Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage

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ABSTRACT
SAUNDERS, M. J., M. D. KANE, and M. K. TODD. Effects of a Carbohydrate-Protein Beverage on Cycling Endurance and Muscle Damage. Med. Sci. Sports Exerc., Vol. 36, No. 7, pp. 1233–1238, 2004. Introduction: The purpose of this study was to determine whether endurance cycling performance and postexercise muscle damage were altered when consuming a carbohydrate and protein beverage (CHO+P, 7.3% and 1.8% concentrations) versus a carbohydrate-only (CHO; 7.3%) beverage. Methods: Fifteen male cyclists (mean VO₂peak = 52.6 ± 10.3 mL·kg⁻¹·min⁻¹) rode a cycle ergometer at 75% VO₂peak to volitional exhaustion, followed 12–15 h later by a second ride to exhaustion at 85% VO₂peak. Subjects consumed 1.8 mL·kg⁻¹ BW immediately after exercise. Beverages were matched for carbohydrate content, resulting in 20% lower total caloric content per administration of CHO beverage. Subjects were blinded to treatment beverage and repeated the same protocol seven to 14 d later with the other beverage. Results: In the first ride (75% VO₂peak), subjects rode 29% longer (P < 0.05) when consuming the CHO+P beverage (106.3 ± 45.2 min) than the CHO beverage (82.3 ± 32.6 min). In the second ride (85% VO₂peak), subjects performed 40% longer when consuming the CHO+P beverage (43.6 ± 12.5 min) than when consuming the CHO beverage (31.2 ± 8.7 min). Peak postexercise plasma CPK levels, indicative of muscle damage, were 83% lower after the CHO+P trial (216.3 ± 122.0 U·L⁻¹) than the CHO trial (1318.1 ± 1935.6 U·L⁻¹). There were no significant differences in exercising levels of VO₂, ventilation, heart rate, RPE, blood glucose, or blood lactate between treatments in either trial. Conclusion: A carbohydrate beverage with additional protein calories produced significant improvements in time to fatigue and reductions in muscle damage in endurance athletes. Further research is necessary to determine whether these effects were the result of higher total caloric content of the CHO+P beverage or due to specific protein-mediated mechanisms. Key Words: CYCLING, RECOVERY, SUBSTRATE UTILIZATION, SPORTS DRINKS

Investigators have consistently documented improved endurance performance in athletes using carbohydrate beverages, compared with water or other placebo beverages (6). Typically, athletes have demonstrated increased performance time to fatigue, or increased power output during the later stages of prolonged exercise. It is generally believed that these performance benefits are derived because carbohydrate beverages augment blood glucose levels during the later stages of prolonged exercise, when muscle glycogen levels are significantly reduced (6).

Recently, it has been suggested that the inclusion of small amounts of protein (typically 20% of total calories) in a carbohydrate beverage may produce benefits over traditional carbohydrate-only beverages (10,20). Limited evidence suggests that these carbohydrate and protein (CHO+P) beverages increase performance time to fatigue (11,20), reduce postexercise muscle damage (16), and enhance muscle glycogen repletion (20) versus carbohydrate-only (CHO) beverages, but there are few peer-reviewed papers supporting or refuting these claims. There is general agreement in the literature that increased CHO levels above 6–10% concentration in sports beverages do not produce additional performance benefits (6,7). Thus, the addition of extra protein calories to CHO beverages is practically important if it produces additional performance or recovery benefits. The current study was designed to address the following research questions: 1) Is cycling time to fatigue improved when subjects consume a CHO+P beverage versus an isocarbohydrate (but not iso-caloric) CHO beverage? and 2) Does the consumption of a CHO+P beverage attenuate muscular damage compared with an isocalorific (but not iso-carbohydrate) CHO beverage after exhaustive cycle ergometry?

