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Macronutrient supplementation for endurance athletes

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Macronutrient Supplementation for Endurance Athletes

An Honors Program Project Presented to
the Faculty of the Undergraduate
College of Health and Behavioral Studies
James Madison University

by Jonathan Bryan Hurst

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Accepted by the faculty of the Department of Kinesiology, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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Dedication

To my loving family which has given me endless amounts of support and love throughout my academic career.

None of my achievements would be possible without your devotion and commitment to helping me achieve my goals.

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I wish to express my sincere gratitude to Dr. Michael Saunders, who has been with me every step of the way in my journey to a completed Honor's thesis. Our adventure began as a literature review course with the intention of simply learning more about his research and expertise. The outcome has become so much more than I ever thought possible and I sincerely thank Dr. Saunders for his guidance and encouragement.

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Abstract

Purpose: The present study addressed two questions related to macronutrient supplementation during endurance exercise. Firstly, the effects of carbohydrate and protein co-ingestion on time trial (TT) performance were compared to carbohydrate alone. Secondly, the effects of isolated protein ingestion on TT performance were compared to a placebo.

Methods: Six trained cyclists (Age: 22 ± 1 years; Height: 167 ± 12 cm; Weight: 60 ± 10 kg; VO_{2max} : 62 ± 7 ml/kg/min) completed four experimental trials, consisting of constant-load cycling for two hours ($55\% W_{max}$) immediately followed by a 30-km simulated time trial. During the trials, subjects consumed one of four experimental beverages at regular intervals during exercise: a non-caloric placebo (PL), a protein-only beverage (PR: 15 g/hr), a carbohydrate-only beverage (45 g/hr), or a carbohydrate and protein beverage (CP: 45 g/hr CHO + 15 g/hr PRO). Physiological measurements (VO_2 , VE, HR, RER, blood glucose, and blood lactate) and subjective measurements (GI distress and RPE) were assessed throughout both the constant-load and TT exercise phases. Trials were completed in a randomly-counterbalanced order. Mean \pm 90% confidence intervals were calculated for all measures, and magnitude-based qualitative inferences were used to assess treatment effects.

Results: In comparison to PL (62.8 ± 8.1 min), both CHO and CP provided ‘possible’ benefits in TT performance (58.9 ± 6.5 min; 59.2 ± 9.4 min respectively) while no clear effects of PRO on performance were observed (61.0 ± 8.0 min). Furthermore, CP had no clear effect on performance versus CHO.

Conclusions: In our sample, the addition of protein to a moderate-dose of carbohydrate did not result in meaningful improvements in time trial performance versus carbohydrate alone. Similarly, protein consumption alone provided no ergogenic effects versus a placebo.

Chapter I: Introduction

In the world of competitive sports, the smallest improvements in performance can make the difference between making a team or being cut; making a diving catch or coming up short, or even the difference between winning and losing a championship. Nutrition has long been a variable that athletes manipulate in an effort to maximize performance. For example, numerous studies have reported that carbohydrate ingestion during exercise improves endurance performance through mechanisms relating to overall energy supply and manipulation of the central nervous system (15). It has been found that blood glucose is a key source of energy during prolonged exercise (15). With the intake of supplemental carbohydrates, liver and muscle glycogen stores may be spared, and high rates of carbohydrate oxidation can be maintained for longer durations during exercise (15). Additionally, supplemental carbohydrate ingestion during exercise has been found to provide benefits via the central nervous system, and rinsing the mouth with carbohydrate solutions (even without ingestion) may also, improve endurance performance (15).

The concept of supplementing carbohydrates and fluids during exercise began in 1965 at the University of Florida in an attempt to improve the Gator's on-field performance (5). It was discovered that providing the athletes with a solution rich in carbohydrates and electrolytes helped the athletes perform at a higher level (5). The beneficial effects of carbohydrate sports beverages on metabolism and performance is strongly supported in the scientific studies from the past 35 years (5). As a result, carbohydrate-electrolyte sports beverages are used ubiquitously among modern endurance athletes.

More recently, the potential ergogenic effects of supplemental protein in sports beverages has been investigated. In 2003, Ivy and colleagues reported that the co-ingestion of carbohydrate and protein (CHO+P) during exercise significantly prolonged time to fatigue during cycling exercise versus a carbohydrate-only beverage (9). Saunders and colleagues reported similar findings in a study conducted in 2004 (14). These initial studies indicated that the addition of protein to a carbohydrate supplement provided additional performance benefits over carbohydrates alone. However, the generalizability of these findings were limited by two issues. Firstly, both studies used Time-to-Exhaustion (TTE) exercise protocols which required subjects to cycle at a specified intensity, for as long as possible. Although an important outcome, improvements in TTE are not directly applicable to athletic performance, as cycling events are generally performed with the goal of completing a fixed distance in the fastest time possible. A second limitation of the aforementioned studies relates to the concentrations of carbohydrate (CHO) and protein (PRO) in the experimental beverages. Both studies compared CHO+P beverages versus CHO beverages that were matched for carbohydrate content. As a result, the CHO+P beverages contained additional calories, and it could not be determined if the observed improvements in performance were due to a unique benefit from protein per se.

Numerous studies have subsequently been conducted to determine the effects of CHO+P beverages on endurance performance. The topic remains controversial, and generalizations are difficult due to the wide variety of exercise protocols and beverage comparisons among these studies. However, beverage composition has an important influence on performance outcomes, and a better understanding of the literature can be obtained by examining the existing studies based on the type of experimental beverages examined in each study, as discussed below.

