CARBOHYDRATE AND PROTEIN HYDROLYSATE CO-INGESTION IMPROVES LATE-EXERCISE TIME-TRIAL PERFORMANCE

Michael J Saunders¹*, Rebecca W Moore¹, Arie K Kies², Nicholas D Luden¹, Casey A Pratt¹.

¹Department of Kinesiology, MSC 2302, James Madison University, Harrisonburg, VA 22807.

²DSM Food Specialties, Postpunt 699-0330, P.O. Box 1, 2600 MA Delft, The Netherlands

*Corresponding author

Running Head: Carb/Protein Hydrolysate & Time-Trial Performance
Abstract

**Purpose:** This study examined if a CHO + casein hydrolysate (CHO+ProH) beverage improved time-trial performance versus a CHO beverage delivering ~60 g CHO·hr⁻¹. Markers of muscle disruption and recovery were also assessed. **Methods:** Thirteen male cyclists (VO₂peak = 60.8±1.6 mL·kg⁻¹·min⁻¹) completed two computer-simulated 60 km time-trials, consisting of three laps of a 20 km course concluding with a 5 km climb (~5% grade). Subjects consumed 200 ml of CHO (6%) or CHO+ProH beverage (6% + 1.8% protein hydrolysate) every 5 km, and 500 ml of beverage immediately post-exercise. 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-hours following cycling. **Results:** Mean 60 km times were 134.4±4.6 and 135.0±4.0 min for CHO+ProH and CHO beverages, respectively. All of the time differences between treatments occurred during the final lap, with protein hydrolysate ingestion explaining a significant (p<0.05) proportion of between-trial 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 following the CHO trial (161±53, 399±175 U·L⁻¹; 15.8±5.1; 37.6±5.7 mm), but not the CHO+ProH trial (115±21, 262±88 U·L⁻¹; 20.9±5.3; 32.2±7.1 mm). **Conclusions:** Late-exercise time-trial performance was enhanced with CHO+ProH beverage ingestion compared to a beverage containing carbohydrate provided at maximal exogenous oxidation rates during exercise. CHO+ProH ingestion also prevented increases in plasma CK and muscle soreness following exercise.

Key Words: Sports beverages, Fatigue, Endurance, Recovery
**Introduction**

At least three studies have reported that carbohydrate-protein (CHO+Pro) ingestion during prolonged cycling may increase time-to-exhaustion compared to conventional carbohydrate (CHO) sports beverages (Ivy, Res, Sprague, & Widzer, 2003; Saunders, Kane, & Todd, 2004; Saunders, Luden, & Herrick, 2007). CHO beverages in these studies were consumed at intake rates of 37-47 g·hr⁻¹, with 9-12 g·hr⁻¹ of protein added to CHO+Pro beverages. Although these levels exceeded typical ingestion rates of endurance athletes during exercise (Noakes, 1993), carbohydrate content in these beverages were below peak exogenous oxidation rates (Jentjens, Achten, & Jeukendrup, 2004; Jentjens, Moseley, Waring, Harding, & Jeukendrup, 2004; Jentjens, Venables, & Jeukendrup, 2004). So it is unclear whether endurance improvements in the CHO+Pro trials were the result of protein-mediated effects or due to 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 this study contained sub-peak carbohydrate levels (45 g·hr⁻¹), so it is possible that protein provided an independent metabolic benefit, as performance was equal to CHO despite 20% less carbohydrate in the CHO+Pro beverage. Thus, further study is necessary to determine if 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⁻¹) and CHO+Pro (60 g·hr⁻¹ + 20g Pro·hr⁻¹) beverages during a simulated 80 km cycling time-trial. The authors concluded that CHO+Pro may 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 this study did not include an examination of performance differences during the latest stages of exercise, where differences between treatments could potentially be detected with greater sensitivity. As an example, any treatment which 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-minute 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-hour 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 minutes of the trial would be much greater, as 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 following prolonged exercise protocols at a fixed intensity (Mitchell et al., 1989; Zachwieja et al., 1992). In addition, performance differences may not be observed early in exercise due to lag-time related to the timing of beverage ingestion, substrate uptake/availability, and achieving threshold amino acid concentrations which may be required to initiate ergogenic effects. Due to these factors, the proportional benefits of CHO+Pro could potentially be much greater when measured 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 Jr., & Silk, 1986; Grimble et al., 1987). Although evidence of 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 been recently developed which include a proline-specific protease, a unique enzyme that can cleave peptide bonds involving proline residues. These processes effectively ‘debitter’ 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 may elicit metabolic alterations that influence recovery from exercise. Koopman et al. (2004) reported that CHO+ProH ingestion during ultra-endurance exercise produced significant improvements in whole-body net protein balance compared to 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/following exhaustive cycling (Millard-Stafford et al., 2005; Romano-Ely et al., 2006; Saunders et al., 2004). However, none of these studies have examined the effects of CHO+Pro ingestion on markers of muscle disruption following 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 may be very similar to time-to-exhaustion protocols, varied-intensity time trials may elicit altered rates of substrate utilization, and
the higher forces required to climb steep inclines may also impact muscle damage markers to a differing degree from time-to-exhaustion protocols.

