHO-P treatments. Overall, a significant increase in endurance performance was exhibited in the CHO-P trial over the CHO and PLA trials, and in both the CHO-P and CHO trials in comparison to the PLA. Hence, it was concluded that CHO-P supplementation during endurance exercise enhances athletic performance.

In addition, Saunders and colleagues conducted an investigation comparing CHO-P and CHO supplements matched in CHO content, and in opposition to the previous work, oral gels were tested instead of the typically used beverage supplementation.[22] Previous research only assessed male endurance athletes; thus a secondary purpose of this study was to determine any potential differences in outcomes by gender. In this randomized, completely counterbalanced, double-blinded study, 13 recreational cyclists, eight male and five female, participated in two rides to fatigue about 7-14 days apart. The tested supplements were matched in CHO content, 7.3%, but not total caloric content. The CHO-P supplement also contained 1.8% protein.

Throughout each trial, participants were instructed to cycle at an intensity of 75% VO_2max until fatigue; fatigue was defined as the inability to maintain a self-selected cycling cadence for 30 seconds at 75% VO_2max. Gel supplementation was administered every 15 minutes throughout the trial until the participant hit fatigue in 0.146 g/kg body weight servings along with water in 2 mL/kg body weight servings.[22] Within the 24 hours preceding each trial, participants were instructed to keep their diet as consistent as possible, with instructions for maintaining dietary consistency provided, and so participants were not in a state of glycogen

depletion prior to the start of any of the trials. In addition, participants were asked to keep activity consistent, refrain from strenuous exercise and record physical activity within the 48-hour time period prior to each trial; instruction on how to maintain consistency among trials for physical activity was provided as well; however, method to check for compliance for both the dietary and physical activity controls was not reported. Metabolic measures, blood samples, HR, and RPE were measured in 30-minute intervals throughout the exercise bout. Performance was measured through the time it took the participant to fatigue. No significant differences were exhibited between treatments in terms of metabolic measures, blood samples, HR or RPE. Along with these findings, no significance was found in relation to treatment x gender interactions.[22] A significant difference was found in time to fatigue favoring the CHO-P gel trials in comparison to the CHO gel and there was no difference in performance benefits between genders. These findings support CHO-P supplementation over CHO supplementation in terms of enhancing endurance performance.

In contrast, Romano and colleagues compared CHO-P and CHO supplements matched for total caloric content, as opposed to CHO content, over two consecutive days of exercise and examined any resultant influences on performance.[20] In this randomized, blinded study, 14 recreational male cyclists completed two rides to fatigue over consecutive days on two separate occasions.[20] A secondary purpose of this study was to examine performance after consecutive days of exercise while ingesting either a CHO-P or CHO supplement throughout the bouts. The CHO supplement, Gatorade®, contained a CHO concentration of 9.3%, and the CHO-P supplement, Accelerade® (PacificHealth Laboratories), consisted of 7.5% CHO and 1.8% whey-protein. The supplements were made isocaloric by cutting calories from the CHO-P supplement

resulting in 25% less CHO content (45 g CHO/hour) in comparison to the CHO supplement (60 g CHO/hour).

Participants completed the first ride to fatigue at an exercise intensity of 70% VO_2max. Within 24-hours after the first ride, the second ride was conducted at 80% VO_2max. Supplementation was administered every 15 minutes throughout both rides until fatigue in 2 mL/kg body weight servings. Prior to each trial, a 24-hour diet record was analyzed and researchers advised and provided instruction to participants to keep their diets consistent within the 24 hours before each trial. According to Romano and colleagues, a purpose of the first ride was to deplete glycogen stores enough to potentially reduce performance in the second ride.[20] Thereupon, participants entered the second trial in a state of glycogen depletion. The investigators did not report any controls in terms of physical activity prior to each trial. Time to fatigue during the second trial was the primary measure of endurance performance. Overall, Romano and colleagues found no significant difference in time to fatigue between the isocaloric CHO and CHO-P supplements tested.[20]

Additionally, Skillen and colleagues compared CHO-P and CHO supplements matched in caloric content.[24] In this randomized, double-blinded study, 12 competitive male cyclists participated in two trials, where each trial consisted of two consecutive days of exercise bouts. Similar to Romano and colleagues investigation, the purpose of testing each supplement over consecutive days of exercise was to determine which supplement enhances performance not only during one exercise bout but also during multiple exercise bouts over a period of time.[20] Within each trial, a different supplement was tested per participant. The CHO supplement contained a 4.6% CHO concentration, whereas the CHO-P supplement contained 3.6% CHO and 1% protein. The majority of previous research used whey protein as the protein source; however, Skillen and

colleagues used the branched chain amino acids isoleucine, valine, and arginine in equal concentrations.[24]

Trial protocol was as follows: while ingesting one of the tested supplements, participants completed an exercise bout on a cycle ergometer at 75% VO_2max for 90 minutes followed by a ride to fatigue at 85% VO_2max; next participants were asked to consume 1500mL of the same tested supplement consumed in the first exercise bout everyday during training (500 mL ad libitum before, during, and after exercise) for two weeks; after two weeks participants were asked to come back in the lab and complete the same exercise bout for two consecutive days. Between trials, participants engaged in a 2-week "wash-out" period and then began the protocol again for the second supplement. Fatigue during the exercise bouts was defined as being unable to maintain the 85% work rate at 60 revolutions per minute (rpm).[24]

For each trial, supplementation was administered as follows: 7 mL/kg body weight of the test drink before the exercise bout, 1.8 mL/kg body weight per 15 minutes during the exercise bout until fatigue, and 7 mL/kg body weight after exercise. To control for consistency of macronutrient intake, 24-hour diet records were submitted prior to each exercise bout, but instructions in terms of maintaining dietary consistency among trials was not provided. Participants were fed both dinner the night before and breakfast the morning of each scheduled exercise bout; thus each participant entered both trials with adequate and equivalent glycogen stores. Participants were also asked to either rest or only perform light activity within the 24-hour time period before each trial. Instruction was not provided in terms of keeping physical activity consistent among trials. In addition, a method to check for compliance for the physical activity control was not reported.

During exercise the respiratory exchange ratio (RER) was measured at 15-minute intervals. Performance was measured via the time to fatigue during the 85% intensity level. Investigators found no difference between supplements in reference to RER, and RER was significantly lower between CHO trials at exhaustion.[24] No significant difference in performance was found between supplements; however, endurance performance was more consistent between consecutive days of exercise during the CHO-P trials in comparison to the CHO trials.

In addition to the above research comparing isocaloric supplements,[20,24] Martinez-Lagunas and colleagues not only compared two isocaloric CHO-P and CHO supplements, but also assessed a CHO-P supplement containing less calories than both the tested isocaloric supplements and the typically used CHO-P supplement.[17] This second CHO-P supplement was created through changing the macronutrient ratio via decreasing the CHO content of the drink; thus, none of the tested supplements were matched in CHO content. The purpose of comparing these three supplements was to determine if performance would remain the same or improve with the addition of protein and reduction of CHO in these supplements.[17] This blinded, randomized, partially counterbalanced study tested the endurance performance in 12 competitive cyclists, consisting of seven males and five females, over four endurance exercise bouts. Each bout was separated by approximately seven days. Within each trial one of four supplements was tested: a traditional CHO supplement (6% CHO concentration), an isocaloric CHO-P supplement (4.5% CHO, 1.15% PRO) referred to as the CHO-P H, a lower calorie CHO-P supplement (3% CHO, 0.75% PRO) referred to as the CHO-P L, and a sucralose-sweetened PLA.[17]

Within each trial, participants completed a 24-minute warm-up at 55% of their individual VO_2max followed by twelve 8-minute intervals and then ten 3-minute intervals of alternating intensity levels, either 55% or 75% VO_2max. After the intervals of varying intensity were

complete, participants cycled at 80% VO_2max with a pedaling cadence of 70-100 rpm until fatigue.[17] Fatigue was defined as the inability to hold a pedaling cadence above 60 rpm after two attempts.[17] Supplementation was delivered every 20 minutes through out the duration of the trial until fatigue and serving size was dependent on body weight; the mean serving was 255.4 ± 9.1 mL. Before the start of the experimental trials, participants completed a 2-day diet record and 3-day training log in order to assess consistencies between diet and exercise among all trials. Participants were provided with instruction on how to keep diet and exercise consistent among trials; however, method to check for compliance for both the dietary and physical activity controls was not reported. Within the 12-hour time period prior to the start of each experimental trial, participants were instructed to fast; thus, participants entered each exercise bout in a state of glycogen depletion.

HR, RPE, and blood glucose was measured at various time points throughout the exercise bout. Performance was measured via the time to fatigue when participants cycled at 80% of their VO_2max. Overall, among all four trials, no treatment x time or treatment influence was experienced in reference to HR. In comparison to the PLA, RPE was significantly lower in all other trials. During the CHO-P H trial, RPE was significantly lower in comparison to the CHO trial. Blood glucose was significantly higher in the CHO, CHO-P H and CHO-P L in comparison to the PLA. In addition, treatment x time differences among the CHO, CHO-P H, and CHO-P L was experienced for blood glucose, such that during the first set of varying intensity intervals, blood glucose was significantly higher in the CHO trial; during the second set of intervals, blood glucose was significantly higher during both the CHO and CHO-P H trials; at fatigue, blood glucose was significantly higher during the CHO-P H trial.[17] Time to fatigue was significantly

longer in the CHO, CHO-P H, and CHO-P L groups in comparison to the PLA. No significant differences in time to fatigue were found between the CHO, CHO-P H, and CHO-P L trials.[17]

Finally, Valentine and researchers compared both isocarbohydrate and isocaloric CHO and CHO-P supplements during endurance exercise.[26] In this double-blind study, 11 competitive male cyclists completed four separate rides to fatigue. Supplements tested during each ride included a CHO supplement that matched the CHO-P supplement in CHO concentration (7.75%), a CHO-CHO supplement that consisted of extra CHO to match the CHO-P supplement in caloric content, and a non-calorically sweetened placebo (PLA). The CHO-P supplement consisted of 7.75% CHO concentration and 1.94% whey-protein; the CHO-CHO supplement consisted of 9.69% CHO concentration.

For each trial, participants were instructed to cycle on a cycle ergometer at an intensity of 75% of their individual VO_2max at a self-selected cadence > 50 rpm until fatigue.[26] Fatigue was defined as being unable to continue cycling at the self-selected cadence for 20 seconds. Supplementation was administered in 250 mL servings every 15 minutes until fatigue. Prior to the first session, participants completed a 24-hour diet log and were instructed to keep their diet consistent within the 24 hours preceding each trial; instruction was given on how to do so based on each individual's diet 24 hours prior to the first trial. These 24-hour diet records were visually inspected for compliance of dietary control.[26] Dietary consistency provided participants with macronutrient consistency and equivalent glycogen status among all four trials; thus, participants were not in a state of glycogen depletion prior to the start of any of the trials. In addition, participants kept a 48-hour physical activity record prior to each trial and were asked to keep activity consistent 48 hours before each trial; however, no instruction was given to participants in

terms of how to maintain consistency of activity. In addition, no method to check for compliance of the physical activity control was reported.

Metabolic measures, blood samples, RPE, and HR were measured at 0, 30, and 60 minutes, and then at exhaustion during each exercise bout. Among all measures taken, the only significant difference found among trials was blood glucose levels among the CHO, CHO-P, and CHO-CHO trial versus the PLA.[26] In addition, time to fatigue was significantly longer in the CHO-P and CHO-CHO groups in comparison to the CHO and PLA; however, time to fatigue in the CHO-P trials was not statistically different in comparison to the CHO-CHO trials, indicating that previous findings supporting CHO-P supplementation and enhanced endurance performance may be due to the additional calories in the CHO-P supplement as opposed to the presence of protein.

As a whole, these investigations have documented contradicting results in regards to performance enhancement and CHO-P supplementation during endurance exercise. The varying outcomes of these studies are most likely a consequence of differences in study design and protocol. These differences include variances within supplement compositions (isocarbohydrate and/or isocaloric), inclusion of a placebo, controlling for order effects, and glycogen status of participants at the start of the endurance exercise bout. While the total caloric content of the tested supplements appears to be the contributing factor to endurance performance benefits in three of the aforementioned studies,[21,22,26] further research is still needed including direct comparisons of supplementation matched not only for total caloric content but also for CHO concentration in order to confirm which component of the supplement, energy or macronutrient composition, may attribute to endurance performance benefits.

Time Trial Methodology

Recommendations have been made for future research to use the TT method to measure and assess endurance performance. It has been proposed that this methodology may elicit lower variance between repeated trials, as compared with the time to fatigue method, and lead to statistically significant results.[26] A primary purpose of the TT methodology is to mimic a typical workout or competitive exercise bout for the athlete. Within these trials, participants typically pedal an ergometer for a set period of time or distance. Performance is measured by either the time or distance it takes the participant to complete the exercise bout. These trials elicit a competitive environment in the sense that the participant is racing against the clock, whereas in the time to fatigue method time or distance to complete the exercise bout holds no prevalence on measuring performance.

Osterberg and her team of investigators compared commercially used and available CHO-P and CHO supplements on endurance performance.[19] In this randomized, double-blinded study, 13 competitive male cyclists participated in three TT rides testing a CHO-P supplement, CHO supplement and PLA. The PLA consisted of water sweetened by a non-caloric sweetener, and the CHO and CHO-P supplements were not matched for either caloric or CHO content. Gatorade® (6% CHO concentration) and Accelerade® (7.5% CHO and 1.6% protein) were used as the CHO and CHO-P supplements, respectively. According to Osterberg and colleagues, these products were used due to their commercial availability and practical implications.[19]

Within each trial, cyclists participated in a constant load exercise bout, conducted above each individual's lactate threshold (lactate concentration of 1 mmol/L above baseline), on a cycle ergometer for 120 minutes.[19] After 120 minutes of exercise, participants were instructed to complete 7 kilojoules(kJ)/kg body weight of work as quickly as possible. Supplementation was

administered during the constant load exercise bout in 250 mL servings every 15 minutes. Although participants were asked to consume the same diet within the 24 hour time period prior to each TT, participants entered each TT after a 12-hour fast, thus resulting in a state of glycogen depletion. In addition, no instruction was given to participants in terms of how to keep diet consistent within this 24-hour time period, nor was a method to check for compliance of dietary control reported. Control for physical activity was not reported.

HR and RPE were measured in 15 minutes intervals through out each TT. In addition, performance was measured as the amount of time it took each cyclist to complete the set amount of work. No significant differences were found between HR or RPE between trials. It was found that performance in the CHO trial was significantly faster than in the PLA, yet there was no significant difference in performance found in the CHO-P trial in comparison to the CHO or PLA trials. Investigators concluded that no additional performance benefits are gained when consuming a commercially available CHO-P supplement in comparison to a commercially available CHO supplement, both neither isocarbohydrate or isocaloric, during endurance exercise.[19]

Along with this, Saunders and his team of investigators compared CHO-P and CHO supplements matched in CHO content as well. In this randomized, double-blinded, counterbalanced study, 13 recreational male cyclists completed two computer simulated 60 km cycling time trials on separate occasions.[23] One supplement was administered per time trial. Both supplements contained a 6% CHO concentration. The CHO-P supplement included a 1.8% protein concentration of casein hydrolysate.[23]

The 60 km TT consisted of three 20 km laps, where each lap ended with a 5 km climb with an average incline of about 5%.[23] Supplements were administered in 200 mL servings every

5 km throughout the TTs. Within the 48-hour time period before each TT participants were instructed to consume a consistent diet and refrain from heavy exercise, yet no instruction was given in terms of how to maintain dietary and activity consistency between trials.[23] In addition, the method to check for compliance for both the dietary and physical activity controls was not reported. As a result of the dietary control used, participants were not in a state of glycogen depletion prior to the start of any of the TTs.