One group of CHO+P studies have used experimental beverages that were matched for carbohydrate content, with total carbohydrate intake below maximal gastrointestinal uptake rates (and, thus, below the rates theorized to produce optimal ergogenic effects with carbohydrates). Specifically, all studies in this group used experimental beverages ingested at rates below 50 gCHO•hr⁻¹. In 2003, in a study by Ivy, three experimental beverages were used in a time-to-exhaustion exercise protocol (9). Experimental beverages consisted of placebo, a 7.75% carbohydrate solution (CHO), and a 7.75% carbohydrate + 1.94% protein solution (CHO+P). The researchers observed that the addition of protein to the carbohydrate supplement improved time-to-exhaustion versus the placebo and CHO beverages (9). In 2004, Saunders and colleagues compared the effects of a 7.3% carbohydrate solution (CHO) versus a 7.3% carbohydrate and 1.8% protein solution (CHO+P) (14). Similar to Ivy, the study by Saunders found that subjects consuming the CHO+P beverage exercised 29% longer at 75% VO_{2peak} than those consuming the CHO beverage (14). Finally, in 2007, another study by Saunders used gels matched for carbohydrate content below the maximum absorption rate (16). The experimental gels consisted of a 0.15 g carbohydrate per kg of bodyweight solution (CHO) and a 0.15 g carbohydrate per kg of bodyweight + 0.038 g protein per kg bodyweight solution (CHO+P) (16). It was found that those subjects consuming the CHO+P gel rode 13% longer at 75% VO_{2peak} on a cycle ergometer than those that consumed the CHO gel (16). Based on these studies it appears that the addition of protein to carbohydrate sports beverages can elicit significant improvements in TTE versus CHO beverages, when the carbohydrate content of the beverages is below the maximal absorption rate. However, no published studies to date have compared the effects of carbohydrate-matched beverages on time trial performance, so the effects of CHO+P on endurance ‘performance’ under these conditions cannot be quantified.

Following the publication of the aforementioned studies by Ivy and Saunders, subsequent studies examined whether the addition of protein could enhance the efficacy of carbohydrate beverages consumed at maximal gastrointestinal uptake rates (i.e. at rates believed to optimize the ergogenic effects of CHO intake). Specifically, the studies in this group used experimental beverages that containing greater than 60 gCHO•hr⁻¹. In 2009, a study by Saunders used experimental beverages consisting of 6% carbohydrates (CHO) and 6% carbohydrates and 1.8 grams of protein hydrolysate (CHO+P) (17). It was found that late-exercise time trial performance was enhanced by a small, but significant, degree (~30 s over the final 5 km) with consumption of the CHO+P beverage compared to consumption of the CHO beverage (17). In contrast, other studies in this group observed no beneficial effects of supplemental protein. For example, Van Essen and colleagues (2006) used experimental beverages with 6% carbohydrates (CHO), with an additional 2% protein (in the CHO+P beverage) (21). Van Essen observed no differences in performance between beverages (21). In 2010, Breen and colleagues used similar beverages with a time-trial exercise protocol. Experimental beverages consisted of 65 gCHO•hr⁻¹ (CHO) plus an additional 19 gPRO•hr⁻¹ (CHO+P) (1). Breen found that the CHO+P beverage did not improve late-exercise performance versus the CHO beverage (1). Lastly, in 2008, a study by Valentine compared two different carbohydrate-only beverages in addition to a carbohydrate plus protein beverage (20). Experimental beverages consisted of a placebo (PLA), a 7.75% carbohydrate solution (CHO), a 9.69% carbohydrate solution (CHO+CHO), and a 7.75% carbohydrate solution with an additional 1.94% protein solution (CHO+P) (20). In this study, CHO and CHO+P were matched for carbohydrate content while CHO+CHO and CHO+P are matched for caloric content. No significant differences in time-to-exhaustion were observed between CHO+P, CHO or CHO+CHO beverages, although all three experimental beverages

improved performance over the placebo (20). This group of studies has collectively shown that adding protein to carbohydrate beverages consumed at maximal gastrointestinal absorption rates of carbohydrates, appears to have little to no effect on improving endurance performance.

Researchers have also examined whether CHO+P beverages influence performance versus carbohydrate beverages that are matched for total calories. In 2006, Romano-Ely and colleagues used experimental beverages with 9.3% carbohydrates (CHO) and another with 7.5% carbohydrates with an additional 1.9% protein (CHO+P) (13). Subjects cycled at 70% VO_{2peak} until fatigue under each experimental condition. No differences in TTE were observed between CHO and CHO+P (13). In 2008, Valentine and colleagues found similar results during a study in which two different carbohydrate-only beverages were compared in addition to a carbohydrate plus protein beverages (as previously discussed) (20). The results of Valentine's study have indicated that the isocaloric beverages, CHO+CHO and CHO+P were not significantly different in time to exhaustion at the 75% VO_{2peak} intensity (20). Together, the studies by Romano-Ely and Valentine have indicated that CHO+P beverages do not improve endurance performance versus carbohydrate beverages matched for total calories. However, these studies also demonstrate that some carbohydrates can be replaced with protein without adversely affecting endurance performance.

