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Research Article

INFLUENCE OF INGESTING CASEIN PROTEIN AND WHEY PROTEIN CARBOHYDRATE BEVERAGES ON RECOVERY AND PERFORMANCE OF AN ENDURANCE CYCLING TEST

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ABSTRACT

The main aim of this study was to determine if short-term post exercise recovery, cycling performance and blood analysis were altered when consuming three different beverages with the same amounts of calories, a carbohydrate-only beverage (CHO, 9% carbohydrate) a carbohydrate and casein protein beverage (CHO+Pc, 7% carbohydrate and 2% protein) and a carbohydrate and whey hydrolyzed drink (CHO+Pw, 7% + 2 %). Fifteen male cyclists (VO_{2peak}= 63.4±9.6 $ml \cdot kg^{-1} \cdot min^{-1}$) performed three trials using a randomly counterbalanced, double-blind design. In each trial one litre of one of the test drinks was consumed in fasting conditions after 1 hour ride at 75% VO_{2peak}. After a two hours recovery period the cyclists rode 20 km at the rider's maximum speed for this distance. The results showed no significant differences in the 20-km ride when consuming the CHO (1770±210 s), the CHO+Pc drink (1819±185 s) or the CHO+Pw (1803±201). Post-exercise creatine kinase (CK) was not significantly different between treatments. However, serum insulin concentrations were higher during recovery when CHO+Pc and CHO+Pw beverages were consumed ($P<0.05$). Glucagon and lactic acid levels increased more on the CHO than on the CHO+Pc and CHO+Pw treatments ($P<0.05$) at the end of the 20 km test. Within the context of this experimental design, the CHO+Pc and CHO+Pw beverages showed different physiological effects than the CHO drink. One purported mechanism indicates muscle glycogen re-synthesis is enhanced when protein is added to a CHO recovery formula. The CHO+Pw and CHO+Pc drinks could be recommended for improving recuperation from intensive exercise. Although this was not reflected in post-recovery exercise performance in this 20 km test, a harder or longer test may be more affected by the physiological parameters especially in the last kilometres of the test.

Key words**:** whey protein, casein protein, recovery, cycling performance

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INTRODUCTION

Sports nutrition is a complex concept, with characteristics that are unique to each sporting event and each athlete. Although most athletes can satisfy their nutritional requirements before and/or after exercise, long-duration activities require that participants also address their nutritional needs during exercise. Endurance exercise promotes vast increases in energy utilization, with significant increases in carbohydrate and fat oxidation rates. Sizable losses of fluid and electrolytes from sweat may also occur, particularly during prolonged exercise in the heat. As a result, inadequate fluid and nutrient intake during endurance exercise can lead to dehydration, hyponatremia, glycogen depletion, hypoglycemia, and impaired performance. In addition, nutritional deficiencies during prolonged activity may limit the capacity for rapid recovery after exercise, which may affect subsequent performance (Saunders et al., 2009).

Numerous studies have investigated the different nutritional approaches to minimize these issues, resulting in various nutritional strategies that elicit positive effects for endurance athletes. One of the commonest strategies carried out is the consumption of sports beverages containing carbohydrate and electrolytes. These beverages promote the fluid balance and euglycemia and augment performance during prolonged endurance activities (Jeukendrup et al., 1996). Traditional guidelines suggest ingesting sports beverages with 4-8% carbohydrates at regular intervals during exercise to provide approximately 600-1400 ml of fluid and 30-60 g of carbohydrates per hour (American College of Sport Medicine, 2006).

Another nutritional strategy, more and more used by athletes, that improves the endurance exercise performance and reduces the muscle damage indicators and improves recovery after exercise, is the utilization of carbohydrate-protein beverages (CHO +Pro).

It is well accepted that strength and power athletes have a protein requirement that might be at least twice that of sedentary individuals and perhaps 30–50% greater than that of endurance athletes (Lemon et al., 1992; Tarnopolsky et al., 1992). The greater amount of protein needed by these athletes is thought to enhance the recovery and remodeling processes of muscle fibers that have been damaged or disrupted during resistance exercise (Tipton et al., 2007).

Recent investigations have reported a reduction in muscle damage, attenuation of force decrements, and enhanced recovery from resistance exercise in individuals using protein and/or amino acid supplements (Kraemer et al., 2006; Ratamess et al., 2003).

Carbohydrate–protein (CHO+Pro) supplements consumed during prolonged exercise have been reported to improve time to fatigue in a number of recent studies (Ivy et al., 2003; Saunders et al., 2004; Saunders et al., 2007).

In each of those studies, CHO+Pro treatments were compared with carbohydrate (CHO) treatments matched for carbohydrate content, but not total caloric content, suggesting that adding protein to a typical carbohydrate sports drink (6–8% CHO) can improve endurance.

