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Altered carbohydrate and protein content in sports beverages: Influence on recovery from heavy endurance exercise

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Altered Carbohydrate and Protein Content in Sports Beverages: Influence on Recovery from Heavy Endurance Exercise

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Abstract

Purpose: The purpose of this study was to compare the efficacy of different carbohydrate-protein recovery beverages following heavy endurance exercise**. Methods:** Twelve well-trained male cyclists completed a glycogen-depleting trial followed by a 4 hour recovery period before completing a simulated 20-km time trial. During the recovery period, subjects consumed one of three isocaloric beverages [high-carbohydrate/low-protein (HCLP); low-carbohydrate/high-protein (LCHP); carbohydrate (CHO)] at 0h and 2h, as well as immediately following the 20-km time trial. Creatine kinase (CK), muscle soreness, isometric peak torque (MVC), and mental/physical fatigue/energy ratings were measured pre- and post trial. Glucose and lactate were measured during the glycogen depleting phase and subsequent exercise. **Results:** Subsequent exercise performance was not significantly different between treatments *(LCHP 50.3±2.7 min; CHO 48.5±1.5 min; HCLP 48.8±2.1 min).* No significant treatment*time interactions were observed for isometric peak torque (MVC), muscle soreness, or mental/physical energy/fatigue ratings. Creatine kinase levels pre- *(LCHP 153.5±68.1; CHO 132.6±39.9; HCLP 137.0±41.1*) and post exercise *(LCHP 172.4±53.1; CHO 150.8±47.4; HCLP 146.6±27.4)* were not significantly different between treatments. **Conclusion:** Recovery beverages containing equal caloric content and differing proportions of carbohydrate/protein provided similar effects on muscle recovery and subsequent exercise performance in well-trained cyclists.

Chapter I

Introduction

Background

Many endurance athletes compete in events that require high levels of performance over repeated days, or even multiple events on the same day (i.e. soccer tournaments, Tour cycling). This type of intense endurance exercise can result in elevated markers of muscle damage, and decreases in performance during subsequent exercise. Recent studies have suggested that these aspects of muscle recovery may be influenced by the consumption of carbohydrate-protein recovery beverages. For example, various studies have reported that creatine kinase (CK) (Seifert et al, 2005; Millard-Stafford et al, 2005; Cepero et al, 2009; Skillen et al, 2008; Saunders et al, 2009; Luden et al, 2007; Rowlands et al, 2007), myoglobin (Mb) (Valentine et al, 2008), muscle soreness (Flakoll et al, 2003; White et al, 2008; Luden et al, 2007; Saunders et al, 2009; Millard-Stafford et al, 2005; Rowlands et al, 2007) and muscle function (Valentine et al, 2008) have been improved when carbohydrate-protein is consumed following heavy aerobic exercise. Importantly, some studies have shown that consumption of carbohydrate-protein supplements during recovery can also lead to improved performance during subsequent exercise, compared to when carbohydrate-only beverages are consumed (Berardi et al, 2008; Saunders et al, 2009; Saunders et al, 2007; Saunders et al. 2004; Skillen et al, 2008). However, these findings are still controversial, as other studies have found no differences in subsequent performance between carbohydrate-protein beverages and carbohydrate-only beverages (Cermak et al, 2009; Rowlands et al, 2007; Betts et al, 2005; Cepero et al, 2009; Romano-Ely et al, 2006). Thus, the specific exercise conditions and beverage compositions that promote enhanced recovery with carbohydrate-protein ingestion require further investigation.

Purpose of Study

The primary purpose of this study was to compare the efficacy of different recovery beverages following heavy endurance exercise. Specifically, the effect of a carbohydrate-only beverage was compared to isocaloric beverages containing differing proportions of carbohydrate and protein (a high carbohydrate/low protein content beverage and a low-carbohydrate-high-protein beverage).

Need for Study

Endurance trained cyclists, in particular, are an athletic population that can benefit from enhanced recovery following exercise. Highly competitive cyclists often compete in multiple-day events (i.e. stage racing), in which augmented recovery may have a positive influence on performance. Thus, information regarding the effects of varying amounts of carbohydrate/protein in recovery beverages could provide useful information regarding the optimal macronutrient composition of beverages to be consumed by endurance athletes after heavy exercise.

It is known that carbohydrate supplementation following exercise can improve recovery. In addition, the peak rates of carbohydrate ingestion to maximize glycogen replenishment post-exercise are reasonably well understood (van Loon et al, 2000; Zawadzki et al, 1992; Tarnopolsky et al, 1997; Wilson et al, 2007; Howarth et al, 2008). However, there is minimal information regarding the optimal amount of protein to produce optimal recovery after aerobic exercise. Some evidence suggests the addition of protein has no effect on muscle soreness following exercise (Valentine et al, 2008; White et al, 2008), while other studies suggest that protein reduces muscle soreness (Flakoll et al, 2003; Luden et al, 2007; Millard-Stafford et al, 2005; Romano-Ely et al, 2006). Similarly, some studies support the addition of protein to attenuate CK levels following exercise (Luden et al, 2007; Romano-Ely et al, 2006; Rowlands et al, 2007; Saunders et al, 2009; Valentine et al, 2008), while other studies have shown no significant change in CK levels with the protein added to a carbohydrate supplement (Cepero et al, 2009; Millard-Stafford et al, 2005).

The impact that these responses have on subsequent exercise performance is also mixed. Some studies have shown that carbohydrate-protein co-ingestion results in improved subsequent performance (Rowlands et al, 2008; Saunders et al, 2004; Thomas et al, 2009), while others have not (Cermak et al, 2009; Rowlands et al, 2007; Betts et al, 2005; Cepero et al, 2009; Romano-Ely et al, 2006). Some of the varied findings between studies may be due to differing exercise protocols and differing amounts/compositions of the beverages utilized between studies. Thus, there is little evidence supporting an 'optimal' macronutrient composition of carbohydrate-protein beverages following heavy endurance exercise. The present study is designed to provide information regarding the effects of recovery beverages with varied proportions of carbohydrate/protein content, independent of caloric differences between beverages.

Hypotheses

1.) Subsequent exercise performance following ingestion of a low-carbohydrate, high-protein (LCHP beverage) will be significantly impaired versus the high-carbohydrate, low-protein (HCLP) and carbohydrate (CHO) beverage. However, markers of muscle damage (CK, soreness, muscle function) will not be different between these treatments..
2.) Ingestion of HCLP beverage will improve subsequent performance; improve muscle soreness, serum CK and muscle function compared to the LCHP and CHO beverages.

Assumptions

1.) It was assumed that all subjects performed a maximal effort during all isometric peak torque tests.
2.) Subjects were asked to treat the 20km time trial as if it were a race. It was assumed all subjects performed at maximal effort as if they were competing in a road race.
3.) Subjects were asked not to consume any food or beverages (other than water) during the recovery period. It was assumed that all subjects complied with this request.
4.) It was also assumed that subjects did not consume any food or beverages other than the predetermined breakfast before the glycogen depleting phase.

Limitations

1.) The subjects in this study were endurance-trained cyclists from James Madison University and the local area. Therefore the subjects may not be representative of all cyclists.
2.) Female subjects were excluded from this study due to the potential differences in substrate utilization between sexes. Thus, the findings may not be generalized to women.

Definition of Terms

1.) Muscle Soreness – measured using a visual analog scale of 100 mm. A rating of 0 was equivalent to no muscle soreness present, while a rating of 100 indicates impaired physical activity due to muscle soreness.
2.) Muscle Damage – measured indirectly with increased serum creatine kinase (CK) levels, muscle soreness ratings, and decreased muscle function with MVC.
3.) Mental/Physical Energy and Fatigue Ratings - series of four different analog scales measuring Mental Fatigue, Mental Energy, Physical Fatigue, and Physical Energy. The scales ranged from 0 to 300.
4.) Isometric Peak Torque (MVC) – the measurement of maximal force from a seated position using subjects right quadriceps muscle group.

Chapter II

Review of Literature

Cyclists and other endurance athletes frequently perform heavy aerobic exercise. Often these athletes have to compete on consecutive days or multiple times in a single day. Heavy endurance exercise can result in depleted glycogen (Costill et al, 1981; Ivy et al, 2002), increases in markers of muscle damage (Seifert et al, 2005; Millard-Stafford et al, 2005; Cepero et al, 2009; Skillen et al, 2008; Saunders et al, 2009; Luden et al, 2007; Rowlands et al, 2007), increased muscle soreness (Romano-Ely et al, 2006; Millard-Stafford et al, 2005) , and impaired performance in subsequent exercise (Skillen et al, 2008).

Carbohydrate replenishment has been used to promote recovery from heavy endurance exercise. Some studies have found that carbohydrate consumption improves muscle glycogen replenishment compared to beverages containing no carbohydrates (Tarnopolsky et al, 1997; Berardi et al, 2006; Wilson et al, 2007). Other studies have shown that an increase in carbohydrate consumption can further improve glycogen replenishment compared to a smaller sample of carbohydrates (van Loon et al, 2000). There is evidence that adequate carbohydrate supplementation can improve subsequent performance compared to no carbohydrate consumption (Achten et al, 2004; Halson et al, 2004).

Recently, the effects of carbohydrate and protein co-ingestion have been investigated, to determine if protein promotes further benefits with respect to muscle recovery and subsequent performance. The addition of protein to carbohydrate has been shown in some studies to improve glycogen synthesis rates (Table 1). However, other studies have not reported significant differences between carbohydrate and carbohydrate-protein treatments.

The ingestion of carbohydrate-protein beverages may also influence markers of muscle damage following heavy exercise. For example, consumption of carbohydrate-protein beverages may decrease muscle soreness compared to when carbohydrate-only beverages are consumed (Table 2). Similarly, a majority of studies show significantly lower post-exercise CK levels following carbohydrate-protein intake versus carbohydrate-only beverages (Table 3).

Carbohydrate-protein intake following endurance exercise may also increase protein synthesis (Table 4) and post-exercise insulin responses (Table 5), although some studies have not reported differences versus carbohydrate-only treatments. Furthermore, some studies have reported that carbohydrate-protein intake may influence blood lactate (Table 6) and glucose (Table 7) responses post-exercise.

As a potential result of the metabolic effects mentioned above, various studies have reported that carbohydrate-protein ingestion may enhance performance in subsequent exercise (Table 8). However, a similar number of studies have conversely reported that protein does not improve subsequent exercise performance (Table 9). The specific reasons for the discrepancies between these studies remain to be elucidated.