HR, RPE, RER, blood glucose, and lactate levels were taken at various time points throughout the TTs.[23] Performance was measured via the time it took each participant to complete the 60 km course. No significant treatment differences or treatment x time interactions were found among any of these measures. Significant differences were found in favor of the CHO-P supplement for both the time to complete the final 20 km and 5 km of the trial; however, no significant difference between tested supplements was found in reference to time to complete the overall distance, 60 km TT.

In addition, Breen and his team of investigators also assessed isocarbohdyrate CHO-P and CHO supplements during endurance exercise.[14] In this crossover, double-blind study, 12 competitive male cyclists participated in two endurance TTs separated by 7-14 days. Supplements tested included a CHO supplement (6% CHO) and a CHO-P supplement (6% CHO, 1.8% protein hydrolysate) matched in CHO content only.

All exercise bouts consisted of 120 minutes of steady-state exercise at 50% of the individual's maximal power output (Wmax) followed by a TT ride lasting about an hour with a targeted amount of work set at 70% of the individual's Wmax.[14] For all participants the mean amount of work to be completed during the TT was 880 ± 27 kJ.[14] This targeted amount of work was selected based on a formula used in previous research in order to produce an exercise bout

lasting about an hour.[14] Supplementation was administered in servings of 270 mL every 15 minutes throughout the 120-minute steady state exercise bout. Within the 48-hour time period prior to each trial an individualized diet was provided to each participant. All trials were conducted in a fasted state, and so participants were glycogen depleted at the start of all exercise bouts.[14] Length of fast in reference to depleting glycogen stores was not reported. Participants were also instructed to refrain from any training/physical activity 48-hours before each scheduled trial. Method to check for compliance for both the dietary and physical activity controls was not reported

Throughout each steady state exercise bout, RER, HR and RPE were collected every 30 minutes. During the TT, HR, RPE, and power output was measured at 25%, 50%, and 75% of the total energy target and at about 10 seconds prior to completion of the TT.[14] Performance was measured based on the time it took to complete the TT ride. During all measured time points of the steady state exercise bout no significant differences were exhibited between supplements for all physiological measures. During the TTs, HR and RPE increased significantly at all time points, yet this was seen during both treatments and no significant difference was experienced between treatments.[14] Average power output was not significantly different between supplement types; consequently, no significant differences were experienced in time to complete the TT between both supplements. Overall, these results demonstrate how even in a glycogen depleted state CHO-P supplementation may not result in greater endurance performance over an isocarbohydrate CHO supplement.

Similarly, Van Essen and Gibala compared isocarbohydrate CHO-P and CHO supplements, and also included a PLA.[27] In this double-blind, cross-over, partially counterbalanced study, 10 competitive male cyclists participated in three separate 80 km TTs,

each within one week from the other. The tested CHO and CHO-P supplements were matched for CHO content (6% CHO concentration), but not total caloric content; the CHO-P supplement contained 2% whey-protein isolate. The PLA consisted of a non-caloric sweetener.

All TT rides consisted of four, 20 km laps via a computer software program where the course was projected on a screen while the participant cycled on a cycle ergometer.[27] Within each TT ride, one of the aforementioned supplements was administered in 250 mL increments every 15 minutes until the end of the trial. Participants were advised to keep diet as consistent and habitual as possible throughout the duration of the study and were provided food to consume within the 24-hour period prior to each trial based on their individual food preferences.[27] As a result of the dietary control within the 24-hours prior to the start of each trial, participants did not enter any of the TTs in a glycogen-depleted state. In addition, cyclists were instructed to continue their regular training regimen within the 24 to 48-hour time period prior to each trial and refrain from exercise 24 hours before all trials. The method used to check for compliance of following a habitual diet throughout the duration of the study and following physical activity control was via self-report. Blood samples were measured every 20 km during each TT. Time to finish the specified distance, 80 km, was used to measure performance.

Overall, significant differences in blood glucose and plasma free fatty acid concentration were found between the CHO and CHO-P supplements versus the PLA during the TTs. A significant difference was also found in TT performance favoring both the CHO and CHO-P trials versus the PLA; however, no significant difference was displayed in time to complete the 80 km TT between the CHO and CHO-P trials. Therefore, it was concluded that in a competitive athletic event, there are no additional performance benefits in using a CHO-P supplement in comparison to a CHO supplement.

In contrast, Toone and Betts aimed to compare two isocaloric supplements, yet these supplements were tested during endurance exercise lasting less than 90 minutes, whereas all aforementioned research used an exercise bout greater than 90 minutes.[25] Within this randomized, completely counterbalanced, double-blinded study, 12 competitive male tri-athletes and/or cyclists completed two cycling trials on a cycle ergometer approximately 5 to10 days apart. The tested CHO-P supplement contained a 6.8% CHO concentration with a 2.2% addition of whey-isolate (3:1 CHO-PRO ratio). The CHO supplement consisted of 9% CHO concentration. Additional CHO was added to this drink in order to match the CHO-P drink in calories, thus supplements were not matched in CHO content.

The exercise trials consisted of a 10-minute warm-up followed by three, 15-minute intervals of varying intensity consisting of intensity levels between 60-90% of each individual's VO_2max. The varying intensity exercise bout was followed by a short rest period, less than one minute, prior to the start of the 6 km TT. The purpose of the rest period was simply to reset the flywheel on the cycle ergometer before starting the time trial.[25] Fifteen minutes prior to the start of the warm-up, participants were given a 7 ml/kg body weight serving of the tested supplement. Supplementation was then administered in servings of 2.5 ml/kg body weight at the end of the warm-up, in between each 15-minute interval, and at the start and finish of the 6-km TT.[25] Supplementation was not administered during the TT. Consumption of water was permitted ad libitum during the first trial; the quantity consumed was then matched during the individual's second trial.[25] Participants were asked to record dietary intake within the 24-hour time period before the first trial. Participants were then expected to consume a similar diet within the 24-hours prior to the start of the second trial. Both trials were held after a 12-hour overnight fast, resulting in a glycogen depleted state at the start of each exercise trial. In addition to dietary

controls, participants were informed to continue their typical training regimen throughout the duration of the study and refrain from strenuous exercise 24-hours prior to each trial. Participants were not provided with any further instruction on how to maintain consistent diet and exercise. Method to check for compliance for both the dietary and physical activity controls was not reported.

At the end of the warm-up, in between each 15-minute interval, and at the start and finish of the 6 km TT, HR, RPE, and blood samples were measured. Performance was measured via the time it took each participant to complete the 6-km TT. All in all, no treatment x time interaction was experienced in relation to blood glucose levels and RPE.[24] Blood glucose concentration was lower and RPE was significantly higher during the CHO-P trial versus the CHO trial as experienced by all participants.[25] For all participants, no difference was experienced in average HR at all time points measured during both trials. Finally, significance was found in the time to complete the 6-km TT between the CHO and CHO-P supplements such that the CHO supplement resulted in a faster time to complete the 6-km distance, indicating greater endurance performance.

As with the time to fatigue trials, the findings from the above research[14,19,23,25,27] vary in terms of performance benefits from CHO-P supplementation during endurance exercise. This may be a consequence of differences in study design and protocol such as variances within supplement compositions (isocarbohydrate and/or isocaloric), inclusion of a placebo, control for order effects, and glycogen status of participants at the start of the TTs.

CONCLUSION

Summary

As a whole, mixed results have been found in regards to performance benefits associated with CHO-P supplementation during endurance exercise. Among the studies that have exhibited significant findings in performance benefits from CHO-P supplementation the tested supplements were matched in CHO content but not caloric content.[15,21,22] Along with this, only some, but not all, of the previously mentioned research included a placebo as one of the tested supplements.[15,17,19,26,27] Some studies have also failed to try and control for order effects.[14,19,20,24,26] In addition, glycogen status of participants at the start of the endurance exercise bouts is inconsistent among all study protocols. In terms of methodology, the time to fatigue method does not mimic competitive activities as well as the TT method.[26] Additionally, the TT method has shown to be more reliable in comparison to the time to fatigue method such that the calculated coefficient of variance for the time to fatigue method among several studies has shown to range from 5.2-55.9% whereas as the TT method has demonstrated a variation of 1-5%.[19] Consequently, to address the gaps in the literature in the area of investigating the influence of CHO-P vs. CHO supplements on endurance performance, more research needs to be conducted with the TT method, using supplements that are matched in both CHO and caloric content, including a placebo and controlling for order effects in the study design, and considering glycogen status upon the start of the exercise bouts. Therefore, the aim of this study was to address these identified gaps to help determine whether potential performance benefits from ingesting a CHO-P supplement during endurance exercise, as assessed using the TT method, is attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to the tested CHO supplements.

Twelve male recreational runners, aged 18-55 years old, ran four 12-mile TT runs on four separate occasions; this is the first study to date to use runners as participants. Using a latin-square design, four supplements were tested, with all participants receiving all four supplements. Supplements consisted of a CHO and CHO-P supplement, matched for CHO content, a CHO-CHO supplement matched to the CHO-P supplement for caloric content, and a placebo. Each TT run was conducted at an exercise intensity of 75% race pace. Participants entered each exercise bout with adequate and equivalent glycogen status per an individualized diet prescription based on their habitual diet. Performance was measured via the time it took to complete the 12-mile run, as well as time it took to complete the last 1.2 miles of the run, in which participants were instructed to run at maximal effort, 100% race pace, for this portion of the TT.

Specific Aims

This study assessed the influences of four different supplements (CHO, CHO-P, CHO-CHO, PLA) on endurance performance in order to determine if any potential performance benefits from supplementation are attributed to caloric content or macronutrient composition of the supplements. It was hypothesized that performance benefits from ingestion of the CHO-P supplement during endurance exercise would be attributed to the extra total calories contained in the supplement and not due to the presence of protein in the supplement (macronutrient composition); thus the timed runs would be significantly faster for CHO-P and CHO-CHO supplements that are matched for energy content in comparison to the CHO supplement. The following aims were assessed.

1.) Timed runs will be significantly lower (faster) for the CHO, CHO-P, and CHO-CHO supplements as compared to the PLA.

2.) Timed runs will be significantly lower (faster) for CHO-P and CHO-CHO supplements matched in caloric content as compared to the CHO supplement.

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CHAPTER 2

MANUSCRIPT

ABSTRACT

Heterogeneous results in terms of performance benefits have been found when comparing carbohydrate (CHO) and carbohydrate-protein (CHO-P) supplementation during endurance exercise. Thus this study assessed performance from three different supplements (CHO-P, CHO, double carbohydrate [CHO-CHO]) as compared to a placebo (PLA) during a time trial (TT) run. Twelve male recreational runners (age = 32.4 ± 9.5 yrs; BMI = 22.7 ± 1.5 kg/m^2; VO_2max = 59.68 ± 7.53 mL/kg/min; 100% white) individually completed four, 12-mile TT runs, 7-10 days apart, at about75% of their race pace. Dietary and physical activity consistency within the 24-hour time period prior to each run was controlled via individualized diet and activity prescriptions based on the diet consumed and the physical activity engaged in prior to TT 1. Throughout each TT run, participants consumed a 600 mL load of one of the four aforementioned supplements. Supplement order was counterbalanced with a latin-square design. Endurance performance was measured by time to complete the 12-mile run, and time to complete the last 1.2 miles of the run, where participants were instructed to run at maximal effort, 100% race pace. A main effect of time occurred during the TTs for perceived exertion (RPE) and heart rate (HR). RPE (Borg 10-point scale) significantly increased from the mid-point of the TT to completion of the run (4.7 ± 0.7, 9.7 ± 0.9, $p < 0.001$); HR significantly increased from the start to the start of the maximal effort, and was significantly higher at completion of the effort (84.4 ± 14.5 bpm, 166.0 ± 8.3 bpm, 178.8 ± 7.4 bpm, $p < 0.001$). No significant difference was found in overall time to complete the 12-mile run or maximal effort between the supplements (PLA = 88.6 ± 11.6 min, CHO = 89.1 ± 11.3 min, CHO-P = 89.1 ± 11.8 min, CHO-CHO = 89.6 ± 11.9 min; PLA = 8.3 ± 1.2 min, CHO = 8.2 ± 1.2 min, CHO-P = 8.2 ± 1.2 min, CHO-CHO = 8.4 ± 1.5 min). These findings suggest that type of supplementation (CHO, CHO-

CHO, CHO-P) consumed during an endurance exercise bout has no influence on enhancing endurance performance in male recreational runners during TT runs less than 100 minutes.

INTRODUCTION

During endurance exercise oxygen is present in adequate amounts and so the body utilizes the aerobic metabolic processes in order to generate enough ATP for fuel in the working muscles. The intensity of the endurance exercise determines which primary substrate, carbohydrate (CHO) or lipid, is applied more abundantly to produce energy.[1] The concept of energy flux in relation to exercise intensity, also known as the crossover concept, serves as a major factor in indicating which substrate will be used during endurance exercise.[2] For example at low-intensity endurance exercise, ≤ 40% VO_2max, lipids serve as the preferred energy source while CHO is utilized at a lesser extent; at higher intensities, > ~60% VO_2max, CHO via muscle glycogen then liver glycogen (as muscle glycogen stores begin to deplete) is preferred over lipids. CHO oxidation occurs at a much quicker and more efficient rate in comparison to lipids, hence CHOs are considered the most important substrate for moderate- to high-intensity endurance exercise.[1] Thus, ingestion of CHO during moderate- to high-intensity endurance exercise will increase the amount of exogenous CHO oxidation and decrease the contribution of CHO being used from muscle and liver glycogen.[1] Since muscle and liver glycogen stores are limited, addition of oral CHO supplementation during endurance exercise conducted at the aforementioned intensity level should positively influence athletic performance.[1]

According to the American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada, the benefits of carbohydrate consumption from sport drinks, such as Gatorade® or Powerade®, during prolonged endurance exercise of moderate- to high-intensity

lasting greater than one hour helps sustain performance, as well as fluid and electrolyte balance.[3] Consistently research has shown that in comparison to water, CHO sport drinks containing a 6-8% CHO concentration along with the addition of ~20-30 meq/L sodium and chloride (chloride as the anion) and ~2-5 meq/L of potassium benefit athletic performance during prolonged exercise at moderate- to high-intensity averaging about 70% VO_2max.[4]

Recently research has investigated the addition of protein to CHO sport drinks, carbohydrate-protein (CHO-P) supplements, and any resultant influences on endurance performance.[5-18] Typically, protein is ingested after the exercise bout to enhance and expedite muscle recovery; during exercise it is understood that protein only contributes about 5-10% to the body's total energy demands, regardless of exercise intensity.[19] However, it has been proposed that CHO-P supplementation during endurance exercise may alter substrate utilization such that lipid oxidation decreases and both protein and CHO oxidation increases. This consequently minimizes muscle and liver glycogen depletion and increases circulating blood glucose concentrations resulting in a faster and more efficient source of energy leading to greater endurance performance.