A final group of studies has used beverages not matched for carbohydrate content or calories. These studies are harder to interpret, as potential differences in performance between treatments cannot be attributed to differences in individual macronutrients and/or calories. Nevertheless, McCleave and colleagues (2011) investigated the effects of a CHO+P beverage containing 3% carbohydrates and 1.2% protein, versus a CHO beverage containing 6% carbohydrates (11). Subjects completed a protocol consisting of 3 hours of varied-intensity

cycling following immediately by a ride to exhaustion at $\sim 75\%$ VO_{2max} . TTE was significantly greater with consumption of CHO+P compared to CHO (20). In 2010, a study by Martinez-Lagunas and colleagues used three beverages, none of which were matched for carbohydrate or caloric content (10). Beverage CHO+PRO H contained 4.5% carbohydrates and 1.15% protein, beverage CHO+PRO L contained 3% carbohydrates and 0.75% protein, and beverage CHO contained 6% carbohydrates. Subjects cycled at intensities between 55% and 75% VO_{2max} for 2.5 hours before completing a ride at 80% VO_{2max} until fatigue. No significant differences in TTE were found between CHO, CHO+PRO H, or CHO+PRO L (10). Similar to the aforementioned findings from McCleave and colleagues, Martinez-Lagunas showed that a beverage (CHO+PRO L) lacking in carbohydrate content, protein content, and total calories was able to elicit similar to results to the beverages containing more macronutrients. A final study, by Schroer and colleagues (2014), examined the effects of protein intake (without carbohydrate co-ingestion) on performance. The study compared three different treatment beverages: a placebo (PLA), a beverage containing 45 g/L protein (PRO), and a beverage containing 15 g/L alanine an amino acid present in protein, which has been speculated to have possible influences on performance (18). Subjects performed 120 minutes of cycling at 55% W_{max} before completing a 30 km time trial. Both ALA and PRO beverages ‘possibly’ harmed time trial performance compared to PLA (18). The results of this study (as well as those from the aforementioned investigations in this section) suggest that the previously published improvements in performance with CHO+P co-ingestion are not the simple result of additional calories from protein. Instead, it is possible that protein may be impacting endurance performance via another mechanism, such as a protein-specific synergistic influence on the ingested carbohydrate. However, it should be noted that the protein intake rates in the Schroer study (45 g/hr whole protein) greatly exceeded the amounts of

protein co-ingested with CHO in studies that have reported performance benefits with CHO+P (typically 10-20 g/hr), which likely contributed to the possible detriments in performance versus CHO. It is not currently known whether protein ingestion at these lower rates has any impact on endurance performance.

In summary, at least three studies have reported that CHO+P ingestion at moderate intake rates (< 50 gCHO/hr) results in substantial improvements (13-36%) in TTE versus CHO beverages containing equal carbohydrate content (9, 14, 16). Additional calories in the CHO+P beverages of these studies (due to the supplemental protein) have been cited as a criticism of these investigations. However, there is no evidence to date that the ingestion of protein alone has any impact on endurance performance and one recent study reported that relatively high protein intake during exercise may actually impair performance. Thus, it is possible that CHO+P ingestion may be impacting endurance performance via another mechanism, such as a protein-specific synergistic influence on the ingested carbohydrate.

A number of studies have reported that CHO+P ingestion has no influence on endurance performance in cycling time trials (1, 12, 21), which represent athletic performance more closely than TTE protocols. However, each of these studies utilized beverages consumed at very high rates of CHO ingestion (> 60 gCHO/hr), in which additional macronutrient intake has little or no impact on performance. As a result, it remains unknown whether CHO+P ingestion at moderate intake rates (< 50 gCHO/hr) results in meaningful improvements in cycling performance, in addition to TTE.

As illustrated above, there are numerous unanswered questions regarding the influences of CHO+P ingestion on endurance performance. Specifically, it remains to be determined how varying amounts of carbohydrate and protein intake (alone, and co-ingested) influence

performance during prolonged cycling time trials. Our laboratory is currently conducting a study investigating two questions on this topic:

1) Does CHO+P ingestion (at 45 gCHO/hr + 15 gPRO/hr) improve cycling performance versus a CHO beverage matched for carbohydrate content (45 gCHO/hr)?

2) Does the ingestion of 15g/hr of protein ingestion improve cycling performance versus a placebo (PL)?

Chapter II: Methodology

Participants

Study participants were recruited and selected based on three primary criteria. First, all subjects were required to be between 18 and 45 years of age. Secondly, each subject, following their $\text{VO}_{2\text{peak}}$ measurement, was required to have a $\text{VO}_{2\text{peak}}$ greater than 55 ml/kg/min or 4.5 L/min. Finally, each selected subject was characterized as “low risk” for exercise complications using criteria from the American College of Sports Medicine’s Guidelines for Exercise Testing and Prescription (9th Ed., ACSM, 2014).

Twelve subjects were recruited for the current study and based on their completion of the criteria mentioned previously. Of the twelve recruited subjects, five (3 males and 2 females) completed all experimental trials while the final subject completed only three experimental trials (Age: 22 ± 1 years; Height: 167 ± 12 cm; Weight: 60 ± 10 kg; $\text{VO}_{2\text{max}}$: 62 ± 7 ml/kg/min).

Study Design

Selected subjects completed a total of six trials, each of which being separated by 5-7 days. Specifically, the following trials were completed: 1 pre-testing trial, 1 familiarization trial, and 4 trials containing experimental treatments. With each experimental trial, subjects consumed one of the following four beverages. Subject either received a non-caloric placebo (PLA), a protein-only beverage (15 g/hr – PRO - whey), a carbohydrate-only beverage (45 g/hr – CHO - dextrose), or a combination of carbohydrate and protein (45 g/hr CHO + 15 g/hr PRO – CP), all of which were matched for flavor.

