Carbohydrate and Protein Hydrolysate Coingestion’s Improvement of Late-Exercise Time-Trial Performance

Michael J. Saunders, Rebecca W. Moore, Arie K. Kies, Nicholas D. Luden, and Casey A. Pratt

This study examined whether a carbohydrate + casein hydrolysate (CHO+ProH) beverage improved time-trial performance vs. a CHO beverage delivering ~60 g CHO/hr. Markers of muscle disruption and recovery were also assessed. Thirteen male cyclists (VO\textsubscript{2peak} = 60.8 ± 1.6 ml · kg\textsuperscript{-1} · min\textsuperscript{-1}) completed 2 computer-simulated 60-km time trials consisting of 3 laps of a 20-km course concluding with a 5-km climb (~5% grade). Participants consumed 200 ml of CHO (6%) or CHO+ProH beverage (6% + 1.8% protein hydrolysate) every 5 km and 500 ml of beverage immediately postexercise. Beverage treatments were administered using a randomly counterbalanced, double-blind design. Plasma creatine phosphokinase (CK) and muscle-soreness ratings were assessed immediately before and 24 hr after cycling. Mean 60-km times were 134.4 ± 4.6 and 135.0 ± 4.0 min for CHO+ProH and CHO beverages, respectively. All time differences between treatments occurred during the final lap, with protein hydrolysate ingestion explaining a significant (p < .05) proportion of between-trials differences over the final 20 km (44.3 ± 1.6, 45.0 ± 1.6 min) and final 5 km (16.5 ± 0.6, 16.9 ± 0.6 min). Plasma CK levels and muscle-soreness ratings increased significantly after the CHO trial (161 ± 53, 399 ± 175 U/L; 15.8 ± 5.1, 37.6 ± 5.7 mm) but not the CHO+ProH trial (115 ± 21, 262 ± 88 U/L; 20.9 ± 5.3, 32.2 ± 7.1 mm). Late-exercise time-trial performance was enhanced with CHO+ProH beverage ingestion compared with a beverage containing CHO provided at maximal exogenous oxidation rates during exercise. CHO+ProH ingestion also prevented increases in plasma CK and muscle soreness after exercise.

Keywords: sport beverages, fatigue, endurance, recovery

At least three studies have reported that carbohydrate-protein (CHO+Pro) ingestion during prolonged cycling might increase time to exhaustion more than conventional carbohydrate (CHO) sport beverages (Ivy, Res, Sprague, & Widzer, 2003; Saunders, Kane, & Todd, 2004; Saunders, Luden, & Herrick, 2007). CHO beverages in those studies were consumed at intake rates of 37–47 g/hr, with 9–12 g/hr of protein added to CHO+Pro beverages. Although these levels exceeded

Saunders, Moore, Luden, and Pratt are with the Dept. of Kinesiology, James Madison University, Harrisonburg, VA 22807. Kies is with DSM Food Specialties, 2600 MA Delft, The Netherlands.
typical ingestion rates of endurance athletes during exercise (Noakes, 1993), carbohydrate levels in the beverages were below peak exogenous oxidation rates (Jentjens, Achten, & Jeukendrup, 2004; Jentjens, Moseley, Waring, Harding, & Jeukendrup, 2004; Jentjens, Venables, & Jeukendrup, 2004). It is thus unclear whether endurance improvements in the CHO+Pro trials were the result of protein-mediated effects or additional caloric content. Romano-Ely, Todd, Saunders, and St. Laurent (2006) reported no differences in time to exhaustion between isocaloric CHO and CHO+Pro beverages. However, the CHO+Pro beverage in that study contained subpeak carbohydrate levels (45 g/hr), so it is possible that protein provided an independent metabolic benefit, because performance was equal to CHO despite 20% less carbohydrate in the CHO+Pro beverage. Thus, further study is necessary to determine whether performance benefits with CHO+Pro are observed when carbohydrate ingestion occurs at maximal rates during exercise.