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Romano-Ely et al. (2006), however, reported no differences in time to exhaustion between isocaloric CHO+Pro and CHO beverages. This suggests that a primary factor for the benefits of CHO+Pro is the additional availability of calories in CHO+Pro beverages. These findings could alternatively support a protein-mediated benefit of CHO+Pro ingestion, because performance in the CHO+Pro trial equaled that in the CHO trial, despite 20% lower carbohydrate content in the CHO+Pro beverage.

Previous studies reporting ergogenic effects of CHO+Pro compared beverages delivered at rates of 37–47 g CHO/hr (Ivy et al., 2003; Saunders et al., 2004, 2007), below the maximal oxidation rates of exogenous carbohydrate. Thus, it is unclear whether adding protein to a beverage containing carbohydrate provided at peak exogenous oxidation rates (60–90 g CHO/hr; Jentjens, et al., 2004) will elicit further improvements in performance.

Williams et al. (2003), reported marked increases in blood glucose, insulin response and glycogen storage with carbohydrate-protein (CHO+P) supplementation, indicating the potential to improve time trial performance and recovery. In this study, the beverages were mixed according to the manufacturer's directions, and the CHO+P beverage contained more carbohydrate and total calories than the carbohydrate-only (CHO) beverage. These factors suggest that the reported benefits may be independent of the protein that was added to the beverages.

Ivy et al. (2003) and Saunders et al. (2004) compared CHO and (CHO+P) beverages that were matched for carbohydrate calories. A greater time to fatigue was found in these studies as well. Although the carbohydrate content was matched, the additional protein provided 25% greater caloric intake during exercise and recovery in the carbohydrate-protein trials. Because protein contributes up to 15% of total energy expenditure in prolonged bouts of exercise (Lemmon, 1998), the protein calories in the CHO+P beverage may account for the improvements in performance. To better understand how adding protein affects endurance performance, CHO and CHO+P beverages should be matched for total calories.

Studies by Van Loon et al. (2000) in which insulinotopic protein hydrolisate was used and others by Zawadzki et al. (1992), in which a supplement combination of CHO and milk serum protein compared with a CHO-only supplement was used, demonstrated that the ingestion of some proteins and/or amino acids in combination with moderate CHO intakes (~0.8 g/kg/h) carries higher speeds in muscle glycogen synthesis compared with the ingestion of the same amount of CHO without protein and /or amino acids.

CHO+P beverages have also been associated with the attenuation of exercise-induced muscle damage. In Saunders et al. (2003), post exercise creatine kinase (CK) was lower in the carbohydrate-protein trials than in CHO trials. The co-ingestion of protein and CHO has been considered advantageous when consumed immediately after exercise compared to CHO alone (Ivy et al., 2003; Saunders et al., 2006). One purported mechanism indicates muscle glycogen re-synthesis is enhanced when protein is added to a CHO recovery formula (Ivy et al., 2003). Insulinmic responses are elevated with CHO+P feedings after exercise compared to CHO (Jentjens et al., 2004).

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Nevertheless, Cepero et al. (2009), comparing the effects of CHO and CHO+P beverages, found that serum insulin concentrations were higher during recovery when CHO+P beverage was consumed ($P<0.05$). The CHO+P drink showed different physiological effects than the CHO drink, so that the CHO+P drink can be recommended for improving recuperation from intensive exercise.

Furthermore, in other similar studies, casein hydrolysate was added to CHO beverages, showing similar benefits in the performance, improvements of muscle damage indicators and in the recovery after exercise (Saunders et al., 2006; Saunders et al., 2009).

Valentine et al. (2008) carried out another study to assess whether the improvement in the endurance exercise performance and the improvement of muscle damage rates with CHO+Pro beverage ingestion are because of the total intake of energy or because of specific effects of the protein. For this, the authors examined effects of CHO+Pro on time to exhaustion and markers of muscle disruption compared with placebo (PLA) and carbohydrate beverages matched for carbohydrate (CHO) and total calories (CHO+CHO). The test was carried out with cyclists and consisted of 4 races to exhaustion at 75% VO_{2peak} in which participants ingested 250 ml of PLA, CHO (7.75%), CHO + CHO (9.69%), or CHO + Pro (7.75% / 1.94%) every 15 minutes. The results in time to exhaustion were significantly higher with CHO+Pro (126.2 + / -25.4 min) and CHO+CHO (121.3 + / -36.8) beverages than with PLA (107.1 + / -30.3). CHO (117.5 + / -24.2) and PLA results were not significantly different. No considerable differences were found between CHO+Pro beverage and the ones with CHO and CHO + CH, so that they concluded that the improved performance with CHO+Pro beverage could be caused for the different amounts of calories. They did obtain important differences in the muscle damage indicators with CHO+Pro beverage ingestion, but they were obtained only with intakes during the exercise.