Experimental Trial Design

Subjects completed four experimental trials using an electronically braked cycle ergometer. Two exercise phases were completed within each trial. The first phase consisted of 120 minutes of steady-state cycling at 55% W_{max} . The second phase of the exercise protocol consisted of a simulated 30-km time trial (~50 minutes).

Experimental Treatments

Treatments were supplied to subjects using a randomly counterbalanced, double-blinded, placebo design. Beverages were provided to subjects before the exercise protocol began, throughout the steady-state exercise phase, and throughout the time trial. Prior to exercise, subjects received a bolus dose (600 ml) of their specific beverage. During the steady-state exercise phase, subjects received 150 ml every 15 minutes. Finally, during the time trial, subjects received 150 ml at three specific distance points, those being 7.5 km, 15 km, and 22.5 km. Each beverage was consumed within two minutes during exercise.

Dietary and Exercise Controls

Subjects were given a “food log” to record all dietary intake 24 hours prior to their first experimental trial. The subject was told to replicate this dietary intake prior to each experimental trial thereafter. Dietary logs were then obtained following each experimental trial.

Subjects were told to refrain from any form of heavy exercise 48 hours prior to each experimental trial. Additionally, subjects were asked to record all physical activity 72 hours preceding each experimental trial. All subjects were asked to continue exercise habits throughout

the duration of the study with consideration towards the final 48 hours prior to each experimental trial.

Subjects performed each experimental trial being fed prior to the initiation of exercise. Standardized meals were given to each subject 1-2 days prior to each trial. The night before each trial, subjects consumed a liquid meal replacement (Ensure Shakes). Two hours prior to the experimental trials, subjects then consumed a standardized meal of ~500 kcals.

Measurements

Performance Time and Mean Power Output: were used to measure exercise performance, measured during phase 2.

Metabolic Measurements: A Moxus Modular Metabolic System recorded metabolic measurements at the following times during exercise: minutes 15, 35, 55, 75, 95, and 115 of phase 1, and at 20 km and 30 km of phase 2.

Blood Glucose and Lactic Acid: finger stick blood samples were obtained at the following times: minutes 20, 40, 60, 80, 100, and 120 of phase 1, and at 20 km and 30 km of phase 2. Glucose and lactate levels was determined using an automated analyzer.

Heart Rate: was assessed at the same times as blood glucose and lactic acid using a heart rate monitor. Average heart rate of the 30 km time trial was also recorded.

Ratings of Perceived Exertion (RPE): subjective ratings of exertion was collected using a Borg RPE scale measured 6-20. Measurements were obtained at the times mentioned for blood glucose and lactic acid.

Gastrointestinal Distress Scale: subjects were asked to complete a questionnaire at minutes 30, 60, 90, and 120 of phase 1, and at 20 km and 30 km of phase 2. The questionnaire contains questions regarding the presence of the following GI problems: stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, vomiting, and urge to urinate or defecate. The items were then scored on a 10-point scale (1 = not at all, 10 = very, very much).

Data Analysis

Probabilistic magnitude-based inferences, using methods described by Hopkins and colleagues, were used to analyze collected data for the present study (7). Many recently published studies have utilized this method of analysis, especially those investigating the effects of nutritional supplementation on endurance performance. This approach has several advantages over null-hypothesis testing as the Hopkins method uses effect-magnitudes, estimate precision, and interpretive descriptors in order to qualify the probability of an important experimental effect. The present study maintained a 90% confidence interval to illustrate uncertainty within treatment effects, as this confidence interval represents an ‘unclear’ effect with a >5% chance of being either negative or positive (7). Additionally, threshold values indicating a substantial change were calculated as $0.2 \times \text{SD}$ (Standard Deviation), from the placebo trial. A spreadsheet (6), developed by Hopkins and colleagues was utilized in order to classify treatment effects as either beneficial (positive), harmful (negative), or trivial (negligible) (5). The following qualitative inferences were used to describe the likelihoods of reaching substantial change threshold values: <1%: most unlikely, 1-5%: very unlikely, 5-25%: unlikely, 25-75%: possible, 75-95%: likely, 95-99%: very likely, and >99%: most likely. An ‘unclear’ inference was applied to measurements that contained values within the 90% CI that exceeded threshold for both positive and negative effects.

Chapter III: Results

30-km Time Trial Performance

Mean performance times, power outputs, and qualitative inferences for comparisons between treatments are summarized in Figure 1, Figure 2, and Figure 3. Most notably, both CHO and CHO+PRO were shown to have ‘possible benefits’ over PL ($-3.9 \pm 5.0\%$ and $-3.6 \pm 5.4\%$ respectively). No clear effects were observed between other treatments.