Van Essen and Gibala (2006) recently reported no difference in performance between CHO (60 g/hr) and CHO+Pro (60 g CHO/hr + 20 g protein/hr) beverages during a simulated 80-km cycling time trial. They concluded that CHO+Pro might not improve endurance performance when beverages are ingested at high rates of carbohydrate intake or when assessed during time-trial tasks. However, the exercise protocol used in that study did not include an examination of performance differences during the latest stages of exercise, when differences between treatments could potentially be detected with greater sensitivity. As an example, any treatment that promotes supplemental energy utilization late in exercise (i.e., when muscle glycogen is depleted) could theoretically improve performance times without influencing early-exercise power output. For example, a 1-min improvement in time-trial performance has great practical significance to competitive cyclists. However, the sensitivity to detect this difference is quite small if measured over a 2-hr trial (<1% difference) and could only be reliably detected in studies with exceedingly large sample sizes. However, if this ergogenic effect were the result of delayed fatigue in late exercise, the sensitivity to detect this same effect over the final 15 min of the trial would be much greater, because it could represent a difference of up to 6.7% between trials. Similarly, other investigators have examined the efficacy of CHO beverages using time trials immediately after prolonged exercise protocols at a fixed intensity (Mitchell et al., 1989; Zachwieja et al., 1992). In addition, performance differences might not be observed early in exercise because of lag time related to the timing of beverage ingestion, substrate uptake and availability, and achieving threshold amino acid concentrations that might be required to initiate ergogenic effects. Because of these factors, the proportional benefits of CHO+Pro could potentially be much greater in late exercise.

To date, no published studies have examined the effects of protein hydrolysates on endurance performance. Hydrolysates containing small peptides (di- and tripeptides) are absorbed faster than free amino acids or amino acids from intact protein and hydrolysates containing larger peptides (Grimble, Keohane, Higgins, Kaminsky, & Silk, 1986; Grimble et al., 1987). Although enhanced availability of amino acids from small peptides has been known for over 20 years, testing of hydrolysates containing such peptides was hampered by the bitter taste. Hydrolysis production processes have recently been developed that include a proline-specific protease, a unique enzyme that can cleave peptide bonds involving proline residues.
These processes effectively “debitter” the hydrophobic peptides known to be the main source of bitterness (Edens et al., 2005), allowing the potential benefits of carbohydrate-protein-hydrolysate beverages (CHO+ProH) to be examined during exercise in beverages with an acceptable taste.

In addition to potential improvements in endurance performance, CHO+ProH consumption might elicit metabolic alterations that influence recovery from exercise. Koopman et al. (2004) reported that CHO+ProH ingestion during ultraendurance exercise produced significant improvements in whole-body net protein balance compared with CHO ingestion. In addition, a few recent studies have reported attenuated markers of sarcolemmal disruption and muscle soreness when CHO+Pro beverages are consumed during and after exhaustive cycling (Millard-Stafford et al., 2005; Romano-Ely et al., 2006; Saunders et al., 2004). However, none of these studies examined the effects of CHO+Pro ingestion on markers of muscle disruption after time-trial protocols. Unlike time-to-exhaustion trials at a fixed workload, the intensity of time trials varies considerably within the exercise session, especially during hilly trials. Thus, although total work during prolonged time trials might be very similar to that during time-to-exhaustion protocols, varied-intensity time trials might elicit altered rates of substrate utilization, and the higher forces required to climb steep inclines might also affect muscle-damage markers to a differing degree from time-to-exhaustion protocols.

The primary purpose of the current study was to determine whether a CHO+ProH beverage improved performance during a prolonged cycle time trial, compared with a CHO beverage. Beverages were matched at maximal rates for exogenous carbohydrate oxidation (~60 g/hr), and the performance assessment included an examination of late-exercise performance, when the putative effects of CHO+ProH beverages were hypothesized to be greatest. A secondary purpose of this study was to determine whether CHO+ProH ingestion reduced markers of muscle disruption after simulated cycling competition versus a CHO beverage.