Within research testing CHO-P versus CHO supplementation during endurance exercise, conflicting results have been found in terms of the use of these supplements and performance benefits which may be a consequence of differences in CHO and caloric content of the supplements. Some of the investigations have only controlled for CHO content of the supplements (isocarbohydrate supplements),[5,6,12-14,18] while others have only controlled for the caloric content of the supplements (isocaloric supplements).[8,11,15,16] Only one investigation controlled for both of these factors and compared both isocaloric and isocarbohydrate supplements.[17] Thus, it is yet to be determined whether reported performance benefits are

attributed to the extra total calories in the CHO-P supplement, as a consequence of the addition of protein, or the presence of protein independent from total calories. In addition, amidst all research protocols, glycogen status of participants at the start of the exercise trials has varied, which may have held an impact on the research outcomes. Some protocols required participants to enter the exercise bout in a glycogen-depleted state,[5,6,8,12,16] while others had participants enter the bout in a non-depleted state.[11,13-15,17,18] Along with this, participants in all but three investigations were competitive endurance athletes, indicating the need to assess any potential influences on performance from supplementation on recreational athletes.[11,13,14]

Additionally, among all studies investigating CHO-P supplementation during endurance exercise, two methods, time to fatigue or time trial (TT), have been used to measure endurance performance. When comparing these methods, the time to fatigue method does not mimic competitive activities as well as the TT method.[17] Additionally, the TT method has shown to be more reliable in comparison to the time to fatigue method such that the calculated coefficient of variance for the time to fatigue method among several studies has shown to range from 5.2-55.9% whereas as the TT method has demonstrated a variation of 1-5%.[10] Thus, it has been suggested that the TT method may be the best method for assessing endurance performance since it is the most reliable and reproducible of the two methods.[10] Therefore, the purpose of this study was to determine whether the performance benefits from ingesting a CHO-P supplement during endurance exercise, as assessed using the TT method, is attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to the tested CHO supplements in recreational endurance athletes who were not glycogen depleted. Twelve males, aged 18-55 years old who were recreational runners, ran four 12-mile TTs at 75% of race pace. Using a latin-square design, four supplements were tested, with all participants receiving all four

supplements. Supplements consisted of a placebo, a CHO supplement, a CHO-P supplement, and a CHO-CHO supplement. The CHO and CHO-P supplements were matched for CHO content, while the CHO-CHO and CHO-P supplement were matched for total caloric content. Participants entered each exercise bout with adequate and equivalent glycogen status per an individualized diet prescription based on their habitual diet. Performance was measured via the time it took to complete the 12-mile run, as well as the time it took to complete the last 1.2 miles of the run, in which participants were instructed to run at maximal effort. It was hypothesized that performance benefits from ingestion of a CHO-P supplement as compared to a CHO supplement during endurance exercise would be attributed to the extra total calories contained in the CHO-P supplement thus the timed runs would be significantly faster for the CHO-P and CHO-CHO supplements in comparison to the CHO supplement. Additionally, it was hypothesized that timed runs would be significantly faster for all supplements containing calories (CHO, CHO-P, CHO-CHO) in comparison to the non-caloric PLA.

EXPERIMENTAL DESIGN & METHODOLOGY

Study Design:

In order to determine whether or not CHO-P supplementation enhances endurance performance, in comparison to the traditional CHO supplement, this study tested a PLA, CHO, CHO-P, and CHO-CHO supplement during four separate endurance exercise bouts. A 4 x 4 mixed factorial design was used, with order (a latin-square design was used to control for order influences) as the between-subjects factor, and supplement type (PLA, CHO, CHO-CHO, and CHO-P) as the within-subjects factor. Participants were randomly assigned to an order and blinded to the type of supplement they received at each trial. Total time to complete each TT, as

well as time to complete the last 1.2 miles of the course, were the primary dependent variables. This study was registered through ClinicalTrials, NCT00972387.

Participants:

Twelve recreational male runners volunteered to participate in this study. Runners were recruited from both the Knoxville Track Club (KTC) and The University of Tennessee-Knoxville. Flyers were posted both around campus and on the message board of the KTC website, ktc.org. In addition, the flyer was sent to all members of the KTC via the organization's e-mail list. Those interested were directed to call the Healthy Eating and Activity Laboratory (HEAL) and underwent a brief phone screen in order to determine eligibility for participation.

Sample Size

To examine the influence of supplement type on endurance performance, twelve participants were needed. Sample size calculations presumed 2-sided hypothesis testing, with type one error rate (alpha) equal to 0.05. To reject with 80% power the null hypothesis of no supplement difference versus the alternative that the supplement difference is 3.90 or greater (effect size from Valentine and colleagues' work) for greater endurance performance for CHO-P versus PLA, 8 males were needed.[17] The standard deviation of endurance performance was expected to be approximately 4.34 minutes, thus we would have been able to detect differences between these two supplements of about 16.9 minutes.[17] To reject with 80% power the null hypothesis of no supplement difference versus the alternative that the supplement difference is 1.84 or greater (effect size from Valentine and colleagues' work) for greater endurance performance for CHO-P versus CHO, 12 males were needed.[17] The standard deviation of endurance performance was expected to be approximately 4.34 minutes, thus we would have

been able to detect differences between these two supplements of about 7.98 minutes.[17] So that there was adequate power to test both research aims, a sample size of 12 was used.

Eligibility Criteria

Participants had to meet the following criteria-

1.) Males aged 18-55 years old
2.) BMI in the healthy range, 18.5-24.9
3.) Run 45-90+ minutes at least 4 days per week
    a. Engage in running this frequency and duration for at least 4 weeks prior to the phone screen
    b. Engage in a run consisting ≥ 10 miles in length for at least 2-4 occasions per month
4.) No previous history of heart conditions
5.) No shortness of breath or chest pain experienced during running or daily activities
6.) No bone or joint problems experienced during running or daily activities

Criteria upon which participants were excluded-

1.) Females and males under the age of 18 and over the age of 55
2.) BMI below 18.5 or greater than 24.9
3.) Allergies to products containing milk, soy, or aspartame
    a. Severe allergies to eggs, wheat, tree nuts, fish, crustaceans, shellfish products (Accelerade, the CHO-P supplement, is made in a facility that processed these products)
4.) Refusal to consume any of the supplements, and/or extreme dislike of the supplements

5.) Refusal to complete the specified distance of the time trials

Between August 2009 to April 2010, 43 individuals expressed interest participating in this study. Two individuals expressed interest in participating after completion of the study. 41 individuals were phone screened for both interest and eligibility for participation. Out of the 41 individuals screened, 24 did not meet eligibility for the following reasons: 7 individuals had a conflict with the time commitment required by the study, 6 individuals had a BMI above the normal range, 5 individuals were no longer interested in participating after learning more about the study during the phone screen, 3 individuals were wait-listed and then unable to be contacted, 2 individuals had transportation conflicts to the TT course, and 1 individual had a food allergy to one of the ingredients in the CHO-P supplement. Informed consent was collected from 17 participants deemed eligible. Of the 17, five participants withdrew from the study for the following reasons: 3 individuals had a conflict with the time commitment required by the study, 2 individuals experienced athletic injuries unrelated to their participation in the study. Overall 12 individuals remained and completed all portions of the study.

Consent:

Following the phone screen, participants who were deemed eligible were given more information about the study and asked if they were interested in participating. At the start of the initial session, upon meeting the primary investigator (PI), the informed consent form, approved by the University of Tennessee- Knoxville Institutional Review Board (IRB), was verbally read to all participants. Participants were given two copies of this form, one for the PI's records and one for the participant's records. While reading through the document, participants were instructed to initial the top of each page, indicating they understood the contents contained on

each page, and to sign and date the final page of the document. Before collecting the signed copy of the informed consent, participants were given the opportunity to ask any further questions in regards to the study.

Procedure:

Upon completing the phone screen, eligible participants interested in participating in the study were scheduled individually for the initial session. At this time, participants were instructed to follow the standard pre-test protocol for both the body composition test and the maximal test prior to meeting for the session. Approximately 48 hours prior to the initial session, the PI emailed participants a reminder about their appointment and provided instructions regarding standardized pre-test protocols.

Initial Session

This session was conducted at the Health, Physical Education and Recreation Building on the University of Tennessee- Knoxville campus. Following obtainment of the informed consent, participants removed shoes and socks and stood in good posture against a portable stadiometer in order to measure height. Once height was recorded and the participant changed into appropriate attire for the body composition measurement procedure (the BOD POD), the participant stood on the electronic scale associated with the BOD POD machine in order to measure body weight. After body weight was measured body composition was assessed with the BOD POD test. After changing into appropriate attire for the maximal test, the participant was escorted to the appropriate room for this test. At this time, the participant was briefed on the protocol of the treadmill VO_2max test, and completed the test. Prior to the conclusion of the initial session, once the maximal test was complete, the date and time for the first TT was scheduled. The participant

was also given a blank 24-hour food record and structured activity log. The participant was instructed to complete both forms for the 24-hour time period prior to the first scheduled TT and returned completed forms upon meeting for the first TT. The participant was then reminded of the requirement to keep diet and exercise 24-hours prior to each TT consistent, as discussed during the phone screen. Consistency of diet for trials 2 to 4 were based on the Diabetic Exchange System that was used to analyze the first 24-hour diet record from trial 1. Consistency of structured activity was based on the duration of exercise conducted in the 24-hour time period prior to trial 1; this time frame set the precedent for amount of time allotted for structured activity 24 hours prior to trials 2 to 4.

Time Trials

Participants completed four TTs, consisting of 12-miles in length per trial. Participants ran each TT individually, not in groups. TTs were separated by approximately seven days. The PI attempted to keep day of the week and time of the TT consistent for each participant throughout the study. Days and times varied pending extreme exceptions and circumstances, such as inclement weather conditions or illness. In this case, length between trials varied no longer than up to ten days, and time of day varied no more than +/- three hours from the original time the first trial was run.

The 12-mile TT course was held on the Springbrook Corporate Center Trail in Alcoa, Tennessee. This closed trail was 0.63 miles in length and consisted of paved and board walk grounds encircling Springbrook Lake. Participants completed 19 laps in order to achieve the 12-mile distance for each TT.

Upon meeting for the first TT, the participant submitted his 24-hour diet record and structured activity log forms to the PI. The participant was instructed to keep his diet and

structured activity 24-hours prior to each TT thereafter consistent with what was done prior to the first TT. After the first TT session, the investigator analyzed both the individual's diet, based on the quantified serving sizes in the Diabetic Exchange List, and the amount of exercise reported by the participant. The diet and activity prescription, per the participant's diet and exercise 24-hours before the first TT, was sent to the participant immediately after the run via email. Upon meeting for TTs 2, 3, 4, before the start of the run, the participant submitted his 24-hour diet record and structured activity log to the PI to check for compliance. Participants were instructed that if diet and exercise did not match the individual's diet and activity prescription, the TT would need to be rescheduled for another day; however, all participants were compliant with their individual diet and exercise prescriptions.

While the participant was warming up for the TT, weather conditions, such as temperature, relative humidity, and average wind speed was measured and recorded. Prior to the start of each TT run, the participant was instructed to run at 75% of his race pace, providing a maximal, all-out, effort for the last two laps, 1.2 miles, of the trial. Exercise intensity was measured and assessed using two tools. The participant was asked to rate his effort on the Borg 10-point scale of perceived exertion (0-10) both at the approximate mid-point of the course, 5.6-mile marker (end of lap 9), and at the finish.[20] At each point, the participant simply yelled out or held up the appropriate number of fingers signifying his rating based on the scale. The scale was introduced to the participant at the start of each TT and presented at both points measured on the course. In addition, heart rate was measured and recorded at the start of the TT, prior to the start of the 1.2-mile maximal effort, and at the finish of the TT. The PI read and recorded the participant's heart rate from all three points of the course via the results from the downloadable

heart rate monitor. Time to complete the TT was recorded at the finish of the course. In addition, time to complete the all-out, 1.2-mile maximal effort was also recorded.

Supplements were administered throughout the route of the course in four fluid-ounce servings. Approximately five minutes before the start of the TT, one serving of the supplement was consumed. Supplements were administered in 2.5-mile increments (at the end of every 4th lap), which took participants at least 15 minutes to complete, throughout the 12-mile course (end of laps: 4, 8, 12, 16). The 15-minute increment is based on previous research that has used this time frame in their protocol.[10-13,15,17,18] Supplement administration was based solely off of distance, completing 2.5 miles/4 laps, as opposed to time. No supplements were given at the finish of the trial; however, participants were allowed to drink water ad libitum at this time. Administration emulated water stations typically used in marathons and other long distance races. Supplements were stored in 4 ounce plastic cups. Upon passing the supplement table, participants were instructed to take a cup and drink the contents at once while running. Participants had one-tenth of a mile to consume all contents in the cup and were instructed to toss the empty cup to their right side when finished and continue running.

Prior to TT 1, all participants were randomized, using a random number table, to one of four orders (Appendix A, Table 2). The purpose of this was to mitigate any potential ordering effect of supplements on performance among trials. Supplement content was blinded to participants.

Compensation:

Upon completing the initial session and first two TT runs, participants were compensated a $10.00 Pilot gas card. After completing the final two TT runs participants received a $40.00

Pilot gas card and their individualized 3-day diet analysis based on their food records submitted at the start of TTs 1-3.

Supplement Composition:

Supplements were in the powder form and were mixed with water. Supplements also consisted of the same color, flavor (fruit punch), consistency, and mouth-feel. The consistency and mouth-feel of the supplements was assured by mixing the powder supplement with water in a blender. All drinks were made following manufacturer instructions.

The placebo used was Crystal Light®. The CHO supplement used was Gatorade® and the CHO-P supplement used was Accelerade®. Both the CHO and CHO-P supplements were matched for CHO content, a 6% CHO concentration, consisting of 7grams CHO per 4 fluid ounce serving. The CHO-P supplement also contained 1.7 grams of protein per 4 fluid ounces, consisting of Accelerade's patented 4:1 CHO to protein ratio. The CHO-CHO supplement was Gatorade®; however, this supplement contained an additional 4.2 grams powder per 4 fluid ounce serving to match total energy content in the CHO-P drink. The CHO-CHO drink also contained 11.2 grams CHO per serving and contained 40 kcal per serving, matching the CHO-P supplement for total caloric content (Appendix A, Table 3).

Measures:

Anthropometrics

Height was recorded to the nearest half-inch using a stadiometer; all participants were barefoot when this measurement was conducted. Weight was recorded to the nearest half-pound using the electronic scale associated with the BOD POD machine; participants were dressed in

standard BOD POD protocol attire while this measurement was conducted. Body composition was measured using the whole body air-displacement plethysmography technique, the BOD POD (Life Measurement Inc., Concord, CA). Prior to weight and body composition measurements, standard procedure for calibrating the BOD POD machine was followed.[21]

The BOD POD's 750-liter volume dual chamber system used both the initial volume of the empty chamber, and volume of the chamber with a participant inside in order to calculate body density from the participant's measured body mass and body volume.[21] Once body density was calculated, the machine used the Siri equation to convert the calculated body density to body fat percentage.[21] In order to obtain the most accurate measurement, participants were instructed to follow standard BOD POD protocol which included: refraining from intense exercise lasting at least 1 hour, refraining from eating for at least 3 hours before the test, consuming minimal amounts of water/fluids, wearing appropriate attire- Speedo or spandex cycling shorts and a swim cap. Swim caps were provided if participants did not own one.

