**SHORTER DURATION TIME TRIAL PERFORMANCE AND RECOVERY IS NOT IMPROVED BY INCLUSION OF PROTEIN IN A MULTIPLE CARBOHYDRATE SUPPLEMENT**

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**ABSTRACT**

Wolfe, AS, Brandt, SA, Krause, IA, Mavison, RW, Aponte, JA, and Ferguson-Stegall, LM. Shorter duration time trial performance and recovery is not improved by inclusion of protein in a multiple carbohydrate supplement. *J Strength Cond Res* 31(9): 2509–2518, 2017—Ingesting multiple carbohydrate (CHO) types during exercise can improve endurance performance compared with single CHO only. Adding protein to a multiple CHO beverage has been shown to increase cycling time to exhaustion (TTE) compared with a single CHO beverage. However, it is unclear if improvements were due to multiple CHO or protein, and TTE protocols are not representative of typical race events. This study investigated whether adding protein to a multiple CHO beverage improved performance and recovery in 2 same-day cycling time trials (TTs) compared with isocaloric multiple CHO only. Ten cyclists (37.4 ± 8.9 years; V̇O₂max 54.6 ± 6.5 ml·kg⁻¹·min⁻¹) performed a familiarization and 2 randomized, crossover, double-blinded experimental trials consisting of pretrial leg strength testing, 40-km TT, 30-min recovery, 10-km TT, and posttrial leg strength testing. Seven 275 ml doses of multiple CHO (MCO) or multiple CHO+protein (MCP) were ingested during the protocol. Blood glucose, lactate, heart rate (HR), and rating of perceived exertion (RPE) were also measured. Continuous variables were analyzed with paired t-tests, and repeated measures with repeated-measures analysis of variance. No differences existed between MCO and MCP in 40-km TT time (81.6 ± 2.8 vs. 81.9 ± 2.9 minutes, respectively, $p = 0.94$), or in 10-km TT time (24.0 ± 0.9 vs. 23.9 ± 1.0 minutes, $p = 0.97$). Blood glucose was higher before 10-km TT in MCO compared with MCP (3.78 ± 0.20 vs. 3.31 ± 0.19 mmol·L⁻¹, $p = 0.002$). No treatment differences were found for lactate, HR, RPE, or strength recovery. When using a protocol and performance measures that replicate realistic, shorter duration events, adding protein to a multiple CHO beverage does not improve performance compared with multiple CHO only.

**KEY WORDS** cycling, supplementation, second bout

**INTRODUCTION**

The ergogenic effects of carbohydrate (CHO) ingestion during cycling endurance exercise have been well documented (6,7,19,23). Previous studies have demonstrated improvements in time to exhaustion (TTE) and power output with CHO ingestion compared with water only, due to increased blood glucose levels during later stages of exercise (6,7,9,10,23). According to Jeukendrup (25), the maximal rate of CHO oxidation is approximately 1.0–1.1 g·min⁻¹ when a single type of CHO (i.e., glucose only) is consumed; however, when multiple CHO types (i.e., glucose + fructose, or glucose + fructose + maltodextrin) are ingested, the rate of oxidation can increase substantially, reaching as high as 1.75 g·min⁻¹. This is most likely due to an optimization of multiple intestinal transporters, leading to an increase in the rate of absorption beyond that of a single CHO alone (10,21,25). Along with increased absorption, the increased oxidation rate is important for many reasons. During exercise, energy expenditure typically exceeds that of energy intake; therefore, ingesting CHOs to use as fuel is essential for exercise to continue, especially if muscle glycogen levels are low or depleted. This exogenous intake of CHOs may spare liver glycogen (22), prevent a decrease in blood glucose levels (7,22,23), and sustain the rate of CHO oxidation needed to maintain exercise intensity (22,23).