The primary purpose of the present study was to determine if a CHO+ProH beverage improved performance during a prolonged cycle time-trial, compared to a CHO beverage. Beverages were matched at maximal rates for exogenous carbohydrate oxidation (~60 g·hr\(^{-1}\)), and the performance assessment included an examination of late-exercise performance, where the putative effects of CHO+ProH beverages were hypothesized to be greatest. A secondary purpose of this study was to determine if CHO+ProH ingestion reduced markers of muscle disruption following simulated cycling competition versus a CHO beverage.

**Methods**

**Subjects**

Thirteen recreationally competitive male cyclists participated in the study. Inclusion criteria for the study included a self-reported weekly cycling frequency of >3 days per week over the preceding 2 months, and a laboratory-tested VO\(_{2peak}\) of >45 mL·kg\(^{-1}\)·min\(^{-1}\). Prior to testing, subjects completed informed consent and a comprehensive medical questionnaire to determine the presence of any risk factors associated with coronary artery disease. All subjects 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. Subject demographics are provided in Table 1. [Insert Table 1 Here]

**Procedures**
Prior to the study intervention, subjects 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, subjects were instructed to adjust the fit of the cycle to their desired specifications, using horizontal and vertical adjustments of the seat and handlebars. These measurements were recorded and replicated in subsequent trials. Using previously described procedures (Saunders et al., 2004), the VO$_2$ 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 gasses of known concentrations and a 3.0 L calibration syringe. VO$_2$ peak was recorded as the highest 30 s mean oxygen uptake value obtained during the test. Subject height and weight were recorded using a stadiometer and digital physician’s scale, respectively. Each subject was measured in their cycling clothing without shoes and socks.

**Endurance Time-Trials.** All subjects completed two computer-simulated 60 km time-trials on the Velotron cycle ergometer, separated by 7-10 days. Each trial consisted of 3 simulated laps of a 20 km course (see Figure 1), with over 407 vertical meters of climbing each lap (1222 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. It was hypothesized that performance differences between treatments would be most noticeable during the late-stages of exercise, as described in the introduction of the paper.
Subjects arrived at the lab 2-3 hours following a light meal, and were instructed to eat the same pre-trial meal prior to both trials. Subjects 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-hours prior to each trial, and to refrain from consuming unfamiliar foods or alcohol during this period. The time-of-day of the trials varied between subjects, but remained consistent for each subject, to limit the potential impact of circadian rhythms between treatments. Subjects were instructed to treat the trials as a competitive event, and refrain from heavy exercise (including resistance training) for 48 h prior to each exercise session.

Subjects received familiarization with bicycle ergometer operation during the VO$_{2\text{peak}}$ testing, but did not perform a formal practice trial of the course prior to their first time-trial. Subjects could freely alter the workload of the ergometer at any time throughout the trials, by utilizing a simulated gear-shifter. The ergometer was electrically-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 express <0.2% variation between repeated trials. As a motivational strategy, subjects were permitted to view their elapsed performance times on the computer screen during the trials, as well as simulated gearing and distance travelled.