VO_2max

During the initial session, participants completed a maximal, graded exercise test in order to determine maximal oxygen consumption. As long as the participant was in good health, the test was conducted during the initial session. If the participant claimed to be ill the test was rescheduled for another date and time. The purpose of this test was to enable investigators to assess each participant's individual fitness level. Standard pre-test protocol included: refraining from engaging in exercise and consuming alcoholic and/or caffeinated beverages the day of the test, and refraining from eating 4-6 hours before the start of the test.[22] Prior to starting the test, participants were allowed five minutes, or longer if needed, to warm-up on the motorized treadmill (Quinton Medtrack ST, Quinton Instrument Co., Bothell, WA) and stretch any

necessary muscle groups.[22] Once the participant was ready to begin the test, the Hans Rudolph valve, equipped with rubber mouthpiece tubing and headpiece for support, was fitted to the participant.

During the VO_2max test, ventilation, carbon dioxide production, oxygen consumption, and RER were obtained continuously via expired gas from the subject and analyzed using the ParoMedics TrueMax 2400 Metabolic Cart **(**Consentius Technologies, Sandy, UT) with electronic flow meter and oxygen and carbon dioxide analyzers. Expired gases were collected via a mouthpiece attached to a Hans Rudolph (Kansas City, MO) two-way non-breathing valve (2700 series, large)**.** Before each test, the metabolic cart was calibrated using a 3.0 L calibration syringe and the gas analyzer was calibrated against known gas concentrations.

The participant self-selected the speed ran to complete the test. The speed was a comfortable pace that elicited a heart rate of approximately 75% of the individual's age-predicted maximum heart rate. This self-selected speed remained constant throughout the duration of the test. The test began at 0% grade and increased 1% in one-minute increments until the subject was unable to continue. At the end of each minute, participants were asked to rate their perceived exertion using the Borg 15-point scale (6-20).[20] A respiratory exchange ratio of $>$ 1.10 and a change in oxygen consumption of < 0.2 liters/minute over the previous work rate was used as the criteria to determine the subjects VO_2max. This criterion was modeled from the same criteria used in previous studies.[6,23]

Diet Record & Activity Log

Participants were instructed to record all caloric foods and beverages consumed from the first eating occasion to the last occasion of the full 24-hour period leading up to their TT. Participants completed and submitted a 24-hour diet record upon meeting for every TT. Diet

records were analyzed using the NDS-R computer diet analysis program version 2008 (NCC, University if Minnesota, Minneapolis, Minnesota). The purpose of the diet record was to determine consistency of energy and macronutrient intake among each time trial.

After the first record was collected at the start of trial 1, the PI analyzed the individual's diet using the quantified serving sizes from the Diabetic Exchange List, developed by the American Dietetic Association. The individual's record from trial 1 set the precedent for his diet 24-hours before each TT in the study. Since participants were asked to maintain a consistent diet 24 hours prior to each TT, the exchanges calculated from the first record were the basis for individual dietary compliance for the remainder of the trials. The exchanges, per the participant's diet 24 hours before the trial, were sent to the participant after the first TT run via email. This email informed the participant of what his diet needed to consist of 24-hours before the next scheduled TT. Records from trials 2, 3, 4 were required to either be exactly the same as the record from trial 1 or within +/- 2 exchanges (or servings) from baseline (trial 1 exchange analysis) in order to have been deemed consistent. If the participant's diet for subsequent trials was not consistent with trial 1, the trial needed to be rescheduled; however, all participants followed their individual diet prescriptions and so no trials were cancelled for this reason. The purpose of using the exchange system was to ensure consistent macronutrient intake and equivalent glycogen status at the start of all trials.

Participants were also instructed to record all structured activity in this 24-hour period. If the daily workout was performed prior to the start of the 24-hour period, it had to be noted and included in the activity log along with the designated hour of the day the exercise occurred. The purpose of the activity log was to assess both the training intensity and duration 24 hours prior to the time trial and any potential resultant influences on TT performance. Along with the diet

record, the structured activity log was also submitted upon meeting for each trial. The structured activity log submitted at trial 1 set the precedent for the individual's structured activity prior to each trial. For trials 2 to 4, participants were allowed a +/- 30-minute variance in structured activity time from the amount of activity engaged in prior to trial 1. If the amount of structured activity for these subsequent trials was not within the specified range from baseline (trial 1 activity time), the TT needed to be rescheduled; however, all participants complied with their activity prescription and thus no runs were cancelled for this reason.

Perceived Exertion

The Borg 10-point scale of perceived exertion (RPE) was used at the finish of each trial and at mile 5.6, the approximate midpoint of the trial.[20] At each point, participants simply yelled out or held up the appropriate number of fingers signifying their rating of perceived exertion based on the scale, which was introduced to them at the start of each TT and presented at each point measured on the course. The purpose of this subjective measure was to ensure a challenge was presented and assess the participants' contributing work, which had to be at least 75% of their race pace. If participants' rating was too low (< 4), they were instructed to increase their running pace until their rating was ≥ 4; however, this never occurred in this investigation.

Heart Rate

Heart rate (HR) monitors were worn throughout the duration of each TT. HR was measured via the downloadable Polar s625x HR monitor (Polar Electro Inc., Lake Success, NY). Prior to the start of the TT, participants were instructed to push the large red button on their HR monitor watch at the start of the TT, at the start of the 1.2-mile maximal effort, and at the finish of the TT. Prior to the start of the 1.2-mile maximal effort and immediately at the finish of the TT run, participants were reminded to push the large red button on their HR watches. When the

TT was complete, the PI recorded the participant's HR from each point during the run via the HR monitor watch.

Weather Conditions

The investigators recognized weather conditions present the potential to play a role in participants' performance, especially since the TTs occurred during the end of the fall and early winter months in East Tennessee. During the fall months temperatures and humidity levels may become very high; during the early winter months temperatures may drop very low, below freezing. In reference to endurance exercise in hot and humid conditions, Ely and colleague's findings indicate that endurance running progressively slows down and performance is negatively influenced in hot and humid conditions.[24] Due to the variance in performance elicited by weather conditions, it was imperative for each participant to run in similar weather conditions for all of the TTs.

Temperature, humidity levels, and average wind speed was recorded, via the Speedtech SM-28 Skymaster Windmeter (Ambient Weather, Chandler, AZ) handheld device, prior to the start of each TT run. Since participants were being recruited locally and were already recreationally active (as defined under the eligibility criteria) they were acclimated to the daily heat and humidity and/or cold conditions in which they were running in. As mentioned by Binkley and Colleagues, once the endurance runner is acclimatized to the weather conditions, the influence on performance decreases.[25] According to the American College of Sports Medicine, continuous activity should be cancelled when temperatures are between 82.1-86.0 °F in order to prevent exertional heat stroke;[26] therefore, in this study, when temperatures during the time of the scheduled trial fell within this range, the trial was cancelled and rescheduled for another day. In addition, for each participant, a variance of +/- 5 °F was allowed for trials 2, 3, 4 based on the

recorded temperature from trial 1 under the condition that the addition of ≤ 5 ºF from this temperature did not fall within the 82.1-86.0 ºF range. If the temperature during the scheduled TT did not meet this criterion, the TT was cancelled and rescheduled for a different day. If it was raining on the day of a scheduled TT, or the course was flooded due to previous rainfall, the trial was cancelled and rescheduled for a different day. Out of the 48 total trials run by eligible participants: two trials were cancelled and rescheduled on account of rain and poor weather conditions, two trials were cancelled and rescheduled due to previous rain and flooding of the course, and one trial was cancelled and rescheduled due to the temperature deviating from the specified range.

Time to Complete the Time Trial &1.2-Mile Maximal Effort

At the finish of each trial, each participant's total time to complete the course and 1.2-mile maximal effort time was recorded to the nearest hundredth of a second using a stopwatch. Participant's time to run the 12-mile course was the primary measure for performance. Traditionally a shorter time to complete a given distance (in miles) equals better performance.

Statistical Analyses:

Baseline characteristics were analyzed using a one-way analysis of variance (ANOVA) to determine if there were differences within each order group. A 4 x 4 mixed repeated measures ANOVA test, with order (1, 2, 3, or 4) as the between-subjects factor and supplement type (PLA, CHO, CHO-CHO, and CHO-P) as the within-subjects factor, was used to analyze dietary intake, physical activity, and weather conditions. RPE was analyzed using a 4 x 4 x 2 mixed repeated measures ANOVA test, with the between-subjects factor of order (1, 2, 3, or 4) and the within-subjects factors of supplement type (PLA, CHO, CHO-CHO, and CHO-P) and time (mid-

point of the run and end of the run). Additionally, HR was analyzed using a 4 x 4 x 3 mixed repeated measures ANOVA test, with the between-subjects factor of order (1, 2, 3, or 4) and the within-subjects factors supplement type (PLA, CHO, CHO-CHO, and CHO-P) and time (start of the run, start of the max. effort, and end of the run).

A significant difference was found between supplement type for the percent of total calories consumed from protein in the 24-hour time period. To control for this difference, a regression analysis was conducted on the primary dependent variables, time to complete the TT and time to complete the maximal effort, using the percent of total calories from protein as the independent variable. Four sets of residualized values, one for each trial, were used in the 4 x 4 mixed, repeated measures ANOVA test, with the between-subjects factor of order, and the within-subject factor of supplement. Probability levels were based on the Greenhouse-Geisser test to control for sphericity in the mixed-factor ANOVAs where appropriate. In addition, post hoc comparisons with Bonferroni corrections were used for significant outcomes. Data were analyzed using SPSS Statistics version 18.0, with significance level set at 0.05.[27]

RESULTS

Participant Characteristics:

A description of the participants may be found in Appendix A, Table 4. There were no significant differences between the orders in demographics, anthropometric measurements, and VO_2max values ($p > 0.05$). All participants were white (100%) and had a mean age of 32.4 ± 9.5 years. Participants were 68.8 ± 2.9 inches tall, weighed 152.6 ± 19.5 pounds, had a BMI of 22.7 ± 1.5 kg/m^2, and had a body composition consisting of 88.7 ± 5.8 percent fat free mass and 11.2 ± 5.8 percent body fat. Fitness levels, or VO_2max, of participants was 59.7 ± 7.5 mL/kg/min.

Diet & Physical Activity:

A description of participant dietary intake and physical activity within the 24-hour time period prior to each trial may be found in Appendix A, Tables 5 & 6. There were no significant main effects or interaction for total caloric intake (2372.1 ± 738.7 calories; $p > 0.05$), and percent total calories from carbohydrate (50.2 ± 3.9%; $p > 0.05$) or fat (31.2 ± 4.1%; $p > 0.05$). However, a significant main effect of supplement was found in terms of percent total calories from protein between the CHO-P and CHO-CHO supplement within a participant's diet ($F(3,24) = 4.078$, $p = 0.042$) such that the percent total calories from protein consumed was significantly greater in the CHO-CHO supplement (19.2 ± 2.7%, $p = 0.042$) over the CHO-P supplement (16.1 ± 3.6%, $p = 0.042$); with no difference found between the other supplements. For physical activity, there were no significant main effects or interaction found (53.3 ± 67.9 minutes; $p > 0.05$).

Weather Conditions:

A description of weather conditions measured on-site, prior to the start of each TT, may be found in Appendix A, Table 7. No significant main effects or interaction in temperature (53.5 ± 13.0 °F; $p > 0.05$), percent relative humidity (57.8 ± 12.5%; $p > 0.05$), or wind speed (0.6 ± 0.5 mph; $p > 0.05$) was found.

Rating of Perceived Exertion & Heart Rate:

A significant main effect of time was found for perceived exertion (RPE) ($F(1,8) = 395.5$, $p < 0.001$). RPE significantly increased from mid-point to completion of the TT (4.7 ± 0.7, 9.7 ± 0.9, $p < 0.001$) (Appendix A, Figure 1).

A significant main influence of time was found for heart rate (HR) ($F(2,16) = 581.1$, $p < 0.001$). HR significantly increased from the start of the TT to the start of the maximal effort, and was again significantly higher at completion of the effort as compared to the start of the maximal effort (84.4 ± 14.5 bpm, 166.0 ± 8.3 bpm, 178.8 ± 7.4 bpm, $p < 0.001$ for all 3 time points). (Appendix A, Figure 2).

Time to Complete the Time Trial & 1.2-mile Maximal Effort:

There were no significant main effects or interaction on time to complete the 12-mile TT run by trial ($p > 0.05$) (Appendix A, Figure 3). In addition, there were no significant main effects or interaction to complete the 1.2-mile maximal effort by trial ($p > 0.05$) (Appendix A, Figure 4). Participants completed the 12-mile run in 89.1 ± 11.6 minutes and the maximal effort in 8.3 ± 1.3 minutes.

DISCUSSION

The purpose of this study was to evaluate the influence of four different supplements (CHO, CHO-P, CHO-CHO, and PLA) during an endurance TT run in order to determine whether any resultant performance benefits could be attributed to caloric content or macronutrient composition of the supplements in male recreational runners. It was hypothesized that performance benefits from ingestion of the CHO-P supplement would be attributed to the extra total calories contained in the supplement and not due to the presence of protein; thus, timed runs would be significantly faster for the supplements matched in caloric content, CHO-P and CHO-CHO, in comparison to the supplements matched in CHO content, CHO-P and CHO supplement. Additionally, it was hypothesized that all supplements containing calories (CHO,

CHO-P, and CHO-CHO) would produce greater endurance performance over the non-caloric supplement (PLA). Overall, no differences in endurance performance, either the 12-mile TT or the 1.2-mile maximal effort, were found among the tested supplements.

This investigation found no differences in outcomes for the isocaloric supplements, CHO-P and CHO-CHO, or the isocarbohydrate supplements, CHO-P and CHO, which is similar to previous research.[5,8,11,14-18] However, this study also found no influence of any of the supplements as compared to the PLA on endurance performance. In previous research, regardless of whether the tested supplements were isocaloric and/or isocarbohydrate, significant differences favoring the tested caloric supplements over the PLA have been found.[6,8,17,18] While these findings are inconsistent with findings from the present study, three of the four previous investigations testing a PLA also used the time to fatigue methodology.[6,8,17] Additionally, two of these three investigations tested participants in a state of glycogen depletion.[6,8] Thus one may attribute the inconsistencies between these three previous studies and the present study to be on account of inconsistencies within participant glycogen status and different methodologies used to assess endurance performance.