Previous investigations have demonstrated that the addition of protein (Pro) to a CHO beverage may further improve endurance performance (14,20,29,33,36). For example, Saunders et al. (36) demonstrated a 29% increase in TTE with a CHO + Pro beverage compared with a single-type CHO beverage.
beverage, whereas Ivy et al. (20) reported an increase of 36% in TTE when protein was added to a CHO beverage. Along with improvement in single-bout endurance performance, some studies have also reported an increase in recovery and second-bout performance (13,18,33,36,42). This added recovery benefit is believed to stem from enhanced insulin release (33,42), an increase in muscle glycogen storage (18,42), decreased muscle damage (36), and an increase in signaling for protein synthesis (13).

Two recent investigations using trained cyclists compared a CHO + Pro beverage containing multiple CHO (dextrose, fructose, and maltodextrin) and a small amount of whey protein to an isocaloric, single CHO (dextrose) beverage and demonstrated a significant increase in endurance performance, assessed by TTE (14,29). While intriguing, the results of their studies have left 3 important questions unanswered. First, was it the ingestion of multiple CHO or the inclusion of protein that improved TTE? Given that multiple CHO have been shown to provide an increase in oxidation compared with a single CHO (22,25), and given that the multiple CHO + Pro treatment was compared with a single CHO-only treatment, it is difficult to determine if the reported performance and recovery benefits were due to ingestion of multiple CHO, or to the inclusion of protein. Second, although TTE is a commonly used endurance protocol (9,14,18,29,36), it may not be the most realistic measure of performance for most competitive cyclists, because cycling races (i.e., road races, time trials [TTs], and criteriums) are typically won by completing a fixed distance in the shortest possible time. It has been suggested that a TT, an event in which athletes cycle a fixed distance as fast as possible, is a more realistic and practical measure of endurance performance (41), and therefore, may be a more accurate assessment of performance than TTE. Lastly, the long duration of the cycling bouts (>3 hours) and recovery period (4 hours) does not reflect what occurs in every type of competitive cycling event. For example, criterium races, some circuit road races, and most TTs are often completed in <90 minutes, and when cyclists compete in multiple events in 1 day, the recovery time between events can be as little as 20–30 minutes.

The benefits of CHO and CHO + Pro ingestion are most clear in long duration events because maintaining blood glucose is well known to be most challenging with prolonged duration (7). However, it is important to determine the effectiveness of CHO and CHO + Pro supplementation for athletes who compete in events of shorter duration. It is also common for cyclists to compete in more than one cycling event in a day when events are relatively short, and to have very limited recovery time in between events. To our knowledge, there are no investigations that compare supplementation types using an experimental protocol that replicates events such as these. Therefore, to better elucidate the potential benefits of the addition of protein to a multiple CHO beverage in same-day events of shorter duration, this study compared a multiple CHO beverage with whey protein (MCP) to an isocaloric multiple CHO-only beverage (MCO), using 2 same-day TTs as the measures of performance. We hypothesized that the MCP beverage would (a) improve TT performance in both bouts, and (b) aid in leg strength recovery, compared with the MCO beverage.

**METHODS**

**Experimental Approach to the Problem**

This investigation sought to apply a protocol intended to better replicate back-to-back race events of shorter durations (<90 minutes) in determining if adding protein to a multiple CHO beverage could improve performance and recovery. We chose this approach because longer duration events and recovery periods have been well studied, yet comparatively little is known about the performance effects of supplementation in shorter duration events. Therefore, there is a practical need to investigate this type of competitive scenario for the many athletes who compete in this manner.

Two examples of the practical relevancy of our design are (a) during a stage race, a circuit race lasting 60–90 minutes may be followed by short (10 km or less) TT, with only ~30 minutes rest between the 2 events, and (b) in criterium racing, a cyclist may compete in 2 back-to-back races separated by only 20–30 minutes. Thus, we aimed to (a) use shorter duration TTs (<90 minutes) and (b) use a protocol that simulates racing in back-to-back race events that are separated by a brief recovery. Using a randomized, double-blinded, repeated-measures crossover design, we compared a 4.8% multiple CHO (MCO) beverage to an isocaloric 1.2% whey protein +3.6% multiple CHO (MCP) beverage in a standardized cycling and recovery protocol.