There is not an exact knowledge of which type of protein has better results yet. There are no enough studies that have compared in a direct way the endurance performance measures among CHO beverages containing different types of proteins (Saunders et al., 2007). 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 et al., 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 et al. (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 (Saunders et al., 2009).

The main objective of this study has been to determine whether the sport performance, the recovery after the effort and the blood biochemistry are changed by the intake of three different sports beverages: CHO-only (9%), CHO+ casein Protein (7% carbohydrates and 2% proteins)

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and CHO+ whey protein, lactoserum, (7% carbohydrates and 2 % proteins) beverages. The study hypothesis indicates that the addition of casein and whey protein in the carbohydrate beverage could increase, on the one hand, the physiologic values that determine the sport performance and, on the other hand, the sport performance itself. Including protein in a carbohydrate solution may accelerate both the rate of glycogen storage and the restoration of exercise capacity following prolonged activity.

METHODS

Experimental Approach to the Problem
The experimental protocol was designed in three phases to determine the differences in performance and recuperation after the ingestion of two drinks with different recuperation. In the first phase the participants were informed of the type of test to be carried out and the procedures involved and signed their consent to take part in this study.

In the second phase a medical examination was made and a test of VO_{2max} undertaken with the aim of determining their state of health and maximum performance. In the third phase the cyclists arrived at the test site after fasting for ten hours and then pedaled for one hour at 75% of their maximum capacity with the object of depleting muscular glycogen reserves (Williams et al., 2003; Betts et al., 2007).

After this hour of pedaling, the cyclists drunk one liter of beverage in a double blind experimental design and rested for two hours with blood samples taken every 15 minutes. After this recuperation time, the cyclist was encouraged to perform 20 km as fast as possible in a test similar to that of Betts et al. (2007).

Participants
Fifteen male cyclists (age 39.0±9.8 years, height 1.76±0.06 m and body mass 74.4±7.2 kg) completed this experimental research study. This number of participants exceeded the minimum sample size needed to detect differences in 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 (Lipsey, 1990).

All volunteers (n=15) were trained cyclists who trained at least 3 days' cycling per week, 2-5 hours per session, and possessed a VO_{2peak} of 65.5±10.3 $ml \cdot kg^{-1} \cdot min^{-1}$ determined on a cycle ergometer. 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 participants could cycle at 75% VO_{2peak} for over an hour.

Testing Procedures
Phase 1: Preliminary Measurements
The potential risks and benefits associated with participation in the experiment were explained to all the participants. They completed a comprehensive medical questionnaire and underwent a medical examination to determine the presence of any risk factors associated with coronary artery disease before participating in the study. The participants signed an informed consent letter. All procedures and protocols were approved by the Ethical Committee of the University of

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Granada (Spain) for use of human subjects and were in accordance with current Spanish law on the matter.

Phase 2: Cardio-respiratory fitness (VO_{2peak})
Participants who passed the initial screening completed an assessment of their cardiorespiratory fitness, height and body mass. These data were used to determine the exercise intensities used for testing in phase 3 of this study. Body mass was measured using a physician's scale and was recorded to the nearest tenth of a kilogram; participants were measured in their cycling shorts and without shoes.

Cardiorespiratory fitness tests were administered to determine each participant's maximal oxygen uptakes on an electrically braked cycle ergometer (Ergoline 900, SensorMedics, Yorba Linda, CA). Before testing, participants performed a 5 min warm-up at 100 W to prepare for maximal exercise.

Participants then performed a graded exercise test to determine their peak oxygen uptake. The initial work load for the test was 100 W and workload was uniformly increased from this initial level by 25 w each 2 minutes during the test; participants were encouraged to cycle at a selected cadence of > 40 rpm either until they were unable to maintain this minimum cadence for a 30 s time period, at which point the test was terminated, or until exhaustion. Workload, heart rate and ratings of perceived exertion were obtained at the end of each 60-s period during the test. Heart rate was obtained via a Polar heart-rate monitor S 610 I (Kempele, Finland), and VO_{2peak} was finally calculated for each subject using Arts and Kuipers' (1994) regression equation, $\%VO_{2max} = 12.1 + 0.866 \cdot \%W_{max}$, the correlation for this equation is 0.98 ($p<0.001$).

Phase 3: Experimental Design and Protocol
All cyclists arrived in the laboratory between 8 and 8:30 am following a 10 hours' overnight fast and having eaten the same dinner for each day before the test. Each participant's body mass was recorded before a cannula was inserted into an antecubital vein and a 15 ml resting venous blood sample obtained. The cannula was kept open throughout each trial by frequent flushing with isotonic saline.