In contrast, the fourth aforementioned investigation, conducted by Van Essen and Gibala,[18] favored caloric supplementation over a non-caloric PLA and was similar to the present study such that the TT method was used and participants entered each trial in a non-depleted glycogen state. The three differences between these investigations include supplement administration, length of the TTs, and type of exercise used (running –vs- cycling). In terms of supplement administration, Van Essen and Gibala[18] did not provide a bolus of the supplement prior to the start of the TT, whereas the present investigation did. While it is theorized that a bolus of CHO prior to the start of exercise may elevate insulin levels, consequently increase

utilization of muscle glycogen stores at the start of exercise, and result in rebound hypoglycemia later in the exercise bout potentially inhibiting performance, the bolus of CHO given in the present study (7 grams) prior to the start of the TT has not previously produced this effect.[28] In terms of length of the TTs, the TT in Van Essen and Gibala's study was 50 miles in length,[18] while the present study used a TT that was 12 miles in length. Thus the TT for Van Essen and Gibala was more than twice the length of the current trial. The difference in the length of the TT can be seen in the time required to complete the TT: for PLA, the mean time for participants to complete the 50-mile TT ride in Van Essen and Gibala's investigation was 141 ± 3 minutes,[18] whereas in the present study, participants completed the 12-mile TT run in 88.6 ± 11.6 minutes.[18] Therefore, the amount of energy required to maintain athletic performance in the 50-mile TT would be much greater, regardless of type of exercise (cycling or running), than in the 12-mile TT, which would make the difference in performance more apparent between a non-energy containing supplement versus those that do contain energy. The differences in outcomes in the two investigations suggests that an exercise bout lasting less than 100 minutes may not present enough energy demand on the body to require supplementation in order to sustain or enhance athletic performance.

Strengths of the current study include the findings of increased HR and RPE during the trials, indicating that the TT was indeed run at higher exertion levels. Additionally, the study used a TT that was conducted outside, simulating a real-life, competitive situation, which increases the external validity of the findings. This study also controlled for several factors that could influence performance, such as weather conditions, the previous day's dietary intake and physical activity, and used a latin-square design to control for order effects.

The present investigation has some limitations in the application of the results. The study sample consisted of white, non-Hispanic, recreationally active male runners, aged 18-55, within normal BMI range, and of similar fitness levels. Although this homogenous study population provides controls for confounding variables, such as age, gender, weight status, and fitness level, it limits generalizability to other groups of differing characteristics, such as adolescents, women, overweight runners, sedentary individuals, etc. In addition, the previous day dietary and physical activity data were self-reported, and the type of CHO contained among the tested supplements was not identical.

In conclusion, this investigation found no significant differences in performance among CHO-P and CHO supplements matched in both total energy and carbohydrate content during an exercise bout at moderate- to high-intensity lasting approximately 90 minutes in white, normal-weight, male recreational runners. Additionally, no significant differences in endurance performances were found between all tested caloric-containing supplements and the PLA. These results suggest that for male recreational athletes in a non-glycogen depleted state, for improving endurance performance, caloric supplementation may only be necessary for exercise bouts lasting greater than 100 minutes at the 75% effort of race pace. Outcomes from this investigation may differ from previous research on account of variations in glycogen status, methodology, and length of experimental trials. To address the limitations discussed in previous research, further research is needed assessing participants with adequate glycogen status at the start of the exercise bout using the TT method. Additionally, within the studies that tested a PLA, all PLAs were artificially sweetened. It may be helpful to include water as a second PLA in future research to determine if flavor, specifically a sweet flavor which may cue a physiological response,[29] plays a role in the relationship between supplementation and endurance performance. To address the

limitations from the present study, further research is needed using endurance running, comparing genders, comparing participants of varying fitness levels, and comparing supplements consisting of the same CHO content. Additionally, in order to confirm that caloric supplementation does not enhance endurance performance lasting less than 100 minutes in length, further research is needed testing the aforementioned supplements and using exercise bouts of varying time frames, including those less than 100 minutes in length as compared to those that are greater than 100 minutes in length.

Findings from this investigation are most relevant to male recreational endurance athletes. Prior to competition, it is common for athletes to partake in some type of carbohydrate-loading regimen in order to maximize glycogen storage and optimize performance. While fluid intake during endurance exercise is still an important component of sustaining performance, as long as a carbohydrate-loading regimen is followed, caloric supplementation during a competitive endurance running event lasting less than 100 minutes does not appear to enhance performance greater than that of simply consuming a non-caloric supplement.

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CONCLUSION

As a whole, mixed results have been found in regards to performance benefits associated with CHO-P supplementation during endurance exercise. To address the gaps in the literature, more research is needed using the TT method, using supplements that are matched in both CHO and caloric content, including a placebo, controlling for order effects in the study design, and considering glycogen status upon the start of the exercise bouts. Therefore, the aim of this study was to address these identified gaps to help determine whether potential performance benefits from ingesting a CHO-P supplement during endurance exercise, as assessed using the TT method, is attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to the tested CHO supplements. It was hypothesized that performance benefits from ingestion of the CHO-P supplement would be attributed to the extra total calories contained in the supplement and not due to the presence of protein; thus, timed runs would be significantly faster for the supplements matched in caloric content, CHO-P and CHO-CHO, in comparison to the supplements matched in CHO content, CHO-P and CHO supplement. Additionally, it was hypothesized that all supplements containing calories (CHO, CHO-P, and CHO-CHO) would produce greater endurance performance over the non-caloric supplement (PLA).

Overall, this investigation found no significant differences in performance among CHO-P and CHO supplements matched in both total energy and carbohydrate content during an exercise bout at moderate- to high-intensity lasting approximately 90 minutes in white, normal-weight, male recreational runners. Additionally, no significant differences in endurance performances were found between all tested caloric-containing supplements and the PLA. These results suggest that for male recreational athletes in a non-glycogen depleted state, in reference to improving endurance performance, caloric supplementation may only be necessary for exercise

bouts lasting greater than 100 minutes at the 75% effort of race pace. Outcomes from this investigation may differ from previous research on account of variations in glycogen status, methodology, and length of experimental trials. To address the limitations discussed in previous research, further research is needed assessing participants with adequate glycogen status at the start of the exercise bout and using the TT method. Additionally, within the studies that tested a PLA, all PLAs were artificially sweetened. It may be helpful to include water as a second PLA in future research. To address the limitations from the present study, further research is needed using endurance running, comparing genders, comparing participants of varying fitness levels, and comparing supplements consisting of the same CHO content. Additionally, in order to confirm that caloric supplementation does not enhance endurance performance lasting less than 100 minutes in length, further research is needed testing the aforementioned supplements and using exercise bouts of varying time frames, including those less than 100 minutes in length as compared to those that are greater than 100 minutes in length.

APPENDICES

APPENDIX A

TABLES & FIGURES

Table 1: Research Comparing CHO & CHO-P Supplementation During Endurance Exercise

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Time to Fatigue Methodology										
Ivy et al., 2003	DB, R, CB*	Competitive cyclists; 9 males	CHO: 7.75% [CHO], CHO-P: 7.75% [CHO], 1.94% [P], PLA; IsoCHO	180 min. cycling at varying intensity followed by a ride at 85% VO_2max until fatigue	200 ml servings; one serving before the trial & one serving every 20 min until the start of the ride at 85% VO_2max	Time to fatigue during the ride at 85% VO_2max	YES-12-hour fast	Informed to eat the same meals two days prior to each trial; no instruction provided in terms of how to keep diet consistent; compliance not reported	Provided standard workout for the two days prior to each trial; compliance not reported	CHO-P > CHO; CHO-P, CHO > PLA
Martinez-Lagunas et al., 2010	B, R, CB*	Competitive cyclists; 7 males, 5 females	CHO: 6% [CHO], CHO-PH: 4.5% [CHO], 1.15% [P] CHO-PL: 3% [CHO], 0.75% [P], PLA; Isocaloric-CHO-PH & CHO	150 min. cycling at varying intensity followed by a ride at 80% VO_2max until fatigue	Standardized based on BW, on average 255.4 ± 9.1 ml fluid every 20 min until fatigue	Time to fatigue during the ride at 80% VO_2max	YES-12-hour fast	2-day diet records; instruction provided in terms of how to keep diet consistent; compliance not reported	3-day training log; instruction provided in terms of how to keep activity consistent; compliance not reported	CHO-PH, CHO-PL, CHO > PLA; CHO-PH = CHO-PL = CHO

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Romano-Ely et al., 2006	B, R	Recreational cyclists; 14 males	CHO: 9.3% [CHO], CHO-P: 7.5% [CHO], 1.8% [P]; Isocaloric	Two rides to fatigue, first ride @ 70% VO_2max, 24-hours later a second ride @ 80% VO_2max; Purpose of two rides 24-hours apart is to access affects after repeated bouts of exercise	2 ml/kg BW servings given every 15 min throughout each ride until fatigue	Time to fatigue after the second ride	NO for first ride; YES for second ride First ride forced participants into a degree of glycogen depletion for the start of the second ride	24-hour diet record; instruction provided in terms of how to keep diet consistent; compliance reported	None reported	CHO = CHO-P

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Saunders et al., 2004	DB, R, CB	Competitive cyclists; 15 males	CHO: 7.3% [CHO], CHO-P: 7.3% [CHO], 1.8% [P]; IsoCHO	Two rides to fatigue per trial, first ride @ 75% VO_2max, 12-15 hours later a second ride @ 85% VO_2max	1.8 ml/kg BW servings every 15 min throughout each ride until fatigue	Time to fatigue after the second ride	YES- First ride depleted glycogen stores for second ride, second ride held 12-15 hours after first	None Reported	None Reported	CHO-P > CHO
Saunders et al., 2007	DB, R, CB	Recreational cyclists; 8 males, 5 females	CHO: 7.3% [CHO], CHO-P: 7.3% [CHO], 1.8% [P]; IsoCHO CHO-P & CHO gels mixed with water	Cycled at 75% VO_2max until fatigue	0.146 ml/kg BW gel + 2 ml/kg BW water every 15 min throughout the ride until fatigue	Time to fatigue	NO	Informed to keep diet the same 24-hours prior to each trial; instruction provided in terms of how to keep diet consistent; compliance not reported	48-hour activity record, informed to refrain from strenuous exercise during this time; instruction provided in terms of how to keep activity consistent; compliance not reported	CHO-P > CHO

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Skillen et al., 2008	DB, R	Competitive cyclists; 12 males	CHO: 4.6% [CHO], CHO-P: 3.6% [CHO], 1% [P]; Isocaloric	90 min at 75% VO_2max then 85% VO_2max until fatigue	7 ml/kg BW before the trial, 1.8 ml/kg BW every 15 min during the trial until fatigue	Time to fatigue during the ride at 85% VO_2max	NO	24-hour diet record collected, dinner the night before and breakfast the morning of each exercise trial was provided; no instruction provided in terms of how to keep diet consistent; compliance reported	Informed to rest or only engage in light activity 24-hours prior; no instruction provided in terms of how to keep activity consistent; compliance not reported	CHO = CHO-P

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Valentine et al., 2008	DB	Competitive cyclists; 11 males	CHO: 7.75% [CHO], CHO-P: 7.75% [CHO], 1.94% [P] CHO-CHO: 9.69% [CHO], PLA; Isocaloric-CHO-CHO & CHO-P, IsoCHO-CHO & CHO-P	Cycled at 75% VO_2max until fatigue	250 ml servings every 15 min until fatigue	Time to fatigue	NO	24-hour diet record; instruction provided in terms of how to keep diet consistent; compliance reported	48-hour activity record; no instruction provided in terms of how to keep activity consistent; compliance not reported	CHO-P, CHO-CHO > CHO, PLA; CHO-P = CHO-CHO
Time Trial Methodology										
Breen et al., 2010	DB, CS	Competitive cyclists; 12 males	CHO: 6% [CHO], CHO-P: 6% [CHO], 1.8% [P]; IsoCHO	120 min steady state exercise @ 55% Wmax followed by ride at 70% individual Wmax, ~880 ± 27 kJ of work	270 ml servings every 15 min throughout the steady state exercise; no supplement given during the ride at 70% VO_2max	Time to complete the ride at 70% Wmax, ~880 ± 27 kJ of work	YES- but depletion protocol not reported	Provided diet 48-hours prior to each trial, but each trial was ran in a fasted state (hours of fast not reported); compliance not reported	Refrain from activity 48-hours prior to each trial; compliance not reported	CHO = CHO-P

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Osterberg et al., 2008	DB, R	Competitive cyclists; 13 males	CHO: 6% [CHO], CHO-P: 7.5% [CHO] 1.6% [P], PLA; Neither isocaloric or isoCHO, Used commer-cially available supplements	120 min constant load exercise set at each person's own lactate threshold (1 mmol/L above baseline) followed by an exercise bout at 7 kJ/kg BW	250 ml servings every 15 min during constant load exercise only, no supplements given thereafter	Time to complete the amount of work measured as 7 kJ/kg BW per individual	YES-12-hour fast	24-hour diet record; no instruction provided in terms of how to keep diet consistent; compliance not reported	None reported	CHO > PLA; CHO-P = CHO, PLA
Saunders et al., 2009	DB, R, CB	Recreational cyclists; 13 males	CHO: 6% [CHO], CHO-P: 6% [CHO], 1.8% [P]; IsoCHO	60 km ride	200 ml servings every 5 km throughout the 60 km ride	Time to complete 60 km	NO	Informed to keep diet the same 48-hours prior to each trial; no instruction provided in terms of how to keep diet consistent; compliance not reported	Refrain from heavy exercise 48-hours prior to each trial, compliance not reported	CHO = CHO-P

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Toone & Betts, 2010	DB, R, CB	Competitive tri-athletes and/or cyclists; 12 males	CHO: 9% [CHO] CHO-P: 6.8% [CHO], 2.2% [P]; Isocaloric	55 min cycling at varying intensity followed by a 6 km ride	7 ml/kg BW given 15 min before the warm-up, 2.5 ml/kg BW given after the warm-up and every 15 min throughout the exercise bout, 2.5 ml/kg BW given at the start and end of the 6 km TT, no supplements given during the TT	Time to complete 6km	YES- 12-hour overnight fast	24-hour diet record; no instruction provided in terms of how to keep diet consistent; compliance not reported	Informed to continue typical training and refrain from strenuous exercise 24-hours before each trial; no instruction provided in terms of how to keep activity consistent; compliance not reported	CHO > CHO-P

Table 1 continued

Authors	Study Design	Participants	Supplements	Trial Protocol	Supplement Administration	Endurance Performance Measurement	Glycogen Depletion	Dietary Control	Physical Activity Control	Results
Van Essen & Gibala, 2006	DB, CS, CB*	Competitive cyclists; 10 males	CHO: 6% [CHO], CHO-P: 6% [CHO], 2% [P], PLA; IsoCHO	80 km ride	250 ml servings every 15 min throughout the 80 km ride	Time to complete 80 km	NO	Provided diet 24-hours prior to each trial; compliance reported	Asked to partake in the same workout 24-48 hours before each trial and refrain from exercise 24-hours prior to each trial; compliance reported	CHO-P, CHO > PLA; CHO = CHO-P

Note. DB = Double-blind; R = Randomized; CB = Partially Counter-balanced; CHO = Carbohydrate; CHO-P = Carbohydrate-Protein; P = Protein; PLA = Placebo; IsoCHO = Isocarbohyrate; min = Minutes; ml = Millileters; B =Blind; CHO-PH = Carbohydrate-Protein high; CHO-PL = Carbohydrate-Protein Low; BW = Body Weight; kg =Kilogram; CB = Completely Counter-balanced; CS = Cross-over; Wmax = Power Output; kj = Kilojoules; mmol/L = Millimole per Liter; km = Kilometers.*

Fast is defined as no food or drink containing calories consumed within the given time-period.