Methods

Participants

Thirteen recreationally competitive male cyclists participated in the study. Inclusion criteria for the study included a self-reported weekly cycling frequency of >3 days/week over the preceding 2 months and a laboratory-tested VO\textsubscript{2peak} of >45 ml · kg\textsuperscript{-1} · min\textsuperscript{-1}. Before testing, participants completed informed consent and a comprehensive medical questionnaire to determine the presence of any risk factors associated with coronary artery disease. All participants were asymptomatic and possessed fewer than two risk factors using ACSM guidelines (American College of Sports Medicine, 2006). All procedures and protocols were approved by the James Madison University Institutional Review board. Participant demographics are provided in Table 1.

Procedures

VO\textsubscript{2peak}. Before the study intervention, participants completed a graded cycling test on a Velotron Dynafit Pro cycle ergometer (RacerMate, Inc., Seattle, WA).
During a 5-min warm-up at 100 W, they were instructed to adjust the fit of the cycle to their desired specifications, using horizontal and vertical adjustments of the seat and handlebars. These settings were recorded and replicated in subsequent trials. Using previously described procedures (Saunders et al., 2004), the \( \text{VO}_{2\text{peak}} \) protocol was initiated at a workload below lactate threshold and increased 25 W every minute until volitional exhaustion. Oxygen uptake was assessed continuously throughout the test using a SensorMedics Vmax Spectra metabolic cart (Yorba Linda, CA). The metabolic cart was calibrated before each test using medical-grade gases of known concentrations and a 3.0-L calibration syringe. \( \text{VO}_{2\text{peak}} \) was recorded as the highest 30-s mean oxygen-uptake value obtained during the test. Participant height and weight were measured using a stadiometer and digital physician’s scale, respectively. Participants were measured in their cycling clothing without shoes and socks.

**Endurance Time Trials.** All participants completed two computer-simulated 60-km time trials on the Velotron cycle ergometer, separated by 7–10 days. Each trial consisted of three simulated laps of a 20-km course (see Figure 1), with over 407 vertical meters of climbing in each lap (1,222 m total). Each lap concluded with a 5-km climb of ~5% average grade (8% maximum grade). The course was designed as such to allow an assessment of performance during the overall trial (60 km), final 20 km, and final 5-km climb. Performance times were recorded for each of these trial segments. We hypothesized that performance differences between treatments would be most noticeable during the late stages of exercise, as described in the introduction of the article.

Participants arrived at the laboratory 2–3 hr after a light meal and were instructed to eat the same pretrial meal before the next trial. They were asked to refrain from consuming caffeine or any pain medications during all trial dates. Participants were also instructed to consume a consistent diet for 48 hr before each trial and to refrain from consuming unfamiliar foods or alcohol during this period. The time of day of the trials varied between participants but remained consistent for each participant, to limit the potential impact of circadian rhythms between treatments. Participants were instructed to treat the trials as a competitive event and refrain from heavy exercise (including resistance training) for 48 hr before each exercise session.

Participants were familiarized with bicycle-ergometer operation during the \( \text{VO}_{2\text{peak}} \) testing but did not perform a formal practice trial of the course before their first time trial. They could freely alter the workload of the ergometer at any time.

### Table 1  Participant Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>( M \pm SEM )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.3 ± 2.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.9 ± 1.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.0 ± 2.6</td>
</tr>
<tr>
<td>( \text{VO}_{2\text{peak}} ) (L/min)</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>( \text{VO}_{2\text{peak}} ) (ml · kg(^{-1}) · min(^{-1}))</td>
<td>60.8 ± 1.6</td>
</tr>
</tbody>
</table>
throughout the trials by using a simulated gear shifter. The ergometer was electronically braked, such that any change in “gearing” was offset by a proportional change in flywheel resistance. Changes in cycling cadence or course topography were similarly offset by changes in flywheel resistance. Ergometer workloads were reported by the manufacturer to have <0.2% variation between repeated trials. As a motivational strategy, participants were permitted to view their elapsed performance times on the computer screen during the trials, as well as simulated gearing and distance traveled.