All participants performed two trials within 16 days, since a greater time delay increases measurement error resulting from potential variations in motivational factors and training status of participants. They undertook a one hour ride at 75% VO_{2max} in each trial with the objective to reach the glycogen-depleted state, and then consumed one liter of one of the test drinks in fasting conditions. After a two hours´ recovery period, during which the evolution of recovery was analyzed, the cyclists rode 20 km as fast as possible. The simulated race was made by each cyclist on his own competition bicycle assembled on a computerized ergometer roller Elite Digital Mag Elastogel CRONO MAG from Elite (Italy). This is a magnetic-type training roller with five different constant-speed resistance levels, and in this study the resistance level was set at level 3 with a slope value of 1.6%. The resistance on the DIGITAL CRONO MAG is generated by powerful magnets placed on the flywheel and two discs that cross its magnetic field. The cyclists were familiarized with the study procedures and with the 20 km time-trial at least twice before the trials. The cyclists performed three main trials separated by at least one

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week in a randomized, counterbalanced design. During the tests the cyclists received information of their heart rate, time and distance recorded, but no encouragement was given.

The resting venous blood sample of 10 ml was obtained every 15 minutes during the 2 h recovery period and at the end of the 20 km ride. Blood variables (insulin, glucagon, glucose, CK, and lactic acid) were measured, and the time needed to ride 20 km was recorded. Plasma CK was obtained as an indicator of muscle damage. Approximately 17 mL of blood were collected using venous-blood draws from the antecubital vein, and whole blood was spun in a centrifuge at 7000 rpm to separate plasma. Plasma samples were frozen at <–18 °C, brought to room temperature (22 °C), and mixed through gentle inversion before analysis. Plasma CK was analyzed using a Johnson and Johnson Vitro DT 6011. Before analyses, the measurement device was calibrated using a reconstituted lyophilized calibration standard purchased. The order and timeline of testing for this study is illustrated in Figure 1.

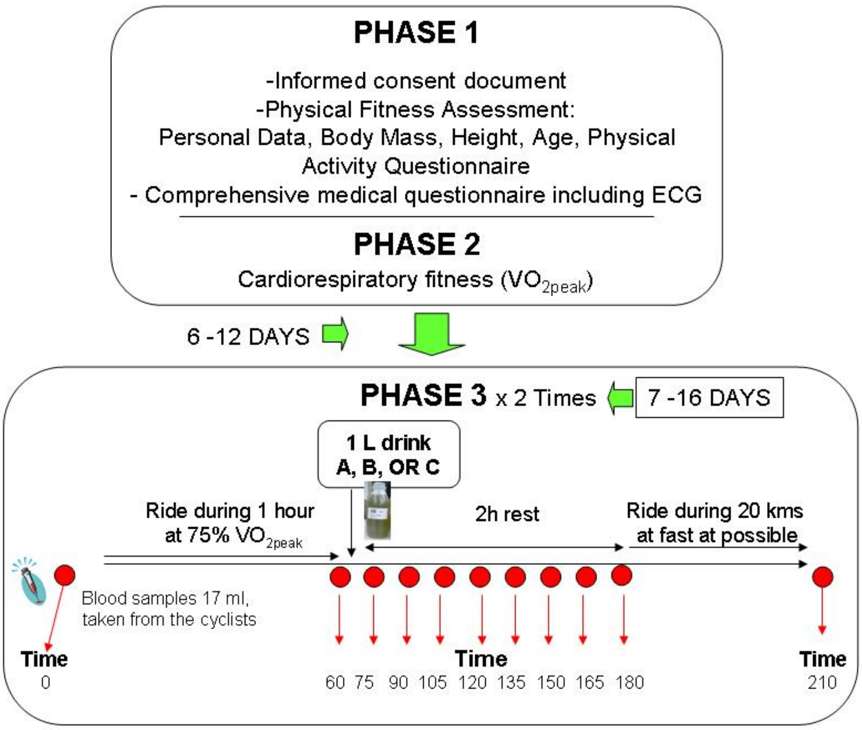

Figure 1. Schematic time course of study protocol.

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Beverage formulation
Three prototypes of isotonic drinks were developed. The drinks have acceptable organoleptic characteristics after UHT treatment and are isotonic (osmolality of about 300 mOsm/kg). The beverages were fortified with vitamins C and E even though there is evidence that these antioxidants may protect against muscle damage (Romano-Ely et al., 2006). Reducing the quantity of CHO included in a supplement and replacing it with protein may not represent an effective nutritional strategy when the supplement is ingested during exercise. This may reflect the central ergogenic influence of exogenous CHO during this activity (Toone and Betts, 2010). The nutrient information and characteristics of these products are provided in Table 1.