METHODS
Subjects. Fifteen male volunteers completed this experimental research study. This number of subjects exceeded the minimum sample size needed to detect differences in...
both primary dependent measures with a power of 0.80, based on an estimated effect size of 1.0 SD units (from pilot data), a two-tailed alpha level of 0.05, and an intraclass correlation of 0.80 between repeat measures (13). All subjects were trained cyclists who performed at least 3 d of cycle training per week and possessed a cycle ergometer VO\textsubscript{2peak} of ≥ 40 mL·kg\textsuperscript{−1}·min\textsuperscript{−1}. These entrance criteria were used so that the findings of the study could be appropriately generalized to competitive athletic populations and to increase the likelihood that all subjects could perform a cycle bout of over 1 h at 75% VO\textsubscript{2peak} (i.e., long enough that reduced muscle glycogen levels would be a contributing factor to fatigue).

All subjects completed an informed consent document, Physical Activity Readiness Questionnaire, and a comprehensive medical questionnaire to determine the presence of any risk factors associated with coronary artery disease before participating in the study. All subjects were asymptomatic and possessed fewer than two risk factors using ACSM guidelines (1). All procedures and protocols were approved by the Institutional Review Board of James Madison University.

Testing procedures. Figure 1 illustrates the order and timeline involved in the testing of each subject.

Phase 1: physical fitness assessment testing. Subjects who passed the initial screening completed an assessment of their cardiorespiratory fitness and body mass. These data were used for demographic purposes and to determine the exercise intensities used for testing in phases 2 and 3 of the study.

Body mass. Body mass was measured using a physician’s scale and was recorded to the nearest tenth of a kilogram; subjects were measured in their cycling shorts without shoes or socks.

Cardiorespiratory fitness (VO\textsubscript{2peak}). Before testing, subjects performed a 3-min warm-up on an electrically braked cycle ergometer (Ergoline 800S, SensorMedics, Yorba Linda, CA) at 100 W to prepare for maximal exercise. Subjects then performed a graded exercise test on the same device to determine their peak oxygen uptake (VO\textsubscript{2peak}). The initial workload for the test was subjectively determined during the warm-up as a wattage at which the rider felt he could maintain for a prolonged ride of “easy-moderate intensity.” In pilot subjects, this self-selected wattage produced an initial workload that was consistently below lactate threshold but minimized excessive test duration that can occur in more rigidly standardized protocols. Self-selected initial workloads varied from 60 to 150 W with a mean of 95 W (SD ± 24 W). Workload was uniformly increased from this initial level by 20 W each minute during the test. Subjects were encouraged to cycle at a self-selected cadence of >50 revolutions per minute (rpm) until they were unable to maintain this minimum cadence for a 30-s time period, at which point the test was terminated. The following measurements were obtained during this test.

Metabolic measures. Metabolic measurements including VO\textsubscript{2}, CO\textsubscript{2}, RER, and ventilation were obtained continuously during the VO\textsubscript{2peak} test. Heart rate was obtained via a Polar heart-rate monitor (Brooklyn, NY). Subjective ratings of exertion were obtained using Borg’s 6- to 20-point RPE scale (1).

Phase 2: experimental rides with blinded treatment 1 (two sessions). Each subject performed two prolonged bouts of cycle ergometry to fatigue with a 12- to 15-h rest period between rides. In the first ride, subjects rode at 75% of VO\textsubscript{2peak} at a self-selected cadence of > 50 rpm until they were unable to maintain this minimum cadence for 30 s. Twelve to 15 h after the initial exhaustive bout, each subject returned and repeated the exercise bout under the same conditions, except at a slightly higher intensity (85% VO\textsubscript{2peak}). The following measurements were obtained during both performance rides.

Metabolic measures, heart rate, and ratings of perceived exertion. Metabolic measures (VO\textsubscript{2}, CO\textsubscript{2}, ventilation), heart rate, and ratings of perceived exertion (RPE) were obtained every 30 min of exercise using the methods described above.