Lactate threshold (LT) and maximal oxygen consumption (\(\text{V}O_2\text{max}\)) testing took place in a preliminary visit to determine eligibility for participation. During the second visit, subjects performed a familiarization trial in which the experimental protocol was performed, but only water was provided and no blood samples were taken. Each subject then completed 2 randomly ordered experimental trials, separated by at least 5–7 days. The protocol for all trials is shown in Figure 1. All trials consisted of pretrial leg strength testing, a 40-km TT, a 30-minute recovery period, a 10-km TT, and posttrial leg strength testing. These TT distances were chosen because they are common distances for competitive TT events. Outcome measures of interest were TT performance (time and average power output), changes in blood glucose, blood lactate, and salivary cortisol, and posttrial leg strength recovery.

**Subjects**

Ten trained cyclists and triathletes were admitted to the study (8 men and 2 women; 37.4 ± 8.9 years). Subject characteristics are listed in Table 1. Subjects were recruited through emails to area cycling racing teams, and fliers were handed out at local races. Each subject had trained consistently and competed in cycling events for at least the last 5
years. Each subject served as his or her own control and performed the same protocol as shown in Figure 1 for each trial. All subjects provided voluntary written informed consent, and the study was approved by the Hamline University Institutional Review Board.

**Procedures**

**Preliminary Testing.** To determine eligibility criteria for admission to the study, and to determine wattages for the warm-ups in the 3 subsequent trials, subjects performed LT and \( V_O^2_{\text{max}} \) tests on an electrically controlled ergometer (Velotron Dynafit Pro; RacerMate, Seattle, WA, USA). The same ergometer was used for the preliminary testing and all subsequent trials. During both tests, subjects breathed through a Hans Rudolph valve, and expired gases were directed to a mixing chamber for the analysis of oxygen and carbon dioxide (ParvoMedics TrueOne 2400; Parvo Medics, Sandy, UT, USA). The LT test preceded the \( V_O^2_{\text{max}} \) test, and the 2 were separated by a 5-minute cool down. Lactate threshold was determined using 5-minute stages, beginning at 70 Watts (W) for men and at 50 W for women. Watts were incrementally increased by 25 W (men) and 15 W (women) for the first 3–4 stages, followed by increases of 15 W (men) and 10 W (women) for the last 2–3 stages. Blood was collected through finger stick and analyzed using a Lactate Plus lactate meter (Nova Biomedical, Waltham, MA, USA) during the final minute of each stage. Lactate threshold was defined as the breakpoint when lactate levels begin to rise above baseline.

After the 5-minute cool down, each subject performed a \( V_O^2_{\text{max}} \) test. The protocol consisted of a 4-minute warm-up beginning at 125 W for men and 75 W for women. The workload increased by 50 W (men) and 30 W (women) for three 2-minute stages, followed by increases of 25 W (men) and 15 W (women) every minute until \( V_O^2_{\text{max}} \) was reached or the subject could not continue pedaling despite verbal encouragement. \( V_O^2_{\text{max}} \) was established using the criteria of a plateau in \( V_O^2 \) despite increasing workload, and a respiratory exchange ratio > 1.10.

**Training and Diet.** Subjects were asked to keep an exercise and dietary intake log for 72 hours before their familiarization trial, in which they recorded their training (duration, mode, and intensity) and all food and beverage intake, including portion sizes. They were also asked to avoid high-intensity exercise for 24 hours before the trial. They were then required to replicate their training and dietary intake exactly as was done the 72 hours before the familiarization trial for the 72 hours before both experimental trials. The logs were examined and compliance verified with each subject by the same investigator before each experimental trial began. All subjects complied with the exercise and dietary consistency requirements. Diets were not standardized across all subjects, as each served as his/her own control. Subjects were not required to fast before the trial because athletes typically do not compete in race events in the fasted state. All subjects were provided with a Kirkland Signature Complete Nutritional Shake to consume 2–3

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**Table 1.** Subject characteristics.*

<table>
<thead>
<tr>
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<th>( n = 10 )</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>37.4 ± 8.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.5 ± 10.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.5 ± 11.7</td>
</tr>
<tr>
<td>( V_O^2_{\text{max}} ) (L·min(^{-1}))</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>( V_O^2_{\text{max}} ) (mL·kg(^{-1})·min(^{-1}))</td>
<td>54.6 ± 6.5</td>
</tr>
<tr>
<td>( V_O^2 ) at lactate threshold (L·min(^{-1}))</td>
<td>3.5 ± 0.7</td>
</tr>
</tbody>
</table>

*Values are mean ± SD.
hours before each trial to ensure that pretrial energy intake was consistent across all subjects. The shake contained 30 g CHO, 9 g protein, 6 g fat, and 230 total kcals.