Table 1. Beverage formulation

	A **Control drink CHO**	**B** **Whey hydrolysate drink CHO+Pw**	**C** **Casein hydrolysate drink CHO+Pc**
Energy	36 kcal/100 ml	36 kcal/100 ml	36 kcal/100 ml
Protein	0%	2 % Whey hydrolysate	2% Casein hydrolysate
Fat	0%	0%	0%
Carbohydrates	9%	7%	7%
Vitamins B, E, C, D	25% DRI per L	25% DRI per L	25% DRI per L
Folic Acid	25% DRI per L	25% DRI per L	25% DRI per L
Minerals	Isotonic	Isotonic	Isotonic
Taste/Color	Lemon-green	Lemon-green	Lemon-green
Treatment	UHT	UHT	UHT

Statistical Analysis
A two factor (treatment by time) ANOVA with repeated measures was used to compare means from the three beverages. The Tukey Post Hoc test was applied to identify significant difference between means. Differences in the 20 km time trial performance, CK, insulin, glucose glucagon, and lactic acid were analyzed. An alpha level of 0.05 was used to indicate statistical significance. The data are presented as means ± SD.

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RESULTS

Cycling Performance

Participants performed three times a 20-km bicycle ride as fast as possible after drinking one liter of beverage and resting for 2 hours (Figure 1, Phase 3).The results showed no significant differences in time taken in performing the 20-km ride when consuming the CHO beverage (1770±210 s), the CHO+Pc (1819±185 s) or the CHO+Pw drink (1803±201).

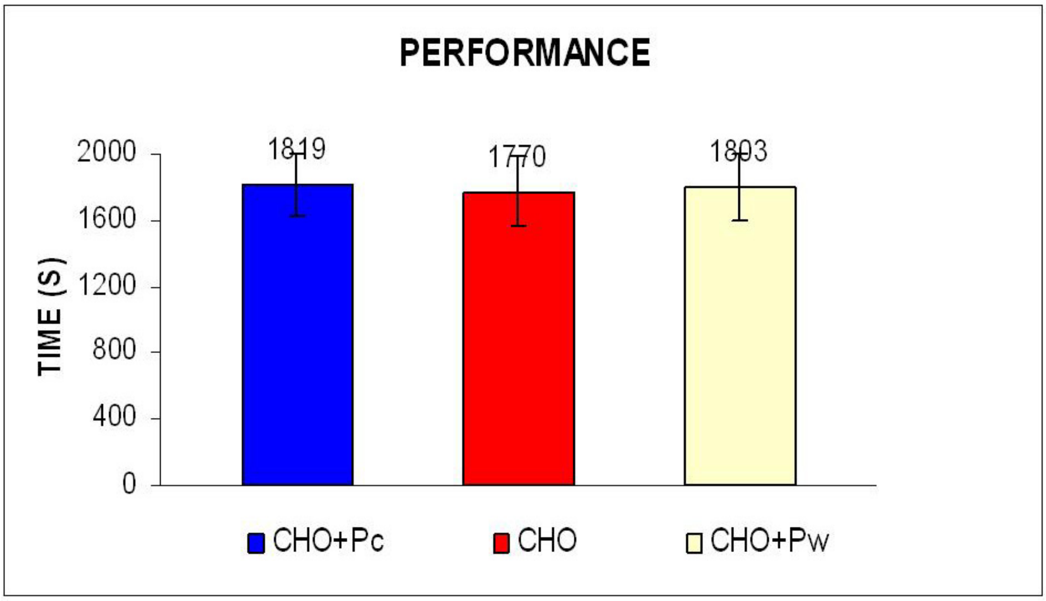

Figure 2. Performance of the 20-km ride after consuming CHO+Pc, CHO+Pw or CHO.

Blood Parameters

The blood parameters of the subjects are listed in Table 2. Post exercise muscle damage was indirectly assessed using plasma CK levels among the three beverage conditions and was not significantly affected by treatment.

Table 2. Blood parameters differences between treatments at the time points indicated for Figure 1.