Blood glucose, CPK, and lactic acid levels. Blood samples were obtained at rest, and every 30 min during exercise to determine glucose and lactic acid levels. The sample consisted of approximately 5–10 drops of blood obtained from the fingertips using finger sticks. Glucose and lactate levels were determined using an automatic glucose/lactate analyzer (YSI 2300 STAT). Samples to be analyzed for plasma CPK were obtained immediately before and 12–15 h after the first prolonged exercise bout. CPK was determined from blood plasma using a Vitro DT60II (Johnson and Johnson). Before CPK analysis, the Vitro DT60II was calibrated using a reconstituted lyophilized calibration standard purchased from the manufacturer. Concentrations

FIGURE 1—Time course of study protocol.
of CPK in the standard were 45, 525, and 1700 U·L⁻¹. The specific time course for peak CPK levels after physical activity is greatly varied. Previous research on runners indicated time to peak accumulation could be 6–24 h after exercise (2,9). Based on three pilot subjects, we determined that peak CPK levels occurred 12–15 h after our exercise protocol. Based on these data, we provided subjects with a 12- to 15-h rest period (held constant for each subject) between phase 2 and 3 of the study, so that the preride 2 CPK measurements would be an appropriate representation of the peak CPK after ride 1.

Fluid replacement protocol. Subjects consumed 1.8 mL·kg⁻¹ of preconstituted treatment fluid (CHO+P drink or CHO drink) every 15 min of exercise. This was equivalent to approximately 127 mL of fluid every 15 min for a 70-kg male. Subjects also consumed 10 mL·kg⁻¹ of treatment fluid within 30 min after the exercise bouts. The order of testing was randomly counter-balanced, such that half of the subjects consumed the CHO+P drink first and half consumed the CHO beverage first. Both drinks were orange-flavored and orange-colored. A double-blind design was used such that the subjects and investigators did not know which drink was administered until completion of the study.

The CHO+P beverage was prepared according to manufacturers instructions (PacificHealth Laboratories, Inc. Woodbridge, NJ) with a 4:1 carbohydrate/protein ratio, such that the beverage had a carbohydrate content of 26 g of carbohydrate (~7.3%) and 6.5 g of whey protein (~1.8%) per 355 mL of water. Following the protocol discussed above, a hypothetical 70-kg cyclist received 37.2 kcal of CHO energy and 9.2 kcal of protein energy every 15 min during the performance rides. In addition, this same cyclist received 205.2 kcal of CHO energy and 51.3 kcal of protein energy during the postexercise administration of beverage. The CHO beverage (Gatorade, Inc., Chicago, IL) was identically matched in carbohydrate content with the CHO+P beverage (i.e., 26 g of carbohydrate per 355 mL of water) but lacked the protein calories. Thus, the CHO beverage had slightly higher carbohydrate content than manufacturer’s instructions (7.3% vs 6%). However, osmolality of the CHO beverage was still within the range considered to be optimal for sports drinks (6,7).

Phase 3: experimental rides with blinded treatment 2 (two sessions). Subjects returned after a 7- to 14-d washout period and repeated the two performance rides discussed in phase 2. The only difference in the aforementioned protocol was the administration of the alternate beverage. For example, if the subject consumed the CHO+P drink during treatment 1, they consumed the CHO drink during treatment 2.

Approach to the problem. Whereas CHO beverages have clearly been associated with improved endurance performance versus water or placebo, the addition of carbohydrate above 6–10% concentration does not appear to elicit further performance benefits (6,7). A few recent studies have suggested that the addition of protein to CHO beverages may produce performance and recovery benefits (10,11,20). Thus, the current study was designed to examine whether the addition of protein to a CHO beverage produced benefits to performance and recovery/damage compared with a CHO beverage matched for carbohydrate content. This approach is highly generalizable, as commercially available CHO and CHO+P beverages tend to be very similar in total carbohydrate content but not in total calories (i.e., protein is added to CHO beverages of 6–10% concentration). To examine differences between beverages in this study, the first performance ride served two purposes. The main purpose was to provide a primary comparison of endurance performance (i.e., time to fatigue) between the two treatments. Based on the proposed benefits of CHO+P beverages (including improved glycogen repletion and decreased muscle damage), we believed any potential differences in performance between the beverages would be more pronounced in a subsequent bout of exercise. Thus, a secondary purpose of the first performance ride was to create a considerable degree of glycogen depletion in the working muscles of the subjects, such that they started a subsequent exercise bout with a decrement in performance potential.