Experimental Beverages. The MCO beverage consisted of 1.6% each of dextrose, fructose, and maltodextrin, and the MCP beverage contained 1.2% of dextrose, fructose, and maltodextrin, plus 1.2% whey protein isolate. The energy and macronutrient content of the beverages are shown in Table 2. Two hundred seventy-five ml doses of each beverage were provided at 7 time points during the protocol, as shown in Figure 1. The CHO ingestion rate for MCO was 81 g $h^{-1}$ (1.35 g $min^{-1}$) and 58 g $h^{-1}$ for MCP (0.97 g $min^{-1}$), both of which are near the rate considered optimal for improving endurance performance (60 g $h^{-1}$ or 1 g $min^{-1}$) (22). The ingredients were obtained in powder form and mixed in the laboratory by a laboratory member not involved in data collection. Green apple flavor and coloring was added to each beverage so that the 2 experimental treatments were identical in color, taste, and consistency. In addition, 3 electrolyte capsules (SaltStick Capsules, Toker Engineering, Thousand Oaks, CA) were dissolved into the total beverage volume to ensure that adequate electrolytes were provided (Table 2).

Cycling Protocols. Three to 5 days after preliminary testing, subjects reported to the laboratory to perform a familiarization trial. This allowed for verification of warm-up workloads, and followed exactly the same protocol as the experimental trials except that subjects only received water with electrolytes, and no blood samples were taken. On arrival to the laboratory, each subject’s exercise and diet was examined and verified, resting blood pressure and body weight was measured, and the subject was fitted with a heart rate (HR) monitor. Pretrial isometric leg strength of the knee extensors was measured (explained below), and then a 10-minute warm-up was performed at 55% of $W_{max}$. The Watts were determined using the following formula adapted from Astrand and Rodahl (2):

$$W = \left(\frac{[V_{O2max} \times %V_{O2max} \text{ desired}]}{12.5 \text{ W} \cdot \text{ml}^{-1} \text{O}_2}\right) - 300 \text{ ml O}_2$$

As shown in Figure 1, the first cycling bout was a 40-km TT, and the second was a 10-km TT. The TT courses were designed to include rolling hills with an uphill finish, using RacerMate Interactive 3D software (RacerMate). Subjects were blinded to elapsed time, power output, rating of perceived exertion (RPE), HR, and kilometers per hour, and all laboratory and personal timing devices were removed from sight. Subjects were able to watch their progress along the course on a computer screen located approximately 1.5 m in

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Time trial (TT) performance. A) Time trial time to completion for the 40-km and 10-km TTs. B) Average power output (W) in the 40-km and 10-km TTs. Values are mean ± SE. MCP = multiple CHO + protein; MCO = multiple CHO.

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**Table 2.** Energy and macronutrient content of the experimental beverages (per 100 ml).*

<table>
<thead>
<tr>
<th></th>
<th>MCO</th>
<th>MCP</th>
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<tbody>
<tr>
<td>Kcals</td>
<td>16.8</td>
<td>16.8</td>
</tr>
<tr>
<td>% total CHO</td>
<td>4.8</td>
<td>3.6</td>
</tr>
<tr>
<td>% dextrose</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>% fructose</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>% maltodextrin</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>% Pro</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td>Ratio of CHO:Pro</td>
<td>3:1</td>
<td></td>
</tr>
<tr>
<td>CHO (g)</td>
<td>11.55</td>
<td>8.25</td>
</tr>
<tr>
<td>Pro (g)</td>
<td>0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* MCO = multiple CHO; MCP = multiple CHO + protein; CHO = carbohydrate; Pro = protein.