[Insert Figure 1 Here]
**Physiological Measurements.** During each of the time-trials, VO\textsubscript{2}, respiratory exchange rate (RER), heart rate, ratings of perceived exertion (RPE), blood glucose and lactate were obtained at 10, 30, and 50 km, as described below. In addition, plasma creatine phosphokinase (CK) and muscle soreness ratings were assessed immediately before and 24-hours following cycling.

\textit{VO}_2, RER

These measurements were obtained using a SensorMedics Vmax Spectra metabolic cart (Yorba Linda, CA). Subjects breathed through a mass-flow sensor connected to the metabolic cart for five minutes at each of the time-points. Following two minutes of equilibration, mean values for VO\textsubscript{2} and RER were calculated as the three-minute average from 2-5 minutes of data collection at each time-point.

Heart Rate, RPE

Heart rate was obtained using a Polar heart-rate monitor (Brooklyn, NY). Heart rate was recorded as the one-minute average for each time-point. Subjective ratings of exertion were obtained using Borg’s 6-20 point RPE scale, following subject instructions regarding use of the scale (American College of Sports Medicine, 2006).

Blood Glucose and Lactate

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, Ohio), following pre-trial calibration.
Plasma CK

Plasma CK was obtained prior and 24 h following the time-trials as an indicator of sarcolemmal disruption. Venous blood draws from the antecubital vein were utilized to obtain approximately four milliliters of blood. Whole blood was spun in a centrifuge at 7000 rpm to separate plasma, and plasma was frozen at -80 °C. Prior to analysis, samples were thawed to room temperature (22 °C) and mixed through gentle inversion. Plasma CK was analyzed using a Johnson and Johnson Vitro DT 6011, as described previously (Luden, Saunders, & Todd, 2007; Saunders et al., 2004). In addition, the 24-h post-exercise timing was utilized to allow an appropriate comparison to previous studies, which have utilized time-points ranging from 12-15 hours (Saunders et al., 2004, 2007) to 24 h post exercise (Luden et al., 2007; Romano-Ely et al., 2006).

Muscle Soreness

Subjective ratings of muscle soreness were obtained prior and 24 h following the time-trials, using a 100 mm visual analog scale (VAS).

Beverage Treatments. Subjects 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⁻¹,
which approximates upper-limits of exogenous carbohydrate oxidation during exercise (Jentjens et al., 2004, 2004, 2004). Therefore, potential performance differences between treatments could be directly attributed to protein, as 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\(^{-1}\) of vanilline 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 and final 5 km). As previously described, subjects were permitted to view their elapsed performance times during the trials, in order to provide motivation for peak performance. However, because subjects were competing against their prior trial, this characteristic of the protocol contributed to a significant ‘order-effect’, whereby subjects performed significantly faster (p<0.05) in their second trial, independent of the treatment utilized. Therefore, the following statistical model was used to correct for this order-effect:

$$\text{Trial Difference} = \alpha_i + \beta + \text{error}$$
where \( \alpha_i \) was the effect of treatment order, and \( \beta \) was the difference due to 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, thus did not require such a correction.

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

All values are presented as mean±SEM and all hypothesis testing was conducted using an alpha level of \( p <0.05 \). In the case of directional hypotheses between treatments (i.e. improvements in performance and muscle disruption/soreness with CHO+ProH ingestion) a one-tailed alpha was utilized. Two-tailed alpha tests were utilized to test 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 (\( p <0.05 \)) 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, blood glucose and lactate. In addition, there were no significant treatment*time interactions in any of the above measures. Main effects from the ANOVA model were examined to determine if 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, blood lactate and glucose all changed significantly over time (Table 3).

Plasma CK and Muscle Soreness

No significant treatment*time interactions were observed for plasma CK and muscle soreness levels. Post-exercise 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-exercise to post-exercise in the CHO trial (from 161 to 399, p <0.05), but not in the CHO+ProH trial (from 115 to 262 U.L$^{-1}$, p = 0.08).

Similarly, post-exercise muscle soreness was not significantly different between treatments (Figure 2), but increased significantly (p <0.05) in the CHO trial (15.8±5.1 to 37.6±5.7 mm) and not in the CHO+ProH trial (20.9±5.3 to 32.2±7.1 mm, p = 0.19).