The purpose of the second performance ride was to provide a measure of performance in a partially depleted state. Based on the proposed benefits of the CHO+P beverages, we hypothesized that differences in performance between beverages would be greater during this ride. In addition, by measuring plasma CPK levels before the onset of the second ride, we were able to obtain a postexercise measurement of muscle damage 12–15 h after the first performance ride.

RESULTS

All 15 subjects completed all testing in the study. Demographic data for these subjects are displayed in Table 1.

Dependent t-tests were used to determine whether performance times were different between the CHO and CHO+P beverages. During the first performance ride, time to exhaustion at 75% of VO₂peak was 29% longer (P < 0.05) during the CHO+P trial (with additional protein calories) than during the CHO trial. In addition, during the subsequent ride at 85% of VO₂peak, subjects rode 40% longer (P < 0.05) when consuming the CHO+P beverage than when consuming the CHO beverage (Fig. 2).

Postexercise muscle damage was indirectly assessed using plasma CPK levels. A two-way (condition × time) repeated-measures ANOVA with Tukey HSD posthoc analyses was utilized to determine whether differences in CPK levels existed between beverage conditions before and after the first performance ride. Whereas preexercise CPK levels were not different between trials, postexercise CPK levels (12–15 h after the first performance ride) were significantly different between treatments. Thus, the current study was designed to examine whether the addition of protein to a CHO beverage produced benefits to performance and recovery/damage compared with a CHO beverage matched for carbohydrate content. This approach is highly generalizable, as commercially available CHO and CHO+P beverages tend to be very similar in total carbohydrate content but not in total calories (i.e., protein is added to CHO beverages of 6–10% concentration). To examine differences between beverages in this study, the first performance ride served two purposes. The main purpose was to provide a primary comparison of endurance performance (i.e., time to fatigue) between the two treatments. Based on the proposed benefits of CHO+P beverages (including improved glycogen repletion and decreased muscle damage), we believed any potential differences in performance between the beverages would be more pronounced in a subsequent bout of exercise. Thus, a secondary purpose of the first performance ride was to create a considerable degree of glycogen depletion in the working muscles of the subjects, such that they started a subsequent exercise bout with a decrement in performance potential.

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
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<tr>
<td>Age (y)</td>
<td>20.9 ± 3.3</td>
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<tr>
<td>Weight (kg)</td>
<td>73.3 ± 6.4</td>
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<tr>
<td>VO₂p (mL·kg⁻¹·min⁻¹)</td>
<td>52.6 ± 10.3</td>
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<tr>
<td>Ride 1 workload (W)</td>
<td>227 ± 35</td>
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<tr>
<td>Ride 2 workload (W)</td>
<td>248 ± 33</td>
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lower ($P < 0.05$) after the CHO+P trial (with additional protein calories) than the CHO trial (Fig. 3). Because of the sizable differences in within-group variability between the postexercise trials, a Wilcoxon signed ranks test was used to compare these CPK levels, which were also statistically significant between treatments.

Dependent $t$-tests were utilized to examine differences in steady-state levels of blood glucose, lactate, ventilation, $\dot{V}O_2$, heart rate, and RPE during exercise between the two beverage conditions. These results are presented in Table 2. Because time to exhaustion varied considerably between subjects, these variables were calculated from data gathered at minute 30 of each ride to maintain consistency. It should also be noted that analyses for the second performance ride contains only 14 data points, as one of the subjects did not reach 30 min of exercise duration during the high intensity ride. None of the variables in Table 2 were significant different ($P < 0.05$) between the treatments for either ride.

**DISCUSSION**

The primary objectives of this study were to determine whether CHO+P beverages produced improvements in performance time to fatigue and muscle damage compared with CHO beverages in endurance athletes. The beverages used for this comparison were matched for total carbohydrate content but not total calories. This approach is highly generalizable, because commercially available CHO+P beverages have typically added protein to beverages that already contain 6–10% carbohydrate (a typical level for CHO-only beverages). Mechanistically, this approach is advantageous because the additional protein content of the CHO+P drink is the only energy substrate difference between the CHO+P and CHO beverages. By matching the carbohydrate portion, any difference in performance or recovery can be attributed to something other than the absolute carbohydrate content of the fluids. A limitation of this approach is that the increased availability of total calories from the CHO+P beverage may have contributed to differences between trials. To minimize the effects of caloric or carbohydrate intake between trials, subjects in this study were asked to maintain a consistent diet before each performance bout. Subjects also provided dietary records for 3 d before each performance ride, which verified the consistency of dietary macronutrients and total caloric content between trials.