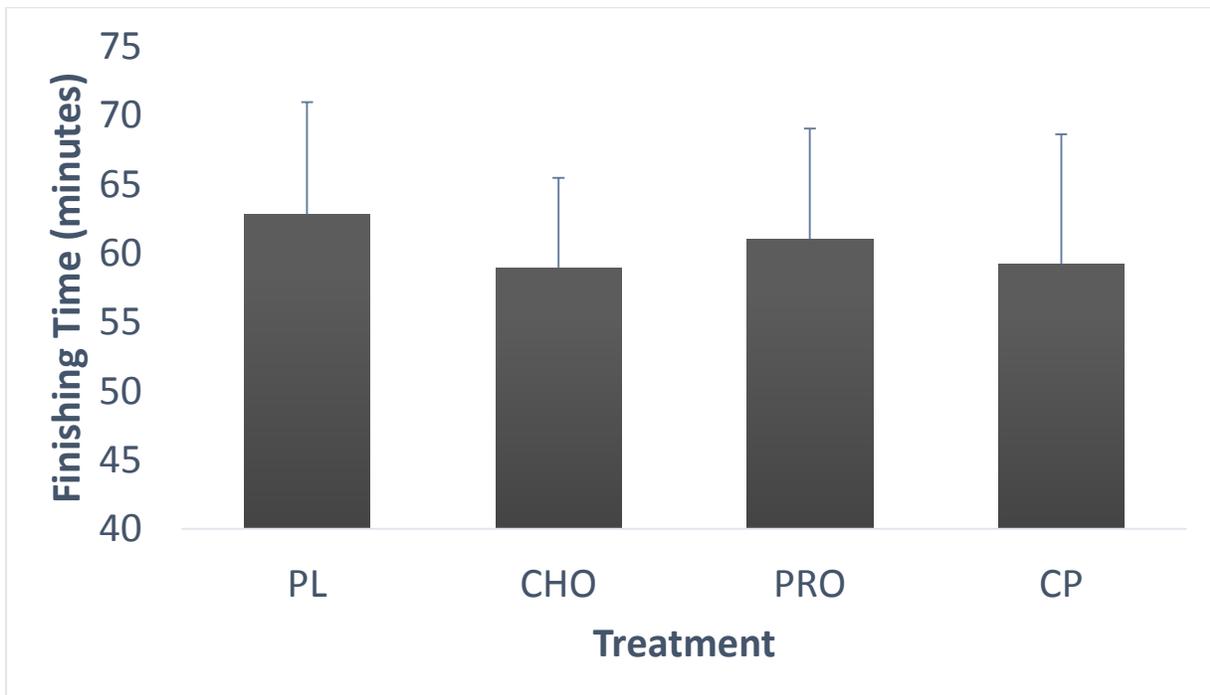


Figure 1. Mean time trial performance measurements (in minutes) for each experimental beverage. Y-axis error bars represent one standard deviation of the mean.

Physiological Measurements during Constant-Load Cycling

Measured values of VO_2 , RER, blood glucose and lactate, and RPE (and qualitative inferences for between-treatment differences) during the constant-load phase are summarized in Table 2. Differences in steady-state responses between treatments were generally ‘unclear’, or small in magnitude. The most consistent observation was that blood glucose levels tended to be slightly higher in the trials containing carbohydrate (CHO and CP) versus other trials (PL and PRO).

Physiological Measurements during the Time-Trial

Physiological measurements obtained during the time trial are summarized, in addition to qualitative inferences, in Table 3. Although there were some ‘unclear’ comparisons between individual treatments, VO_2 , RER, blood glucose and lactate levels tended to be generally higher in the CHO and CP trials versus the PL and PRO trials, which was likely a reflection of the higher power outputs during the CHO and CP trials. RPE was similar across treatments.

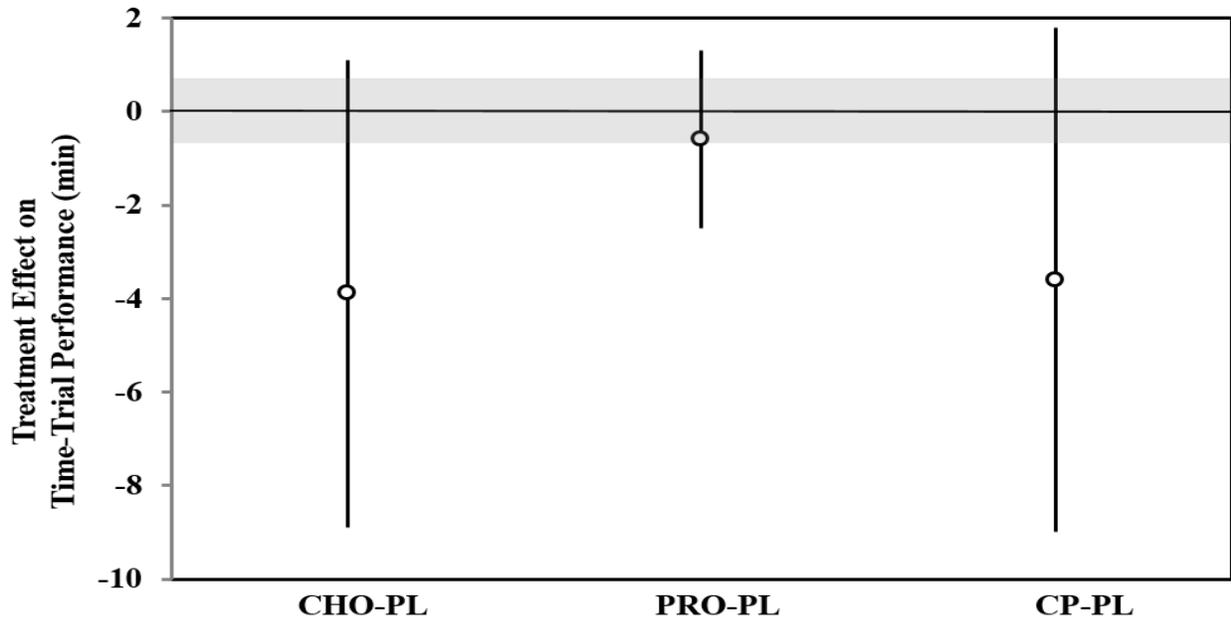


Figure 2. Comparison of mean time trial performance between experimental treatments.

