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Carbohydrate-Protein Coingestion Enhances Cycling Performance with Minimal Recovery Time between Bouts of Exhaustive Intermittent Exercise

Original Research

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Abstract

Introduction: The addition of protein to a carbohydrate solution has been shown to effectively stimulate glycogen synthesis in an acute setting and enhance exercise performance in a subsequent bout of exhaustive exercise. This study examined the effects of carbohydrate-protein (CHO-P), carbohydrate (CHO), and placebo (PLA) within a 2-hour recovery period on subsequent high-intensity exercise performance.
Methods: This was a randomized, single-blind between-subject design. Participants (n = 25) were assigned to consume one of three beverages during a 2-hour recovery period: PLA, CHO (1.2 g/kg bm), or CHO-P (0.8 g/kg bm CHO + 0.4 g/kg bm PRO). During Visit#1, participants completed graded exercise testing (VO_2peak; cycle ergometer). Familiarization (Visit#2) consisted of 5 x 4 min intervals at 70-80% of peak power output [PPO, watts] with 2 min of active recovery at 50W, followed by time to exhaustion [TTE] at 90% PPO. The same high-intensity interval protocol with TTE was conducted pre-and post-beverage consumption on Visit#3.
Results: The ANCOVA indicated a significant difference among the group means for the posttest TTE ($F_{2,21}$=8.248, p=.002, η^2=.440) and RER ($F_{2,21}$=6.811, p=.005, η^2=.393) values after adjusting for the pretest differences.
Conclusions: Carbohydrate-protein co-ingestion was effective in promoting an increase in TTE performance with limited time to recover.

Key Words: glycogen, post-exercise, fatigue

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Introduction

Glucose is the key energy substrate used by the body to fuel exercise at an intensity that is equal to or greater than approximately 70% of an individual's aerobic capacity [1,2]. Glucose availability is therefore paramount to sustain high-intensity endurance (e.g., triathlon, cycling, running) performance of prolonged duration [3]. Team sports such as soccer, hockey and basketball are intermittent in nature, comprised of short bursts of explosive type exercise (sprinting, passing, and shooting the ball) over the course of 60-90 min which also rely on a sufficient amount of muscle and liver glycogen and plasma glucose to fuel the intensity and duration of this activity [4]. Glycogen can provide enough glucose to sustain high-intensity endurance exercise for approximately two hours [5]. Williams and Rollo (2015) suggest a 50-60% reduction of muscle glycogen stores following a competitive soccer match [4].

Recently, Namma-Motonaga and colleagues (2022) reported that a carbohydrate intake of 5 g/kg of body mass (bm) was not sufficient to restore muscle glycogen to pre-exercise values within a 24-hour period, as compared to consuming 7 g/kg bm and 10 g/kg bm [6]. Of interest, muscle glycogen was significantly less during the 4-12 h recovery period when collegiate endurance athletes consumed carbohydrate in the amount of 5 g/kg bm as compared to 7 g/kg bm and 10 g/kg bm [6]. These results have important implications for athletes who train more than once within a 24-h period or compete in multiple heats and matches within a day, as often there is little time (~hours) to consume adequate carbohydrate-containing foods to the degree necessary that glycogen and exercise capacity is sufficiently restored.

Many studies support an immediate post-exercise intake of 1.0-1.2 g/kg bm of carbohydrate to stimulate muscle glycogen resynthesis during an acute recovery phase of ~4-6 hours [7,8]. The addition of protein (0.2 – 0.4 g/kg bm) to a carbohydrate (0.8 g/kg bm) solution has also been shown to effectively stimulate glycogen resynthesis in an acute setting (≤ 6 h) and even enhance exercise performance in a subsequent bout of exhaustive exercise [7,9,10]. Few studies [11,12] have examined the effect of carbohydrate intake on recovery within 2 hours of glycogen-depleting exercise. The first purpose of the study is to replicate a recent investigation that examined carbohydrate intake in the short-term [12]. The second objective is to examine the effects of carbohydrate-protein co-ingestion and carbohydrate and placebo within a 2-hour recovery period on subsequent high-intensity exercise performance. Adding whey protein to a commercially available sports beverage could substantially reduce the amount of carbohydrate that an athlete needs to consume while sufficiently restoring muscle glycogen and exercise capacity within a 2-hour window.