Discussion
The primary purpose of the present study was to determine if 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⁻¹). The small difference in overall 60 km performance was not statistically different between treatments. However, as hypothesized in the introduction of this paper, all of the performance improvement with CHO+ProH was observed in the final 20 km of the trial and most of the difference 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 over the final 5 km of the trial. These findings have substantial relevance for competitive athletes, as most cycling races are determined by time differences of considerably less than 30s. 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 30s treatment difference in final 5km times represented a 3% improvement with CHO+Pro, while the 42s treatment difference in 60km times was a 0.5% difference. As described earlier in the manuscript, 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 present study, each of these studies compared CHO+Pro beverages to CHO beverages matched for carbohydrate-content. However, because carbohydrate
intake rates in these studies (37-47 g·hr\(^{-1}\)) 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 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 these studies (13-36%) could be explained by the relatively small differences in calories between treatments. The present 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, we observed that the ergogenic effects of CHO+ProH may be observed during time-trial protocols, even when beverages are ingested at very high (~60 g·hr\(^{-1}\)) levels of carbohydrate intake.

Only one prior study has compared CHO+Pro and CHO beverages using a time-trial protocol. In contrast to the present 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 differing findings from the present study are unclear. Apparently, the protocol of Van Essen and Gibala (2006) mimicked a flat time-trial, and did not examine time differences for the latest stages of exercise, where the potential benefits of performance from CHO+Pro beverages may be most apparent. As previously discussed, the assessment of late-exercise performance in the present study may 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 may have been influenced by the sources of protein utilized in the beverages. The three prior studies reporting improved endurance with CHO+Pro all utilized intact whey protein (Ivy et al., 2003; Saunders et al., 2004, 2007). However, it is difficult to directly compare our results to these studies as they each measured performance using time-to-exhaustion protocols. The present investigation, and the study from Van Essen and Gibala (2006) both examined time-trial performance, and both utilized similarly high rates of fluid (1000 ml·hr⁻¹), carbohydrate (60 g·hr⁻¹) and protein (15-20 g·hr⁻¹) compared to the aforementioned studies (508-600 ml·hr⁻¹, 37-47 g·hr⁻¹, and 9-12 g·hr⁻¹, respectively). However, Van Essen and Gibala (2006) used intact whey protein, while the present study utilized 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 and colleagues (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 following consumption of intact protein or long-chain protein hydrolysates versus 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 utilized in the present study (PeptoPro®) contained mainly di- and tripeptides, which, in an animal model, was shown to exhibit lower endogenous protein losses than its native protein source, casein (Moughan et al., 2007). As a consequence, more protein is available for, e.g., muscle synthesis.

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

Including the present findings, four of the six studies examining CHO+Pro ingestion during endurance exercise have reported performance benefits versus CHO. The mechanism(s) by which CHO+Pro may 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 substrates, and augmented insulin stimulation. In addition, Betts, Williams, Boobis, and Tsintzas (2008) recently reported that CHO+Pro consumed immediately following 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 post-exercise levels of plasma CK or ratings of muscle soreness between treatments. However, both variables were significantly elevated from pre-exercise to post-exercise in the CHO trial, but not in the CHO+ProH trial. Several studies have reported CHO+Pro ingestion during and/or following endurance exercise may reduce post-exercise 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., 2007; Millard-Stafford et al., 2005; Romano-Ely et al., 2006) compared to CHO ingestion. These effects have been reported when CHO+Pro and CHO beverages were matched for carbohydrate content (Luden et al., 2007; Millard-Stafford et al., 2005; Saunders et al., 2004; Valentine et al., 2008) or total calories (Romano-Ely et al., 2006; Valentine et al., 2008). The absence of a significant treatment difference in the present study may have been the result of differences in exercise protocols, subject samples, and differences in statistical power related to these varied factors. For example, Luden et al. (2007) recently reported significant reductions in plasma CK levels with CHO+Pro supplementation despite mean treatment differences that were very similar to the present study. However, the study by Luden and associates (2007) included a sample of 23 subjects, providing the statistical power to observe more subtle differences between treatments than the present 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 may 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 may be the most relevant measure of muscle recovery for athletes. In a recent investigation, Valentine et al. (2008) reported plasma CK values following exhaustive cycling that were quite similar to the present study (373±417 U·L\(^{-1}\) following a CHO trial, versus 192±149 U·L\(^{-1}\) for CHO+Pro). This relatively small attenuation in plasma CK was accompanied by significantly improved muscle function 24-hours following exercise. Based on the findings of the present 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 the inclusion of protein in the beverage may have provided a protective effect from muscle disruption following heavy exercise.