Treatment effects (mean difference \pm 90% CI) for each experimental beverage is compared to the placebo. Open circles represent the mean value while the vertical lines represent the range of individual values.

Probabilities of benefit/trivial/harm and Qualitative Inferences:

CHO-PL: 'possible' benefit (4/23/72) for CHO; PRO-PL: 'unclear' (10/48/92); CP-PL:

'possible' benefit (2/30/68) for CHO.

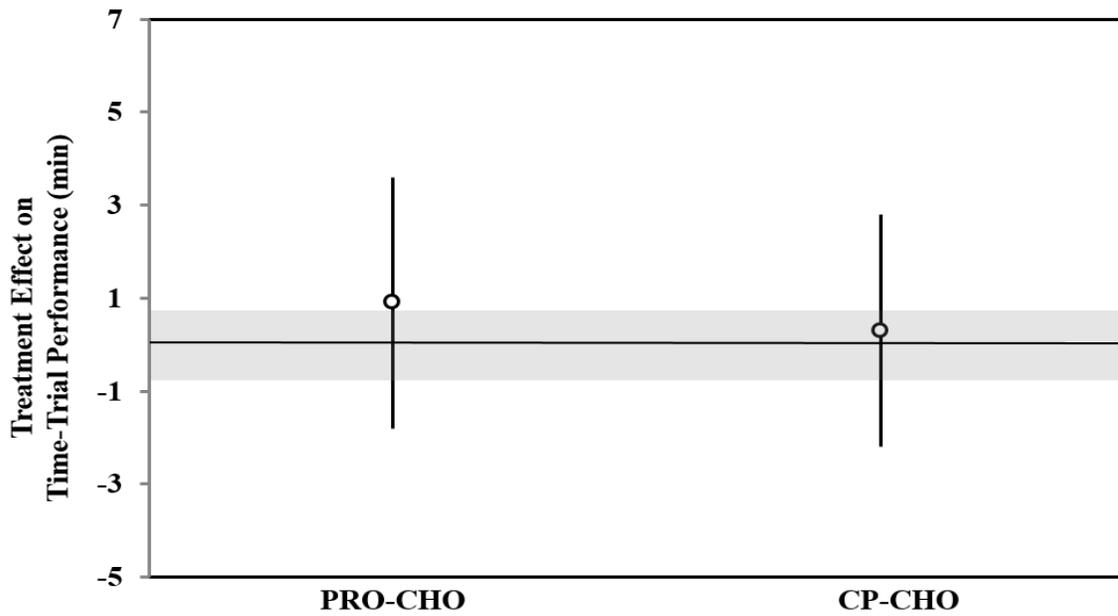


Figure 3. Comparison of mean time trial performance between experimental treatments.

Treatment effects (mean difference \pm 90% CI) for each experimental beverage is compared to the carbohydrate-only beverage. Open circles represent the mean value while the vertical lines represent the range of individual values.

Qualitative Inferences:

PRO-CHO: 'unclear' (51/36/13); CP-CHO: 'unclear' (39/36/25).

Table 1. Constant Load Measurements.

Variable	Time	Mean \pm SD				Treatment Differences				
		PL	CHO	PRO	CP	CHO-PL	PRO-PL	CP-PL	PRO-CHO	CP-CHO
VO ₂ (ml·min ⁻¹)	20	2548 \pm 515	2491 \pm 459	2455 \pm 424	2506 \pm 518	-58 \pm 120 3/70/27 Possible	14 \pm 148 14/80/6 Unclear	-43 \pm 115 4/65/31 Possible	77 \pm 35 16/84/0 Likely Trivial	15 \pm 77 5/89/6 Likely Trivial
	120	2681 \pm 509	2591 \pm 484	2624 \pm 564	2639 \pm 542	-89 \pm 128 3/44/53 Possible	61 \pm 117 22/75/3 Likely Trivial	-41 \pm 158 7/52/41 Unclear	166 \pm 182 76/23/1 Likely	48 \pm 92 13/83/4 Likely Trivial
RER	20	0.90 \pm 0.02	0.88 \pm 0.01	0.89 \pm 0.01	0.91 \pm 0.03	-0.02 \pm 0.02 3/3/94 Likely	-0.02 \pm 0.01 1/3/96 Very Likely	0.00 \pm 0.02 33/29/38 Unclear	0.01 \pm 0.02 75/17/8 Unclear	0.03 \pm 0.02 88/5/6 Unclear
	120	0.85 \pm 0.03	0.86 \pm 0.01	0.85 \pm 0.03	0.88 \pm 0.03	0.01 \pm 0.02 61/30/9 Unclear	-0.01 \pm 0.01 2/53/45 Possible	0.02 \pm 0.02 90/7/3 Likely	-0.01 \pm 0.02 5/17/78 Likely	0.02 \pm 0.02 74/19/7 Unclear
Glucose (mg·dL ⁻¹)	20	76 \pm 11	80 \pm 9	74 \pm 5	81 \pm 7	-4 \pm 9 51/29/20 Unclear	-5 \pm 10 11/22/67 Unclear	5 \pm 10 42/37/21 Unclear	-7 \pm 5 1/4/95 Likely	1 \pm 5 13/55/31 Unclear
	120	66 \pm 7	75 \pm 11	70 \pm 5	75 \pm 7	9 \pm 11 80/8/12 Unclear	2 \pm 4 70/26/4 Possible	10 \pm 7 92/4/4 Likely	-6 \pm 12 15/12/73 Unclear	1 \pm 6 43/29/28 Unclear
Lactate (mmol·L ⁻¹)	20	1.9 \pm 0.9	1.9 \pm 0.7	1.5 \pm 0.4	1.5 \pm 0.3	0.0 \pm 0.8 45/23/32 Unclear	-0.6 \pm 1.0 11/12/77 Unclear	-0.4 \pm 0.6 5/13/82 Unclear	-0.6 \pm 0.5 2/5/92 Likely	-0.4 \pm 0.6 4/10/86 Likely
	120	1.4 \pm 0.2	1.6 \pm 0.6	1.7 \pm 0.9	1.5 \pm 0.5	0.2 \pm 0.5 84/6/10 Unclear	0.3 \pm 0.7 56/9/35 Unclear	0.1 \pm 0.5 42/9/49 Unclear	0.0 \pm 1.0 31/7/62 Unclear	-0.1 \pm 0.5 8/5/87 Unclear
RPE (6-20)	20	12.3 \pm 1.0	11.5 \pm 1.4	12.2 \pm 0.8	12.2 \pm 1.2	-0.8 \pm 0.6 3/7/90 Likely	-0.4 \pm 0.9 10/22/67 Unclear	-0.2 \pm 0.6 17/32/51 Unclear	0.4 \pm 1.1 66/19/15 Unclear	0.7 \pm 0.8 76/14/10 Unclear
	120	14.8 \pm 1.9	14.0 \pm 1.1	14.2 \pm 0.8	14.0 \pm 1.1	-0.8 \pm 1.4 11/55/34 Unclear	0.0 \pm 1.2 28/27/25 Unclear	-0.8 \pm 1.2 10/37/52 Unclear	0.2 \pm 1.4 42/38/21 Unclear	0.0 \pm 1.0 19/41/39 Unclear