**Physiological Measurements.** During each of the time trials, VO$_2$, respiratory-exchange rate (RER), heart rate, ratings of perceived exertion (RPEs), blood glucose, and lactate were obtained at 10, 30, and 50 km. In addition, plasma creatine phosphokinase (CK) and muscle-soreness ratings were assessed immediately before and 24 hr after cycling.

VO$_2$ and RER measurements were obtained using a SensorMedics Vmax Spectra metabolic cart. Participants breathed through a mass-flow sensor connected to the metabolic cart for 5 min at each of the time points. After 2 min of equilibration, mean values for VO$_2$ and RER were calculated as the 3-min average from 2 to 5 min of data collection at each time point.

Heart rate was obtained using a Polar heart-rate monitor (Brooklyn, NY). It was recorded as the 1-min average for each time point. Subjective RPEs were obtained using Borg’s 6–20 point scale, after participants were instructed regarding its use (American College of Sports Medicine, 2006).

Blood samples were obtained using finger sticks to acquire ~0.2 ml of blood at each sampling. Glucose and lactate concentrations were determined in duplicate.
from whole blood using a YSI 2300 STAT automated glucose/lactate analyzer (Yellow Springs, OH) after pretrial calibration.

Plasma CK was obtained before and 24 hr after the time trials as an indicator of sarcolemmal disruption. Venous blood draws from the antecubital vein were used to obtain approximately 4 ml of blood. Whole blood was spun in a centrifuge at 7,000 rpm to separate plasma, which was frozen at –80 °C. Before analysis, samples were thawed to room temperature (22 °C) and mixed through gentle inversion. Plasma CK was analyzed using a Johnson & Johnson Vitro DT 6011 as described previously (Luden, Saunders, & Todd, 2007; Saunders et al., 2004). In addition, the 24-hr postexercise timing was used to allow an appropriate comparison with previous studies, which have used time points ranging from 12–15 hr (Saunders et al., 2004, 2007) to 24 hr postexercise (Luden et al.; Romano-Ely et al., 2006).

Subjective ratings of muscle soreness were obtained before and 24 hr after the time trials, using a 100-mm visual analog scale.

**Beverage Treatments**

Participants consumed 200 ml of treatment beverage every 5 km during the time trials. The CHO beverage consisted of a 6% carbohydrate solution containing equal amounts of glucose and maltodextrin. The CHO+ProH beverage contained identical carbohydrate ingredients but also included 14.4 g of protein from a specific casein protein hydrolysate (PeptoPro, DSM Food Specialties, Delft, The Netherlands). During each 60-km trial, 132 g of carbohydrate was ingested, with an additional 32 g of protein consumed in the CHO+ProH trial. Thus, carbohydrate levels were matched between treatments at a level that provided approximately 60 g CHO/hr, which approximates the upper limits of exogenous carbohydrate oxidation during exercise (Jentjens, Achten, & Jeukendrup, 2004; Jentjens, Moseley, et al., 2004; Jentjens, Venables, & Jeukendrup, 2004). Therefore, potential performance differences between treatments could be directly attributed to protein, because additional carbohydrate would not be likely to provide additional benefits in performance (Jeukendrup & Jentjens, 2000). Cyclists consumed an additional 500 ml of beverage within 30 min of trial completion. Beverages were treated with 0.5 g/L of vanillin to provide an identical vanilla flavor to both treatments. Beverages were administered in a randomly counterbalanced, double-blind design.