In conclusion, ingestion of a CHO+ProH beverage during endurance cycling produced significant improvements in late-exercise time-trial performance compared to a CHO beverage. These findings are particularly relevant for athletes, as the beverages were matched at theoretically maximal levels of exogenous carbohydrate oxidation (~60 g·hr\(^{-1}\)), above which further performance benefits would be unlikely with additional carbohydrate content. The magnitude of performance differences (3% during the final 5
km climb) were smaller than previous studies of CHO+Pro using time-to-exhaustion protocols, but remain highly important for competitive athletes.

CHO+ProH ingestion during and following a cycling time-trial also prevented increases in plasma CK and muscle soreness ratings, which were observed in the CHO trial. These findings support previous research suggesting that CHO+ProH beverages consumed during and immediately following exercise may be advantageous for performance and muscle recovery in endurance athletes.
Carb/Protein Hydrolysate & Time-Trial Performance

References


Acknowledgements

The authors wish to thank DSM Food Specialties, Inc. for supporting this project with a research grant. Dr. Arie Kies is an employee of DSM Food Specialties, Delft, The Netherlands. 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.
Figure Captions

Figure 1 – Course & Measurement Profile

[Insert Figure 1 here]

60km Time-trial = 3 consecutive laps of 20km course
Physiological Measures = VO$_2$, RER, heart rate, RPE, glucose & lactate

Figure 2 – Pre and post-exercise Plasma CK levels and Muscle Soreness Ratings

[Insert Figure 2 here]

Data reported are means/standard error
[* = significantly higher (p<0.05) than pre-exercise]
Table 1 - Subject Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</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>VO$_2$peak (L·min$^{-1}$)</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>VO$_2$peak (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>60.8±1.6</td>
</tr>
</tbody>
</table>
Table 2 - Performance Differences Between Treatments

<table>
<thead>
<tr>
<th>Measurement Period</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
</tr>
<tr>
<td>60 km</td>
<td>135.0±4.0</td>
</tr>
<tr>
<td></td>
<td>(135.1±4.1)</td>
</tr>
<tr>
<td>Final 20 km*</td>
<td>45.0±1.6</td>
</tr>
<tr>
<td></td>
<td>(45.1±1.6)</td>
</tr>
<tr>
<td>Final 5 km*</td>
<td>16.9±0.6</td>
</tr>
<tr>
<td></td>
<td>(17.0±0.7)</td>
</tr>
</tbody>
</table>

Reported mean±SEM are corrected for a significant order-effect, with raw scores in parentheses. 
(* = significant difference between treatments; p<0.05)
### Table 3 - Physiological Reponses During Exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>10 km</th>
<th>30 km</th>
<th>50 km</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
<td>CHO + ProH</td>
<td>CHO</td>
</tr>
<tr>
<td>VO(_2) (mL·kg·min(^{-1}))</td>
<td>43.7±2.1</td>
<td>44.7±2.2</td>
<td>42.5±2.5</td>
</tr>
<tr>
<td>RER*</td>
<td>0.99±0.01</td>
<td>0.99±0.01</td>
<td>0.96±0.01</td>
</tr>
<tr>
<td>Heart rate (bt·min(^{-1}))*</td>
<td>159.8±4.7</td>
<td>157.4±5.1</td>
<td>161.5±4.7</td>
</tr>
<tr>
<td>RPE*</td>
<td>12.2±0.3</td>
<td>12.3±0.6</td>
<td>13.5±0.5</td>
</tr>
<tr>
<td>Glucose (mg·dL(^{-1}))*</td>
<td>82.2±3.1</td>
<td>81.8±3.3</td>
<td>87.1±3.4</td>
</tr>
<tr>
<td>Lactate (mmol·L(^{-1}))*</td>
<td>3.1±0.4</td>
<td>3.0±0.4</td>
<td>2.5±0.4</td>
</tr>
</tbody>
</table>

Data reported are Mean±SEM
* = significant main-effect for time (p<0.05)