Table 1: Carbohydrate + protein and Muscle Glycogen

Article	Problem Studied	Participants	Procedure	Treatment	Findings
The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. (Costill et al, 1981)	Effect of the type, amount, and the frequency of feeding of carbohydrates on muscle glycogen resynthesis.	Ten trained male runners. 6 males in phase 1 group. 4 males in phase 2 group.	Subjects completed 16.1-km run followed by 3x 1 minute sprints.	4 CHO diets over the next 48 hrs. 70% CHO, 20% fat, 10% pro. Phase 2 consisted of meals gradually increasing in CHO%	Increasing feedings of CHO/per day did not increase muscle glycogen compared to high CHO ingestion within 2 meals.
Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. (Zawadzki et al, 1992)	Whether ingestion of a CHO-Pro supplement after endurance exercise would have a synergistic effect on plasma insulin and enhance rate of muscle glycogen storage compared to a CHO supplement.	Nine male cyclists. Avg VO2max = 66.6 ± 2.9 ml/kg/min	Subjects completed 3 separate muscle glycogen depleting rides of 2 hrs each. After exercise and 2hrs into recovery, subjects consumed 1 of 3 drinks (CHO, PRO, or CHO-Pro).	CHO (112g CHO); Pro (40.7g Pro); CHO-P (112g CHO, 40.7 Pro)	CHO and CHO-Pro beverages had a greater plasma glucose and insulin responses than the PRO. CHO-Pro also had muscle glycogen storage significantly faster than CHO.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. (Tarnopolsky et al, 1997)	Effect of isoenergetic CHO and CHO-Pro supplements on muscle glycogen resynthesis following 90 minutes of endurance exercise.	16 subjects. (8 males; 8 females) men minimum VO2peak = 55ml/kg/min Females = 50ml/kg/min	All subjects completed 90 minutes of endurance exercise at 65% VO2peak; following exercise subjects received 1 of 3 supplements; CHO (.75g/kg) + Pro (0.1g/kg) + Fat (.02g/kg), CHO (1 g/kg), or placebo.	CHO-Pro-Fat (0.75g-kg CHO, 0.1g-kg Pro, 0.02g-kg Fat); CHO (1kg-g), PLA (artificial sweetner)	The CHO-Pro-Fat and CHO supplement groups showed an increase in glycogen resynthesis compared to the placebo. No difference between genders.
Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. (van Loon et al, 2000)	Whether increased CHO intake, CHO + Pro hydrolysate and amino acids, or both result in higher postexercise muscle glycogen synthesis compared to CHO.	Eight trained male cyclists. (mean age: 24.0 ± 0.6 yrs)	Each subject completed 3 separate glycogen-depletion protocols. Subjects consumed one of the three (CHO, CHO-Pro, or CHO-CHO) beverages every 30 minutes.	CHO (0.8g-kg CHO), CHO-P (0.8g-kg CHO, 0.4g-kg Pro), CHO-CHO (1.2g-kg CHO)	Muscle glycogen synthesis was higher in subjects consuming CHO-Pro and CHO-CHO compared to the CHO drink.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. (Levenhagen et al, 2001)	The timing of postexercise nutrient ingestion affects whole body and leg protein dynamics.	Ten healthy adults. (5 males; 5 females)	Subjects were tested twice. First test was a supplement following 60 minutes of moderate exercise. Second, same supplement 3 hours after 60 minutes of moderate exercise.	Oral sample (10g Pro, 8g CHO, 3g fat)	Leg glucose uptake was significantly higher with supplement following exercise. Leg blood flow and circulating concentration of glucose, amino acids, and insulin were similar in both trials.
Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. (Jentjens et al, 2001)	Whether coingestion of an insulinotropic amino acid mixture and a high rate of CHO intake would increase the rate of muscle glycogen resynthesis.	Eight endurance trained male cyclist. (mean age 27.1±2.6 yrs)	Subjects cycled until exhaustion at certain workloads, they then consumed either a CHO or a CHO-Pro supplement.	CHO (1.2g-kg CHO); CHO-P (1.2g-kg CHO, 0.4g-kg Pro)	The addition of protein amino acid mixture with carbohydrates did not result in increased muscle glycogen synthesis.
Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. (Ivy et al, 2002)	A CHO-Pro supplement will enhance muscle glycogen storage after vigorous exercise. Compared to HCHO and LCHO.	Seven male cyclists.	Cycled on stationary bike for 2 hours at 65-75% of VO2 max. Following 2 hours, a series of 1 min sprints at max effort with 1 minute rest between sprints.	CHO *(80g CHO, 28g Pro, 6g Fat);* LCHO *(80g CHO, 6g Fat);* HCHO *(108g CHO, 6g Fat)*	The CHO-Pro supplement had significantly greater muscle glycogen storage after vigorous exercise compared to LCHO and HCHO.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effects of carbohydrate supplementation on performance and carbohydrate oxidation after intensified cycling training. (Halson et al, 2004)	Metabolic effects of a CHO supplement and the impact of CHO availability before, immediately after, and during recovery of cycling.	Six male endurance athletes (mean age 29.7±1.5 yrs)	Subjects completed two 4 week training periods. 1 week of normal training, 1 week of intense training, and 2 weeks of recovery. Subjects drank either a Low-CHO or High-CHO supplement.	HCHO (6% sol before and during trial; 20% sol at 1 h post); LCHO (2% sol, before, during, and 1 h post)	Both groups showed a decrease in muscle glycogen oxidation following the intense week of training.
Postexercise Muscle Glycogen Recovery Enhanced with a Carbohydrate-Protein Supplement. (Berardi et al, 2006).	Liquid CHO-Pro supplement will enhance muscle glycogen resynthesis during recovery compared to an isoenergetic liquid CHO supplement.	6 male cyclists. (mean age = 23.8 ± 3.5 yrs)	Subjects completed a 60min time trial. Following exercise they consumed 1 of 3 drinks (CHO-Pro, CHO, or Placebo) at 1h and 2h postex. 4hrs postex they were given a solid meal, 6hrs postex they completed the same time trial.	CHO-P (0.8g-kg CHO, 0.4g Pro, 4.8 kcal); CHO (1.2g-kg CHO, 4.8kcal); PLA (no energy)	The CHO-Pro supplement, following exercise, enhanced glycogen resynthesis relative to CHO and Placebo. Performance was not affected.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Glycogenin protein and mRNA expression in response to changing glycogen concentration in exercise and recovery. (Wilson et al, 2007)	High CHO ingestion would alter muscle glycogen submaximal resynthesis following submaximal exercise.	Ten healthy active males (mean age = 22.6 ± 0.8 yrs)	2 hrs prior to exercise subjects consumed a breakfast (55% CHO, 30% Fat, 15% pro). Subjects cycled at 65% VO2max until exhaustion. Trial 1 subjects consumed Gatorade after exercise and every hr after, for 5 hrs. Trial 2, consumed water.	CHO (1g-kg CHO); PLA	After 5 hrs of recovery, glycogen was resynthesis to about 60% of rest in the CHO trial, while it was unchanged in the water trial.
Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. (De Bock et al, 2008)	Comparing the effects of endurance training in a fasted state with a carbohydrate supplemented state on resting and exercise induced muscle gene, protein expression and energy substrate selection.	20 physically active men. (mean age 21.2 ± 0.4 yrs)	Subjects completed endurance training over a 6 wk period; 3 days a week for 1-2 hrs at 75% VO2peak. 10 subjects performed exercise a fasted state; 10 after CHO-intake. During exercise, subjects were fed 1g/kg/wt of CHO.	CHO state (standardized diet with additional CHO; 1g-kg-body wgt); F (fasted state, standard diet but no additional CHO)	Training in the fasted state did not result in an increase of fat oxidation with CHO intake. Glycogen breakdown was lower in fasted training state compared with CHO state.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. (Howarth et al, 2008)	Determine if ingesting protein and CHO during recovery from prolonged exercise would increase mixed skeletal muscle protein fractional synthetic rate (FSR) and improve whole body protein balance when compared to CHO.	6 healthy men; recreationally active, including running, cycling, weightlifting, and intramural sports.	Subjects completed 2 hrs of cycling followed by a 4 hr recovery period in which they were randomly given 1 of 3 drinks; high CHO (HCHO), low CHO (LCHO), and CHO + Pro (CHOP). Drinks were administered in 15 min intervals for the first 3 hrs of recovery.	LCHO (1.2g-kg CHO), Pro-CHO (1.2g-kg CHO, 0.4g-kg Pro), HCHO (1.6g-kg CHO)	CHOP did not enhance glycogen resynthesis during recovery.
Increased carbohydrate oxidation after ingesting carbohydrate with added protein. (Betts et al, 2008)	Examine metabolic impact of including protein in a post-exercise carbohydrate supplement when ingested in between running bouts.	Six healthy male subjects. (mean age = 22±1 yrs)	Each subject completed a 90 minute run at 70% VO2max followed by a 4 hr recovery period and then a 60 min run at same intensity. During recovery period, every 30 min subjects consumed either a CHO or a CHO-Pro solution.	CHO (0.8g-kg CHO); CHO-P (0.8g-kg CHO, 0.3g-kg whey protein)	There was no difference between groups with muscle glycogen resynthesis. Carbohydrate oxidation during the second run was significantly higher with the CHO-Pro treatment than with the CHO drink.

Table 2: Muscle Soreness

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Post-exercise protein supplementation improves health and muscle soreness during basic military training in marine recruits. (Flakoll et al, 2003)	The impact of post-exercise protein supplementation on muscle soreness and function during the stress of basic training.	387 healthy male subjects. Mean age = 18.9 ± 0.1 yrs.	Subjects were divided into 3 groups. Group 1 (n=128) received a placebo tablet; group 2 (n=129) received CHO + Fat (CON); and group 3 (n=130) received same amount of fat, CHO but the addition of Protein (Pro). All subjects completed the same workouts for 54 days. Muscle soreness was measure on a scale 1-10.	PLA *(0g, 0g, 0g)*, control *(8g CHO, 0g Pro, 3g Fat)*, supplement *(8g CHO, 10g Pro, 3g Fat)*	PRO group had 33% fewer medical visits, 37% fewer due to muscle/joint problems; 83% fewer due to heat exhaustion. Muscle soreness immediately postexercise was reduced in PRO compared to PLA and CON. Protein supplementation may impact health, muscle soreness, and hydration during exercise.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Recovery from Run Training: Efficiency of a Carbohydrate-Protein beverage? (Millard-Stafford et al, 2005)	To determine if added protein or increased carbohydrate affects performance.	8 runners; (mean VO2max = 56.5 ± 5.9 ml/kg/min)	Subjects completed a 21km run followed by a run to fatigue (RTF) at 90% VO2max, 2 hr recovery and a 2nd RTF at 90% VO2max. A 5km trial was completed 24 hrs post initial exercise.	CHO6 (6% CHO); CHO-P (8% CHO, 2% Pro); CHO10 (10% CHO)	Perceived muscle soreness was lower with CHO-P than with CHO10.
Effect of an Isocaloric Carbohydrate-Protein-antioxidant Drink on Cycling Performance. (Romano-Ely et al, 2006)	Does CHOPA (CHO-Protein with Vit C and E) alter time to fatigue during prolonged exercise compared to an isocaloric CHO drink?	Fourteen male volunteers. Ages 18-33 yrs.	Subjects comp 2 phases of 2 rides to fatigue at 70% and 80% VO2 peak. 22-24 hrs btw ride 1 and 2. 6-14 days btw phases.	CHO-PA (7.5% CHO + 1.8% Pro); CHO-only (9.3% CHO)	Baseline values were similar between groups. Muscle soreness ratings were significantly higher with CHO compared to CHO-PA.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Postexercise Carbohydrate-Protein-Antioxidant Ingestion Decreases Plasma Creatine Kinase and Muscle Soreness. (Luden et al, 2007)	Compare effects of 6 days of post-exercise CHO and CHOPA beverage on muscle damage, soreness, and subsequent performance.	36 NCAA D-1 cross country runners. Data from 23 (11 male, 12 female) were used.	Two 6 day intervention periods. First intervention subjects completed normal training, consuming CHO or CHOPA 30 min after training. Second intervention subjects consumed the other drink following exercise.	CHO *(1.46g-kg CHO);* CHO-P-AA *(1.46g-kg CHO, 0.365g-kg whey-protein)*	5 days of post-exercise CHOPA supplementation significantly attenuated muscle soreness compared to CHO.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of Protein-Rich Feeding on Recovery After Intense Exercise. (Rowlands et al, 2007)	Determine the effect of enriching the protein content of a carbohydrate-rich food on muscle damage, soreness, and performance.	10 male cyclists; men VO2max = 64.5 ± 9.3 ml/kg/min	Subjects cycled 12 min warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Feedings were 4h post. 10 power sprints performed next day.	Protein-enriched (418g Pro, 435g CHO, 79g Fat); control (34g Pro, 640g CHO, 79g Fat)	Protein-enriched diet reduced tiredness and leg-soreness.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Influence of Carbohydrate-Protein Beverage on Cycling Endurance and Indices of Muscle Disruption. (Valentine et al, 2008)	Effects of CHO-Pro on time to exhaustion and markers of muscle disruption compared with placebo (PLA), CHO, and CHO-CHO.	11 men between 18-26 yrs. VO2peak > 45 ml/kg/min	Each subject completed 4 rides to exhaustion at 75% VO2peak. Every 15 minutes, subjects consumed either PLA, CHO, CHO-CHO, or CHO+Pro.	CHO (7.75g* 100ml CHO, 0 g* 100ml Pro, 0.3g Fat); CHO-CHO (9.69 g* 100ml CHO, 0 g* 100ml Pro, 0.30 g* 100ml Fat); CHO-P (7.75 g* 100ml CHO, 0.30 g* 100ml Fat, 1.94 g* 100ml Pro), PLA	There was no significant difference with muscle soreness ratings between groups.
Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage. (White et al, 2008)	Whether timing of a supplement would have an effect on muscle damage, function and soreness.	27 untrained men, (21±3 yrs)	Two beverages; CHO-Pro and a placebo. Subjects were split into 3 groups; 1- CHO-Pro pre-exercise and placebo post, 2- placebo pre-ex and CHO-Pro post-ex, 3- pla both pre- and post-exercise. Subjects completed a MVC followed by a 15 min ex.	CHO-Pro (75g CHO, 23g whey protein, mixed with 300ml water); Placebo.	Timing of CHO-Pro supplement had no effect on muscle damage, loss of strength, and soreness.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Carbohydrate and Protein Hydrolysate Coingestion's Improvement of Late-Exercise Time-Trial Performance. (Saunders et al, 2009)	Whether a CHO with Casein Hydrolysate (CHO+ProH) beverage will improve time-trial performance compared to a CHO beverage.	13 male cyclists, mean VO2peak = 60.8 ± 1.6 ml/kg/min	Subjects completed two 60km time trials; consuming either a CHO-ProH or a CHO beverage every 5km. beverages contained an equal amount of CHO (6%) with the CHO-ProH containing 14.4g/200ml of protein.	CHO (6% CHO), CHO-P (6% CHO, 1.8% Pro)	Muscle soreness was not significantly different between trials but did increase in the CHO trial compared to the CHO-P trial.

Table 3: Creatine Kinase

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Muscle Damage, Fluid Ingestion and Energy Supplementation During Recreational Alpine Skiing. (Seifert et al, 2005)	To examine fluid ingestion and energy supplementation on indices of muscle damage.	18 alpine skiers(9 male; 9 female)	Subjects consumed a CHO-P drink, a Placebo, or No Fluid (NF) during and after skiing. Skiing took place on 1 of 6 selected slopes, ranging from 2469 to 3026m above sea level.	CHO-P *(360ml contained 21g CHO; 5g Pro)*; PLA *(no CHO or Pro)*	Creatine Kinase was maintained in the CHO-P group 2H post-skiing, compared to the PLA and NF. Results showed that ingestion of a CHO-Protein beverage can reduce muscle damage indices.
Recovery from Run Training: Efficiency of a Carbohydrate-Protein beverage? (Millard-Stafford et al, 2005)	To determine if added protein or increased carbohydrate affects performance.	8 runners; (mean VO2max = 56.5 ± 5.9 ml/kg/min)	Subjects completed a 21km run followed by a run to fatigue (RTF) at 90% VO2max, 2 hr recovery and a 2nd RTF at 90% VO2max. A 5km trial was completed 24 hrs post initial exercise.	CHO6 *(6% CHO);* CHO-P *(8% CHO, 2% Pro);* CHO10 (*10% CHO)*	CK levels were not significantly different among groups.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of an Isocaloric Carbohydrate-Protein-antioxidant Drink on Cycling Performance. (Romano-Ely et al, 2006)	Does CHOPA (CHO-Protein with Vit C and E) alter time to fatigue during prolonged exercise compared to an isocaloric CHO drink.	Fourteen male volunteers. Ages 18-33 yrs.	Subjects comp 2 phases of 2 rides to fatigue at 70% and 80% VO2 peak. 22-24 hrs btw ride 1 and 2. 6-14 days btw phases.	CHO-PA (7.5% CHO + 1.8% Pro); CHO-only (9.3% CHO)	CK values were elevated post-exercise and 24h-post in the CHO group, but not in CHO-Pro. Baseline values were similar between groups.
Postexercise Carbohydrate-Protein-Antioxidant Ingestion Decreases Plasma Creatine Kinase and Muscle Soreness. (Luden et al, 2007)	Compare effects of 6 days of post-exercise CHO and CHOPA beverage on muscle damage, soreness, and subsequent performance.	36 NCAA D-1 cross country runners. Data from 23 (11 male, 12 female) were used.	Two 6 day intervention periods. First intervention subjects completed normal training, consuming CHO or CHOPA 30 min after training. Second intervention subjects consumed the other drink following exercise.	CHO (1.46g-kg CHO); CHO-P-AA (1.46g-kg CHO, 0.365g-kg whey-protein)	5 days of post-exercise CHOPA supplementation significantly attenuated plasma CK compared to CHO.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. (Rowlands et al, 2007)	Determine the effect of post-exercise protein content imposed over a high-carbohydrate background on subsequent exercise.	12 male cyclists; mean VO2max 4.9 ± 0.6 L/min	Subjects cycled for a 12 minute warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Sprint power tests performed 15 h post and 60 h post.	Protein-enriched (1.4g-kg CHO, 0.7g-kg Pro, 0.26g-kg Fat); Control (2.1g-kg CHO, 0.1g-kg Pro, 0.26g-kg Fat)	CK activity was significantly reduced prior to exercise at 60h-post.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of Protein-Rich Feeding on Recovery After Intense Exercise. (Rowlands et al, 2007)	Determine the effect of enriching the protein content of a carbohydrate-rich food on muscle damage, soreness, and performance.	10 male cyclists; men VO2max = 64.5 ± 9.3 ml/kg/min	Subjects cycled 12 min warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min rec was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Feedings were 4h post. 10 power sprints performed next day.	Protein-enriched (418g Pro, 435g CHO, 79g Fat); control (34g Pro, 640g CHO, 79g Fat)	Protein-enriched group reduced CK levels by 33% on next day.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Influence of Carbohydrate-Protein Beverage on Cycling Endurance and Indices of Muscle Disruption. (Valentine et al, 2008)	Effects of CHO-Pro on time to exhaustion and markers of muscle disruption compared with placebo (PLA), CHO, and CHO-CHO.	11 men between 18-26 yrs. VO2peak > 45 ml/kg/min	Each subject completed 4 rides to exhaustion at 75% VO2peak. Every 15 minutes, subjects consumed either PLA, CHO, CHO-CHO, or CHO+Pro.	CHO *(7.75g* 100ml CHO, 0 g* 100ml Pro, 0.3g Fat);* CHO-CHO *(9.69 g* 100ml CHO, 0 g* 100ml Pro, 0.30 g* 100ml Fat);* CHO-P *(7.75 g* 100ml CHO, 0.30 g* 100ml Fat, 1.94 g* 100ml Pro),* PLA	CK levels were lower in the CHO-Pro group post-exercise compared to the other trials. CK levels were significantly higher with PLA, CHO, and CHO-CHO but not with CHO-Pro.

