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## Research Article

### **EFFECTS OF A CARBOHYDRATE AND A CARBOHYDRATE AND CASEIN PROTEIN BEVERAGES ON RECOVERY AND PERFORMANCE OF ENDURANCE CYCLING CAPACITY**

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#### **ABSTRACT**

The main aim of this study was to determine if short-term post exercise recovery, cycling performance and muscle damage were altered when consuming a carbohydrate-only beverage (CHO, 7% carbohydrate) versus a carbohydrate and casein protein beverage (CHO+P, 7% carbohydrate and 4% protein). Fifteen male cyclists ( $VO_{2peak} = 63.4 \pm 9.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) performed two trials using a randomly counterbalanced, double-blind design. In each trial one liter of one of the test drinks was consumed in fasting conditions after 1 hour ride at 75%  $VO_{2peak}$  determined by Arts and Kuipers' regression equation. 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 ( $1770 \pm 210 \text{ s}$ ) or the CHO+P drink ( $1819 \pm 185 \text{ s}$ ). Post-exercise creatine kinase (CK) was not significantly different between treatments. However, serum insulin concentrations were higher during recovery when CHO+P beverage was consumed ( $P < 0.05$ ). Glucagon and lactic acid levels increased more on the CHO than on the CHO+P treatment ( $P < 0.05$ ) at the end of the 20 km test. Within the context of this experimental design, 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. 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.

**Key words:** *Ergogenic supplement, casein protein, recovery, cycling performance*

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## INTRODUCTION

Sport beverages containing carbohydrate and protein have gained popularity among athletes, in part because of evidence suggesting that the added protein improves endurance performance and recovery. Some of these beverages are designed as a supplement to be used during exercise, while others, which typically have higher nutrient concentrations, are taken immediately after exercise to facilitate recovery and improve subsequent performance.

When examining previous literature on carbohydrate supplementation during resistance exercise, the results are mixed, with some studies showing no effect on exercise performance (Osterberg et al., 2008; Van Essen and Gibala, 2006; Niles et al., 2001) and others showing an improvement in performance (Saunders et al., 2007; Saunders et al., 2006; Saunders et al., 2004; Ivy et al., 2003). Strenuous exercise performed over consecutive days without sufficient dietary carbohydrate can deplete muscle glycogen and adversely affect exercise performance (Jentjens and Jeukendrup, 2003). Muscle glycogen resynthesis rates are optimized when sufficient carbohydrates (1.0-1.85 g/kg/h) are ingested soon after exercise compared to a delay of 2 h (Saunders et al., 2006). The addition of certain amino acids or proteins can increase muscle glycogen synthesis rates enough to make a difference in performance (Jentjens and Jeukendrup, 2003).

Van Loon (2007) demonstrated that the ingestion of a beverage containing a mixture of wheat protein hydrolysate in combination with a carbohydrate-only (CHO) beverage resulted in very high insulin levels, improving recovery, without causing gastrointestinal discomfort, and further that ingestion of this beverage resulted in increased muscle glycogen synthesis rates compared with the ingestion of CHO only.

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 CHO. 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 also found in these studies. 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 (Lemon, 1998), the protein calories in the CHO+P beverage may account for the improvements in performance (Betts et al., 2007). To understand more accurately how adding protein affects endurance performance, CHO and CHO+P beverages should have similar amounts of total calories.

CHO+P beverages have also been associated with the attenuation of exercise-induced muscle damage. In Saunders et al., (2004), 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 (Saunders et al., 2004; Ivy et al., 2003). One purported mechanism indicates muscle glycogen re-synthesis is enhanced when protein is added to a CHO recovery formula (Saunders et al.,

2004). Insulin responses are elevated with CHO+P ingestion after exercise compared to CHO (Jentjens et al., 2004).

The main aim of this study was to determine if endurance cycling performance, post-exercise recovery and muscle damage were altered when consuming a carbohydrate-only beverage (CHO, 7% carbohydrate) versus a carbohydrate and casein protein beverage (CHO+P, 7% carbohydrate and 4% protein). The casein protein tested was a hydrolyzed casein preparation. Including protein in a carbohydrate solution may accelerate both the rate of glycogen storage and the restoration of exercise capacity following prolonged activity.

## MATERIAL AND 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  $39.0 \pm 9.8$  years, height  $1.76 \pm 0.06$  m and body mass  $74.4 \pm 7.2$  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  $65.5 \pm 10.3$  ml·kg<sup>-1</sup>·min<sup>-1</sup> 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 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 cardio-respiratory 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.