Table 2: Supplement Order Sequence

	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**
Order 1	CHO	CHO-P	CHO-CHO	PLA
Order 2	CHO-P	CHO-CHO	PLA	CHO
Order 3	CHO-CHO	PLA	CHO	CHO-P
Order 4	PLA	CHO	CHO-P	CHO-CHO

Note. CHO = Carbohydrate; CHO-P = Carbohydrate-Protein; CHO-CHO = Double Carbohydrate; PLA = Placebo.

Table 3: Supplement Composition per 4 Fluid Ounce Serving

	Components	Total Calories	CHO (grams)	Protein (grams)
CHO	Sucrose/dextrose	25	7	0
CHO-P	Sucrose/trehalose/fructose & Whey protein concentrate	40	7	1.7
CHO-CHO	Sucrose/dextrose	40	11.2	0
PLA	Aspartame	2.5	0	0

Note. CHO = Carbohydrate; CHO-P = Carbohydrate-Protein; CHO-CHO = Double Carbohydrate; PLA = Placebo.

Table 4: Participant Anthropometric and VO_2max Measurements (M ± SD)

	Order 1 n=3	**Order 2 n=3**	**Order 3 n=3**	**Order 4 n=3**
Age (years)	26.6 ± 4.0	26.6 ± 1.1	34.0 ± 14.0	42.3 ± 6.4
Height (inches)	67.8 ± 1.7	70.6 ± 3.3	66.3 ± 3.5	70.6 ± 0.2
Weight (pounds)	147.0 ± 6.2	155.0 ± 28.6	137.2 ± 17.2	171.3 ± 2.5
BMI (kg/m^2)	22.7 ± 1.8	21.9 ± 2.2	22.0 ± 0.8	24.2 ± 0.2
%FFM	89.7 ± 7.6	90.8 ± 3.1	89.4 ± 0.8	85.0 ±9.4
%BF	10.3 ± 7.6	9.2 ± 3.1	10.6 ± 0.8	14.9 ± 9.4
VO_2max (mL/kg/min)	62.0 ± 7.3	61.0 ± 6.1	61.1 ± 10.7	54.6 ± 7.3

Note. kg/m^2 = Kilograms per Meters Squared; %FFM = Percent Fat Free Mass; %BF = Percent Body Fat; mL/kg/min = Millileters per Kilogram per Minute.

Table 5: Dietary Intake for All Participants 24-hours Prior to Each Time Trial (M ± SD)

	CHO n=12	**CHO-P n=12**	**CHO-CHO n=12**	**PLA n=12**
Total Calories	2301.2 ± 735.8	2484.9 ± 837.4	2517.3 ± 732.2	2185.1 ± 827.9
%Kcals CHO	50.9 ± 7.0	51.1 ± 4.8	48.7 ± 5.1	50.5 ± 6.2
%Kcals Protein	17.3 ± 4.4^{ab}	16.1 ± 3.6^{b}	19.2 ± 2.7^{a}	18.4 ± 4.6^{ab}
%Kcals Fat	30.5 ± 6.3	31.5 ± 4.4	31.8 ± 4.8	31.1 ± 6.2

Note. CHO = Carbohydrate; CHO-P = Carbohydrate Protein; CHO-CHO = Double Carbohydrate; PLA = Placebo; %kcals = Percent Calories from; different superscripts indicate significant differences ($p < 0.05$).

Table 6: Structured Activity for All Participants 24-hours Prior to Each Time Trial (M ± SD)

	CHO n=12	**CHO-P n=12**	**CHO-CHO n=12**	**PLA n=12**
Activity (minutes)	57.1 ± 73.9	55.2 ± 71.8	52.3 ± 64.6	48.4 ± 63.3

Note. CHO = Carbohydrate; CHO-P = Carbohydrate-Protein; CHO-CHO = Double Carbohydrate; PLA = Placebo.

Table 7: Weather Conditions During Each Time Trial (M ± SD)

	CHO n=12	**CHO-P n=12**	**CHO-CHO n=12**	**PLA n=12**
Temperature (ºF)	53.6 ± 12.2	51.8 ± 15.4	53.9 ± 13.4	54.8 ± 12.7
% Relative Humidity	59.2 ± 17.3	55.1 ± 15.2	61.9 ± 18.4	55.2 ± 17.5
Wind Speed (mph)	0.7 ± 1.1	1.1 ± 1.1	0.1 ± 0.3	0.6 ± 1.1

Note. CHO = Carbohydrate; CHO-P = Carbohydrate-Protein; CHO-CHO = Double Carbohydrate; PLA = Placebo.

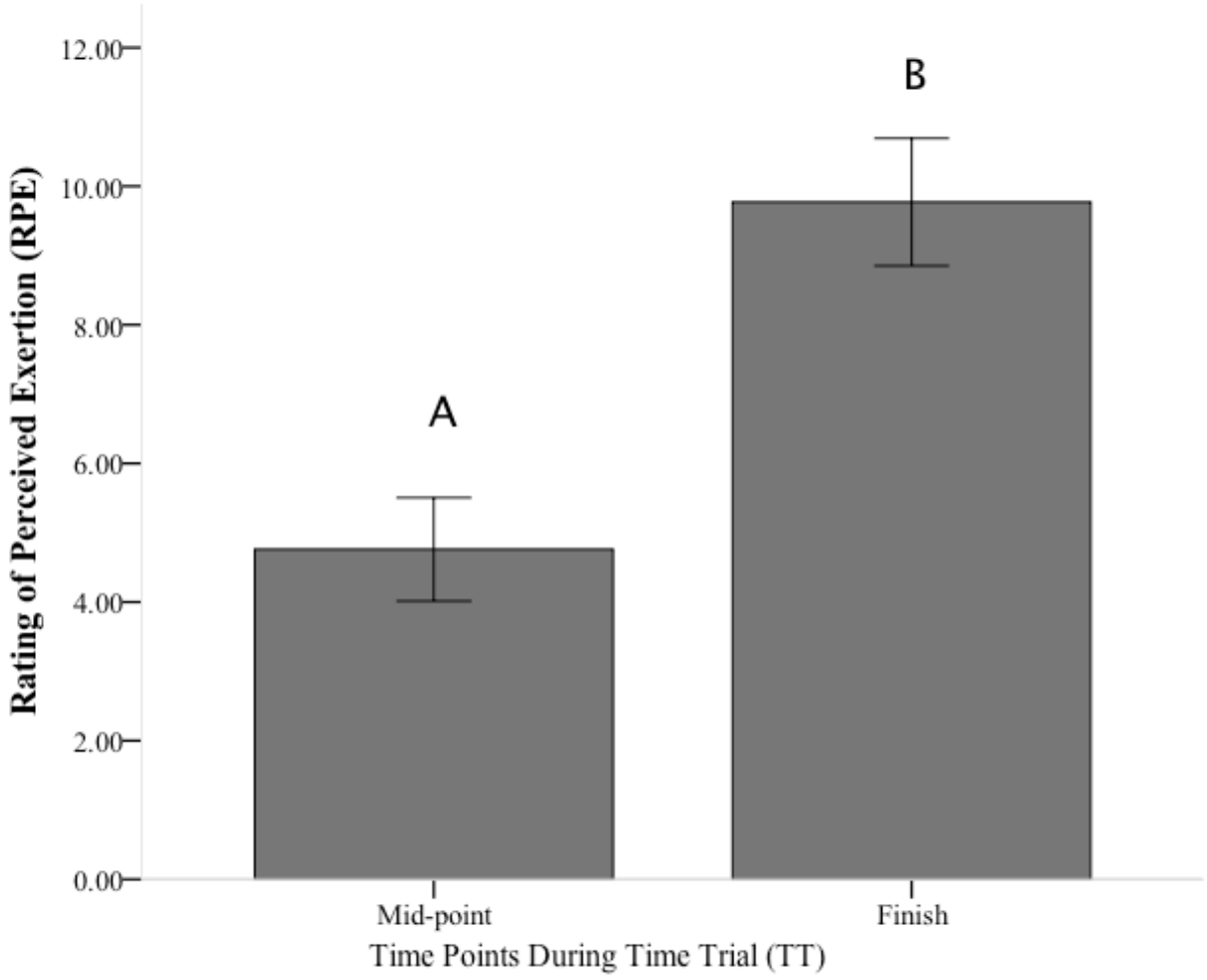

Figure 1: Rating of Perceived Exertion in Time Trials (M ± SD)

Different superscripts indicate significant differences ($p < 0.05$).

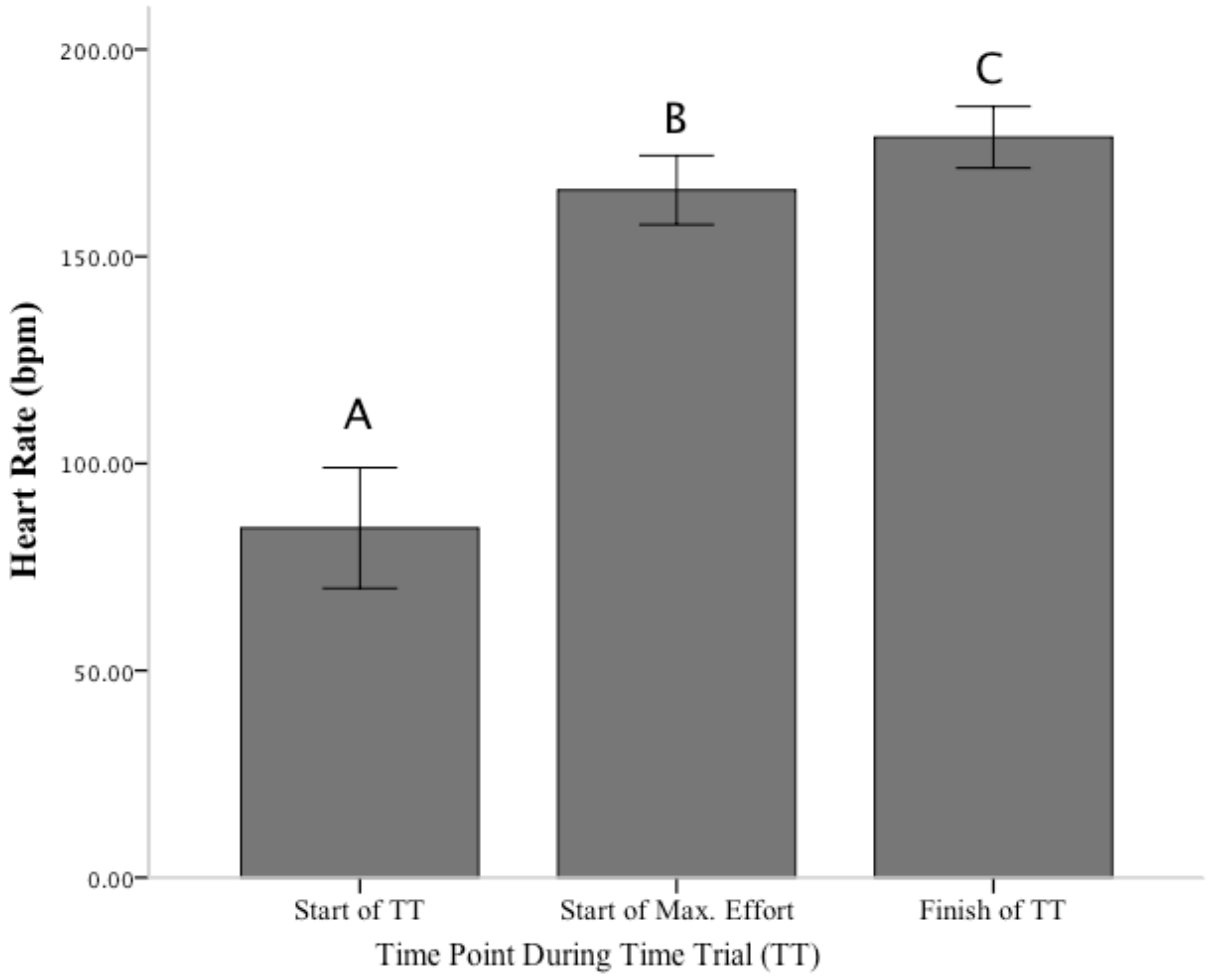

Figure 2: Heart Rate During Time Trials (M ± SD)

Different superscripts indicate significant differences ($p < 0.05$).

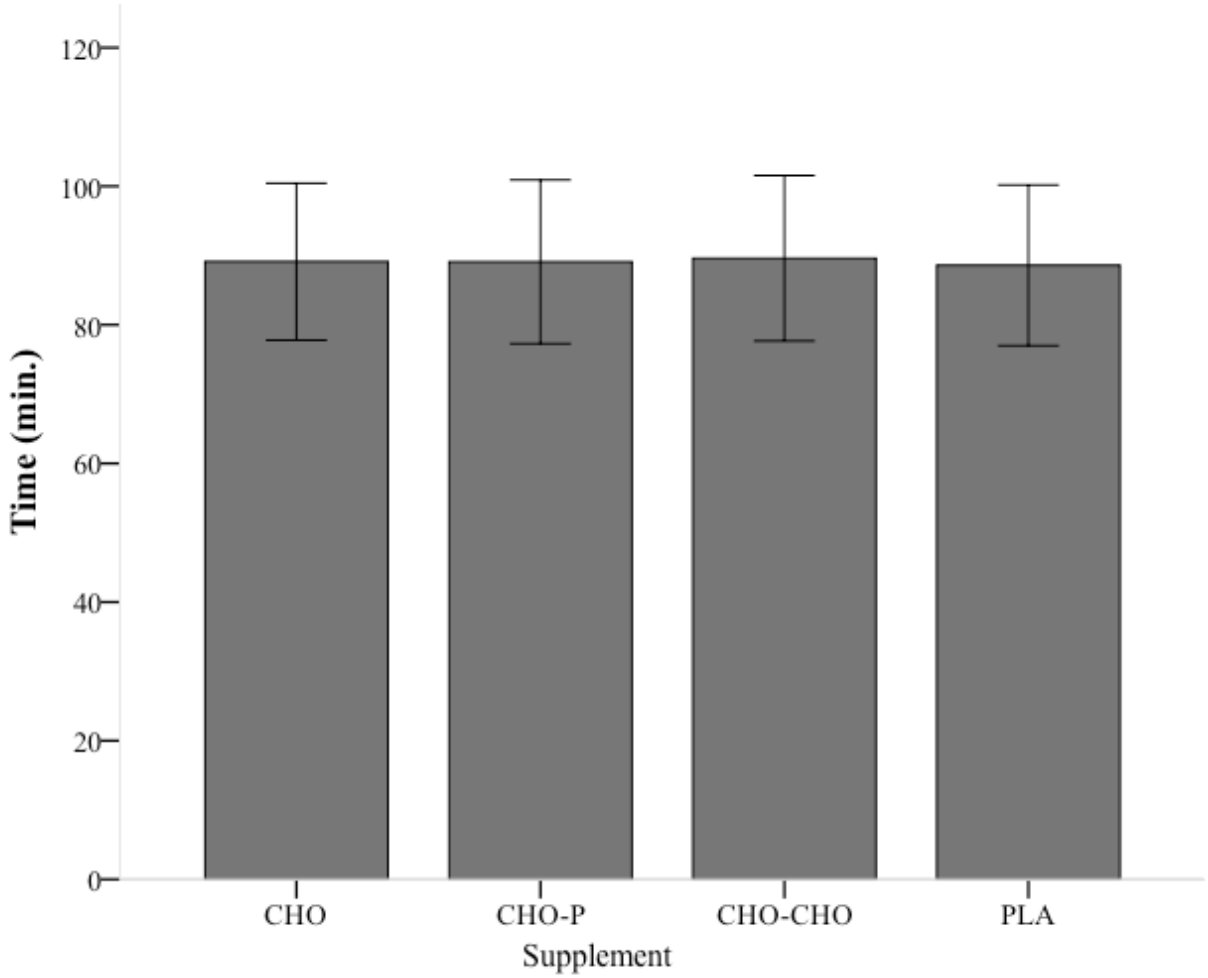

Figure 3: Overall Time to Complete Time Trials for Each Supplement (M ± SD)