† Two hundred seventy-five milliliters was provided for each dose during the experimental protocol. Both treatments contained the same amount of electrolytes Na⁺, K⁺, Mg²⁺, and Ca²⁺.
front of the ergometer. The laboratory temperature was maintained at approximately 21°C, and a fan was directed toward the subject to reduce thermal stress. Music was played from a laboratory computer. The same playlist chosen by each subject was used for each trial, but was played in random order in an attempt to reduce any time-related sense of pacing. Subjects were instructed to complete the TT as they normally would in race conditions, and consistent verbal encouragement was given to all subjects by the same investigators during each trial.

Heart Rate and Rating of Perceived Exertion. Heart rate and RPE were recorded every 5 minutes during both TTs. Heart rate was recorded using an HR monitor (Polar Electro Oy, Kempele, Finland). Rating of perceived exertion was assessed using a 1–10 scale, with 1 being no effort at all and 10 indicating maximal effort. A color-coded chart was displayed on a clipboard and explained in detail during the familiarization trial. This allowed subjects to relay their RPE both verbally and physically by pointing to ensure accuracy.

Blood and Saliva Sampling. Capillary blood samples were taken from the fingertips during the TTs using an Accu-Chek Safe-T-Pro lancet (Roche Diagnostics, Indianapolis, IN, USA). Blood glucose was measured in duplicate by a glucose meter (Freedom Lite, Freestyle, Alameda, CA, USA), and blood lactate was analyzed using a Lactate Plus lactate meter (Nova Biomedical). Saliva samples were collected using an oral swab method at 4 time points (Figure 1). Before the saliva collections, subjects were given water and asked to rinse their mouth thoroughly. Then samples were collected using Salimetrics Oral Swabs (Salimetrics LLC, Carlsbad, CA, USA). Samples were frozen and stored at −20°C until analysis. Salivary cortisol levels were measured by competitive enzyme-linked immunosorbent assay according to the manufacturer’s protocol using the Salimetrics Salivary Cortisol 3102-5 Enzyme Immunoassay Kit (Salimetrics LLC). All samples were run in duplicate with an acceptable coefficient of variation of 5.5%.

Recovery Period. As described previously, the recovery period was 30 minutes long to simulate the very brief recovery periods that often occur between cycling events during the same day, such as in stage races or back-to-back criteriums. During this time, subjects sat comfortably in a reclining chair. As shown in Figure 1, subjects were instructed to drink a serving of the experimental beverage at 10 minutes into the

Figure 3. Blood glucose (A and B) and lactate concentrations (C and D) were measured at 4 time points during the 40-km time trial (TT) (Pre, 20, 40 minutes, and Finish), and immediately before (Pre) and at the end (Finish) of the 10-km TT. *p = 0.002, MCO vs. MCP immediately before the 10-km TT. A significant time effect was present in both treatments for glucose and lactate in both TTs (all p ≤ 0.05). Values are mean ± SE.
recovery period, and at 25 minutes, a saliva sample was taken after rinsing the mouth thoroughly with water.

**Leg Strength Recovery.** Before the 40-km TT and after the 10-km TT, isometric maximal voluntary contraction (MVC) strength of the knee extensors (quadriceps) was determined using an Isometric Leg Strength System (ILSS), which was custom built previously by our laboratory. Briefly, the ILSS is a knee extension machine fitted with a strain gage, and the voltage output is converted by an attached power meter into force measurements. Subjects were seated with their backs resting against the back pad of the unit, and adjustments were made to the position so that the knees were at a 90° angle, and ankles were comfortably placed under the ankle pads. Subjects were asked to firmly grip the handgrips beside the seat for stability and support during each contraction effort, and to hold their MVC for 3 seconds. After a familiarization trial, the maximum force of 2 trials was used for analysis. Each trial was separated by 60 seconds. The percent change from pre- to post-experimental protocol was calculated as the leg strength recovery outcome measure.