Article	**Problem Studied**	**Participants**	**Procedure**	**Treatment**	**Findings**
Carbohydrate and Protein Hydrolysate Coingestion's Improvement of Late-Exercise Time-Trial Performance. (Saunders et al, 2009)	Whether a CHO with Casein Hydrolysate (CHO+ProH) beverage will improve time-trial performance compared to a CHO beverage.	13 male cyclists, mean VO2peak = 60.8 ± 1.6 ml/kg/min	Subjects completed two 60km time trials; CHO-ProH or a CHO beverage every 5km. Beverages contained an equal amount of CHO (6%) with the CHO-ProH containing 14.4g/200ml of protein.	CHO *(6% CHO)*, CHO-ProH *(6% CHO, 1.8% Pro)*	CHO-ProH prevented plasma CK increase.
Muscle Metabolism during exercise with Carbohydrate or Protein-Carbohydrate ingestion. (Cermak et al, 2009)	Testing a CHO verse CHO-PRO ingestion and its affect on muscle energy metabolism as by way of glycogen catabolism, increase muscle pool of TCAI and reduced phosphocreatine.	Eight males (mean age 29±3 yrs). All subjects had background in road cycling, triathlon, or duathlon.	Subjects completed heavy intervals for approximately 90 min on a cycle ergometer. Beverages were consumed every 15 min during exercise. 24 hr post intervals, subjects completed a 20km time trial.	CHO *(6% CHO);* CHO-PRO *(6% CHO + 2% PRO)*	Plasma CK levels were not significantly different between groups.

Table 4: Protein Synthesis

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. (Koopman et al, 2004)	Determine whole body protein synthesis and degradation rates at rest, during exercise and recovery.	Eight well trained males. (mean age 31 ± 3 yrs)	Each subject completed 6 hrs of exercise (2.5 hrs of cycling, 1 hr running, 2.5 hrs of cycling) @ 50% VO2max. Subjects consumed CHO drink or CHO-Pro every 30 minutes.	CHO *(0.7g-kg CHO);* CHO-P *(0.7g-kg CHO, 0.25g-kg Pro)*	The CHO-Pro supplement improved protein balance at rest, exercise and during recovery compared to CHO alone.
Milk Ingestion Stimulates Net Muscle Protein Synthesis following Resistance Exercise. (Elliot et al, 2006)	Designed to determine the response of net muscle protein balance following resistance exercise.	24 subjects (16 males; 8 females)	The beverages were consumed 1 hr after leg resistance exercise.	Three different milk drinks were used, 237g fat free; 237g whole; 393g fat-free-isocaloric with WM.	Threonine uptake relative to ingest was significantly higher in whole milk than fat-free milk.
Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. (Howarth et al, 2008)	Determine if ingesting Pro and CHO during recovery from prolonged exercise increase mixed skeletal muscle pro fractional synthetic rate (FSR)	6 healthy men; recreationally active, including running, cycling, weightlifting, and intramural sports.	Subjects completed 2 hrs of cycling followed by a 4 hr recovery period randomly given 1 of 3 drinks; high CHO (HCHO), (LCHO), (CHOP). Drinks were in 15 min intervals 3 hr	LCHO (1.2g-kg CHO), Pro-CHO (1.2g-kg CHO, 0.4g-kg Pro), HCHO (1.6g-kg CHO)	Subjects that consumed Pro-CHO beverage had a positive whole body net protein balance (WBNB) and a reduced rate of protein breakdown.

Table 5: Serum Insulin

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. (van Loon et al, 2000)	Whether increased CHO intake, CHO + Pro hydrolysate and amino acids, or both result in higher postexercise muscle glycogen synthesis compared to CHO.	Eight trained male cyclists. (mean age: 24.0 ± 0.6 yrs)	Each subject completed 3 separate glycogen-depletion protocols. Subjects consumed one of the three (CHO, CHO-Pro, or CHO-CHO) beverages every 30 minutes.	CHO (0.8g-kg CHO), CHO-P (0.8g-kg CHO, 0.4g-kg Pro), CHO-CHO (1.2g-kg CHO)	Insulin levels increased in the first 2h post-ex. Levels in CHO-P trial decreased during the last hour. Insulin levels in the CHO and CHO-P were significantly higher than control group.
Early post-exercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. (Ivy et al, 2002)	A CHO-Pro supplement will enhance muscle glycogen storage after vigorous exercise. Compared to HCHO and LCHO.	Seven male cyclists.	Cycled on bike for 2 h at 65-75% of VO2 max. Following 2 hours, a series of 1 min sprints at max effort with 1 min rest between sprints.	CHO (80g CHO, 28g Pro, 6g Fat); LCHO (80g CHO, 6g Fat); HCHO (108g CHO, 6g Fat)	Insulin levels were not significantly different among groups.
Recovery from Run Training: Efficiency of a Carbohydrate-Protein beverage? (Millard-Stafford et al, 2005)	To determine if added protein or increased carbohydrate affects performance.	8 runners; (mean VO2max = 56.5 ± 5.9 ml/kg/min)	21km run followed by a run to fatigue at 90% VO2max, 2 hr recovery and a 2nd RTF at 90% VO2max. A 5km trial completed 24 hrs post	CHO6 (6% CHO); CHO-P (8% CHO, 2% Pro); CHO10 (10% CHO)	Insulin levels with CHO10 were higher compared to CHO-P and CHO6.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of Protein-Rich Feeding on Recovery After Intense Exercise. (Rowlands et al, 2007)	Determine the effect of enriching the protein content of a carbohydrate-rich food on muscle damage, soreness, and performance.	10 male cyclists; men VO2max = 64.5 ± 9.3 ml/kg/min	Subjects cycled for a 12 minute warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Feedings were 4h post. 10 power sprints.	Protein-enriched (418g Pro, 435g CHO, 79g Fat); control (34g Pro, 640g CHO, 79g Fat)	No clear effect on plasma insulin.
Effects of a CHO and a Carbohydrate and Casein Protein Beverages on recovery and performance of endurance cycling capacity. (Cepero et al, 2009)	Determine whether short-term exercise recovery, muscle damage, and cycling performance altered after consuming either CHO-only or a CHO-P	15 male cyclists (vo2peak = 63.4 ± 9.6 ml/kg/min)	Each subject consumed 1 liter of the beverage after 1 hour of cycling at 75% VO2peak. After 2 hrs of recovery, subjects completed a 20km trial.	CHO-only (7% CHO); CHO-P (7% CHO, 4% Pro)	Serum insulin concentration was higher during recovery with CHO-P.

Table 6: Lactic Acid

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. (Rowlands et al, 2007)	Determine the effect of post-exercise protein content imposed over a high-carbohydrate background on subsequent exercise.	12 male cyclists; mean VO2max 4.9 ± 0.6 L/min	Subjects cycled for a 12 minute warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Sprint power tests performed 15 h post and 60 h post.	Protein-enriched *(1.4g-kg CHO, 0.7g-kg Pro, 0.26g-kg Fat);* Control *(2.1g-kg CHO, 0.1g-kg Pro, 0.26g-kg Fat)*	Lactate concentration was not significantly different between groups on days 1 and 2 of recovery. During exercise on day 2 (15h-post), lactate was significantly higher (15%) in control group.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Muscle Metabolism during exercise with Carbohydrate or Protein-Carbohydrate ingestion. (Cermak et al, 2009)	Testing a CHO verse a CHO-PRO ingestion and its affect on muscle energy metabolism as by way of glycogen catabolism, increase muscle pool of TCAI and reduced phosphocreatine.	Eight males (mean age 29±3 yrs). All subjects had background in road cycling, triathlon, or duathlon.	Subjects completed heavy intervals for approximately 90 min on a cycle ergometer. Beverages were consumed every 15 min during exercise. 24 hr post intervals, subjects completed a 20km time trial.	CHO (6% CHO); CHO-PRO (6% CHO + 2% PRO)	There was no significant change in lactate levels between trials.
Effects of a Carbohydrate and a Carbohydrate and Casein Protein Beverages on recovery and performance of endurance cycling capacity. (Cepero et al, 2009)	Determine whether short-term exercise recovery, muscle damage, and cycling performance were altered after consuming either CHO-only or a CHO-P beverage.	15 male cyclists (vo2peak = 63.4 ± 9.6 ml/kg/min)	Each subject consumed 1 liter of the beverage after 1 hour of cycling at 75% VO2peak. After 2 hrs of recovery, subjects completed a 20km time trial.	CHO-only *(7% CHO);* CHO-P *(7% CHO, 4% Pro)*	Lactic Acid levels were higher with CHO than with CHO-P following 20km trial.

Table 7: Glucose synthesis

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Recovery from Run Training: Efficiency of a Carbohydrate-Protein beverage? (Millard-Stafford et al, 2005)	To determine if added protein or increased carbohydrate affects performance.	8 runners; (mean VO2max = 56.5 ± 5.9 ml/kg/min)	Subjects completed a 21km run followed by a run to fatigue (RTF) at 90% VO2max, 2 hr recovery and a 2nd RTF at 90% VO2max. A 5km trial was completed 24 hrs post initial exercise.	CHO6 (6% CHO); CHO-P (8% CHO, 2% Pro); CHO10 (10% CHO)	Blood glucose levels were higher with CHO10 compared to CHO-P and CHO6.
Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. (Rowlands et al, 2007)	Determine the effect of post-exercise protein content imposed over a high-carbohydrate background on subsequent exercise.	12 male cyclists; mean VO2max 4.9 ± 0.6 L/min	Subjects cycled 12 min warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. Inters 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min rec 50%); finished workout w 3 x 5 min inters at 70% (rec 50%). Sprint pwr tests 15 h post and 60 h post.	Protein-enriched (1.4g-kg CHO, 0.7g-kg Pro, 0.26g-kg Fat); Control (2.1g-kg CHO, 0.1g-kg Pro, 0.26g-kg Fat)	Glucose concentration was reduced by 15% at day 1 of recovery with Pro-enriched. Glucose had a 24% decrease at day 2 of recovery.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Muscle Metabolism during exercise with Carbohydrate or Protein-Carbohydrate ingestion. (Cermak et al, 2009)	Testing a CHO verse CHO-PRO ingestion and its affect on muscle energy metabolism as by way of glycogen catabolism, increase muscle pool of TCAI and reduced phosphocreatine.	Eight males (mean age 29±3 yrs). All subjects had background in road cycling, triathlon, or duathlon.	Subjects completed heavy intervals for approximately 90 min on a cycle ergometer. Beverages were consumed every 15 min during exercise. 24 hr post intervals, subjects completed a 20km time trial.	CHO (6% CHO); CHO-PRO (6% CHO + 2% PRO)	There was no significant change in glucose levels between trials.
Effects of a Carbohydrate and a Carbohydrate and Casein Protein Beverages on recovery and performance of endurance cycling capacity. (Cepero et al, 2009)	Determine whether short-term exercise recovery, muscle damage, and cycling performance were altered after consuming either CHO-only or a CHO-P beverage.	15 male cyclists (vo2peak = 63.4 ± 9.6 ml/kg/min)	Each subject consumed 1 liter of the beverage after 1 hour of cycling at 75% VO2peak. After 2 hrs of recovery, subjects completed a 20km time trial.	CHO-only (7% CHO); CHO-P (7% CHO, 4% Pro)	Glucagon levels were higher with CHO than CHO-P following the 20km trial.

Table 8: Carbohydrate + Protein and improved subsequent performance

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effects of a carbohydrate-protein beverage on Cycling endurance and muscle damage. (Saunders et al, 2004)	Endurance cycling performance and postexercise muscle damage were altered when consuming a carbohydrate and protein beverage.	15 male cyclists. (mean VO2peak = 52.6 ± 10.3 ml/kg/min)	First trial subjects rode on a cycle ergometer at 75% VO2peak until exhaustion. Second trial, at 85% VO2peak. Every 15min during exercise subjects consumed 1 of 2 drinks, CHO-only or CHO-Pro.	CHO-only (7.3% CHO); CHO-P (7.3% CHO, 1.8% Pro)	The CHO-Pro showed a significant decrease in time to fatigue when compared to the CHO drink alone.
Chocolate Milk as a Post-Exercise Recovery Aid. (Karp et al, 2006)	Test the efficacy of chocolate milk as a recovery aid after glycogen-depleting exercise when compared to a fluid replacement and a CHO beverage.	9 male trained cyclists. Mean VO2max = 65.0 ml/kg/min	Subjects completed interval workout followed by a 4 hr recovery and a trial to exhaustion at 70% VO2max.	CM (70g CHO, 19.1g Pro, 5.3g Fat, 381.8 kcal); FR (29.7g CHO, 0g Pro, 0g Fat, 106.1 kcals); CR (70g CHO, 18.5g Pro, 1.5g Fat, 381 kcals)	Time to Exhaustion was greater with the CM and fluid replacement drink compared to the CHO drink.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
The influence of carbohydrate and protein ingestion during recovery from prolonged exercise on subsequent endurance performance. (Betts et al, 2007)	The addition of protein to a CHO drink may improve subsequent exercise capacity relative to the CHO drink but not more effective than the isoenergetic amount of CHO.	Six males. (mean age 21 yrs.) at least 6 hrs of endurance running a week.	Subjects completed a 90 min treadmill run at 70% of VO2max, followed by a 4 hr recovery and then a second run to exhaustion at 70% VO2max. During recovery, one of three drinks were consumed every 30 minutes. CHO-Pro, CHO, or CHO-CHO.	CHO-P *(CHO-0.8g-kg, whey Pro-0.3g-kg);* CHO-CHO *(1.1g-kg CHO)*	Exercise capacity during run 2 was greater when CHO-Pro and CHO-CHO drinks were consumed compared to the CHO. There was no difference between the CHO-Pro and the CHO-CHO.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. (Rowlands et al, 2007)	Determine the effect of post-exercise protein content imposed over a high-carbohydrate background on subsequent exercise.	12 male cyclists; mean VO2max 4.9 ± 0.6 L/min	Subjects cycled for a 12 minute warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. The intervals consisted of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery was at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Sprint power tests performed 15 h post and 60 h post.	Protein-enriched *(1.4g-kg CHO, 0.7g-kg Pro, 0.26g-kg Fat);* Control *(2.1g-kg CHO, 0.1g-kg Pro, 0.26g-kg Fat)*	Sprint Power was 4.1% higher on day 4 (60h post). Not significantly different on day 2 (15h post).