**Statistical Analyses**

Treatment differences in time were compared between treatments for each trial segment (60 km, final 20 km, and final 5 km). As previously described, participants were permitted to view their elapsed performance times during the trials to provide motivation for peak performance. However, because they were competing against their prior trial, this characteristic of the protocol contributed to a significant order effect, whereby participants performed significantly faster ($p < .05$) in their second trial, independent of the treatment used. Therefore, the following statistical model was used to correct for this order effect:

$$\text{Trial difference} = \alpha_i + \beta + \text{error}$$
where $\alpha_i$ is the effect of treatment order and $\beta$ is the difference resulting from treatment beverages. This is a simple regression model, with order of treatments as the dependent variable. To facilitate comparisons with mean values in other published studies, mean values for time are provided in Table 2 with and without correction for the order effect. All other measured variables showed no trial-order effect, so they did not require such a correction.

Physiological measurements obtained during exercise (VO$_2$, RER, heart rate, RPE, and blood glucose and lactate), plasma CK, and muscle-soreness ratings were examined using two-way (Treatment $\times$ Time) repeated-measures ANOVAs for each variable. Because CK results were not normally distributed, the results were analyzed after log transformation of the post- and preexercise CK-level ratios. To preserve comparability with other work, results are presented in their original or back-transformed units.

All values are presented as $M \pm SEM$, and all hypothesis testing was conducted using an alpha level of $p < .05$. In the case of directional hypotheses between treatments (i.e., improvements in performance and muscle disruption or soreness with CHO+ProH ingestion), a one-tailed alpha was used. Two-tailed alpha tests were used for all other statistical tests.

**Results**

**Time-Trial Performance**

Treatment differences in performance times for each trial segment are displayed in Table 2, with and without correction for the order effect. The presence of protein hydrolysate in the beverage explained a significant ($p < .05$) amount of variance in performance times between trials during the final 20 and 5 km of the time trial.

**Physiological Data**

Physiological responses to the exercise trials are included in Table 3. No significant treatment differences were observed between CHO and CHO+ProH trials for VO$_2$, RER, heart rate, RPE, or blood glucose and lactate. In addition, there were

<table>
<thead>
<tr>
<th>Measurement period</th>
<th>CHO</th>
<th>CHO+ProH</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 km</td>
<td>135.0 ± 4.0 (135.1 ± 4.1)</td>
<td>134.4 ± 4.6 (134.3 ± 4.5)</td>
</tr>
<tr>
<td>Final 20 km*</td>
<td>45.0 ± 1.6 (45.1 ± 1.6)</td>
<td>44.3 ± 1.6 (44.2 ± 1.6)</td>
</tr>
<tr>
<td>Final 5 km*</td>
<td>16.9 ± 0.6 (17.0 ± 0.7)</td>
<td>16.5 ± 0.6 (16.5 ± 0.6)</td>
</tr>
</tbody>
</table>

*Significant difference between treatments, $p < .05$.
no significant Treatment $\times$ Time interactions in any of those measures. Main effects from the ANOVA model were examined to determine whether physiological measures changed over the course of the trial, independent of treatment effects. No significant changes were observed in VO$_2$ between 10 and 50 km. However, heart rate, RPE, RER, and blood lactate and glucose all changed significantly over time (Table 3).

**Plasma CK and Muscle Soreness**

No significant Treatment $\times$ Time interactions were observed for plasma CK and muscle-soreness levels. Postexercise CK levels were not significantly different between CHO (399 ± 175) and CHO+ProH (262 ± 88) treatments (Figure 2). However, CK levels increased significantly from pre- to postexercise in the CHO trial (from 161 to 399, $p < .05$) but not in the CHO+ProH trial (from 115 to 262 U/L, $p = .08$). Similarly, postexercise muscle soreness was not significantly different between treatments (Figure 2) but increased significantly ($p < .05$) in the CHO trial (from 15.8 ± 5.1 to 37.6 ± 5.7 mm) and not in the CHO+ProH trial (from 20.9 ± 5.3 to 32.2 ± 7.1 mm; $p = .19$).