**Statistical Analyses**

Power analysis was performed using G-Power 3.1.9.2 software developed at Dusseldorf University, Germany (12) before beginning subject recruitment. The minimum number of subjects needed for a 2-tailed alpha level of 0.05 and desired power value of 0.80 was 10. Interval variables (TT performance outcome measures and leg strength recovery) were analyzed using 2-tailed paired t-tests. Repeated measures (blood glucose, blood lactate, and salivary cortisol) were analyzed using 2-way repeated-measures analysis of variance (treatment by time), and post hoc analysis was performed using a Bonferroni correction when significance was found. The significance level for all analyses was determined at $p \leq 0.05$. All data were expressed as mean ± SE. SPSS Version 22 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

**Results**

**Time Trial Performance**

As shown in Figure 2A, no significant differences in TT time existed between MCO and MCP in either the 40-km ($81.6 \pm 2.8$ vs. $81.9 \pm 2.9$ minutes, respectively, $p = 0.94$) or the 10-km ($MCO, 24.0 \pm 0.9$ vs. MCP, $23.9 \pm 1.0$ minutes, $p = 0.97$) TTs. Average power output (W) was also not significantly different between MCO and MCP for the 40-km ($236.1 \pm 17.5$ vs. $235.6 \pm 17.9$ W, respectively, $p = 0.98$), or the 10-km ($MCO, 241.9 \pm 18.0$ vs. MCP, $243.2 \pm 18.5$ W, $p = 0.96$) TTs (Figure 2B).

**Heart Rate and Rating of Perceived Exertion.** Average HR during TTs was not significantly different between treatments for either the 40-km ($MCO, 150.7 \pm 3.6$ vs. MCP, $149.7 \pm 4.2$ b-min$^{-1}$, $p = 0.68$) or the 10-km ($MCO, 153.7 \pm 4.1$ vs. MCP, $153.9 \pm 3.8$ b-min$^{-1}$, $p = 0.90$) TTs. Average RPE was also not different for either the 40-km ($MCO, 6.7 \pm 0.3$ vs. MCP, $6.6 \pm 0.3$, $p = 0.82$) or the 10-km ($MCO, 7.5 \pm 0.3$ vs. MCP, $7.5 \pm 0.3$, $p = 0.85$) TTs.

**Blood Glucose and Lactate.** The rise in blood glucose during the 40-km TT was significant across time ($p = 0.04$), but there were no significant treatment or treatment by time differences (Figure 3A; all $p > 0.05$). As shown in Figure 3B, blood glucose rose significantly during the 10-km TT in both treatments (time effect, $p = 0.000$). The blood glucose concentration was significantly higher in MCO than that in MCP immediately before the 10-km TT (Figure 3B; $p = 0.002$), although at TT end, there was no difference between treatments ($p = 0.31$).
As shown in Figure 3C, D, blood lactate levels rose significantly across time during each TT (40 km, \( p = 0.000 \); 10 km, \( p = 0.001 \)). There were no significant treatments or treatment by time differences in blood lactate for either the 40-km or the 10-km TT (Figure 3C, D, respectively; all \( p > 0.05 \)).

**Salivary Cortisol.** No significant treatment (\( p = 0.66 \)) or treatment by time (\( p = 0.57 \)) differences were observed in salivary cortisol levels. A significant time effect was found (\( p = 0.005 \)) (see Figure 4 for graphical results).

**Leg Strength Recovery.** Leg strength declined significantly from pre- to post-experimental protocol in both treatments (\( P > 0.05 \), Figure 5), but no significant differences were found between treatments in leg strength recovery, which was calculated as percent change from pre- to post-experimental protocol measures (\( p = 0.13 \)).

**DISCUSSION**

The primary purpose of this study was to determine if a multiple CHO plus whey protein beverage could improve TT performance in comparison with an isocaloric multiple CHO-only beverage. To our knowledge, this is the first investigation to directly compare treatment beverages containing the same 3 CHOs (dextrose, fructose, and maltodextrin) with and without protein, using shorter duration (<90 minutes) TTs as the measure of realistic cycling performance. The most noteworthy finding was that including protein in a multiple CHO beverage (MCP) did not improve TT performance compared with the multiple CHO-only (MCO) beverage. Importantly, our results help to clarify the conditions in which inclusion of protein in a CHO beverage may not provide performance benefits over that of CHO only.