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Recovery from a cycling time trial is enhanced with CHO-Pro supplementation vs. isoenergetic CHO supplementation. (Berardi et al, 2008)	Whether a CHO-Pro supplement during recovery following time trial would result in improved time compared to a CHO supplement.	15 trained male cyclists.	Subjects completed a 60 min time trial in the AM. Subjects consumed either CHO-Pro or CHO drink at 10, 60, and 120 minutes following ride. 6 hrs post AM ride, subjects repeated time trial.	CHO-P *(4.8 kcal-kg, 0.8g-kg CHO, 0.4 g-kg P);* CHO *(4.8 kcal-kg, 1.2 g-kg CHO);* PLB *(no energy)*	All subjects had reduction in performance in the second time trial. The CHO group had a much greater decrease in performance and power in the second trial compared to the CHO-Pro.
Effects of an Amino Acid-Carbohydrate Drink on Exercise Performance After Consecutive-Day Exercise Bouts. (Skillen et al, 2008)	Effect of amino acids in a CHO beverage on cycling performance. Specifically reducing muscle damage and fatigue.	12 male athletes (mean age = 28.5 ± 2.1 yrs); VO2peak > 50 ml/kg/min	Subjects cycled for 90 minutes at 75% VO2peak, followed by a ride to exhaustion at 85%. 2 weeks later, subjects completed 2 more rides to exhaustion on consecutive days. Consuming either 3.6% CHO + 1% amino acid (AA); or 4.6% CHO-only (CHO).	AA (3.6% CHO, 1.0% amino acids); CHO (4.6% CHO)	AA added to a CHO beverage maintained performance compared with the CHO beverage.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sport drinks. (Thomas et al, 2009)	Effect of 3 recovery drinks on endurance performance following glycogen-depleting exercise.	9 trained male cyclists.	Each subject performed 3 trials, glycogen depleting trial, 4-h recovery, cycle to exhaustion at 70% power at VOmax. At hours 0 and 2 in the recovery, subjects consumed one of three drinks; chocolate milk, CHO drink, or a fluid replacement drink.	Choc Milk (CHO-62.9 g, Pro-14.2 g, Fat- 9.2 g, Na-312.2mg); Fluid Repl. (CHO-30.7g, Pro-0g, Fat- 0g, Na-242.2mg); CHO-replace (CHO-72.5g, Pro-18.9g, Fat-1.6g, Na-321mg)	Subjects cycled 51% and 43% longer when ingesting Chocolate Milk (CM) than the CHO drink or the fluid replacement. Despite similar caloric intake in the CHO and CM, CM subjects cycled longer.

Table 9: Carbohydrate + Protein and no improvement in subsequent performance

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Recovery from Run Training: Efficiency of a Carbohydrate-Protein beverage? (Millard-Stafford et al, 2005)	To determine if added protein or increased carbohydrate affects performance.	8 runners; (mean VO2max = 56.5 ± 5.9 ml/kg/min)	Subjects completed a 21km run followed by a run to fatigue (RTF) at 90% VO2max, 2 hr recovery and a 2nd RTF at 90% VO2max. A 5km trial was completed 24 hrs post initial exercise.	CHO6 *(6% CHO);* CHO-P *(8% CHO, 2% Pro);* CHO10 (*10% CHO)*	There was no significant difference in performance between groups with RTF or 5km trial.
Recovery of Endurance Running Capacity: Effect of Carbohydrate-Protein Mixtures. (Betts et al, 2005)	Determine ingestion of a carbohydrate-protein mixture during recovery from prolonged exercise results in greater recovery than a carbohydrate-only supplement.	16 recreationally active males.	Subjects performed two trials. Each trial consisted of 90 min treadmill run followed by 4 h recovery. Supplements were consumed during recovery. After recovery, subjects ran to exhaustion at 85% VO2max.	CHO-P *(9.3% CHO + 1.5% Pro)*; CHO *(9.3% CHO)*	There was no significant difference in run to exhaustion times between groups.

Article	Problem Studied	Participants	Procedure	Treatment	Findings
Effect of an Isocaloric Carbohydrate-Protein-antioxidant Drink on Cycling Performance. (Romano-Ely et al, 2006)	Does CHOPA (CHO-Protein with Vit C and E) alter time to fatigue during prolonged exercise compared to an isocaloric CHO drink.	Fourteen male volunteers. Ages 18-33 yrs.	Subjects completed 2 phases of 2 rides to fatigue at 70% and 80% $VO_{2\ peak}$. 22-24 hours between ride 1 and 2. 6-14 days between phases.	CHO-PA (*7.5% CHO + 1.8% Pro*); CHO-only (*9.3% CHO*)	Time to Fatigue was not different between treatments.
Effect of Protein-Rich Feeding on Recovery After Intense Exercise. (Rowlands et al, 2007)	Determine the effect of enriching the protein content of a carbohydrate-rich food on muscle damage, soreness, and performance.	10 male cyclists; men VO2max = 64.5 ± 9.3 ml/kg/min	Subjects cycled 12 min warm-up at 30% Wmax, 5min at 40%, and 5 min at 50%. Intervals of 10 x 2 min at 90% Wmax, 12 x 2 at 80% Wmax, (2 min recovery at 50%); finished workout with 3 x 5 min intervals at 70% (recovery at 50%). Feedings 4h post. 10 power sprints performed next day.	Protein-enriched *(418g Pro, 435g CHO, 79g Fat);* control *(34g Pro, 640g CHO, 79g Fat)*	No clear effect on next day performance.

Article	**Problem Studied**	**Participants**	**Procedure**	**Treatment**	**Findings**
Muscle Metabolism during exercise with Carbohydrate or Protein-Carbohydrate ingestion. (Cermak et al, 2009)	Testing a CHO verse a CHO-PRO ingestion and its affect on muscle energy metabolism as by way of glycogen catabolism, increase muscle pool of TCAI and reduced phosphocreatine.	Eight males (mean age 29±3 yrs). All subjects had background in road cycling, triathlon, or duathlon.	Subjects completed heavy intervals for approximately 90 min on a cycle ergometer. Beverages were consumed every 15 min during exercise. 24 hr post intervals, subjects completed a 20km time trial.	CHO (6% CHO); CHO-PRO (6% CHO + 2% PRO)	There was no significant change in time trial performance between groups.
Effects of a CHO and a CHO and Casein Protein Beverages on recovery and performance of endurance cycling capacity. (Cepero et al, 2009)	Determine whether short-term exercise recovery, muscle damage, and cycling performance were altered after consuming either CHO-only or a CHO-P beverage.	15 male cyclists (vo2peak = 63.4 ± 9.6 ml/kg/min)	Each subject consumed 1 liter of the beverage after 1 hour of cycling at 75% VO2peak. After 2 hrs of recovery, subjects completed a 20km time trial.	CHO-only (7% CHO); CHO-P (7% CHO, 4% Pro)	No significant difference with 20km ride between groups.

Chapter III

Methods

Subjects

Fifteen endurance-trained male cyclists (18-45 yrs) were recruited from James Madison University and the surrounding area. Inclusion criteria included a minimum of three days a week of cycling for the past 2 months and a peak aerobic capacity (VO_2peak) greater than 45 ml/kg/min. All subjects received a written consent form that described the risks and benefits associated with the study. After a description of the protocols, subjects signed the written consent form. James Madison University Institutional Review Board approved the experiment design before data collection began.

Procedures

The study design consisted of a pre-testing/screening phase, familiarization trial, and three treatment trials.

Pre-testing Phase

All potential subjects that met the study criteria completed the following assessments.

Body Mass and Height

Subjects had their body weight measured to the nearest 0.1kg and height measured to the nearest 0.5cm.

VO_{2peak}/W_{max}

Subjects completed an exercise test to determine their peak oxygen uptake (VO_{2peak}) and associated power output (W_{max}). The test began with subjects riding a cycle ergometer at a self-selected pace, described as a "comfortable but not easy pace for an hour long ride." After subjects selected their initial workload, power output was increased by 25 W every 2 minutes until the subject voluntary requested to stop the test due to fatigue. Peak oxygen uptake and power output were measured during the test to determine if subjects met entry criteria for the study, and to establish intensities for subsequent exercise protocols.

Familiarization Trial

All subjects that met the inclusion criteria performed a familiarization trial within two weeks of the pre-testing assessment. The purpose of the familiarization trial was to determine if the exercise intensities were appropriate for each subject. The familiarization trial also allowed subjects to become adjusted to the protocol and learn an appropriate pace for the time-trial segment. Subjects were tested prior to the treatment phase to ensure the subjects could complete the treatment trial. Another purpose of the familiarization trial was to minimize any effects of learning and the repeated-bout effect, in order to reduce error variance between treatment trials. All subjects received the CHO recovery beverage during the familiarization trial (described below).

Treatment Trials

Treatment trials were similar to the familiarization trial with the addition of the supplementation treatment provided during the trial. The treatment phase consisted of a glycogen-depleting exercise, a recovery period and a subsequent time trial. Repeated treatment trials were completed within 7-14 days. Figure 1 shows a timeline of a trial.

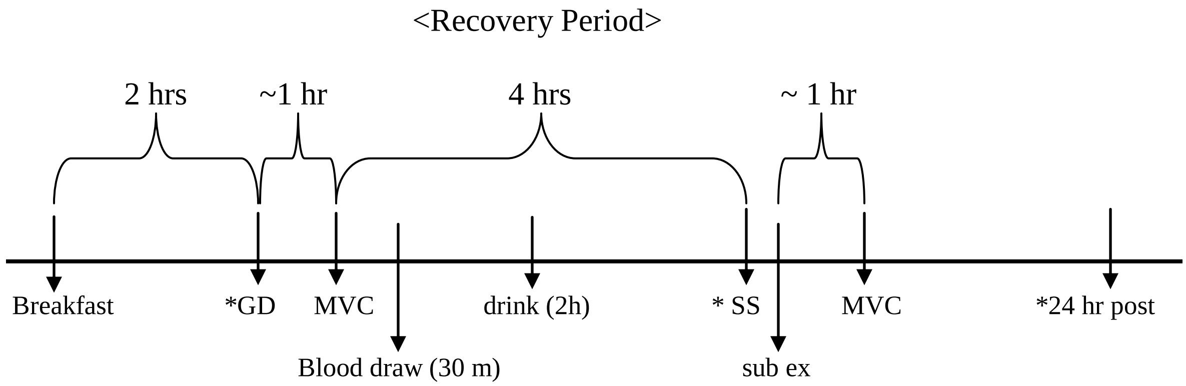

Figure 1: Time line of a trial. * *ME (mental energy), MF (mental fatigue), PE (physical energy), PF (physical fatigue), MS (muscle soreness), and MVC (isometric peak torque) were all measured at these time points. SS = Steady State. Blood draw was also taken 24 hr post.*

Glycogen-depleting exercise

Subjects performed a warm-up/steady state period that consisted of a 10 minute period at 60% W_{max}. After the initial 10 minutes of exercise, subjects completed a series of high-intensity intervals. The high-intensity intervals consisted of 2 minutes at 95% W_{max} followed by 2 minutes at 50% W_{max}. Subjects continued the high-intensity intervals until they were unable to maintain a cadence of 70rpm during the intervals at 95% W_{max}. Once the cadence dropped below 70rpm, the workloads for the high-intensity intervals were decreased to 85% W_{max} until 70rpm could not be maintained. At this point, workload for the high-intensity intervals was further decreased to 75% W_{max}. The test was terminated when subjects failed to maintain a cadence of 70 rpm at this intensity. This protocol was designed to induce muscle glycogen depletion and moderate fatigue. Based on similar findings from Karp et al (2006) and Van Loon et al (2000), the duration of the test were intended to last approximately 50-75 minutes, with 30-45 minutes of high-intensity intervals. All subjects were permitted to consume water *ad libitum* throughout the trial.

Recovery Period

Following the glycogen-depleting exercise there was a 4 hour period of recovery. Subjects consumed 750-ml of a recovery beverage immediately following this trial and again 2-hours post-exercise. (see ‘treatment beverage’ below for description)

Subsequent Exercise Performance

After the 4 hour recovery period, subjects performed an endurance performance trial on a computerized cycle ergometer (VeloTron, Racermate, Inc). The start of the trial consisted of 20 minutes at a steady-state segment, intensity corresponding to 60% W_{max}. The purpose of the steady state segment was to assess steady-state substrate utilization and to reduce muscle glycogen. Following 20 minutes of steady-state exercise, subjects completed a simulated 20-km time trial. At the end of the 20-km course was a 5-km uphill segment (5% elevation). This was included in the protocol to allow an assessment of late-exercise performance.

All trials were performed at ambient room temperature (70-72 ^{o}F). The subjects were asked to void their bladders prior to all trials. A Windmere® fan, set on ‘medium’ speed, was placed 2 meters from the handlebars of the ergometer for cooling purposes during the trial. Water was provided ad libitum throughout all trials. Each subject was asked to provide maximal effort and treat each time trial as a competitive event. Subjects were allowed to listen to music during the steady-state stage but not during the 20-km time trial due to the potential influence on performance. For consistency, no verbal encouragement was provided to the subjects during the time trial. The only feedback subjects received during the trials regarding performance were distance remaining and distance completed during the time trial.

Subjects were given at least one week between trials to ensure complete recovery. Participants were allotted 14 days to complete a trial if unrelated illness had occurred.

Dependent measurements

Subsequent Exercise Performance

Subsequent exercise performance was measured using cycling time for a simulated 20km time trial. Time was also recorded during the final 5-km of the course. Power outputs (average Watts per segment) were recorded for the 20-km trial and the final 5km of the trial.

Physiological Responses during Steady-State Exercise

Metabolic Measurements

Metabolic measurements were obtained during the glycogen-depleting trial and during the steady-state segment of the subsequent performance trial using a SensorMedics® Spectra metabolic cart. These measurements were obtained after 5 minutes of cycling during the glycogen depleting trial (at 60% W_{max}) and after 5 minutes of cycling during the subsequent performance trial (also at 60% W_{max}). Expired respiratory gases were collected for 5 minutes at each time point. Average values were calculated using the final 3 minutes of each time period, following 2 minutes of breathing equilibration. Measurements obtained from the expired gases included oxygen uptake (VO2), ventilation rate (VE), and respiratory exchange ratio (RER; indicating relative contributions of fat and carbohydrate to energy consumption).

Heart Rate

Heart rate was recorded using a Polar® heart rate monitor. Measurements were obtained at minute 5 of the glycogen-depleting trial and the subsequent exercise trial. Average heart was also recorded for the 20-km time trial and the final 5km segment.