Cardio-respiratory 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*

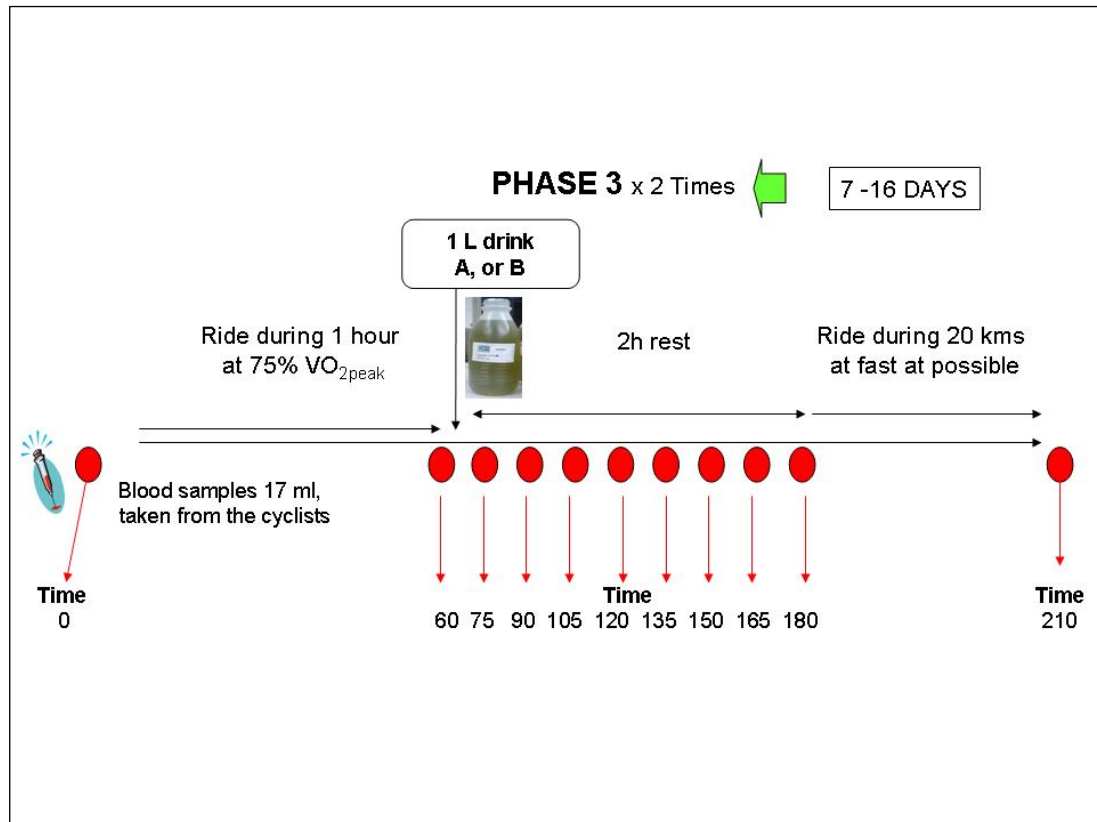
All cyclists arrived in the laboratory between 8 and 8:30 am following a 10 hours' overnight fast and having eaten the same dinner for each day before the test. Each participant's body mass was recorded before a cannula was inserted into an antecubital vein and a 15 ml resting venous blood sample obtained. 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 two main trials separated by at least one 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. The order and timeline of testing for this study is illustrated in [figure 1](#).



*Figure 1. Schematic time course of study protocol*

### ***Beverage formulation***

Two prototypes of isotonic drinks were developed at Puleva Biotech Company in Granada, Spain. 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](#)). The nutrient information and characteristics of these products is provided in [table 1](#).

**Table 1.** Beverage formulation

	Control drink CHO	Casein hydrolysate drink CHO+P
Protein	0g./L. (0%)	40 g./L (4%)
Fat	0g./L (0%)	0g./L (0%)
Carbohydrates	70g./L.(7%)	70g./L (7%)
Vitamins B, E, C, D	25% DRI per L	25% DRI per L
Folic Acid	25% DRI per L	25% DRI per L
Minerals	Isotonic	Isotonic
Taste/Color	Lemon-green	Lemon-green
Treatment	UHT	UHT