**Discussion**

The primary purpose of the current study was to determine whether a CHO+ProH beverage elicited improvements in cycling time-trial performance versus a CHO beverage matched at optimal levels of carbohydrate intake (~60 g/hr). The small difference in overall 60-km performance was not statistically different between treatments. However, as hypothesized in the introduction of this article, all the performance improvement with CHO+ProH was observed in the final 20 km of the trial, and most of it occurred during the final 5-km climb to the finish. As a result, the presence of protein in the beverage explained a significant portion of the variance in performance time for the final 20- and 5-km segments, and CHO+ProH ingestion resulted in a 3% improvement in time for the final 5 km of the trial. These findings have substantial relevance for competitive athletes, because most cycling races are determined by time differences of considerably less than 30 s. Although the total times were not significantly different between treatments, this is probably related to the statistical sensitivity with which differences between treatments can be detected. For example, the 30-s treatment difference in final 5-km times represented a 3% improvement with CHO+Pro, whereas the 42-s treatment difference in 60-km times was a 0.5% difference. As described earlier in the article, a very large sample size would be required to detect this difference with adequate statistical power, even though it represents a difference of considerable practical importance to competitive athletes.

These findings corroborate previous studies reporting significant improvements in time to exhaustion with CHO+Pro ingestion (Ivy et al., 2003; Saunders et al., 2004, 2007). Similar to the current study, each of those studies compared CHO+Pro beverages with CHO beverages matched for carbohydrate content. However, because carbohydrate intake rates in the studies (37–47 g/hr) were below peak exogenous oxidation rates, the ergogenic effects of CHO+Pro could have been related to the additional calories delivered by protein, as opposed to
<table>
<thead>
<tr>
<th>Variable</th>
<th>10 km (CHO)</th>
<th>10 km (CHO+ProH)</th>
<th>30 km (CHO)</th>
<th>30 km (CHO+ProH)</th>
<th>50 km (CHO)</th>
<th>50 km (CHO+ProH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ (mL · kg⁻¹ · min⁻¹)</td>
<td>43.7 ± 2.1</td>
<td>44.7 ± 2.2</td>
<td>42.5 ± 2.5</td>
<td>44.3 ± 2.7</td>
<td>44.9 ± 2.3</td>
<td>45.1 ± 2.2</td>
</tr>
<tr>
<td>Respiratory-exchange rate*</td>
<td>0.99 ± 0.01</td>
<td>0.99 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)*</td>
<td>159.8 ± 4.7</td>
<td>157.4 ± 5.1</td>
<td>161.5 ± 4.7</td>
<td>161.1 ± 3.8</td>
<td>165.2 ± 4.5</td>
<td>166.5 ± 4.5</td>
</tr>
<tr>
<td>Rating of perceived exertion*</td>
<td>12.2 ± 0.3</td>
<td>12.3 ± 0.6</td>
<td>13.5 ± 0.5</td>
<td>13.8 ± 0.4</td>
<td>14.5 ± 0.7</td>
<td>14.9 ± 0.5</td>
</tr>
<tr>
<td>Glucose (mg/dl)*</td>
<td>82.2 ± 3.1</td>
<td>81.8 ± 3.3</td>
<td>87.1 ± 3.4</td>
<td>87.5 ± 2.0</td>
<td>85.7 ± 2.5</td>
<td>87.3 ± 2.0</td>
</tr>
<tr>
<td>Lactate (mmol/L)*</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; CHO+ProH = CHO plus casein hydrolysate.

*Significant main effect for time, p < .05.
protein-specific mechanisms. However, as discussed previously (Saunders et al., 2004), it seems unlikely that the relatively large improvements in time to exhaustion reported in the studies (13–36%) could be explained by the relatively small differences in calories between treatments. The current study did not compare isocaloric treatment beverages, so it is not possible to completely discount the potential effects of the additional calories in the CHO+ProH beverage. However, the current study demonstrates that the ergogenic effects of CHO+ProH are observed during time-trial protocols, even when ingested at very high (~60 g/hr) levels of carbohydrate intake.