One of the main purposes of this study was to determine whether a CHO+P beverage could enhance athletic performance during prolonged endurance bouts to a greater extent than a CHO beverage. When utilizing the CHO+P beverage, subjects maintained an intensity of 75% of their $\dot{V}O_{2\text{peak}}$ 29% longer ($P < 0.05$) than when consuming a CHO beverage. In a comparable study, Ivy et al. (11) compared CHO and CHO+P beverages during an exercise bout that simulated a competitive cycling event. After 180 min of varying intensity cycling, athletes in the CHO+P trial sustained exercise at 85% $\dot{V}O_{2\text{peak}}$ for 36% longer than during a CHO trial (26.9 ± 4.5 vs 19.7 ± 4.6 min). The agreement

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**TABLE 2. Physiological data from performance rides.**

<table>
<thead>
<tr>
<th>Performance Ride</th>
<th>CHO + P</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL⁻¹)</td>
<td>90.3 ± 15.4</td>
<td>91.5 ± 13.5</td>
</tr>
<tr>
<td>Lactate (mmol/L⁻¹)</td>
<td>2.7 ± 1.7</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Ventilation (L/min⁻¹)</td>
<td>89.3 ± 32.1</td>
<td>95.5 ± 17.6</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L/min⁻¹)</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>RPE</td>
<td>14.9 ± 2.3</td>
<td>15.6 ± 2.0</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>17.7 ± 0.8</td>
<td>18.4 ± 0.6</td>
</tr>
<tr>
<td>Pre-Ride 1</td>
<td>166.0 ± 14.0</td>
<td>168.4 ± 13.5</td>
</tr>
<tr>
<td>Post-Ride 1</td>
<td>172.9 ± 9.0</td>
<td>175.3 ± 6.8</td>
</tr>
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FIGURE 2—Time to exhaustion during performance rides. *Significantly greater ($P < 0.05$) than CHO ride.

FIGURE 3—Change in CPK levels. *Significantly lower ($P < 0.05$) than CHO ride.
in findings between these studies is particularly relevant because both studies utilized beverages that contained a 4:1 ratio of carbohydrate to protein, in nearly identical concentrations. In addition, both studies matched beverages for total carbohydrate content but not total caloric content.

It is possible that increased availability of total calories may have contributed to the performance improvements observed in the CHO+P trial. A hypothetical 70-kg cyclist received 37.2 kcal of CHO and 9.2 kcal of protein during the CHO+P trial every 15 min of cycling. The CHO trial was matched for CHO calories with no protein calories. Thus, CHO concentrations. In addition, both studies matched beverages for ratio of carbohydrate to protein, in nearly identical concentrations. This is consistent with the proposed benefits of the CHO+P beverage. A few studies (10,21) have observed significantly faster glycogen resynthesis rates after exercise in those consuming CHO+P versus those consuming CHO. Williams et al. (20) showed significantly greater muscle glycogen storage (128%) after CHO+P consumption, compared with a CHO beverage. These increases in muscle glycogen may have been mediated by elevated postexercise insulin levels observed in two of these studies (20,21), which may facilitate greater glucose uptake via translocation of GLUT4 transporters or activation of glycogen synthase (4,21). Increased muscle glycogen storage could explain why CHO+P beverages have been associated with 21% (14) to 55% (20) increases in postrecovery endurance performance, when compared with CHO beverages.