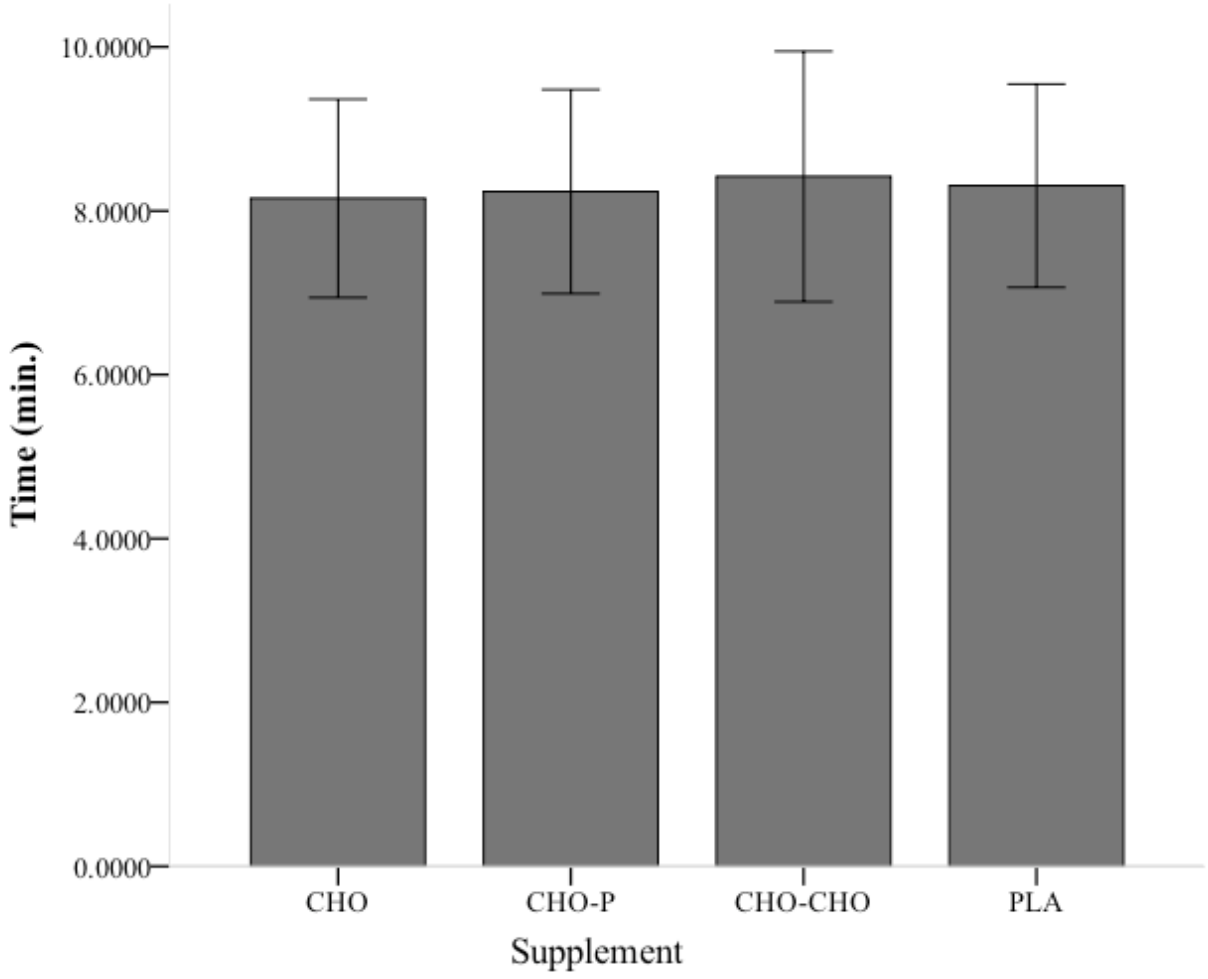

Figure 4: Time to Complete the Maximal Effort for Each Supplement (M ± SD)

APPENDIX B

FLYERS AND FORMS

Recruitment Flyer

Do You Enjoy Distance Running??

If so, you should consider participating in

The Sport Drink Study!

This study involves a FREE fitness test, FREE body composition test, FREE sport drinks, height & weight measurement, Heart Rate measurement, and long distance runs.

$50 gift card compensation for completion of all study requirements.

Eligibility Requirements include:

- ✓ Males 18-55 years of age
- ✓ Normal Weight (BMI 18.5-24.9)
- ✓ Run 45-90+ minutes at least 4 days/week
- ✓ Willing and able to run 12 miles
- ✓ Willing and able to drink Gatorade, Crystal Light, and Accelerade

If interested please contact Adriana (acoletta@utk.edu) at the Healthy Eating and Activity Laboratory 974-0754

Participant Eligibility Phone Screen

Hello, my name is _____ and I am currently a graduate student here at the University of Tennessee-Knoxville. I would like to take a few minutes to briefly tell you about my study, ask you a few questions in order to determine eligibility for participation, and answer any questions you may have about the study.

The purpose of this study is to determine whether the performance benefits from consuming a carbohydrate-protein (CHO-P) supplement, such as Accelerade, during endurance exercise, as opposed to the traditionally used carbohydrate (CHO) supplement, such as Gatorade, are attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to CHO supplements. Numerous studies comparing CHO and CHO-P supplements on endurance performance have found contradicting results in terms of CHO-P supplementation and performance benefits. This study will be comparing 4 different supplements during endurance exercise, a CHO-P supplement, CHO supplement, a double carbohydrate supplement (CHO-CHO), and a placebo (PLA). The CHO and CHO-P supplement will be matched for CHO content, whereas the CHO-P and CHO-CHO supplements will be matched for total caloric content.

The study will take each participant approximately 5 weeks to complete. Participants will be required to meet the investigator for an initial session at the Healthy Physical Education and Recreation (HPER) building on the University of Tennessee-Knoxville's campus. During this session we will collect anthropometric data from you, such as your height and weight, and ask you to complete a body composition test via the BOD POD, and a VO_2max test on a treadmill in order to assess your current fitness

level. The body composition test will take approximately 5 minutes to conduct, whereas the VO_2max test will take approximately 12 minutes to complete. After this session you will be asked to meet the investigator 4 more times, 4 separate occasions, each approximately 7 days apart, at the Springbrook Corporate Center Trail in Alcoa. Within each one of these meetings following the initial session you will run a 12-mile time trial at approximately 75% of your race pace with an all-out maximal effort for the last 1.6 miles of the course. Within each TT, you will be given 1 of 4 of the tested supplements aforementioned, throughout the duration of the trial. You will also be asked to keep your diet and exercise 24 hours prior to the start of each TT run consistent with that of the regimen conducted 24 hours prior to the first run. Participating in this study will provide you with individualized analysis of your diet, offer insight and information in regards to your energy intake and exercise regimen, and inform you of both you body composition and current fitness level (via VO_2max test). In addition, Upon completing all components of the study, initial session and TT runs, you will be compensated with a $50.00 gift card for your participation. If you are still interested in participating in this study do you mind if I ask you a few questions in order to determine eligibility? It will only take about 10 minutes of your time.

****If participant agrees to continue, begin asking questions listed below.**

1. Gender: Male___ Female____

If female: "I'm sorry at this time we are only looking for male participants; therefore you are not eligible for this study.

2. A) Age: _____ B) Date of birth:____/_____/_____ **(must be between 18 and 55)**

If age is not between 18 and 55: "I am sorry, but the age range we are recruiting for is 18-55. Since you are_____ yrs old, you are not eligible for this study.

3. a) Current weight: _____ lbs. b) Height: _____ft _____inches

   c) Current BMI: _____ **(must be between 18.5 and 24.9)**
   BMI=kg/m^2 or (lbs/in^2) x 703

If BMI is < 18.5 or > 24.9: "I am sorry, but we are looking for participants with a BMI in the range of 18.5-24.9. Since your BMI is _____ you do not fall within this range and are not eligible to participate in this study.

QUESTIONS 4-6: If participant answers NO, then he is not eligible. If the participant answers YES, then continue to the next question.

4. Are you currently engaging in runs lasting between 45-90+ minutes at least 4 days per week?

5. Have you been running this frequency and duration for at least the past 4 weeks?

6. Do you engage in runs consisting of ≥ 10 miles in length on at least 2-4 occasions per month?

QUESTIONS 7-20: If participant answers YES to any one of the following questions, then he is NOT ELIGIBLE.

If YES: "I am sorry but due to this medical history, participating in this study may be of harm to your health; therefore, you are no longer eligible to participate in this study."

If the participant answers NO, then continue to the next question.

7. Has you doctor ever said that you have a heart condition and recommend only medically supervised physical activity?

8. Do you frequently have pains in your chest when you perform physical activity?

9. Have you had chest pain when you were not doing physical activity?

10. Have you had a stroke?

11. Do you lose your balance due to dizziness of do you ever lose consciousness?

12. Do you have a bone, joint, or any other health problem that causes you pain or limitations that must be addressed prior to the start of the time trial runs (i.e.- diabetes, osteoporosis, high blood pressure, high cholesterol, arthritis, anorexia, bulimia, anemia, epilepsy, respiratory ailments, back problems, etc.)?

13. Do you have asthma or exercise induced asthma?

14. Do you have low blood sugar levels (hypoglycemia)?

15. Do you have diabetes?

16. Have you had recent surgery?

17. Are you allergic to food products containing milk, soy, or aspartame?

18. Do you experience an allergic reaction to foods prepared in the presence of eggs, wheat, tree nuts, fish, crustaceans, or shellfish products?

19. Would you be opposed to consuming Gatorade, Accelerade, and Crystal Light during the time trial runs?

20. Are you willing and available to meet with the primary investigator on 5 separate occasions, approximately 7 days apart, for an overall time frame of about 5 weeks participation in this study?

IF ELIGIBLE: Congratulations! I am happy to tell you that you meet the eligibility criteria for the sport drink study! At this time I would like to schedule you for the initial session.

First Name:_____ Last Name:_____

E-mail Address:_____

Phone #1: _____mobile/home/other

Phone #2:_____ mobile/home/other

Eligible: No____ Yes_____ If No, Reason:_____ Appointment Date: ___/___/___ Time:_____	Screened by:_____ Date:_____

Informed Consent Statement

Nutritional Ergogenic Aids: The Influences of Carbohydrate-Protein Supplementation on Endurance Exercise

You are invited to participate in a research study assessing the influences of carbohydrate-protein supplements on endurance performance. The purpose of this study is to determine whether the performance benefits from consuming a carbohydrate-protein (CHO-P) supplement, such as Accelerade®, during endurance exercise, as opposed to the traditionally used carbohydrate (CHO) supplement, such as Gatorade®, are attributed to the extra calories in the CHO-P supplement or the presence of protein in comparison to CHO supplements. Numerous studies comparing CHO and CHO-P supplements on endurance performance have found contradicting results in terms of CHO-P supplement consumption during exercise resulting in performance benefits. It has yet to be determined whether performance benefits are attributed to the extra total calories in the CHO-P supplement or the presence of protein independent from total calories.

If you decide to participate in this study you will meet with the investigator on 5 separate occasions. Each occasions will separated by approximately 7 days; it will take at least 5 weeks to complete this study. Upon completing the phone screen and fulfilling the eligibility criteria, you will meet with the investigator at the Health, Physical Education, and Recreation Building on the UTK campus for the initial session.

At the start of the initial session, signed consent forms and any questions in regards to the study will be taken. You will be given a copy of the signed consent form for your records. This session will take no more than one hour maximum to complete. Next, you will be asked to remove your shoes and socks and stand in good posture against a portable stadiometer in order to record your height. Once this measurement is taken, the investigator will leave the room briefly so that you may change into appropriate BOD POD procedure attire, if you are not already in the attire. Once dressed appropriately, you will stand on the electronic scale associated with the BOD POD machine in order to measure body weight. After body weight is measured and recorded, you will have a seat in the BOD POD chamber so that the body composition test may be conducted. Upon completing the BOD POD test, you will be given a few minutes to change, in private, into proper attire for the maximal test. After changing, you will be escorted to the appropriate room for the maximal test. At this time, you will complete the VO_2max test. Prior to the conclusion of the initial session, once the maximal test has been completed, the date and time for the first TT will be scheduled.

Before you leave the initial session you will also be given a blank 24-hour food record and structured activity log. You will be instructed to complete both forms in regards to the 24-hour time period prior to the first scheduled TT and return completed forms upon meeting for the first TT. It is absolutely imperative that you keep your diet and exercise 24-hours prior to each TT consistent.

If you decide to participate in this study, you will complete four 12-

mile time trial (TT) runs on 4 separate occasions. When you meet for the first TT, you will submit your 24-hour diet recall and structured activity log forms to the investigator. After the first TT session, the investigator will analyze both your diet, based off of the serving sizes used in the Diabetic Exchange List, and the amount of exercise you reported. The exchanges and guidelines for structured activity, per your diet and exercise 24-hours before the trial, will be sent to you immediately after the first TT run via your preferred method of contact, e-mail or postal service. Upon meeting for TTs 2,3,4, before you start the run you will submit the 24-hour diet-recall and structured activity log. If this data is not consistent with the data from the first TT, the TT must be rescheduled for another day.

In addition, a TT may also need to be rescheduled in the event of poor weather conditions. A variance of +/- 5°F will be allowed for trials 2,3,4 based off of the recorded temperature from trial 1 under the condition that the addition of ≤5ºF from this temperature does not fall within the 82.1-86.0ºF range. If the temperature during the scheduled TT does not meet this criterion, the TT will be cancelled and rescheduled for a different day. If it is raining on the day of a scheduled TT, the trial will be cancelled and rescheduled for a different day.

You will be instructed to run each TT at 75% of your race pace, providing a maximal, all-out, effort for the last two laps, 1.26 miles, of the trial's course. You will be asked to rate your effort on the Borg 10-point scale of perceived exertion both at the approximate mid-point of the course, 5.6-mile marker (lap 9), and at the finish. At each point, you will simply yell out or hold up the appropriate number of fingers signifying your rating based off of the scale. In addition, you will wear a heart rate (HR) monitor during each TT and HR will be measured and recorded at the start of the TT, prior to the start of the 1.6-mile maximal effort, and at the finish of the TT.

Approximately 5 minutes before the start of the TT, one serving of the supplement will be given to you to drink. Supplements will be administered in 2.52-mile increments (at the end of every 4th lap), approximately 15-20 minutes, through out the 12-mile course (end of laps: 4, 8, 12, 16). No supplements will be given at the finish of the trial. Supplement administration will mimic water stations typically used in marathons and other long distance races. Upon passing the drinking station you will be instructed to take a cup and drink the contents at once while running. It is absolutely imperative that you take a serving of the supplement and drink the entire contents of the cup at the end of the aforementioned laps.