Our findings are in agreement with several previous investigations that compared CHO and CHO + Pro beverages using TTs as the measure of performance and found no significant difference in performance between treatments (4,38,41). Saunders et al. (38) compared CHO vs. CHO + Pro beverages during a 60-km TT in 13 competitive male cyclists, and although postexercise creatine kinase and muscle soreness were lower in the CHO + Pro treatment compared with CHO, there was no difference in TT performance. Similarly, Van Essen and Gibala (41) compared a 6% CHO (sucrose) beverage to a 6% sucrose + 2% protein beverage in 10 trained male cyclists and reported that although both the CHO and CHO + Pro treatments improved 80 km TT performance compared with placebo, there was no difference between the CHO and CHO + Pro trials. Others showed no difference in 1 hour TT performance between CHO and CHO + Pro beverages, even after first completing a 2-hour ride before the TT, during which the treatments were also administered (4).

Importantly, each of the 3 studies cited above provided a CHO ingestion rate of 60–65 g $h^{-1}$ (4,38,41), which is similar to that of this study (MCP, 58 g $h^{-1}$; MCO, 81 g $h^{-1}$). It should be noted, though, that these investigations used longer duration TTs (>90 minutes) than the ones used in this study. However, the results of these studies and ours suggest that when the measure of performance more closely matches the intensity and duration at which cyclists typically compete, and when the ingestion rate of CHO in the treatments is optimal, there is likely to be no performance benefit of added protein compared with multiple CHO only.

Our finding of no difference in performance between the 2 experimental treatments is in contrast to that of several previous investigations which used TTE protocols (14,20,29,33,36). Most notably, our findings differ from that of 2 recent studies that showed significantly improved cycling endurance performance while ingesting a beverage containing the same types of multiple CHO (dextrose, fructose, and maltodextrin) and proteins as we used in this study, compared with a single CHO-only (dextrose) beverage (14,29). However, the differences in experimental design and methodology likely underlie the conflicting findings across studies. Specifically, differences in the measure of...
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endurance performance used (TTE vs. TT) and the duration of the exercise bouts are likely to explain much of the disparate findings between studies of CHO vs. CHO + Pro supplementation and exercise performance.

When evaluating athletic performance, it is critical that the manner in which athletes are assessed closely simulates an event that they typically perform. Many investigations have used TTE as the measure of performance (14,20,28,29,35–37,42), yet the results of improved performance are mixed. Some have reported significantly improved TTE with CHO + Pro compared with CHO (14,20,29,36,37,42), whereas others have demonstrated no TTE difference (28,34,35,40). The recent findings by Ferguson-Stegall et al. (14) and McCleave et al. (29) demonstrated an increase in TTE by 28.7 and 15.2%, respectively, in the CHO + PRO group compared with CHO only. Although the TTE method of cycling at a fixed workload (s) for set periods of time (3 hours) and then continuing for as long as possible at a higher workload until volitional exhaustion may be somewhat applicable to athletes who compete in ultraendurance events, the practical application is quite limited for those who compete in shorter, more intense cycling events, such as circuit races, TTs, and criteriums. In these types of events, cyclists race over a fixed distance and aim to cover that distance in the fastest time possible. Thus, we suggest that the use of the TTs in this study more accurately represents the real-world competitive events of many cyclists.

In addition, when events are relatively short, it is common for cyclists to compete in more than one cycling event in a day. For example, in stage races, a circuit race that may last 60–90 minutes may be followed by short (10 km or less) TT, often with only ~30 minutes rest between the 2. Another example of back-to-back shorter duration events are criterium races, in which many cyclists often choose to compete in multiple races separated by a very brief recovery time (e.g., in a category 3–4 race that lasts ~45 minutes, followed 20–30 minutes later by a Masters category race that lasts approximately 30–40 minutes). To our knowledge, there are no investigations that compare supplementation types using a protocol that replicates events such as these. Therefore, this study sought to use shorter duration TTs (<90 minutes) and use a protocol that simulates racing in back-to-back race events that are separated by a brief recovery to address this knowledge gap.