*Note: One subject did not complete a PRO trial, so mean values (and corresponding treatment differences) were calculated on a sample of 5

Table 2. Time Trial Measurements.

Variable	Mean \pm SD				Treatment Effects				
	PL	CHO	PRO	CP	CHO-PL	PRO-PL	CP-PL	PRO-CHO	CP-CHO
VO₂ (ml·min ⁻¹)	2696 \pm 686	2729 \pm 726	2642 \pm 626	2959 \pm 867	34 \pm 635 4/26/70 Possible	-93 \pm 287 6/64/30 Unclear	264 \pm 480 14/77/10 Unclear	154 \pm 241 54/43/3 Possible	230 \pm 309 72/24/4 Possible
RER	0.81 \pm 0.06	0.84 \pm 0.02	0.82 \pm 0.03	0.87 \pm 0.05	0.03 \pm 0.04 63/30/6 Unclear	-0.01 \pm 0.03 4/40/56 Possible	0.04 \pm 0.01 100/0/0 Most Likely	-0.03 \pm 0.02 1/5/94 Likely	0.03 \pm 0.03 69/24/7 Unclear
Glucose (mg·dL ⁻¹)	63 \pm 7	76 \pm 6	66 \pm 5	73 \pm 6	12.4 \pm 3.3 100/0/0 Most Likely	0.0 \pm 3.5 21/59/20 Unclear	9.8 \pm 6.6 95/3/1 Very Likely	-11.1 \pm 2.9 0/0/100 Most Likely	-2.7 \pm 4.5 4/17/80 Likely
Lactate (mmol·L ⁻¹)	1.5 \pm 0.7	2.0 \pm 0.9	1.2 \pm 0.4	1.7 \pm 0.8	0.5 \pm 0.6 64/26/10 Unclear	-0.4 \pm 0.9 13/15/72 Unclear	0.1 \pm 0.6 9/37/54 Unclear	-0.7 \pm 1.2 10/9/81 Unclear	-0.4 \pm 0.6 10/15/75 Unclear
RPE (6-20)	17.0 \pm 1.7	16.7 \pm 0.8	16.2 \pm 1.3	16.8 \pm 1.1	-0.3 \pm 0.8 20/64/16 Unclear	-0.4 \pm 0.5 2/37/61 Possible	-0.2 \pm 0.6 19/65/16 Unclear	-0.4 \pm 0.5 2/33/65 Possible	0.2 \pm 0.6 18/62/20 Unclear

*Note: One subject did not complete a PRO trial, so mean values (and corresponding treatment differences) were calculated on a sample of 5.

GI Distress Symptoms

Ratings of GI discomfort were low across all treatments. Mean values (1-10 scale) measured during the constant-load phase, and time-trial phase were \leq 1.8 for all GI variables (stomach problems, GI cramping, bloating, nausea, belching, and vomiting). No more than one subject per treatment reported a score $>$ 2 at any particular time-point, and these ratings appeared to be randomly distributed across treatments. No subject reported any GI symptoms greater than 5 (moderate).

Chapter IV: Discussion

A primary purpose of the present study was to determine if the co-ingestion of carbohydrate and protein during exercise enhanced cycling performance versus carbohydrate alone. Performance in a 30 km time-trial (following 120 min at 55% W_{max}) was ‘possibly’ improved by both CP (59.2 ± 9.4 min) and CHO (58.9 ± 6.5 min) versus a non-caloric placebo (62.8 ± 8.1 min), but no clear differences were observed between CP and CHO beverages.