Ratings of Perceived Exertion (RPE)

Ratings of perceived exertion were obtained by having the subjects point to a corresponding level of exertion on the Borg RPE scale (6-20). The RPE recording was made at the same time-points indicated before (5 minute mark during the glycogen-depleting and subsequent exercise). The following statement was read to the subjects to explain the RPE scale: *"Please describe your current level of exertion using the following scale. This level should represent your overall perception of effort, and not localized to a specific group of muscles, etc. For reference, a 6 would represent your effort when you are resting or watching TV, while 20* *would represent the highest level of exertion you are capable of producing during exercise".*

Blood Glucose and Lactic Acid

Blood samples were obtained immediately prior to exercise, during the glycogen-depleting trial (at minute 5), and during the subsequent exercise performance trial (at minute 5). Approximately 0.5 ml of blood was collected during each point via a finger-stick. An automated analyzer (YSI® 2300 STAT glucose/lactate analyzer) determined glucose and lactate levels.

Serum Insulin

Thirty minutes after consuming the recovery beverage, 5 ml of blood was collected using a venous blood draw from an antecubital vein. The blood was collected in SST clot agent Vacutainer® tubes, centrifuged, and serum stored at -80 ^{o}C. Insulin levels were assessed once returned to room temperature, from the serum through a commercially-available ELISA assay.

Muscle Recovery Variables

All of the muscle recovery variables were collected three times; prior to the glycogen depletion trial (PRE), prior to the subsequent exercise performance trial (4HrPost) and 24 hours following the glycogen depletion trial (24HrPost).

Muscle Soreness Ratings

During the familiarization trial subjects received instruction on how to utilize the muscle soreness scale. The following statement was read to the subjects; "please describe your overall level of muscle soreness that you feel currently while performing normal daily activities (i.e. walking up or down stairs)". The soreness rating used a 100mm visual analog scale, 0 indicated no muscle soreness and 100 indicated impaired movement due to muscle soreness.

Mental and Physical Energy/Fatigue Ratings

The Mental and Physical State and Trait Energy and Fatigue Scales (MPSTEFS, O'Conner, 2004) were used to record ratings of mental and physical energy. Separate ratings of physical energy, physical fatigue, mental energy and mental fatigue were obtained using visual analog scales, with potential scores ranging from 0 to 300 for each rating.

Muscle Function Test/Isometric peak torque (MVC)

Peak torque of the knee extensors were assessed to determine treatment differences in muscle function during recovery from exercise. Subjects were seated in a modified chair and asked to push as hard as possible against a shin pad that was connected to a force transducer. Each subject performed 3 to 6, 5-second repetitions at each time-point. There was a 1 minute rest between each repetition. MVC was also assessed immediately following both exercise sessions.

Serum Creatine Kinase (CK)

Serum CK was measured as an indirect indicator of muscle damage. Approximately 5ml of blood was obtained using a venous blood draw from an antecubital vein prior to exercise and 24-hr post exercise. Using a centrifuge, whole blood was spun at 7000 rpm to separate serum and stored in a -80 ^{o} C freezer. Serum CK was analyzed using a Johnson and Johnson Vitro DT6011. The measurement device was calibrated prior to analyses using a reconstituted lyophilized calibration standard purchased from the manufacturer.

Study Treatments:

Recovery beverages were consumed immediately following exercise, 2 hours post-exercise, and immediately following the subsequent exercise performance trial. The study protocols were performed by each subject on three separate occasions (separated by 7-14 days each). Subjects consumed a different beverage for each trial. The beverages were provided in a randomly-counterbalanced, double-blinded design. Beverages were refrigerated at 5 ^{o}C from 2 hours prior to each trial until immediately prior to being consumed. Subjects were informed that the beverages consisted of the same amount of fluid with different nutrient composition, but were not informed the specific composition of the different beverages. Upon completion of the final trial, subjects completed a questionnaire to determine if they could detect the difference in the ingredients of the beverages and a subjective rating of perceived efficacy of the beverages.

Carbohydrate-only Beverage (CHO):

The CHO beverage contained 300 kcal, and 75 g of carbohydrate, per 625 ml serving (12% by volume). The beverage was consumed along with 125 ml of water, such that the total volume of fluid consumed was 750 ml.

High Carbohydrate, Low Protein Beverage (HCLP):

Each 250 ml serving of the HCLP beverage contained 300 kcal, 50 g carbohydrate, 25 g protein, and 0.5 g of fat. In order to provide an isocaloric and isovolumetric (750 ml) comparison to other beverages, the HCLP mixture was consumed along with 500 ml of water.

Low Carbohydrate, High Protein Beverage (LCHP):

Each 250 ml serving of the LCHP consisted of 300 kcal, 10 g of carbohydrate, 55 g of protein, and 10 g of fat. In order to provide iscaloric and isovolumetric comparison to other beverages, the LCHP mixture was consumed along with 500 ml of water.

Dietary and Exercise Controls:

Subjects were asked not to perform any heavy exercise 48 hours prior to each trial. A 48-hour diet and exercise log was completed by each subject preceding each treatment trial, and participants were instructed to maintain a consistent dietary and exercise habit between trials. No less than 12 hours prior to the start of the exercise, subjects consumed their final self-selected meal (dinner the evening prior to testing). After this meal, subjects followed the standardized dietary protocol consisting of the following procedures:

1) No food or beverage intake between dinner and bedtime on the evening prior to the trial (water was consumed *ad libitum*).

2) On the morning of the exercise trials, subjects consumed a standardized breakfast two hours prior to the glycogen-depleting exercise trial. The meal was provided by the researchers, and consisted of 495-510 kcal, including 90-98 g carbohydrate, 8-12 g protein and 4.5-7.5 g fat. In order to account for personal tastes, subjects chose from one of 4 meal choices, but the identical meal chosen was repeated across the four trials for each subject. These dietary restrictions ensured that no differences in nutrient intake occurred between the intervention periods, other than the treatments provided by the researchers.

3) No nutrients were consumed between the *glycogen depleting trial* and the *subsequent exercise performance trial*, other than the recovery beverages provided by the researchers.

4) No nutrients were consumed for 2 hours following the subsequent exercise performance trial, other than the recovery beverages provided by the researchers.

5) Subjects resumed their normal diets from 2 hours following the *subsequent exercise performance trial* through the 24HrPost measurement period. However, subjects recorded their dietary intake during this period, and were instructed to maintain similar dietary habits between the different trials.

Diet Analysis

Nutrition analysis was completed using Diet Analysis Plus 8.0™. Total calories, carbohydrate, protein, and fat were calculated. Subject's diet 12 hours prior to trial (including standard breakfast) and 24 hours post trial were measured.

Data Analysis

The study utilized a double-blind, randomly counterbalanced, within-subject design, to test the differing effects of sports drinks on recovery from heavy endurance exercise. SPSS® Version 17.0 was used to conduct statistical analyses. Repeated Measures Analyses of Variance (RMANOVA) was used to analyze subsequent exercise performance, power output, and insulin levels, with treatment as the within-subject factor. *A priori* comparisons were performed between HCLP and CHO/LCHP treatments, using dependent t-tests. The recovery variables (MVC, muscle soreness and MPSTEFS ratings, plasma CK levels) were analyzed using RMANOVA, with treatment and time as within-subject factors. *A priori* comparisons within individual time-points were conducted using RMANOVA with treatment as the within-subject factor. The steady-state responses during subsequent exercise were analyzed using RMANOVA with the treatment as the within-subject factor.

Chapter IV

Manuscript

ALTERED CARBOHYDRATE AND PROTEIN CONTENT IN SPORTS BEVERAGES: INFLUENCE ON RECOVERY FROM HEAVY ENDURANCE EXERCISE

Christopher A. Boop, Qingnian Goh, Michael J. Saunders, Nicholas D. Luden, and M. Kent Todd

Abstract

Purpose: The purpose of this study was to compare the efficacy of different carbohydrate-protein recovery beverages following heavy endurance exercise**. Methods:** Twelve well-trained male cyclists completed a glycogen-depleting trial followed by a 4 hour recovery period before completing a simulated 20-km time trial. During the recovery period, subjects consumed one of three isocaloric beverages [high-carbohydrate/low-protein (HCLP); low-carbohydrate/high-protein (LCHP); carbohydrate (CHO)] at 0h and 2h, as well as immediately following the 20-km time trial. Creatine kinase (CK), muscle soreness, isometric peak torque (MVC), and mental/physical fatigue/energy ratings were measured pre- and post trial. Glucose and lactate were measured during the glycogen depleting phase and subsequent exercise. **Results:** Subsequent exercise performance was not significantly different between treatments *(LCHP 50.3±2.7 min; CHO 48.5±1.5 min; HCLP 48.8±2.1 min).* No significant treatment*time interactions were observed for isometric peak torque (MVC), muscle soreness, or mental/physical energy/fatigue ratings. Creatine kinase levels pre- *(LCHP 153.5±68.1; CHO 132.6±39.9; HCLP 137.0±41.1*) and post exercise *(LCHP 172.4±53.1; CHO 150.8±47.4; HCLP 146.6±27.4)* were not significantly different between treatments. **Conclusion:** Recovery beverages containing equal caloric content and differing proportions of carbohydrate/protein provided similar effects on muscle recovery and subsequent exercise performance in well-trained cyclists.

Introduction

Many endurance athletes compete in events that require high levels of performance over repeated days, or even multiple events on the same day (i.e. soccer tournaments, Tour cycling). This type of intense endurance exercise can result in muscle damage, muscle soreness, and decreases in subsequent exercise performance. Recent studies have suggested that these aspects of muscle recovery may be influenced by the consumption of carbohydrate-protein recovery beverages. For example, various studies have reported that creatine kinase (CK) (Seifert et al, 2005; Millard-Stafford et al, 2005; Cepero et al, 2009; Skillen et al, 2008; Saunders et al, 2004; Luden et al, 2007; Rowlands et al, 2007), myoglobin (Mb) (Valentine et al, 2008), muscle soreness (Flakoll et al, 2003; White et al, 2008; Luden et al, 2007; Saunders et al, 2009; Millard-Stafford et al, 2005; Rowlands et al, 2007) and muscle function (Valentine et al, 2008) have been improved when carbohydrate-protein is consumed following heavy aerobic exercise. Importantly, some studies have shown that consumption of carbohydrate-protein supplements during recovery can also lead to improved performance during subsequent exercise, compared to when carbohydrate-only beverages are consumed (Berardi et al, 2008; Saunders et al. 2004; Skillen et al, 2008; Niles et al, 2001; Williams et al, 2003; Rowlands et al, 2008). However, these findings are still controversial, as other studies have found no differences in subsequent performance between carbohydrate-protein beverages and carbohydrate-only beverages (Romano-Ely et al, 2006; Betts et al, 2007; Valentine et al, 2007; Betts et al, 2005; Millard-Stafford et al, 2005; Berardi et al, 2006; Luden et al, 2007; Rowlands et al, 2007). Thus, the specific exercise conditions and beverage compositions that may promote enhanced recovery with carbohydrate-protein ingestion require further investigation.

Some of the varied findings between studies may be due to differing exercise protocols and differing amounts/compositions of the beverages utilized between studies. Thus, there is little evidence supporting an 'optimal' macronutrient composition of carbohydrate-protein beverages following heavy endurance exercise. The present study was designed to provide information regarding the effects of recovery beverages with varied proportions of carbohydrate/protein content, independent of caloric differences between beverages.Specifically, the effects of a carbohydrate-only beverage was compared to isocaloric beverages containing differing proportions of carbohydrate and protein (a high carbohydrate/low protein content beverage and a low-carbohydrate-high-protein beverage).

Methods

Subjects

Fifteen endurance-trained male cyclists (18-45 yrs) were recruited from James Madison University and the surrounding area. Inclusion criteria included a minimum of three days a week of cycling for the past 2 months and a peak aerobic capacity (VO_2peak) greater than 45 ml/kg/min. All subjects received a written consent form that described the risks and benefits associated with the study. After a description of the protocols, subjects signed a written consent form. James Madison University Institutional Review Board approved the experiment design before data collection began. Twelve subjects completed all protocols and all statistical analyses were performed on these 12 subjects.

Procedures

The study design consisted of a pre-testing/screening phase, familiarization trial, and three treatment trials.

Pre-testing Phase

All potential subjects that met the study criteria completed the following assessments.

Body Mass and Height

Subjects had their body weight measured to the nearest 0.1 kg and height measured to the nearest 0.5 cm.

VO_{2peak}/W_{max}

Subjects completed an exercise test to determine their peak oxygen uptake (VO_{2peak}) and associated power output (W_{max}). The test began with subjects riding a cycle ergometer at self-selected pace, described as a "comfortable but not easy pace for an hour long ride." After subjects selected their initial workload, power output was increased by 25 W every 2 minutes until the subject voluntary requested to stop the test due to fatigue. Peak oxygen uptake and power output were measured during the test to determine if subjects met entry criteria for the study, and to establish intensities for subsequent exercise protocols.

Familiarization Trial

All subjects that met the inclusion criteria performed a familiarization trial within two weeks of the pre-testing assessment. The purpose of the familiarization trial was to determine if the exercise intensities were appropriate for each subject. The familiarization trial also allowed subjects to become acclimated to the protocol and learn an appropriate pace for the time-trial segment. Subjects were tested prior to the treatment phase to ensure the subjects could complete the treatment trial. Another purpose of the familiarization trial was to minimize any effects of learning and the repeated-bout effect, in order to reduce error variance between treatment trials. All subjects received the CHO recovery beverage during the familiarization trial (described below).

<u>Treatment Trials</u>

Treatment trials were similar to the familiarization trial with the addition of the supplementation treatment provided during the trial. The treatment phase consisted of a glycogen-depleting exercise, a recovery period and a subsequent time trial. Repeated treatment trials were completed within 7-14 days. Figure 1 shows a timeline of a trial.