			TIME										
VARIABLES			**0**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**	**210**
CK Concentration (U/L)	**CHO+Pc**	**M**	124,93	136,27		127,47		124,53					140,87
		SD	44,46	45,79		46,85		48,55					52,12
	CHO+Pw	**M**	136,00	151,87		143,93		142,73					156,27
		SD	76,30	87,04		83,28		77,23					77,85
	CHO	**M**	113,87	127,87		119,40		117,87					132,47
		SD	53,62	53,33		52,66		58,43					63,88
Serum Insulin concentration (mcU/ml)	**CHO+Pc**	**M**	5,42	4,90	19,94	47,43	47,03	30,27	28,40	26,4*	25,18**	17,27**	9,91
		SD	0,87	0,03	9,88	27,72	48,88	20,33	14,90	13,95	8,83	11,00	5,91
	CHO+Pw	**M**	4,95	4,90	8,99**	27,24	27,62	23,62	16,11	13,43	11,87**	8,94**	6,70
		SD	0,18	0,00	6,88	16,88	20,77	25,30	13,01	12,46	10,44	10,10	3,10
	CHO	**M**	5,43	4,93	17,01	39,08	39,88	28,83	21,31	18,54*	11,11**	7,68**	9,11
		SD	1,40	0,10	11,46	19,75	28,33	22,90	19,74	18,86	8,48	6,64	7,14
Plasma Glucose concentration (mg/dl)	**CHO+Pc**	**M**	90,68	100,96	115,60	109,68	92,99*	82,50	78,67	87,74	89,24*	78,42	132,97
		SD	15,01	14,27	18,09	19,49	32,77	18,82	13,10	23,50	16,98	15,32	46,82
	CHO+Pw	**M**	90,78	101,26	111,51	123,54	96,42	80,68	76,10	75,93	78,41	79,62	120,08*
		SD	14,78	15,87	23,63	25,30	35,51	25,32	19,77	20,72	21,86	27,29	28,02
	CHO	**M**	87,33	99,66	115,47	128,65	114,85*	95,55	81,41	75,02	72,09*	65,10	137,80*
		SD	11,77	14,36	24,38	21,61	33,35	33,14	26,17	21,99	20,03	16,32	36,33
Glucagon concentration (pg/ml)	**CHO+Pc**	**M**	61,13	64,73		81,00		80,73					93,93*
		SD	13,36	12,38		10,04		10,98					16,07
	CHO+Pw	**M**	70,33	78,40		93,00		93,33					111,93
		SD	13,69	15,46		19,19		17,31					23,95
	CHO	**M**	70,07	75,79		83,71		82,07					114,53*
		SD	14,71	17,20		15,08		16,96					22,19
Blood lactate concentration (mg/dl)	**CHO+Pc**	**M**	12,82	15,16		12,98		15,42					72,44**
		SD	3,59	4,42		3,17		4,09					20,07
	CHO+Pw	**M**	11,89	14,97		13,55		16,47					89,52
		SD	3,54	8,37		3,77		3,29					29,22
	CHO	**M**	12,22	15,28		13,24		16,48					100,35**
		SD	3,85	8,63		4,62		3,95					31,13

**$P<0.01$ *$P<0.05$

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In the three groups the serum insulin level rose after the beverages were drunk. Serum insulin concentrations were higher during recovery in the CHO+Pc in the final phases of recovery, at 165 and 180 min ($P<0.01$). Blood glucose was significantly elevated at 105 and 165 min during recovery with CHO compared to CHO+Pc and CHO+Pw ($P<0.05$). Glucagon levels increased during the trial, but more with CHO than with the CHO+Pc and CHO+Pw treatment at 210 min ($P<0.05$). Lactic acid levels were stable during the trial, but increased following the 20-km ride and were affected by the beverage.

DISCUSSION AND CONCLUSIONS

The primary objective of this study was to compare the effects of CHO+Pc, CHO+Pw and CHO beverages on time to perform 20 km in a bicycle ride as fast as possible. Time of performance was not different among treatments, a finding that is in agreement with some studies but in contrast to others that compared carbohydrate-protein beverages with CHO.

An often discussed explanation for the performance improvements, in others studies, sometimes seen with carbohydrate–protein beverages is that the added protein may facilitate greater carbohydrate uptake by increasing insulin levels.

Recently published studies have reported significant improvements in endurance when protein is consumed with carbohydrate during prolonged exercise (Saunders et al., 2009). 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+P 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 (Saunders et al., 2009)

Ivy et al (2003) compared the effects of a carbohydrate protein beverage (CHO+P) versus carbohydrate-only (CHO) and placebo beverages. To asses endurance performance, these investigators measured cycling time to exhaustion at 85% VO_{2peak} after 180 min of varied-intensity, sub maximal cycling, which was designed to simulate the variations in intensity typically observed during competitive cycling events. Cyclist rode significantly longer (36%) in the time to exhaustion segment of the CHO+P trial (26.9± 4.5 min) than the CHO trial (19.7 ± 4.6 min), with both sports beverages outperforming a placebo (12.7 ± 3.1 min). Saunders (20) compared endurance performance between CHO+P and CHO beverages in male cyclist during a ride to exhaustion at 75% VO_{2peak}. Cyclist rode 106.3 ± 45.2 min when receiving the CHO+P beverage, compared with 82.3 ± 32.6 with the CHO beverage, a 29% improvement endurance. However, Van Essen and Gibala (2006) examined 80 km time-trial performance between CHO and CHO+P beverages, these investigators observed no significant differences in performance between CHO+P (135 ± 9 min) and CHO (135 ± 9 min) treatments, although both beverages outperformed a placebo beverage (141± 10 min).

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Romano-Ely et al. (2006) and one reported by Millard-Stafford et al. (2005), support that protein typically contributes a small proportion to total energy demands during exercise, utilization of protein added to CHO beverages could spare carbohydrate reserves, allowing athletes to perform for longer periods before exhaustion occurs. In those and in this study there were no significant differences between times to fatigue or performance when the comparison beverages were matched for total calories.