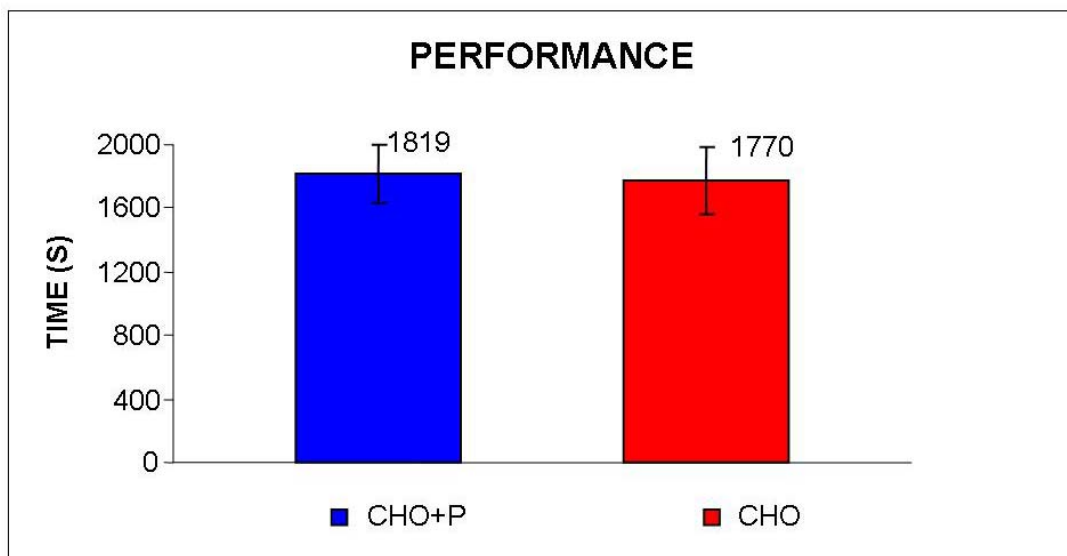
**Statistical Analysis**

A two factor (treatment by time) ANOVA with repeated measures was used to compare means from the two 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.

**RESULTS**

**Cycling Performance**

Participants performed twice 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) or the CHO+P drink (1819±185 s).



**Figure 2.** Performance of the 20-km ride after consuming CHO+P 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 between the two beverage conditions and was not significantly affected by treatment.

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

VARIABLES	TIME												
	0	60	75	90	105	120	135	150	165	180	210		
CK Concentration (U/L)	CHO+P	M	124.93	136.27		127.47		124.53				140.87	
	N = 15	SD	44.46	45.79		46.85		48.55				52.12	
	CHO	M	113.87	127.87		119.40		117.87				132.47	
	N = 15	SD	53.62	53.33		52.66		58.43				63.88	
Serum Insulin concentration (mcU/ml)	CHO+P	M	5.42	4.90	19.94	47.43	47.03	30.27	28.40	26.4*	25.18**	17.27**	9.91
	N = 15	SD	0.87	0.03	9.88	27.72	48.88	20.33	14.90	13.95	8.83	11.00	5.91
	CHO	M	5.43	4.93	17.01	39.08	39.88	28.83	21.31	18.54*	11.11**	7.68**	9.11
	N = 15	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+P	M	90.68	100.96	115.60	109.68	92.99*	82.50	78.67	87.74	89.24*	78.42	132.97
	N = 15	SD	15.01	14.27	18.09	19.49	32.77	18.82	13.10	23.50	16.98	15.32	46.82
	CHO	M	87.33	99.66	115.47	128.65	114.85*	95.55	81.41	75.02	72.09*	65.10	137.80
	N = 15	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+P	M	61.13	64.73		81.00		80.73					93.93*
	N = 15	SD	13.36	12.38		10.04		10.98					16.07
	CHO	M	70.07	75.79		83.71		82.07					114.53*
	N = 15	SD	14.71	17.20		15.08		16.96					22.19
Blood lactate concentration (mg/dl)	CHO+P	M	12.82	15.16		12.98		15.42					72.44**
	N = 15	SD	3.59	4.42		3.17		4.09					20.07
	CHO	M	12.22	15.28		13.24		16.48					100.35**
	N = 15	SD	3.85	8.63		4.62		3.95					31.13

\*\*P<0.01 \*P<0.05

In both groups the serum insulin level rose after the beverages were drunk. Serum insulin concentrations were higher during recovery in the CHO+P 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+P (P<0.05). Glucagon levels increased during the trial, but more with CHO than with the CHO+P 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. The increase was higher with CHO than with CHO+P (P<0.01).

## DISCUSSION AND CONCLUSION

### Performance

One of the objectives of this study was to compare the effects of CHO+P and CHO beverages on the time required to perform a 20-km bicycle ride at the rider's maximum speed for that distance. The time taken was not different between treatments, a finding that is in agreement with some studies (Osterberg et al., 2008; Van Essen and Gibala, 2006; Niles et al., 2001) but in contrast to others that compared carbohydrate-protein beverages with CHO (Saunders et al., 2007; Saunders et al., 2006; Saunders et al., 2004; Ivy et al., 2003).