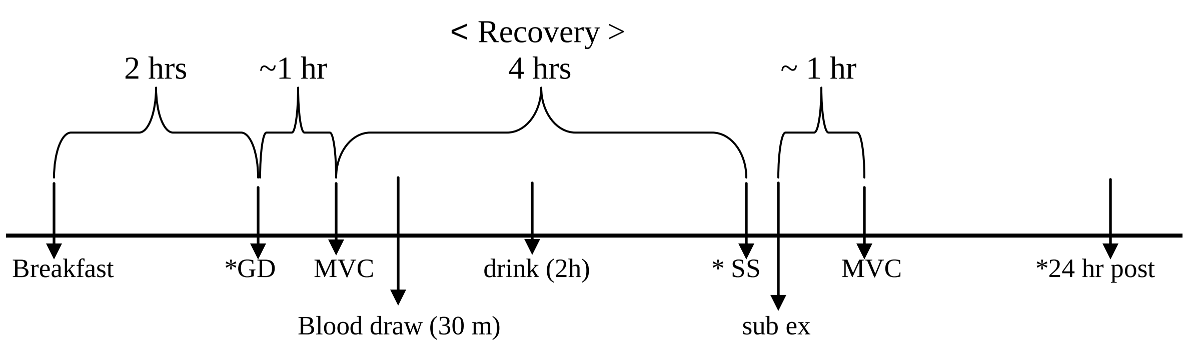

Figure 1: Time line of a trial. * *ME (mental energy), MF (mental fatigue), PE (physical energy), PF (physical fatigue), MS (muscle soreness), and MVC (isometric peak torque) were all measured at these time points. SS = Steady State. Blood draw was also taken 24 hr post.*

Glycogen-depleting exercise

Subjects performed a warm-up/steady state period that consisted of a 10 minute period at 60% W_{max}. After the initial 10 minutes of exercise, subjects completed a series of 2 minute high-intensity intervals. The high-intensity intervals consisted of 2 minutes at 95% W_{max} followed by 2 minutes at 50% W_{max}. Subjects continued the high-intensity intervals until they were unable to maintain a cadence of 70 rpm during the intervals at 95% W_{max}. Once the cadence dropped below 70 rpm, the workloads for the high-intensity intervals were decreased to 85% W_{max} until 70 rpm could not be maintained. At this point, workload for the high-intensity intervals was further decreased to 75% W_{max}. The test was terminated when subjects failed to maintain a cadence of 70 rpm at this intensity. This protocol was designed to induce muscle glycogen depletion and moderate fatigue. Based on similar protocols utilized by Karp et al (2006) and Van Loon et al (2000), the duration of the test was intended to last approximately 50-75 minutes, with 30-45 minutes of high-intensity intervals.

Subjects were allowed to listen to music while they performed the glycogen-depleting phase, however, the music needed to be similar for each trial. All subjects were permitted to consume water *ad libitum* throughout the trial.

Recovery Period

Following the glycogen-depleting exercise there was a 4 hour period of recovery. Subjects consumed 750 ml of a recovery beverage immediately following this trial and again 2-hours post-exercise. (see 'treatment beverage' below for description).

<u>Subsequent Exercise Performance</u>

After the 4 hour recovery period, subjects performed an endurance performance trial on a computerized cycle ergometer (VeloTron, Racermate, Inc). The start of the trial consisted of 20 minutes at a steady-state intensity corresponding to 60% W_{max}. The purpose of the steady state segment was to assess steady-state substrate utilization and to deplete muscle glycogen. Following 20 minutes of steady-state exercise, subjects completed a simulated 20km time trial. At the end of the 20-km course was a 5km uphill segment (5% elevation). This was included in the protocol to allow an assessment of late-exercise performance.

All trials were performed at ambient room temperature (70-72 ^{o}F). The subjects were asked to void their bladders prior to all trials. A Windmere fan, set on ‘medium’ speed, was placed 2 meters from the handlebars of the ergometer for cooling purposes during the trial. Water was provided *ad libitum* throughout all trials. Each subject was asked to provide maximal effort and treat each time trial as a competitive event. Subjects were allowed to listen to music during the steady-state stage but not during the 20-km time trial due to the potential influence on performance. For consistency, no verbal encouragement was provided to the subjects during the time trial. The only feedback subjects received during the trials regarding performance were distance remaining and distance completed during the time trial.

Subjects were given at least one week between trials to ensure complete recovery. Participants were allotted 14 days to complete a trial if unrelated illness occurred.

Dependent measurements

Subsequent Exercise Performance

Subsequent exercise performance was measured using cycling time for a simulated 20-km time trial. Time was also recorded during the final 5-km of the course. Power outputs (average Watts per segment) were recorded for the 20-km trial and the final 5km of the trial.

Physiological Responses during Steady-State Exercise

Metabolic Measurements

Metabolic measurements were obtained during the glycogen-depleting trial and during the steady-state segment of the subsequent performance trial using a SensorMedics Spectra metabolic cart. These measurements were obtained after 5 minutes of cycling during the glycogen depleting trial (at 60% W_{max}) and after 5 minutes of cycling during the subsequent performance trial (also at 60% W_{max}). Expired respiratory gases were collected for 5 minutes at each time point. Average values were calculated using the final 3 minutes of each time period, following 2 minutes of breathing equilibration. Measurements obtained from the expired gases included oxygen uptake (VO2), ventilation rate (VE), and respiratory exchange ratio (RER; indicating relative contributions of fat and carbohydrate to energy consumption).

Heart Rate

Heart rate was recorded using a Polar heart rate monitor. Measurements were obtained at minute 5 of the glycogen-depleting trial and the subsequent exercise trial. Average heart was also recorded for the 20km time trial and the final 5km segment.

Ratings of Perceived Exertion (RPE)

Ratings of perceived exertion were obtained by having the subjects point to a corresponding level of exertion on the Borg RPE scale (6-20). The RPE recording was made at the same time-points indicated before (5 minute mark during the glycogen-depleting and subsequent exercise). The following statement was read to the subjects to explain the RPE scale: *"Please describe your current level of exertion using the following scale. This level should represent your overall perception of effort, and not localized to a specific group of muscles, etc. For reference, a 6 would represent your effort when you are resting or watching TV, while 20 would represent the highest level of exertion you are capable of producing during exercise".*

Blood Glucose and Lactic Acid

Blood samples were obtained immediately prior to exercise, during the glycogen-depleting trial (at minute 5), and during the subsequent exercise performance trial (at minute 5). Approximately 0.5 ml of blood was collected during each point via a finger-stick. An automated analyzer

(YSI® 2300 STAT glucose/lactate analyzer) determined glucose and lactate levels.

Serum Insulin

Thirty minutes after consuming the recovery beverage, 5 ml of blood was collected using a venous blood draw from an antecubital vein. The blood was collected in SST clot agent Vacutainer® tubes, centrifuged, and serum stored at -80 ^{o}C. Insulin levels were assessed once returned to room temperature, from the serum through a commercially-available ELISA assay.

Muscle Recovery Variables

Muscle soreness and MPSTEFS variables were collected three times; prior to the glycogen depletion trial (PRE), prior to the subsequent exercise performance trial (4HrPost) and 24 hours following the glycogen depletion trial (24HrPost). Creatine kinase values were collected PRE and 24hr post exercise. MVC was collected five times; PRE, immediately following glycogen depleting phase (0HrPost), 4HrPost, immediately following subsequent exercise, and 24HrPost.

Muscle Soreness Ratings

During the familiarization trial subjects received instruction on how to utilize the muscle soreness scale. The following statement was read to the subjects; “please describe your overall level of muscle soreness that you feel currently while performing normal daily activities (i.e. walking up or down stairs)”. The soreness rating used a 100 mm visual analog scale, 0 indicated no muscle soreness and 100 indicated impaired movement due to muscle soreness.

Mental and Physical Energy/Fatigue Ratings

The Mental and Physical State and Trait Energy and Fatigue Scales (MPSTEFS, O'Conner, 2004) were used to record ratings of mental and physical energy. Separate ratings of physical energy, physical fatigue, mental energy and mental fatigue were obtained using visual analog scales, with potential scores ranging from 0 to 300 for each rating.

Muscle Function Test/Isometric peak torque (MVC)

Peak torque of the knee extensors was assessed to determine treatment differences in muscle function during recovery from exercise. Subjects were seated in a modified chair and asked to push as hard as possible against a shin pad that was connected to a force transducer. Each subject performed three to six, 5-second repetitions at each time-point, with one minute rest between each repetition.

Serum Creatine Kinase (CK)

Serum CK was measured as an indirect indicator of muscle damage. Approximately 5 ml of blood was obtained using a venous blood draw from an antecubital vein prior to exercise and 24-hr post exercise. Using a centrifuge, whole blood was spun at 7000 rpm to separate serum and stored in a -80 ^{o} C freezer. Serum CK was analyzed using a Johnson and Johnson Vitro DT6011. The measurement device was calibrated prior to analyses using a reconstituted lyophilized calibration standard purchased from the manufacturer.

<u>Study Treatments:</u>

Recovery beverages were consumed immediately following exercise, 2 hours post-exercise, and immediately following the subsequent exercise performance trial. The study protocols were performed by each subject on three separate occasions (separated by 7-14 days each). Subjects consumed a different beverage for each trial. The beverages were provided in a randomly-counterbalanced, double-blinded design. Beverages were refrigerated at 5 ^{o}C from 2 hours prior to each trial until immediately prior to being consumed. Subjects were informed that the beverages consisted of the same amount of fluid with different nutrient composition, but were not informed the specific composition of the different beverages. Upon completion of the final trial, subjects completed a questionnaire to determine if they could detect the difference in the ingredients of the beverages and a subjective rating of perceived efficacy of the beverages.

<u>Carbohydrate-only Beverage (CHO):</u>

The CHO beverage contained 300 kcal, and 75g of carbohydrate, per 625 ml serving (12% by volume). The beverage was consumed along with 125 ml of water to provide a total fluid volume of 750 ml.

<u>High Carbohydrate, Low Protein Beverage (HCLP):</u>

Each 250 ml serving of the HCLP beverage contained 300 kcal, 50g carbohydrate, 25g protein, and 0.5g of fat. In order to provide an isocaloric and isovolumetric (750 ml) comparison to other beverages, the HCLP mixture was consumed along with 500 ml of water.

Low Carbohydrate, High Protein Beverage (LCHP):

Each 250 ml serving of the LCHP consisted of 300 kcal, 10g of carbohydrate, 55g of protein, and 10g of fat. In order to provide iscaloric and isovolumetric comparison to other beverages, the LCHP mixture was consumed along with 500 ml of water.

Dietary and Exercise Controls:

Subjects were asked not to perform any heavy exercise 48 hours prior to each trial. A 48-hour diet and exercise log was completed by each subject preceding each treatment trial, and participants were instructed to maintain consistent dietary and exercise habits between trials. No less than 12 hours prior to the start of the exercise, subjects consumed their final self-selected meal (dinner the evening prior to testing). After this meal, subjects followed a standardized dietary protocol consisting of the following procedures:

1) No food or beverage intake between dinner and bedtime on the evening prior to the trial (water was consumed *ad libitum*).

2) On the morning of the exercise trials, subjects consumed a standardized breakfast two hours prior to the glycogen-depleting exercise trial. The meal was provided by the researchers, and consisted of 495-510 kcal, including 90-98g carbohydrate, 8-12g protein and 4.5-7.5g fat. In order to account for personal tastes, subjects chose from one of 4 meal choices, but the identical meal chosen was repeated across the four trials for each subject. These dietary restrictions ensured that no differences in nutrient intake occurred between the intervention periods, other than the treatments provided by the researchers.

3) No nutrients were consumed between the *glycogen depleting trial* and the *subsequent exercise performance trial*, other than the recovery beverages provided by the researchers.

4) No nutrients were consumed for 2 hours following the subsequent exercise performance trial, other than the recovery beverages provided by the researchers.

5) Subjects resumed their normal diets from 2 hours following the *subsequent exercise performance trial* through the 24HrPost measurement period. However, subjects recorded their dietary intake during this period, and were instructed to maintain similar dietary habits between the different trials.

Diet Analysis

Nutrition analysis was completed using Diet Analysis Plus 8.0™. Total calories, carbohydrate, protein, and fat were calculated. Subject's diet 12 hours prior to trial (including standard breakfast) and 24 hours post trial were measured.

Data Analysis

The study utilized a double-blind, randomly counterbalanced, within-subject design, to test the differing effects of sports drinks on recovery from heavy endurance exercise. SPSS Version 17.0 was used to conduct statistical analyses. Repeated Measures Analysis of Variance (RMANOVA) was used to analyze subsequent exercise performance, power output, and insulin levels, with treatment as the within-subject factor. *A priori* comparisons were performed between HCLP and CHO/LCHP treatments, using dependent t-tests. The recovery variables (MVC, muscle soreness and MPSTEFS ratings, plasma CK levels) were analyzed using RMANOVA, with treatment and time as within-subject factors. *A priori* comparisons within individual time-points were conducted using RMANOVA with treatment as the within-subject factor. The steady-state responses during subsequent exercise were analyzed using RMANOVA with the treatment as the within-subject factor.

Results

Subjects

All subjects in this study met the minimal VO_{2peak} required for entry into the study (>45ml/kg/min). Demographic statistics for the participants are shown in Table 1.

Table 1:Demographics

Variable	**Mean ± SD**
Age (years)	25.5 ± 8.1
Weight (kg)	73.3 ± 2.3
Height (cm)	177.5 ± 7.5
VO_{2peak} (ml·kg-1·min-1)	65.5 ± 7.1
Wpeak (watts)	352 ± 34

Glycogen Depletion Exercise

During the initial glycogen depleting phase of the trial, VO_2, RER, and RPE were measured during the first five minutes of steady-state exercise. Blood was also taken during the final five minutes of steady-state exercise to measure lactate and glucose levels. No significant differences were observed between trials during the glycogen depleting phase for VO_2 (*LCHP 44.3±5.1; CHO 44.2±4.8; HCLP 44.1±5.0 ml/kg/min*), RER (*LCHP 0.93±0.03; CHO 0.93±0.04; HCLP 0.92±0.03*) and RPE (*LCHP 12.5±1.6;*

CHO12.8±1.2; HCLP 12.7±1.3). Lactate levels measured during the glycogen depleting phase were not significantly different between trials (*LCHP 2.7±0.9; CHO 2.6±1.1; HCLP 2.7±1.2*). Glucose levels measured during the glycogen depleting phase were not significantly different between trials (*LCHP 66.0±8.7; CHO 65.8±9.9; HCLP 62.2±6.8*).

Table 2: Glycogen Depleting Exercise Trials

	LCHP	**CHO**	**HCLP**
Time (m)	58.6 ± 16.7	59.2 ± 19.3	60.2 ± 19.3
High-Intensity Intervals	13.0±1.2	13.1±1.4	13.4±1.4
Caloric Expenditure	745.8 ± 263.7	758.5 ± 297.8	774.0 ± 298.4

No significant difference between trials.

Total time and caloric expenditure for glycogen depleting phase are reported in Table 2. The high intensity intervals (95% Wpeak) had a mean W percentage of 94.1%. Intervals for 85% Wpeak and 75% Wpeak had mean percentages of 83.9% and 74.4% respectively. Mean work output for each interval is shown in Table 3. No significant difference was observed in the number of high-intensity intervals completed in each trial (Table 2).

Table 3: Interval Wpeak % (Watts)

Interval	**Mean ± SD**
95% W_{peak}	333.1 ± 36.4
85% W_{peak}	295.4 ± 32.9
75% W_{peak}	261.7 ± 30.5

<u>Subsequent Exercise (Time Trial)</u>

During the steady state portion of the trial (first 10 minutes) there were no significant difference between trials for VO_2 (*LCHP 45.4±4.1 ml/kg/min; CHO 44.6±4.4 ml/kg/min; HCLP 43.8±5.7 ml/kg/min*), RPE *(12.9; 13.4; 13.1)*, RER *(.892; .912; .905)*;

VE *(70.2; 70.0; 67.5 L/min)*; and lactate levels *(1.53; 1.87; 1.92)*. Glucose levels were significantly lower ($p < .05$) for CHO (58.8 ± 9.7) than for HCLP (67.3 ± 6.2) or LCHP (66.8 ± 8.5).