The observation of prolonged time to exhaustion in the studies by Ivy et al. (2003) and Saunders et al. (2004, 2007), suggest a strong potential for ergogenic effects with carbohydrate-protein beverages. However, as demonstrated in this study and by Van Esse and Gibala (2006), improved endurance performance has not been universally observed with carbohydrate-protein ingestion. Thus, questions remain regarding the conditions under which the presence of protein in a sports beverage may improve performance.

Carbohydrate and CHO+P beverages have been compared using time-to-exhaustion (Ivy et al., 2003; Saunders et al., 2007) and long-duration time trials, in Van Essen and Gibala, (2006) and in this study, this could minimize the putative benefits of CHO+P ingestion, because protein oxidation is heightened in late exercise when glycogen levels are depleted (Van Hall et al., 1996). However, Jeukendrup et al. (1996), observed that time-to-exhaustion protocols may evoke relatively high measurement error, reporting a coefficient of variation of >25% over 5 repeated trials and the treatment effects between beverages would need to be quite large to overcome this error variance.

In this study, performance has been measured in a typical race against the clock in 20 km. This kind of time trials exhibit lower error variance between repeated trials (Jeukendrup et al., 1996) and is representative of performance in endurance cycling (St Laurent et al., 2006). However, the relative differences reported between nutritional treatments are typically smaller when using time trials versus time-to-exhaustion protocols, perhaps because time-trial performance is less closely linked to glycogen depletion (Saunders et al., 2007) although in this study the cyclists arrived exhausted to the final.

An often discussed explanation for the performance improvements sometimes seen with carbohydrate-protein beverages is that the added protein may facilitate greater carbohydrate uptake by increasing insulin levels. Ivy et al. (2003) reported elevated insulin levels with CHO+P ingestion compared with water, but these levels were not statistically higher than a CHO trial. In this study, we have obtained greater significant values for serum insulin at 165 and 180 minutes with the CHO+P beverage. This data showed a positive physiological effect although this was not reflected in post recovery exercise performance. Niles et al. (2001) also reported that a carbohydrate–protein beverage was associated with greater postexercise insulin increases than an isocaloric CHO beverage; however, in contrast to the present study, time to fatigue following a glycogen-depleting regime was greater with the carbohydrate–protein beverage.

Fundamental differences in design may explain the discrepancy. The present study was designed to mimic day-to-day training and dietary practices common among competitive cyclist, whereas Niles et al. (2001) appear to have designed a study intended to maximize the treatment effect. Niles et al. (2001) facilitated glycogen depletion with a low-carbohydrate diet (i.e., 35–40% of

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total calories) that began 48 h prior to an exhaustive exercise bout, and the run to exhaustion occurred within 2 h of ingesting the recovery beverage, presumably at a time when insulin levels were estimated to peak (Jeukendrup, 1996). Participants in the present study cycled to exhaustion on two separate days. The first ride was at 70% VO_{2peak}, considerably lower than the intensity used by Niles et al. (2001), and these conditions prior to the second ride were not comparable with the conditions used by these (Niles et al., 2001) or other researchers (Colombani et al., 1999).

Millard-Stafford et al. (2005) compared the effects of a carbohydrate–protein beverage with an isocaloric CHO beverage and reported time to fatigue results similar to those found in the present study, thus supporting the position that a much of the performance difference observed in other research (Colombani et al., 1999) was due to utilization of added protein. An alternate view assumes that when protein calories are substituted for carbohydrates, a resulting attenuation of the insulin response favors greater hepatic glucose output, but the position that the added protein calories are used as an energy substrate is further supported by data from Colombani et al. (1999). These researchers found that amino acid levels, urea, and urinary total nitrogen were elevated with a carbohydrate–protein supplementation during marathon running when compared with a CHO treatment.

In the present study, the CHO+P beverage contained the same number of total calories and 25% fewer carbohydrate calories than the CHO beverage. Under these conditions, performance time during the CHO+P trial was nearly identical to that observed in the CHO trial, thus indicating that when matched for total calories, carbohydrate–protein beverages are equally effective as CHO beverages in providing metabolic benefits during exercise.

The studies discussed suggest that recovery from exercise could be augmented by CHO+P ingestion during exercise. This concept is supported by a number of recent studies that have observed attenuated markers of postexercise muscle damage with CHO+P ingestion. CHO+P has been associated with attenuated postexercise levels of plasma CK (Luden et al., 2007; Romano-Ely 2006) and LDH (Romano-Ely et al., 2006) and subjective ratings of muscle soreness (Luden et al., 2007) compared with CHO ingestion. Furthermore, these benefits have been observed in studies that compared CHO+P and CHO beverages that were matched for carbohydrate content (Luden et al., 2007) or total calories (Romano-Ely et al., 2006).