Although the proposed mechanism for improved postrecovery performance is plausible, it should be noted that some investigators have observed no differences in glycogen storage between CHO+P and CHO treatments (5,18,19). Ivy et al. (10) discussed numerous methodological variances that could explain these inconsistencies, including differences in carbohydrate and protein concentrations in the beverages, beverage administration protocols, and the time period of recovery measurements. It is possible that the improved postrecovery performance in this study was related to the higher total calories consumed in the CHO+P trial, due to the added protein and longer time to fatigue in the initial ride to exhaustion. During and after the first performance ride, the cyclists consumed 190 more calories during the CHO+P trial (581 kcal) than the CHO trial (391 kcal). However, as discussed previously, the added calories consumed do not match the additional calories expended during the longer CHO+P trial. Thus, it is unlikely that the additional calories consumed played a major role in the differences in performance between trials, as the net energy difference between trials (i.e., calories expended vs calories consumed) is in favor of the CHO trial. This hypothesis is supported by data from Ivy et al. (10), who showed that glycogen resynthesis after cycling was improved after consumption of a CHO+P beverage versus an isocarbohydrate CHO beverage or an isocaloric CHO beverage. This suggests that CHO+P supplements may augment muscle recovery through mechanisms that are independent of total calories consumed.

The second purpose of the present study was to determine whether postexercise creatine phosphokinase (CPK) levels, a common indicator of muscular damage, differed between the two beverage trials. CPK levels measured 12–15 h after the CHO+P trial were reduced 83% (P < 0.05) compared with the CHO trial. We are aware of no peer-reviewed studies specifically comparing CPK levels between similar beverage treatments. However, the trends in the present study agree with data presented by Ready et al. (16). These investigators observed that CPK levels 24 h after a run/cycle duathlon were 36% lower when consuming a CHO+P beverage than when consuming a CHO beverage. Although the measured reduction was significantly greater in this study, the exercise methods and CPK measurement times were different between the studies. In a pilot for the present study, postexercise plasma CPK concentration peaked between 12 and 15 h after exercise; thus, this time period was utilized for postexercise CPK measurements. The measurements by Ready et al. (16) were obtained 24 h postexercise, which
may explain some of the variance in magnitude of CPK changes between studies.

The postexercise CPK levels observed in the CHO trial were comparable to those reported by Prou et al. (15) 6 and 24 h after an endurance triathlon that included swimming, cycling, and running. Due to the primarily concentric muscular actions of cycling, more moderate CPK responses are expected after endurance cycling than endurance running (12). However, the seemingly high postcycling CPK levels observed in this study may have been related to the type of endurance protocol utilized. Fatigue was defined as the point at which the cyclist could no longer maintain a cadence of 50 rpm for 30 s. As the subjects began to fatigue over the course of the trial, their cadence dropped from approximately 90–105 rpm to 50 rpm. As cadence decreased, resistance to pedaling increased to maintain a constant workload. This created a somewhat novel cycling environment where muscular force production was considerably higher than cyclists would typically experience for a given workload. In addition, the clipless pedals utilized by the cyclists allowed them to “pull” the pedals through the lower portion of the pedal stroke as cadence decreased, potentially increasing the extent of eccentric muscular contractions. These factors likely explain why the postexercise CPK values were somewhat higher than expected during typical cycling exercise.

The decreased postexercise CPK levels observed in our CHO+P trial indicated reduced muscle damage 12–15 h after exhaustive endurance exercise. The current study did not address specific mechanisms regarding how muscle damage was reduced in the CHO+P trial. However, Bloomer and Goldfarb (3) have suggested that nutritional supplements theoretically function to minimize secondary or delayed onset damage, as opposed to reducing the initial mechanical damage from exercise. It seems plausible that the CHO+P beverage in our study increased protein concentrations outside the cell, potentially driving increased protein synthesis and repair. This and other specific mechanisms should be examined by future investigators.

In summary, administration of a carbohydrate beverage with additional protein calories resulted in significant improvements in cycling time to fatigue and reductions in postexercise muscle damage (measured indirectly using plasma CPK levels) in comparison with a carbohydrate-only beverage. Further research is necessary to determine whether these effects were the result of higher total caloric content in the CHO+P beverage or due to specific protein-mediated mechanisms. Regardless of the specific mechanisms, these findings may have important implications for endurance athletes because it suggests that the effective caloric concentration of sports beverages can be elevated in CHO+P beverages to a greater degree than the 6–8% typically observed in CHO beverages.

REFERENCES