20 km performance times during the subsequent exercise trial were not significantly different between treatments *(LCHP 50.3±2.7 min; CHO 48.5±1.5 min; HCLP 48.8±2.1 min*). In addition, no differences were observed between treatments for performance over the final 5-km of the trial. Performance times are shown in Figure 2.

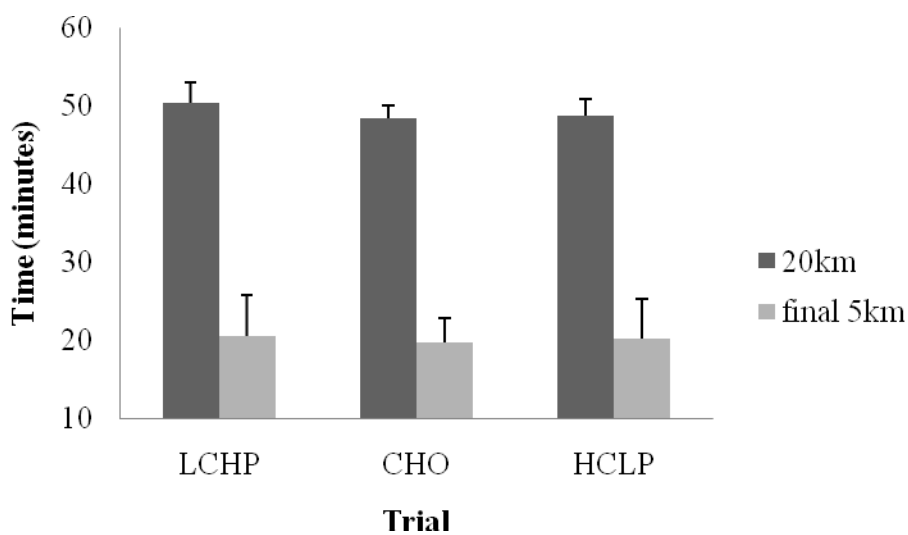

Figure 2: Peformance time in subsequent exercise trial.

Power output (Figure 3) was not significantly different between treatments during the 20-km time trial (*LCHP 197.8±42.5 watts; CHO 203.5±34.3 watts; HCLP 205.0±37.1 watts*). Similarly, power output measured during the final 5km was not significantly different between treatments (*LCHP 203.9±43.6 watts; CHO 209.7±38.3 watts; HCLP 210.4±45.7 watts*).

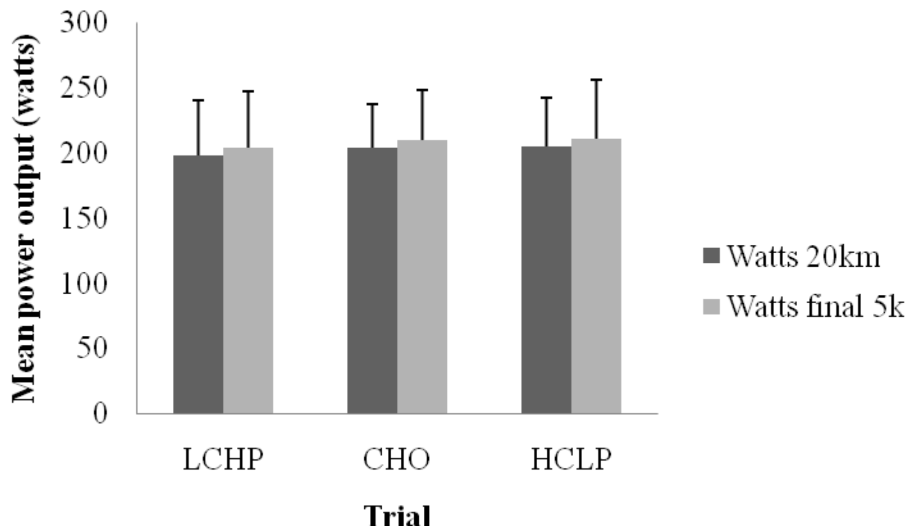

Figure 3: Power output (watts) for 20-km and final 5-km of subsequent exercise trial.

Recovery

Insulin was measured 30 minutes post glycogen depleting phase. Insulin levels were significantly higher with HCLP *(113±31 µU/ml^{-1})* and CHO *(133±63 µU/ml^{-1})* compared to the LCHP *(31±10 µU/ml^{-1})*. Data is shown in Table 4.

Table 4: Insulin (µU/ml^{-1})

Treatment	Mean ± SD
LCHP	31 ± 10
CHO	133 ± 63
HCLP	113 ± 31

A number of insulin values were 'out-of-range' so the values observed represent four subjects with accurate values in all three trials.

Creatine kinase levels were measured prior to exercise and 24 hours post exercise. No significant difference between trials was observed. There was no significant difference between pre and 24 hours post exercise. CK data is shown in Figure 4.

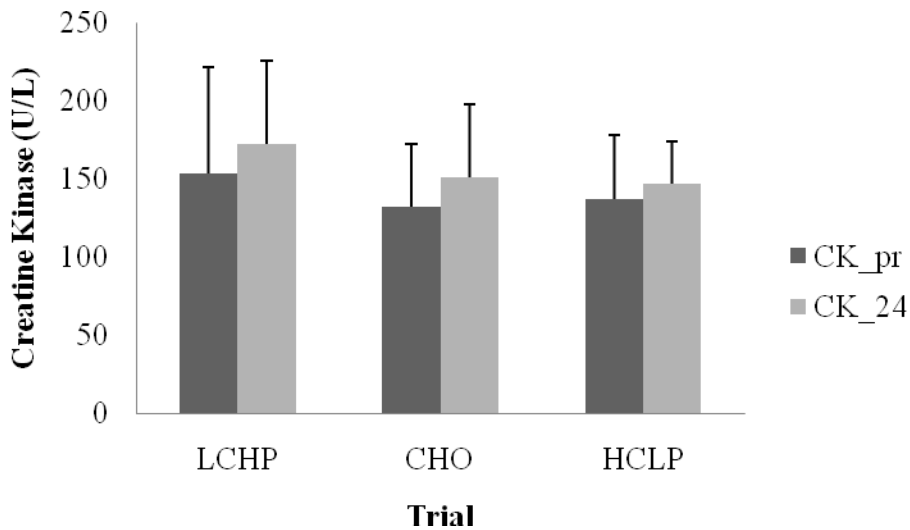

Figure 4: Creatine Kinase levels pre- and post exercise.

Isometric peak torque (MVC) values were measured five times; prior to glycogen depleting phase, immediately following glycogen depleting exercise,prior to subsequent exercise, immediately following subsequent exercise and 24 hours post glycogen depleting exercise. MVC values were significantly decreased immediately following both exercise sessions (i.e. post glyc and post sub were significantly lower than pre glyc and pre sub time-points), but had returned to baseline levels 24-hr post-exercise. There was no significant between-treatment difference in MVC (Table 5).

Table 5: MVC; mean peak torque (watts)

	LCHP	CHO	HCLP
Pre_glyc	482.6 ± 128.6	451.1 ± 103.7	475.7 ± 94.9
Post_glyc	426.2 ± 139.1	427.2 ± 142.7	415.3 ± 97.7
Pre_sub	463.3 ± 90.1	445.9 ± 86.1	471.0 ± 114.7
Post_sub	415.0 ± 112.4	433.4 ± 138.4	420.5 ± 87.4
24-h post	492.5 ± 103.8	489.5 ± 120.1	499.4 ± 101.9

Isometric peak torque differences between treatments. *Pre glyc*: measured before glycogen depleting phase; *post glyc:* measured following glycogen depleting phase; *pre sub:* before subsequent exercise; *post sub:* following subsequent exercise; *24 h p*: measured 24 hours post glycogen depleting phase.

Muscle soreness ratings were measured prior to the glycogen depleting phase and prior to subsequent exercise; as well as 24 hours post exercise. There was a significant difference ($p < .05$) in muscle soreness ratings at the three different time periods (Figure 5). Muscle soreness ratings increased from pre-glycogen depleting phase (*13.15±2.9*) to pre-subsequent exercise (*36.07±3.8*). Twenty-four hours post exercise, ratings decreased to a mean score of 22.97±4.4, which remained significantly elevated from baseline (pre-glycogen depleting phase) levels. There were no significant differences in muscle soreness ratings between treatments.

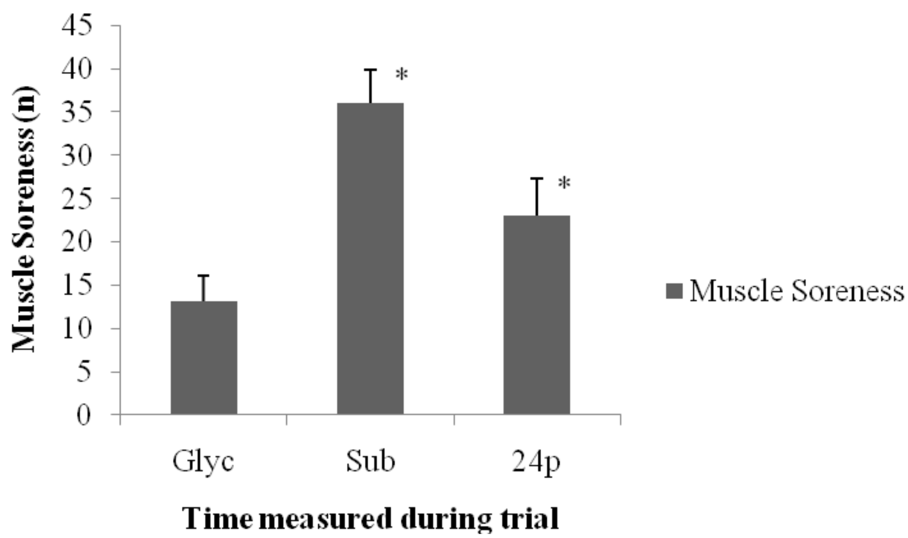

Figure 5: Muscle soreness was measured three times during each trial, prior to the glycogen depleting phase (PRE), prior to the subsequent exercise phase (4HrPost), and 24 hours post exercise (24HrPost). **significant difference from baseline measurements.*

Ratings of Mental and Physcial fatigue and energy were also measured at PRE, 4HrPost, and 24HrPost. There were no significant differences between treatments for any of the ratings. Each rating of mental fatigue (MF), mental energy (ME), physical fatigue (PF) and physical energy (PE) had a significant change from pre-glycogen phase to subsequent exercise followed by a return to near-baseline levels 24 hours post exercise. Ratings of energy and fatigue are shown in Table 6.

Table 6: Physical Energy (PE), Physical Fatigue (PF), Mental Energy (ME), and Mental Fatigue (MF) ratings.

	LCHP			CHO			HCLP		
	Pre	4Hrpost	24Hrpost	Pre	4Hrpost	24Hrpost	Pre	4Hrpost	24Hrpost
PE	192.8	161.0	192.6	200.3	159.7	200.4	193.6	160.3	181.3
	(46.0)	(55.8)	(63.1)	(63.2)	(60.7)	(64.3)	(64.0)	(49.2)	(69.0)
PF	88.2	142.8	90.8	79.2	148.1	87.5	83.4	138.3	106.2
	(46.7)	(56.6)	(54.0)	(58.7)	(65.9)	(60.8)	(59.0)	(53.0)	(62.7)
ME	201.2	168.9	194.3	194.7	174.6	200.6	197.7	171.8	191.0
	(53.9)	(59.9)	(65.9)	(70.3)	(61.5)	(66.6)	(54.3)	(59.4)	(65.8)
MF	88.3	116.8	90.3	94.4	117.5	89.3	81.2	116.5	89.0
	(53.4)	(61.8)	(55.3)	(67.1)	(60.8)	(69.6)	(51.9)	(61.1)	(59.3)

After completing the final trial, subjects were asked to rank from high to low the amount of carbohydrate, protein, and calories in each beverage from the three trials. Subjects perceived the HCLP beverage had the highest amount of protein and the CHO beverage had the highest amount of carbohydrate. Data for the rankings is shown in Figures 6-7.

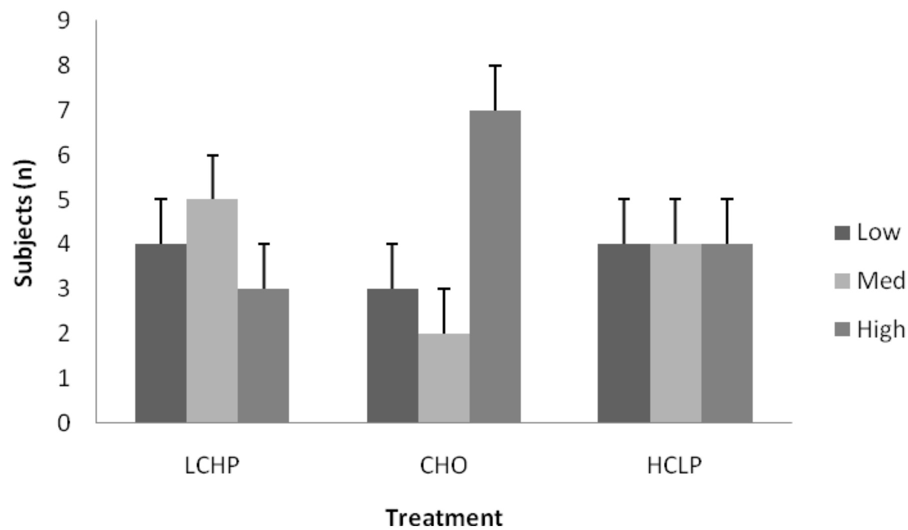

Figure 6: Carbohydrate rankings (low; med; high) per treatment. Subjects suspected highest amounts of carbohydrate in the CHO-only beverage.

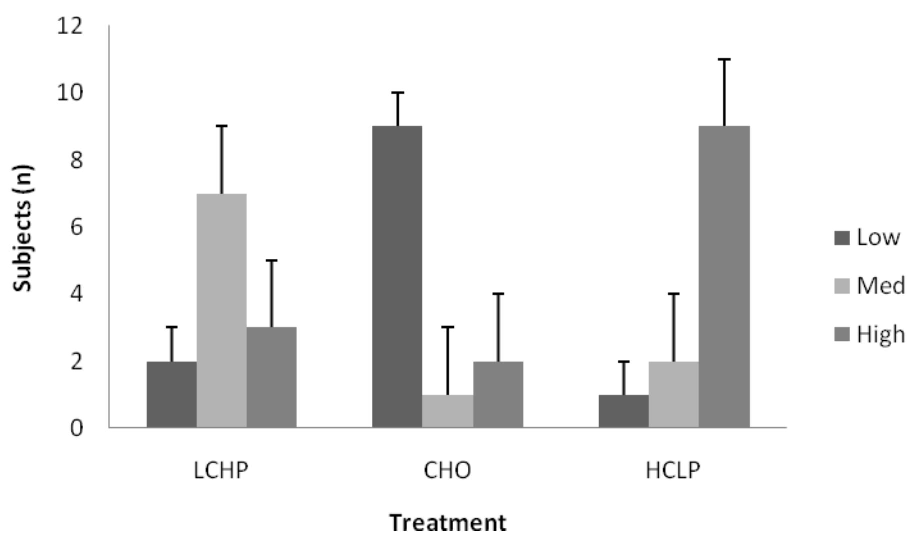

Figure 7: Protein rankings (low; med; high) per treatment. Subjects (9) suspected low protein amounts in the beverage with no protein (CHO) and high amounts of protein in the HCLP beverage.