Only one prior study has compared CHO+Pro and CHO beverages using a time-trial protocol. In contrast to the current findings, Van Essen and Gibala (2006) observed no differences in performance between CHO+Pro (135 ± 9 min) and CHO (135 ± 9 min) treatments during an 80-km time trial. In addition, time splits for each 20-km segment of the trial were not different between treatments. The reasons for the findings differing from those of the current study are unclear.
Apparently, Van Essen and Gibala’s protocol mimicked a flat time trial and did not examine time differences for the latest stages of exercise, when the potential benefits of performance from CHO+Pro beverages might be most apparent. As previously discussed, the assessment of late-exercise performance in the current study might have increased the sensitivity to detect treatment differences, especially with a metabolically challenging climb occurring in the final 5 km of the trial.

The differing results between studies might have been influenced by the sources of protein used in the beverages. The three prior studies reporting improved endurance with CHO+Pro all used intact whey protein (Ivy et al., 2003; Saunders et al., 2004, 2007). However, it is difficult to directly compare our results with those studies because they each measured performance using time-to-exhaustion protocols. The current investigation and the study of Van Essen and Gibala (2006) both examined time-trial performance, and both used similarly high rates of fluid (1,000 ml/hr), carbohydrate (60 g/hr), and protein (15–20 g/hr) compared with the aforementioned studies (508–600 ml/hr, 37–47 g/hr, and 9–12 g/hr, respectively). However, Van Essen and Gibala used intact whey protein, whereas the current study used a casein protein hydrolysate.

It could be that Van Essen and Gibala (2006) failed to observe a performance benefit from protein because there is a limited capacity to digest and absorb intact proteins during prolonged endurance exercise. Grimble et al. (1986, 1987) compared absorption rates of protein hydrolysates and their equivalent amino acid mixtures using jejunal-perfusion techniques. Amino acids were more rapidly absorbed from hydrolysates containing small peptides (Grimble et al., 1986, 1987), and the authors suggested that brush-border hydrolysis of peptides with four or more amino acids limited the rate of absorption (Grimble et al., 1987). In addition, ileal endogenous protein losses are higher after consumption of intact protein or long-chain protein hydrolysates than protein hydrolysates containing di- and tripeptides (Moughan, Fuller, Han, Kies, & Miner-Williams, 2007). Higher endogenous losses result from increased production of digestive enzymes and mucin and greater sloughing of intestinal-tract cells. The protein hydrolysate used in the current study (PeptoPro) contained mainly di- and tripeptides and, in an animal model, was shown to exhibit lower endogenous protein losses than its native protein source, casein (Moughan et al.). As a consequence, more protein is available for, for example, muscle synthesis.

As suggested earlier, hydrolysates containing di- and tripeptides might positively influence amino acid absorption rates and lower endogenous protein production. In addition, Fairclough, Hegarty, Silk, and Clark (1980) suggested that small peptides might provide positive effects on water and electrolyte absorption versus hydrolysates containing larger peptides. Thus, the use of di- and tripeptides might have influenced the positive performance outcome observed in the current investigation.

Including the current findings, four of the six studies examining CHO+Pro ingestion during endurance exercise have reported performance benefits versus CHO. The mechanisms by which CHO+Pro might promote improved endurance are currently unknown. In a recent review of this topic (Saunders, 2007) various potential mechanisms were discussed, including increased protein oxidation (potentially sparing muscle glycogen), improved maintenance of TCA cycle intermediates, attenuation of central fatigue, improved uptake of fluid or other fuel
CHO/Protein Hydrolysate and Time-Trial Performance

substrates, and augmented insulin stimulation. In addition, Betts, Williams, Boobis, and Tsintzas (2008) recently reported that CHO+Pro consumed immediately after a bout of prolonged treadmill running resulted in significant increases in whole-body carbohydrate oxidation during a subsequent bout of exercise, without alterations in muscle glycogen utilization. However, very few studies have examined the influence of CHO+Pro consumption during exercise on these potential mechanisms, and the metabolic influences of CHO+Pro ingestion related to improved endurance performance remain poorly understood at present.