Carbohydrate and CHO+P beverages have been compared using time-to-exhaustion (Saunders et al., 2007; Saunders et al., 2004; Ivy et al., 2003; Roman et al., 2006) and long-duration time trials (Osterberg et al., 2008; Van Essen and Gibala, 2006). In the time-trial studies, 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 20-km race against the clock. This kind of trial gives lower error variance between repeated trials (Jeukendrup and Jentjens, 2000) and is representative of performance in endurance cycling (St. Laurent et al., 2006). In the other hand, 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, 2007) although in this study the cyclists arrived exhausted at the end of the test.

### *Beverage formulation*

Previous studies reporting performance improvements with CHO+P ingestion matched treatment beverages for carbohydrate content, resulting in higher total caloric content in CHO+P beverages (Saunders et al., 2006; Saunders et al., 2004; Ivy et al., 2003; Williams et al., 2003; Betts et al., 2007). This suggests that adding protein to a typical CHO sports drink can improve endurance performance but it is difficult to determine whether differences in performance with CHO+P beverages were specifically protein mediated or calorically mediated.

The lack of performance differences between beverages in this study suggests that the additional availability of calories in previous studies may be a factor in the improved performance with CHO+P beverages. It seems unlikely, however, that such a small difference in calories could explain the comparatively large differences in performance that have been reported.

Although there is growing evidence that CHO+P ingestion may improve endurance performance, protein provides a relatively small contribution to energy production during endurance exercise, perhaps 5-10% of total energy demands compared with carbohydrates and fats (Bloomer and Goldfarb, 2003) but this proportion may increase when exercise is performed in a glycogen-depleted state, as occurs during the late stages of endurance exercise



(Van Hall et al., 1996). Koopman et al., (2004) reported that CHO+P ingestion during prolonged exercise resulted in a 2-fold increase in protein oxidation compared with CHO. Increased protein oxidation with CHO+P ingestion could alter substrate utilization and potentially spare blood glucose and/or muscle glycogen late in exercise (Saunders et al., 2007) and this explains the positive ergogenic effect of the protein including in the CHO+P beverage of this study.

### ***Blood parameters***

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. The greater significant values for serum insulin at 165 and 180 minutes were obtained with the CHO+P beverage. This datum showed a positive physiological effect of the protein because the lower quantity of carbohydrate in the CHO+P beverage. Niles et al., (2001) also reported that a carbohydrate-protein beverage was associated with greater post-exercise insulin increases than an isocaloric CHO beverage and 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 cyclists, whereas Niles et al., (2001) appear to have designed a study intended to maximize the treatment effect. They facilitated glycogen depletion with a low-carbohydrate diet (i.e., 35–40% of 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 and Jentjens, 2000).

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 much of the performance difference observed in others research (Jeukendrup and Jentjens, 2000; Colombani et al., 1999) was due to utilization of added protein.

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 studies that have observed attenuated markers of post-exercise muscle damage with CHO+P ingestion. CHO+P has been associated with attenuated post-exercise levels of plasma CK (Saunders et al., 2004; Romano-Ely et al., 2006; Luden et al., 2007; Laurent et al., 2006). Furthermore, these benefits have been observed in studies that compared CHO+P and CHO beverages that were matched for carbohydrate content (Saunders et al., 2004; Millard-Stafford et al., 2005; Luden et al., 2007) or total calories (Romano-Ely et al., 2006).

Luden et al. (2007) reported that runners completing higher weekly mileages observed the greatest attenuations in post-exercise 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 to improve subsequent performance with the CHO+P treatment.

The data discussed here suggest that CHO+P ingestion may reduce muscle damage markers in endurance athletes. These reductions may produce important effects on subsequent performance if the attenuations in muscle damage are large enough to be of practical importance for muscle function. In agreement with the present study, Millard-Stafford et al. (2005) reported no difference in post-exercise CK values between isocalorically matched carbohydrate-protein and CHO treatments.

Blood lactate concentrations for the 2 treatments were similar before exercise. In contrast with others studies (Baty et al. 2007 and Millard-Stafford et al., 2005) after exercise, a significant group x time interaction was found at the end of the test what indicates a positive effect of the CHO+P beverage in the recovery and muscle.

Within the context of this experimental design, the CHO+P drink showed more explicit physiological effects than the CHO drink, serum insulin concentrations were higher during recovery when the CHO+P beverage was consumed ( $P<0.05$ ). Glucagon and lactic acid levels increased more on the CHO than on the CHO+P treatment ( $P<0.05$ ) at the end of the 20 km test, so that the CHO+P drink can be recommended for improving recovery. Although it was not reflected in the post-recovery exercise performance after a 20 km ride, possibly in longer and more demanding tests, such as biathlon, triathlon, heptathlon and decathlon, the physiological factors would have had a significant effect on performance, as they would aid recovery during the event or competition. Moreover this study involved a comparatively short period and the results may have been more explicit if the trial had taken place over several weeks.

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