The observed improvement in cycling performance in our carbohydrate-containing beverages (CHO and CP) has been reported in numerous prior studies (9, 14, 16). During prolonged endurance exercise, the ergogenic effects of carbohydrate are largely attributed to the maintenance of high rates of carbohydrate oxidation late in exercise (9, 12, 14, 16, 17, 20). In support of this concept, we observed ‘likely’ elevations in steady-state RER and blood glucose with CP supplementation when compared to the placebo. Additionally, CP co-ingested resulted in ‘most likely’ and ‘very likely’ increases in time trial RER and blood glucose respectively, as compared with the placebo.

Prior studies comparing the effects of CP and CHO on endurance performance have provided conflicting findings. Some studies have reported relatively large improvements in performance with CP (9, 14, 16), while others have shown no differences between CP and CHO beverages (1, 12, 20, 21). Studies reporting no effects with CP have utilized beverages with high carbohydrate content (60+ g/hr) and typically used time-trial protocols, suggesting that the addition of protein may have little or no effects on time-trial performance when carbohydrate is consumed at rates that maximize exogenous oxidation rates (1, 12, 21). The studies reporting large improvements with CP have typically compared beverages containing moderate

carbohydrate content (40-50 g/hr), and employed time-to-exhaustion protocols. Because no prior studies have used a time-trial model to examine CP beverages containing moderate carbohydrate, it is unclear whether protein can elicit benefits under these conditions. The current findings provides novel information in this respect, as no clear improvements in time-trial performance were observed with CP (45 gCHO/hr + 15 gPro/hr) versus CHO (45 g/hr).

It is not clear why CP with moderate carbohydrate could enhance time-to-exhaustion (9, 14, 16), but not performance in a long-duration time trial, as shown in the present study. However, it is theoretically possible that the TTE protocol creates a more favorable environment for detecting possible ergogenic effects with CP. For example, some evidence suggests that carbohydrate and protein co-ingestion could shift carbohydrate usage towards exogenous blood glucose potentially delaying the use of endogenous glycogen stores, which could contribute to a delayed onset of fatigue (19). Similarly, there is indirect evidence that CP may impact endurance performance via improved cardiovascular and thermoregulatory responses (4). It could be that the prolonged moderate-intensity exercise of a TTE protocol could produce a metabolic environment in which these factors contribute more directly to fatigue (versus a time trial), thus increasing the likelihood that nutritional interventions, which impact these factors, would produce favorable results. However, this is highly speculative, as the mechanisms responsible for previously reported ergogenic effects with CP are poorly understood. In addition to the potential physiological differences between protocols, Hopkins and colleagues reported that time trial protocols may inherently introduce more error variance (due to differences in pacing), as compared to TTE protocols (6). Therefore, it is possible that the TTE protocol might be more sensitive in detecting small, but meaningful changes in performance compared to a TT protocol.

There are also a few limitations in the existing study which could have impacted our findings. The statistical power of our analyses was negatively affected by our small sample size, as only five subjects completed all exercise protocols (with a sixth completing all but the PRO trial). This decreases the confidence in our statistical conclusions. Furthermore, six additional subjects dropped out of the study prior to completion, likely due to the large number of demanding exercise trials (five three-hour trials over a month-long period, including the familiarization trial). This raises the concern that our subjects may have had difficulty maintaining consistent motivation and/or performance levels over the duration of the study. If so, this would also increase error variance and minimize the likelihood of detecting meaningful treatment effects. Therefore, further study is warranted in larger samples of competitive cyclists.

As indicated previously, the potential mechanisms to explain performance gains with CP in prior studies (9, 14, 16) are not well understood. Some have suggested that ergogenic effects are merely the result of additional calories from the supplemental protein (19, 20), while others have suggested that protein may have synergistic effects with carbohydrate when co-ingested (9, 14, 16). Therefore, a second purpose of our study was to determine if protein ingestion (PRO) alone affected cycling performance in comparison to a non-caloric placebo (PL). To our knowledge, only one prior study has examined the potential ergogenic effects of protein consumed in isolation. Schroer and colleagues (2014) reported that protein ingestion resulted in possible performance impairments compared to placebo. However, these investigators utilized a relatively high rate of protein ingestion (45 g/hr) in order to relate their findings to comparable ingestion rates of carbohydrate. As a result, the possible impairments in performance with protein could have been due to gastrointestinal distress related to malabsorption of the relatively high doses of protein; a concept that was supported by increased incidents of GI discomfort with

protein versus placebo. The present study was designed to examine a lower dose of protein ingestion (15 g/hr), which is directly comparable to the supplemental doses of protein provided in prior studies reporting ergogenic effects with CP co-ingestion (9, 14, 16). This rate of ingestion was effective at minimizing gastrointestinal distress, as there was no evidence of increased gastrointestinal symptoms with PRO, and symptoms were low across all trials. However, PRO ingestion produced no clear benefits in performance (-0.6 ± 1.9 min) versus PL. This provides additional evidence that protein ingestion in isolation has no ergogenic effects; and thus, the previously reported benefits of CP beverages in some studies (9, 14, 16) were possibly the result of synergistic effects with carbohydrate. However, as mentioned previously, these conclusions should be interpreted cautiously due to the low statistical power in the present study.

In summary, co-ingestion of carbohydrate and protein at moderate intake rates (45 gCHO/hr + 15 gPro/hr) had no effect on cycling time-trial performance versus carbohydrate alone (45 g/hr). In addition, protein intake alone (15 g/hr) had no ergogenic effects versus a non-caloric placebo. However, further study of this topic is required, as the present study lacked the statistical power to detect small but athletically-relevant differences in performance between treatments.

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