A secondary purpose of this study was to assess markers of muscle disruption between CHO and CHO+ProH treatments. No significant differences were observed in postexercise levels of plasma CK or ratings of muscle soreness between treatments. However, both variables were significantly elevated from preexercise to postexercise in the CHO trial but not in the CHO+ProH trial. Several studies have reported that CHO+Pro ingestion during and/or after endurance exercise might reduce postexercise plasma CK levels (Luden et al., 2007; Romano-Ely et al., 2006; Saunders et al., 2004; Valentine, Saunders, Todd, & St. Laurent, 2008) and ratings of muscle soreness (Flakoll, Judy, Flinn, Carr, & Flinn, 2004; Luden et al.; Millard-Stafford et al., 2005; Romano-Ely et al.) compared with CHO ingestion. These effects have been reported when CHO+Pro and CHO beverages were matched for carbohydrate content (Luden et al.; Millard-Stafford et al.; Saunders et al., 2004; Valentine et al.) or total calories (Romano-Ely et al.; Valentine et al.). The absence of a significant treatment difference in the current study might have been the result of differences in exercise protocols, in participant samples, and in statistical power related to these varied factors. For example, Luden et al. recently reported significant reductions in plasma CK levels with CHO+Pro supplementation despite mean treatment differences that were very similar to those of the current study. However, Luden et al. used a sample of 23 participants, providing the statistical power to observe more subtle differences between treatments than the current investigation (Lipsey, 1990).

No studies to date have determined whether CHO+Pro ingestion during prolonged exercise attenuates changes in myofibrillar muscle damage. Although inferences regarding muscle damage might be made from changes in plasma CK and muscle-soreness values, these measurements do not always correlate well with direct measures of muscle damage (Beaton, Allan, Tarnopolsky, Tiidus, & Phillips, 2002; Warren, Lowe, & Armstrong, 1999). However, changes in muscle function might be the most relevant measure of muscle recovery for athletes. In a recent investigation, Valentine et al. (2008) reported plasma CK values after exhaustive cycling that were quite similar to those of the current study (373 ± 417 U/L after a CHO trial vs. 192 ± 149 U/L for CHO+Pro). This relatively small attenuation in plasma CK was accompanied by significantly improved muscle function 24 hr after exercise. Based on the findings of the current study alone, we cannot clearly conclude a significant treatment effect on markers of muscle disruption. However, the general trends of the data are consistent with other recent studies of CHO+Pro ingestion and suggest that including protein in the beverage might have provided a protective effect from muscle disruption after heavy exercise.

In conclusion, ingesting a CHO+ProH beverage during endurance cycling produced significant improvements in late-exercise time-trial performance...
compared with a CHO beverage. These findings are particularly relevant for athletes, because the beverages were matched at theoretically maximal levels of exogenous carbohydrate oxidation (~60 g/hr), above which further performance benefits would be unlikely with additional carbohydrate content. The magnitudes of performance differences (3% during the final 5-km climb) were smaller than in previous studies of CHO+Pro using time-to-exhaustion protocols but remain highly important for competitive athletes.

CHO+ProH ingestion during and after a cycling time trial also prevented increases in plasma CK and muscle-soreness ratings that were observed in the CHO trial. These findings support previous research suggesting that CHO+ProH beverages consumed during and immediately after exercise might be advantageous for performance and muscle recovery in endurance athletes.

Acknowledgments

The authors wish to thank DSM Food Specialties, Inc., Delft, The Netherlands, for supporting this project with a research grant. Dr. Arie Kies is an employee of DSM Food Specialties. In addition, the authors are grateful to David Bolton, Adam Clawson, Brian McCarthy, Jamie Munnis, and Melissa Rivers for their assistance with data collection and to Dr. Wim Plugge (DSM Food Specialties) for statistical advice.

References