CHO+P 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+P beverages consumed during and immediately after exercise might be advantageous for performance and muscle recovery in endurance athletes (Saunders et al., 2009)

Saunders (2004) reported significant reductions in postexercise plasma CK levels after CHO+P ingestion, which were accompanied by improvements in subsequent endurance-exercise performance. However, this research have reported no improvements in subsequent performance after CHO+P ingestion. Differences in these findings may be a result of relative differences in muscle damage in these studies, because the postexercise CK response elicited during the non protein trial was much greater in the study reporting a significant improvement in subsequent

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performance (~1300 U/L) (20) than in studies showing no differences in subsequent performance (~300–580 U/L) (Luden et al., 2007). Similarly, Luden et al. (2007) reported that runners completing higher weekly mileages observed the greatest attenuations in postexercise CK with CHO+P, perhaps because of the higher potential for damage associated with increased mileage. These higher mileage athletes also had a greater tendency for improved subsequent performance with the CHO+P treatment.

The data discussed here suggest that CHO+P ingestion may reduce markers of muscle damage in endurance athletes. These alterations may produce important effects on subsequent performance if the attenuations in muscle damage are large enough to be of practical importance for muscle function. Although these studies suggest that CHO+P is potentially important for recovery in endurance athletes, it is difficult to determine whether these benefits were the result of feedings provided during exercise, because the aforementioned studies provided CHO+P postexercise (Luden et al., 2007; Millard et al., 2005) or both during exercise and postexercise (Romano-Ely et al., 2006). However, St Laurent et al. (2006), compared the muscle recovery effects of a CHO+P beverage (78 g CHO/h + 19 g Pro/h) with those of a calorically matched CHO beverage (97 g CHO/h), carbohydrate-matched CHO beverage (78 g CHO/h), and placebo beverage (0 g CHO/h), which were provided during exercise to exhaustion. Although the beverages were provided only during exercise, the CHO+P treatment produced significant reductions in postexercise CK and myoglobin levels compared with all other treatments.

In addition, muscle performance during a leg-extension test 24 h postexercise was significantly higher after the CHO+P trial than all other trials. Collectively, these data suggest that CHO+P ingestion can reduce markers of postexercise damage and potentially improve performance in subsequent exercise. In addition, it appears that these benefits can be elicited by consuming CHO+P beverages during exercise alone.

In agreement to the present study, Millard-Stafford et al. (2005) reported no difference in postexercise CK values between isocalorically matched carbohydrate–protein and CHO treatments.

Within the context of this experimental design, the CHO+P drink showed more explicit physiological effects than the CHO drink, but this was not reflected in post-recovery exercise performance.

There are a number of design factors that should be considered for future research. First, more valid comparisons could be made if three types of beverages (i.e., CHO+P, isocaloric CHO, and isocarbohydrate–CHO) were simultaneously studied. This design would help clarify whether the benefits of carbohydrate–protein beverages are due to the additional calories or somehow attributable to the unique properties of protein.

Secondly, to determinewhether adding protein to carbohydrate drinks attenuates fatigue, these beverages should be studied in conditions where carbohydrate intake and absorption are maximized. In the present study, the subjects tolerated the 9 carbohydrate solution, a concentration that is above the general recommendations. Considering that carbohydrate availability is a primary limiting factor in prolonged exercise, maximal capacity for carbohydrate

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absorption needs to be further addressed. If additional carbohydrate can be reasonably tolerated, a greater amount of energy can be provided during exercise. Furthermore, because protein is absorbed by a separate mechanism in the digestive tract, discovering the upper limit of carbohydrate absorption and then adding protein may prove to be an effective way to maximize performance. Finally, the effect of CHOPA-type beverages on muscle damage should be compared against a control trial that includes exercise to exhaustion without the aid of supplements and against one supplement containing only protein and another supplement containing only antioxidants. A control trial would be of particular value if the protein or antioxidants provided a small but significant benefit that could not be detected when statistically compared with the opposing supplement or CHOPA.

The effects of these nutrients on muscle damage should also be evaluated with direct measures of damage (i.e., muscle biopsies or MRI) as well as biochemical markers specific to oxidative stress. Given the high variability of CK and muscle soreness observed in this and other studies, measures of muscle damage that are more direct and specific may provide better evidence of whether the benefits of CHO+P beverages are attributable to the protein, antioxidants, or a combination of both.

In conclusion, data from this study add to the growing body of evidence indicating that CHO+P beverages consumed during and after exhaustive exercise may attenuate muscle damage, increasing the posterior performance. Also, because time to fatigue was the same between the isocaloric treatments, these data suggest that protein may serve as an important energy substrate when given in combination with CHO beverages during exercise. These results further corroborate data from previous studies showing that performance benefits observed with carbohydrate and protein sports beverages may be due to a carbohydrate-sparing effect related to the oxidation of the additional protein calories.

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