Prior to the first TT run, you will be randomly assigned to one of four different supplement order groups. You will not know the contents of the supplement you will be drinking for any TT until all trials are complete.

RISKS

The risk of participating in this study is no greater than participating in a competitive long distance race. It is important to recognize the rare possibility of an adverse cardiac event, which has an increased occurrence during heavy activity. You may experience

slightly sore or tight muscles throughout the duration of the study; however, this is dependent on the amount of warming up and cooling down/stretching you do independent of what is asked of you by the investigator.

BENEFITS
You will receive personalized information about your diet and physical fitness level; this may benefit you in terms of your overall health. Participating in this study may teach you how to optimize you endurance performance via proper diet and supplement consumption.

CONFIDENTIALITY
All consent forms, personal information, and data collected will be securely stored in locked filing cabinets in the Healthy Eating and Activity Laboratory, room 102 of the Jessie Harris Building located on the University of Tennessee- Knoxville's campus.. This information will only be available to the primary researcher and persons helping in conducting the study. People other than those mentioned will be granted permission to review your information under the condition that permission is given from you in writing. All data entered into the computer will be password protected and only those involved with the study will be able to assess the information.

COMPENSATION
Upon completing the initial anthropometric measurements,VO_2max test, and the four TT runs, you will be compensated a $50.00 gift card for your efforts and participation in this study. . At the approximate halfway point, after completing the second TT run, you will receive a $10.00 gift card. Upon completion of the fourth and final TT, you will be compensated a $40.00 gift card. In addition, participating in this study will provide you with individualized analysis of your diet, offer insight and information in regards to your energy intake and exercise regimen, learn of both your body composition and current fitness level (via VO_2max test).

EMERGENCY MEDICAL TREATMENT
The University of Tennessee will not "automatically" reimburse you for medical claims or other compensation. If physical injury is suffered in the course of research, or for more information, please notify the investigator in charge. Primary Investigator: Adriana Coletta, 865-974-0754.

CONTACT INFORMATION
If you have questions at any time about the study, the study procedures, or you experience adverse influences as a result of participating in this study, please contact the Adriana Coletta at 865-974-0754 or via email acoletta@utk.edu. If you have questions about your rights as a participant, contact Brenda Lawson in the Office of Research Compliance at (865) 974-3466.

PARTICIPATION
Your participation in this study is voluntary; you may decline to participate without penalty. If you decide to participate, you may withdraw from the study at anytime without

penalty and without loss of benefits to which you are otherwise entitled. If you withdraw from the study before data collection is completed your data will be returned to you or destroyed.

CONSENT

I have read the above information. I have received a copy of this form. I agree to participate in this study.

Participant's signature _____ **Date** _____ _

Investigator's signature _____ **Date** _____

SEP 2 9 2009

Body Composition and Maximal Test Protocol

Body Composition (BOD POD) Pre-test Protocol

During the initial session, you will complete the body composition test first followed by the maximal test. Please follow these steps prior to coming in for the initial session:

1. Refrain from intense exercise lasting at least 1 hour prior to your scheduled test time.
2. Refrain from eating for at least 3 hours before your scheduled test time.
3. Try to consume minimal amounts of water/fluids prior to your scheduled test time.
    a. Refrain from consuming alcoholic and/or caffeinated beverages the day of the test.
4. Appropriate attire for the BOD POD test must be worn. Appropriate attire includes a Speedo or spandex cycling shorts and a swim cap. Swim caps will be provided if you do not own one.

Prior to the BOD POD test your height and weight will be measured.

Maximal Test (VO_2max) Protocol

During the initial session, you will complete a maximal, graded exercise test in order to determine your maximal oxygen consumption. The purpose of this test is to enable investigators to learn of your individual fitness level. Please follow the pre-test protocol for the BOD POD test (listed above) for the maximal test as well. Proper maximal test attire includes tennis shoes, gym shorts, and a t-shirt. You will be running during this test so please come prepared with comfortable attire to run in.

Prior to starting the test, you will be allowed 5-minutes, or longer if needed, to warm-up and stretch any necessary muscle groups. Once you are ready to begin the test, the mouthpiece will be fitted to your head. The mouthpiece fits naturally around your head and face and should not provide any discomfort during the test.

The treadmill speed set to complete the test will be determined by you, you may complete the test at a speed you are most comfortable as long as it elicits a heart rate of approximately 75% of your age-predicted maximum heart rate. This self-selected speed will remain constant throughout the duration of the test. The test will begin at 0% grade and will increase 1% in one-minute increments until you are unable to continue. At the end of each minute, you will be asked to rate your perceived exertion using the Borg 10-point scale. Once the test is complete, you will be given 5 minutes, or longer if needed, to cool-down and stretch any necessary muscle groups.

Office Use Only
ID # _____
Trial # _____

Food Record Form

In the table below, please write down a description of what you ate and drank in the past 24 hours. In the description, include the time that you started eating and/or drinking each meal or snack, a complete description of each item that you ate or drank (including how food was cooked or prepared), and the amount that you ate or drank of each item.

Example: At lunch (12:00 pm), Tom ate a turkey sandwich, chips, a soda, and cookies.

Time	**Description of Food and Drink**	**Amount**
12:00	Turkey sandwich	
	White bread	2 slices
	Turkey luncheon meat (Oscar Meyer)	3 slices
	American cheese	2 slices
	Mayonnaise - regular	1 tablespoon
	Lettuce - iceberg	1 leaf
	Lay's regular potato chips	Large grab bag – 2 oz
	Diet coke	20 oz
	Oreo cookies	10

Time	Description of Food and Drink	Amount

Time	Description of Food and Drink	Amount

Office Use Only
ID # _____
Trial # _____

Example of Structured Activity Log

Time:	Activity:
Time:	Activity:
Time:	Activity:
Time:	Activity:
Time:	Activity:
Time:	Activity:

EXCHANGE SYSTEM

MEAT

1 serving = 2-3 oz. of protein

1 oz. meat contains { 0 gms of carbs, 7 gms of protein, 3-8 gms of fat, 45-100 calories

EXAMPLES OF MEAT

Beef	Chicken
Fish	Lamb
Pork	Wild game
Turkey	Veal
Seafood	

MEAT SUBSTITUTES

Cottage cheese	¼ cup
Cheese	1 oz.
Egg	1 egg
Peanut Butter	1 Tbsp.
Tofu	½ cup

EACH PORTION LOOKS LIKE:

- 3 oz. Grilled fish = size of a checkbook
- 3 oz. Chicken = a deck of cards
- 3 oz. Beef patty = palm of a woman's hand
- 1 Tbsp. Peanut butter = 1 thumb

STARCH

1 serving = 15 gms carbs, 80 calories

Potato (large)	¼ (3 oz.)
Bagel (large)	¼ of bagel (1 oz.)
English muffin	½
Hamburger/ hot dog bun	½ (1 oz.)
Popcorn, un-buttered	3 cups
Pita pocket - 6"	½ of pita
Pancake (4" across)	1 pancake
Tortilla, corn - 6"	1 tortilla
Tortilla, flour - 6"	1 tortilla
White or wheat bread	1 slice
Rice, white or brown, cooked	⅓ cup
Peas, green or corn	½ cup
Yam, sweet potato, plain	½ cup

EACH PORTION LOOKS LIKE:

- 1 cup of corn flakes = fist
- 1 pancake = compact disc
- ⅓ cup of cooked rice, pasta = 1 lemon
- ½ cup cooked potato = ½ baseball

VEGETABLE

1 serving = 5 gms of carbs, 25 calories

1 CUP RAW or ½ CUP COOKED

- Beans (green, wax)
- Beets
- Carrots
- Cauliflower
- Celery
- Cucumber
- Greens (collard, kale, mustard)
- Mixed vegetables (without corn, peas, or pasta)
- Mushrooms
- Peppers
- Radishes
- Salad greens
- Summer squash
- Tomato
- Tomatoes, canned
- Tomato/vegetable juice ½ cup
- Zucchini

EACH PORTION LOOKS LIKE:

- 1 cup of raw vegetables = 1 tennis ball
- ½ cup of cooked vegetables = ½ tennis ball

FRUIT

1 serving = 15 gms of carbs, 60 calories

Apple, small	1 - 4 oz.
Banana, small	1 - 4 oz.
Canned fruit (unsweet.)	½ cup
Grapefruit	½ large
Grapes	17 grapes
Melon (cubed)	1 cup
Orange, small	1 (6 ½ oz.)
Raisins	2 Tbsp.
Strawberries, whole	1 ¼ cup

FRUIT JUICE

Apple, orange	½ cup
Grapefruit, pineapple	½ cup
Grape, peach, pear, prune	⅓ cup
Cranberry (juice cocktail)	½ cup

EACH PORTION LOOKS LIKE:

- 1 apple = 1 baseball
- ½ cup of canned fruit = ½ baseball
- 2 Tbsp. of raisins = 1 large marshmallow

MILK

1 serving = 12 gms of carbs, 8 gms protein

FAT-FREE & LOW-FAT = 100 cal.

(0-3 gms fat per serving)

Fat-free (skim) milk	1 cup (8 oz.)
Evaporated, low fat milk	½ cup
1 % milk	1 cup
Soy milk, light	1 cup
Yogurt, with sugar substitute	6 oz.

REDUCED-FAT = 120 calories

(5 gms fat per serving)

2% milk	1 cup
Soy milk, plain	1 cup
Yogurt, plain	6 oz.

WHOLE = 160 calories

(8 gms fat per serving)

Whole milk	1 cup
Evaporated whole milk	½ cup
Yogurt, plain	8 oz.

EACH PORTION LOOKS LIKE:

- 1 cup milk = 1 fist
- 6 oz. yogurt = 1 lightbulb

FAT

1 serving = 5 gms fat, 45 calories

MONOSATURATED

Oil (canola, olive, peanut)	1 tsp.
Almonds/cashews	6 nuts
Peanuts	10 nuts
Pecan	4 halves

POLYUNSATURATED

Margarine:	
- regular	1 tsp.
- lower-fat spread	1 Tbsp.
Oil (corn, safflower, soybean)	1 tsp.
Mayonnaise:	
- regular	1 tsp.
- reduced-fat	1 Tbsp.
Salad dressing:	
- regular	2 tsp.
- reduced-fat	1 Tbsp.

SATURATED

(may raise cholesterol levels)

Butter	1 tsp.
Shortening or lard	1 tsp.
Cream cheese:	
- regular	1 Tbsp.
- reduced-fat	1½ Tbsp.
Sour cream:	
- regular	2 Tbsp.
- reduced-fat	3 Tbsp.

EACH PORTION LOOKS LIKE:

- 1 tsp. butter = 1 thumb tip
- 1 Tbsp. reduced fat mayonnaise = 1 whole thumb

FREE FOOD

UNLIMITED SERVINGS

- Club soda
- Coffee
- Diet soft drink, sugar-free
- Drink mixes, sugar-free
- Garlic
- Gelatin dessert, sugar-free
- Gum, sugar-free
- Herbs, fresh or dried
- Hot pepper sauce
- Mustard
- Sugar substitutes
- Tea
- Tonic water, sugar-free
- Worchestershire sauce

These servings can be taken 3 times per day, but not all at the same time:

Catsup or honey mustard	1 Tbsp.
Cream cheese, fat-free	1 Tbsp.
Creamer, nondairy, liquid	1 Tbsp.
Jam or jelly, light	2 tsp.
Mayonnaise, fat-free	1 Tbsp.
Margarine, spread fat-free	1 Tbsp.
Pickle relish	1 Tbsp.
Salad dressing, fat-free, Italian	1 Tbsp.
Salsa	¼ cup
Syrup, sugar-free	2 Tbsp.
Whipped topping, light or fat-free	2 Tbsp.

Please consult your certified diabetes educator or physician for medical advice. This information is for general education only, and should not replace the relationship you have with your health care professional.

S0015

Office Use Only
ID # _____
Trial # _____

Exchange System
Sample- Exchange System Diet Analysis & Structured Activity Analysis

Diet:
The purpose of this exchange system is to ensure compliance with a consistent diet among trials. Based off of your reported diet 24 hours prior to the first time trial run, the diet listed below is the diet you will need to follow 24 hours prior to the run for all subsequent time trials scheduled. It is absolutely imperative that you consume a diet as close to what is listed as possible. If your diet recall for trials 2,3,4 is within +/- 2 exchanges from that of trial 1, then the trial will need to be rescheduled.

Your Diet 24 hours prior to each time trial hereafter must consist of:

Food Group	Number of Servings
Meat & Beans	
Starches	
Fruits	
Vegetables	
Dairy	
Fats & Oils	

Structured Activity:
The purpose of the structured activity log is to assess both the training intensity 24 hours prior to the time trial and any potential resultant influences your performance. It is absolutely imperative that you maintain consistent structured activity 24 hours prior to each time trial run. If the amount of structured activity for these subsequent trials varies within >+/- 30 minutes from trial 1 in trials 2,3,4 then the time trial will have to be rescheduled.

Your allotted time frame for structured activity 24 hours prior to each time trial hereafter is:

_____ Minutes

Borge 10-Point Scale of Perceived Exertion

0	Nothing at all
1	Very light
2	Fairly light
3	Moderate
4	Some what hard
5	Hard
6	
7	Very hard
8	
9	
10	Very, very hard

VITA

Adriana Coletta is originally from Holland, Pennsylvania. She earned her Bachelor of Science degree in Nutrition Science at The Pennsylvania State University, State College, Pennsylvania in May 2008. During her undergraduate studies, she had several opportunities working in various facets within the field of Nutrition. These opportunities included gaining experience in: teaching general nutrition education in reference to weight loss/maintenance, healthful eating/lifestyle, and sports nutrition; experiencing nutrition within the clinical setting; practicing ServSafe principles in various metabolic kitchens; developing general nutrition education lesson plans/materials.

Upon earning her Bachelors degree, she then moved to Knoxville, Tennessee in August 2008 to earn her Masters of Science degree in Nutrition Science with a minor in Exercise Science at the University of Tennessee- Knoxville (UTK). Adriana has been fortunate enough to have her graduate studies completely funded through a Graduate Teaching Assistantship with Dr. Spence and Lee Murphy MS-MPH RD LDN for an introductory level nutrition course. This assistantship provided her with valuable knowledge and experience in teaching. Adriana was given the opportunity to work with Dr. Spence and McGraw-Hill Higher Education in developing a state of the art, interactive CD-ROM learning tool to be sold with the latest edition of the introductory level nutrition textbook. This opportunity provided valuable experience in using new technology to teach general nutrition concepts. In addition, Adriana spent some time working in Dr. Hollie Raynor's laboratory, the Healthy Eating and Activity Lab (HEAL), where she gained experience in behavioral weight loss management. While working in HEAL, Adriana also assisted with delivering the weight loss intervention in doctoral candidate Jessica Bachman's dissertation project, and further developed her skills in working with behavioral

weight loss management. Dr. Raynor also provided Adriana with the opportunity to work on several projects in coordination with Cherokee Health System disseminating general nutrition education to different populations through out East Tennessee. This opportunity further developed her skills in teaching. After completing the degree requirements for her MS, Adriana will be completing her Dietetic Internship at UTK in 2011. Once she earns her license as a Registered Dietitian, Adriana plans on pursuing a career in sports nutrition.