<u>Nutrition Analysis</u>

Nutrition logs were collected 12 hours prior and 24 hours post trials. Analysis was completed using Diet Analysis Plus 8.0™. No significance was observed between the total calories, carbohydrate, or protein consumption pre- or post trials. Nutrition data is shown in Figures 8-10.

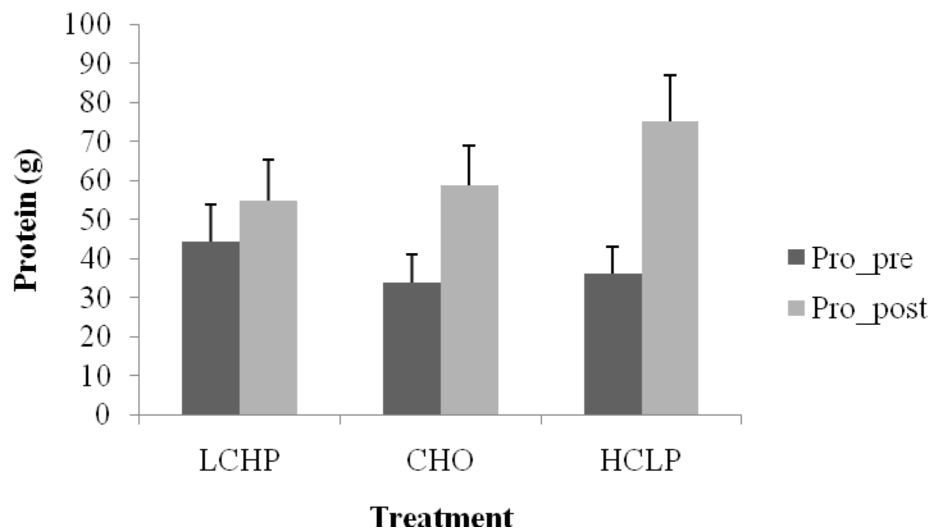

Figure 8: Protein consumption pre- and post exercise.

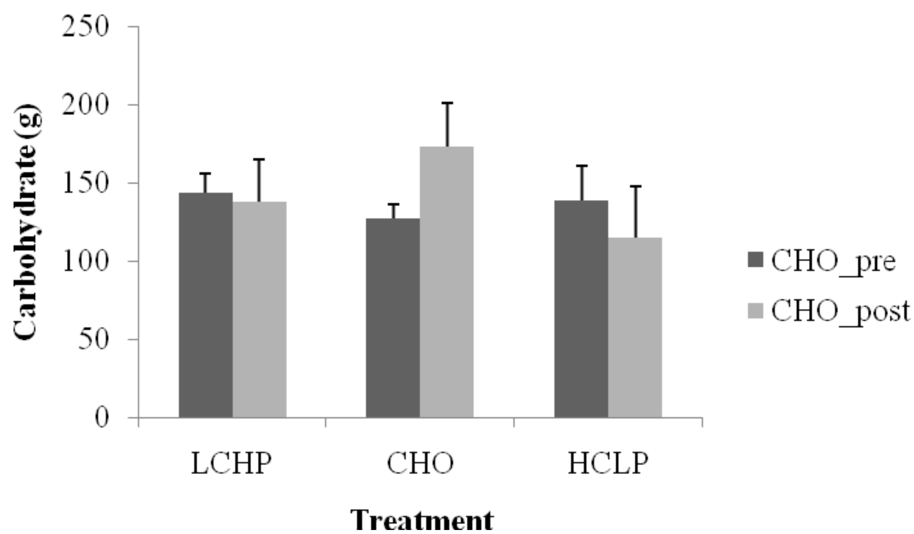

Figure 9: Carbohydrate consumption pre- and post exercise.

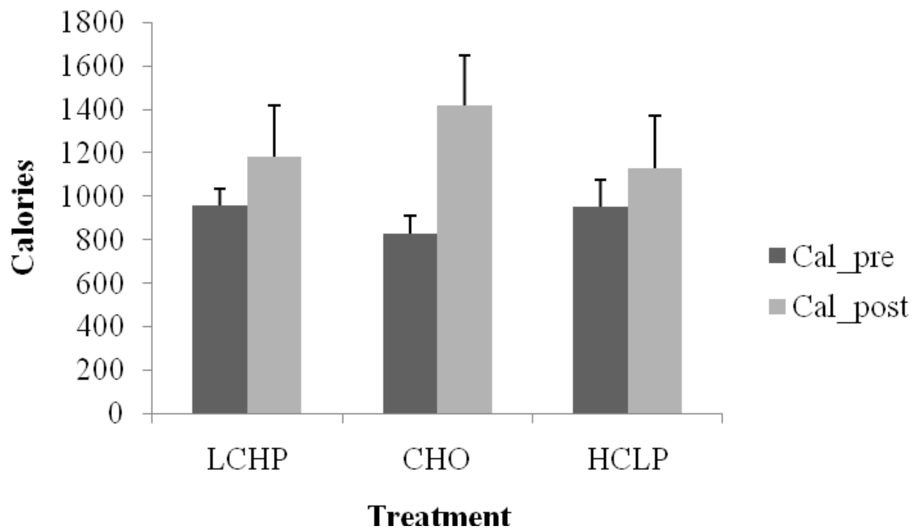

Figure 10: Total calories consumed pre- and post exercise.

Discussion

The purpose of this study was to examine the influence of altered carbohydrate and protein content in sports beverages on recovery from heavy endurance exercise. The three beverages (high carbohydrate-low protein, HCLP; low carbohydrate- high protein, LCHP; carbohydrate CHO), all contained equal caloric values (300 kcal/serving). The HCLP contained 50g of carbohydrate and 25g of protein; the LCHP beverage contained 10g carbohydrate and 55g protein, and the CHO beverage contained 0g of protein and 75g of carbohydrate. We hypothesized that the HCLP beverage would improve subsequent exercise performance and improve markers of muscle recovery compared to the LCHP and CHO beverages. However, following a 4 hour recovery period, there were no significant difference in 20km time or power output between treatments. Other measurements of recovery, including muscle soreness, creatine kinase, lactate, muscle function (MVC), and mental/physical energy ratings were also similar between treatments following 4-hr and 24-hr of recovery.

Some previous studies have shown improvements in subsequent exercise performance with carbohydrate-protein supplements compared to carbohydrate-only supplements (Berardi et al, 2008; Saunders et al. 2004; Skillen et al, 2008; Niles et al, 2001; Williams et al, 2003; Rowlands et al, 2008). However, other studies have not observed significant differences in performance between carbohydrate-protein and carbohydrate-only supplements (Cermak et al, 2009; Rowlands et al, 2007; Betts et al, 2005; Cepero et al, 2009; Romano-Ely et al, 2006). Variations in findings between studies may reflect differences in exercise protocols/participants, recovery periods and amounts/types of treatment beverages utilized. The present study design is most similar to those of Karp et al (2006) and Thomas et al (2009), as these studies used a similar glycogen-depleting exercise protocol as the initial exercise session. The duration of the glycogen-depleting phase in the present study (58.6-60.2 min) was similar to that of Karp et al (2006; 60.8-72.6 min) and Thomas et al (2009; 54-75 min). Recovery time (4 hours) was also identical to that used by Karp (2006) and Thomas (2009). In the present study, there was no significant difference in subsequent exercise performance between the HCLP (48.81±2.1 min), LCHP (50.31±2.7 min), and CHO (48.49±1.5 min) beverages, while the aforementioned studies observed significant differences between treatments. However the prior studies observed larger between-trial variations in exercise duration (in the initial exercise session) than the current study, which suggests the familiarization trial used in the current study was successful in reducing errors of variance between trials. In addition, the studies previously mentioned did not compare isocaloric carbohydrate and carbohydrate-protein beverages, and thus caloric differences between beverages could have explained the observed differences in subsequent performance between treatments.

The present study investigated a number of other markers of muscle recovery such as muscle soreness, muscle function (MVC), serum CK and ratings of mental and physical fatigue. A number of prior studies have reported decreases in muscle soreness ratings with a carbohydrate-protein supplement compared to an isocaloric carbohydrate supplement (Rowlands et al, 2007; Millard-Stafford et al, 2005; Romano-Ely et al, 2006), although not all studies have reported this effect (Valentine et al, 2008; White et al, 2008). Similarly, some (Luden et al, 2007; Romano-Ely et al, 2006; Rowlands et al, 2007; Saunders et al, 2004; Valentine et al, 2008), but not all (Cepero et al, 2009; Millard-Stafford et al, 2005) studies have observed significant attenuations in plasma/serum CK levels with carbohydrate-protein treatments. In the present study, no significant increases in CK levels were observed in any of the treatment trials, so it was not possible to determine if the treatments had a differential effect on post-exercise CK levels. Though muscle soreness ratings were significantly increased from baseline levels following exercise (independent of treatment), the absolute changes were relatively small, and no significant differences were observed between treatments. The reason for no observed significant difference between treatments could be due to the limited disruption in the measured variables, (i.e. muscle soreness, CK).

Serum insulin was measured thirty minutes post glycogen-depleting phase (i.e. after the first beverage feeding). Insulin levels were significantly higher with HCLP *(113±31 µU/ml*$^{-1}$*)* and CHO *(133±63 µU/ml*$^{-1}$*)* compared to the LCHP *(31±10 µU/ml*$^{-1}$*).*

Millard-Stafford et al (2005), observed a significant increase in insulin levels with a carbohydrate beverage one hour following exercise compared to an isocaloric carbohydrate-protein beverage, while Ivy et al (2002) found no significant differences in insulin levels between isocaloric carbohydrate and carbohydrate-protein beverages following exercise. The current study found a significant difference between the HCLP and LCHP beverages. This finding suggests that the influence of carbohydrate-protein beverages on insulin levels is affected by the specific composition of the beverages, and that adequately high carbohydrate levels are required to stimulate optimal post-exercise insulin responses.

Glucose levels in the current study were significantly decreased from pre-glycogen-depleting phase to the initial steady-state phase of the subsequent ride (pre glyc 77.5±14.9; steady state 65.1±8.5; $p < 0.003$). This suggests that glucose availability may have been compromised to some extent during the subsequent exercise trial. Glucose levels assessed 30-min following beverage ingestion were significantly different between treatments in proportion to the amount of carbohydrate in each beverage. However, glucose levels did not remain elevated at the start of the subsequent exercise trial with the carbohydrate group. This could be explained by the significantly higher insulin levels with carbohydrate, which perhaps resulted in increased carbohydrate storage. A potentially decreased carbohydrate availability in the LCHP trial could explain the trend towards decreased lactate levels with this treatment. However, this difference was apparently not important enough to significantly influence subsequent performance levels between treatments.

An isometric peak torque test (MVC) was performed to assess changes in muscle function. MVC levels were significantly lower immediately following each of the exercise trials than the corresponding pre-exercise levels. However, MVC levels 24-hours post-exercise were not significantly different from baseline levels, and there were no significant differences in MVC responses between treatments. Valentine et al (2008), examined muscle function 22-24 hours post exercise when comparing carbohydrate and carbohydrate-protein beverages (using number of repetitions at 70% 1-RM as the measure of muscle function). A significant increase in repetitions was observed 22-24hrs post exercise with carbohydrate-protein ingestion (Valentine et al, 2008). However, post-exercise changes in CK levels were considerably larger in the Valentine study (400 IU/L in carbohydrate trials) versus the present study. This suggests that our exercise protocol created much smaller disruptions in muscle recovery than Valentine et al (2008), which may have limited our ability to observe differences in muscle function between treatments.

There are a variety of extraneous factors that could have influenced recovery outside of the study parameters. For example, post-exercise muscle recovery could have been influenced by inconstant diets among subjects prior to each trial. However, a standardized breakfast was consumed by each subject 2 hours before the glycogen depleting phase. In addition, data from nutrition logs revealed no significant differences between trials in total calories, carbohydrate, or protein consumption prior to the glycogen depleting phase. Nutrition logs were also maintained following each trial leading up to the measurements 24 hours post exercise, and no differences in macronutrient intake were observed. Therefore, it seems unlikely that variations in nutritional intake had a significant influence on muscle recovery between trials. Another possible limitation of this study was whether the glycogen depleting phase was adequately demanding to significantly impair muscle recovery. The glycogen depleting phase showed similar metabolic values between trials, and well as total time and energy expenditure. This suggests that the glycogen depleting trial was highly consistent between the three treatment trials. However, despite the consistency of the glycogen depleting trials, it was apparently not demanding enough to elicit large changes in the measured variables 24-hours post-exercise; i.e. CK, MVC, and muscle soreness. The highly-trained subjects utilized in this study appeared to recover to near-baseline levels by 24-hours post-exercise, regardless of the treatment. As a result, the small post-exercise disruptions in recovery-measurements observed in the study may have significantly limited our ability to detect potential treatment differences under more demanding exercise conditions.

In summary, each of the isocaloric beverages provided similar recovery following heavy aerobic exercise, despite variations in the carbohydrate/protein compositions of the beverages. Thus, following heavy exercise of 1-1.5 hr duration, short-term exercise recovery of well-conditioned cyclists does not appear to be significantly influenced by the macronutrient content of recovery beverages, provided that the caloric content of the beverages is equal. These findings may be limited to well-trained endurance cyclists, as it is possible that populations that incur larger disruptions in muscle recovery may obtain differing results from the treatment beverages. Therefore it is recommended that future research examine the effects of carbohydrate-protein beverages under differing exercise conditions, and with participants of varying training status.

Chapter V

Conclusions

1. High-carbohydrate-low-protein supplementation did not improve subsequent performance compared to a low-carbohydrate-high-protein or carbohydrate-only beverage. There were no significant differences in subsequent 20km time trial performance between the three treatments.
2. Post-exercise changes in muscle soreness, muscle function, and creatine kinase were similar between HCLP, LCHP and CHO beverages.
3. Following heavy exercise of 1-1.5 hr duration, short-term exercise recovery of well-conditioned cyclists does not appear to be significantly influenced by the macronutrient content of recovery beverages, provided that the caloric content of the beverages is equal.

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