A secondary outcome of interest in this study was to determine whether postexercise leg strength recovery could be improved with the inclusion of protein in a multiple CHO beverage. As described earlier, isometric MVC strength of the quadriceps was measured before the first TT and after the second TT, and the percent change from pre- to post-experimental trial was used to compare the 2 treatments. Leg strength declined from pre- to post-experimental trial in both treatments, as would be expected. Although we hypothesized that MCP would yield better strength recov-
before the 10-km TT. However, despite the difference and the mechanism(s) responsible, there was no impact on TT performance.

There are several limitations to this study. First, although this experimental protocol was designed to simulate a back-to-back race scenario, it is difficult to generalize the recovery period length to all such events, as recovery periods during competitive events vary widely (from 15 to 20 minutes to a few hours). In addition, our recovery period was relatively short compared with that of other investigations using a second exercise bout or strength test, as mentioned previously. Given that the combination of CHO and protein may work to improve second-bout performance because of factors, such as reduced muscle damage after much longer recovery periods (36,37), accelerated muscle glycogen storage (18), and faster muscle repair through improved signaling for protein synthesis (13), our 30-minute recovery period may not have been long enough to demonstrate any possible benefits of the added protein on second-bout TT performance or leg strength recovery. However, the short length of our recovery period was chosen to simulate specific conditions experienced by many stage or criterium racers, in which events may be separated by only 20–30 minutes, rather than to determine the effects of much longer recovery periods.

Second, we did not measure markers of muscle damage, such as myoglobin or creatine kinase, as was done in many previous investigations. However, the association between muscle damage and improved cycling performance is not clear. Some have reported improved TTE with CHO + Pro compared with CHO, concomitant with significantly reduced muscle damage (36,37), whereas others have demonstrated significantly increased TTE without any differences in muscle damage markers when comparing CHO + Pro to CHO (14,29,55,40). Therefore, we chose to focus on performance measures as our primary outcome measures, rather than markers of muscle damage.

Finally, we did not include a placebo trial in our experimental trials, nor did we analyze our water-only trial, for 2 key reasons. First, the water-only trial served as the familiarization trial, and secondly, it has been established that ingesting CHO yields better performance than water only in previous studies of cycling endurance performance using bouts of ~60 minutes in duration (1,3,5,11,24). Thus, because we sought to compare the effects of MCO with and without added protein, comparing these 2 treatments directly was of more relevance than comparing them to a placebo treatment.

In summary, this study demonstrates that, when using measures of performance that closely match that of real-world competitive cycling events lasting <90 minutes, there is no difference in 40-km TT performance, second-bout 10-km TT performance, or in leg strength recovery when ingesting an isocaloric multiple CHO beverage compared with a multiple CHO beverage with protein.

**Practical Applications**

Choosing a sports beverage from the many formulations available to use during exercise can be confusing for competitive cyclists and trainers/coaches alike. The results of our study help to clarify the conditions in which protein may not provide any performance or recovery benefit over that of CHO alone. During events of very long duration, such as ultramarathons, ultracycling events, and advanced mountaineering, protein may offer an advantage over CHO alone, as evidenced by the many studies using TTE that show a significant improvement in endurance time (14,20,29,36,37), or in improved protein balance during and after ultradistance exercise (27). However, for the many athletes who compete in events lasting <90 minutes, such as criteriums, TTs, and circuit races, the inclusion of protein in a multiple CHO beverage may offer no additional performance benefit. Furthermore, when the recovery period between events is very brief (~30 minutes), added protein is unlikely to benefit second-bout performance beyond that of a multiple CHO supplement alone.

It is important to note that we did not demonstrate a decrement in performance when protein was added to the beverage; thus, there is no obvious reason to suggest that CHO + Pro would be less favorable than a CHO-only beverage, especially if it contains multiple CHO and delivers approximately 60 g·hour⁻¹ CHO. We therefore suggest that individual preference should be the deciding factor when choosing between a multiple CHO plus protein or multiple CHO-only supplement during cycling events lasting <90 minutes.

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