Scientific Methods

Protocol

Three testing visits were separated by a minimum of 48 hours and completed within two weeks. The timeline for each testing visit is outlined in Figure 1. Participants were asked to a) maintain a consistent diet 24 hours prior to the familiarization and experimental trials and b) keep dietary intake consistent on both the familiarization and experimental testing days. Participants provided a dietary recall for the 24 hours preceding the final day of testing. After assessing height, body mass, and body composition, participants performed a graded exercise test on a cycle ergometer to determine maximal aerobic capacity and peak power output. During the familiarization trial, participants performed 5 x 4 min aerobic intervals (INT) at 70-80% of peak power output (PPO) immediately followed by a time to exhaustion trial (TTE) at 90% PPO[12]. A randomized, single-blind between-subject design was employed where participants were assigned to consume one of three potential recovery beverages during a 2-hour recovery period: placebo, carbohydrate-only (1.2 g/kg bm), or carbohydrate-protein co-ingestion (0.8 g/kg bm carbohydrate + 0.4 g/kg bm protein). All recovery beverages were standardized to one liter (32 oz.) total fluid volume regardless of treatment group. Consumption of the recovery beverage was ad libitum, and participants were asked to consume the beverage in its entirety within 90 min of completing the aerobic intervals and TTE. Participants repeated the INT and TTE assessment immediately following the 2-hour recovery period (Figure 1).

Participants

Twenty-five recreationally active [13] men (n = 20) and women (n = 5) between the ages of 18 and 35 years old were recruited to participate in this research investigation (Table 1). Using the study by McCarthy and Spriet (2020), the calculation of sample size was estimated from the reported effect size (Cohen d_z = 0.82) from decrease in time to exhaustion difference between PL vs CHO groups using power analysis software (G*Power 3.1.9.2, Dusseldorf, Germany)[12] for a treatment X time repeated measures ANOVA design. Participants completed at least 75-300 min moderate-to-vigorous intensity activity through numerous modes of exercise, such as high-intensity functional training (e.g., CrossFit), endurance (triathlon, cycling, running), soccer, wrestling, and weightlifting[13]. After participants signed the informed consent, they completed the Physical Activity Readiness Questionnaire (PARQ+) and a medical and activity history questionnaire (MAHQ). This study was approved by the university's Institutional Review Board. Participants were excluded if they had any recent musculoskeletal injuries or surgeries, metabolic disease, or any chronic illness that required continuous medical care.

Anthropometrics and body composition

Height was assessed using a stadiometer (Health-o-meter Professional Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Body composition was assessed using bio-electrical impedance analysis (InBody 770, Biospace Co, Ltd. Seoul, Korea) with a built-in scale to measure body mass for 52% of participants (13/25). Due to technological complications, bioelectrical impedance spectroscopy analysis (SOZO, Carlsbad, CA, USA) was used to assess body composition in the remaining study participants (12/25), and body mass was measured separately with a scale (Health-

o-meter Professional Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA). Participants were tested wearing minimal clothing and barefoot without socks.

Nutrient intake and dietary recall

The ASA24® is a validated, automated self-administered 24-hour dietary assessment tool developed by the National Cancer Institute (Bethesda, Maryland)[14]. Participants were instructed to record their dietary intake during the 2-hour recovery period on the day of the experimental trial. The ASA24® dietary recall assessment was utilized to estimate mean total energy intake in kilocalories (Kcal) for the 24 hours prior to the final testing session. During the recall, participants received automated prompts that would assist them in quantifying portion sizes, the actual volume of food consumed at each meal or snack, and commonly forgotten items (condiments, supplements, sugar-sweetened beverages)[15]. A total of 21 participants complied with the dietary recall instructions and were included in the data analysis.

Graded exercise test and indirect calorimetry

Participants performed an incremental test to volitional exhaustion on an electromagnetically braked cycle ergometer (Corival, Lode B.V., Groningen, Netherlands). Gas exchange data were collected with a metabolic gas analyzer (Trueone 2400, Parvo Medics, Utah, USA) and used to determine maximal oxygen uptake (VO_2peak). The test consisted of 2-min stages and began at an initial workload of 50W. Participants then performed two 2-min stages at 100W and 150W, respectively, followed by an increase of 25W every 2 minutes until the participant could no longer maintain 60rpm[12]. VO_2peak was determined as the highest peak value achieved during the test if it coincided with at least two of the following three parameters: heart rate (HR) within 10% of age-predicted maximal HR; respiratory exchange ratio (RER) of 1.15 or higher; a plateau in oxygen consumption despite an increase of exercise intensity. Peak power output (PPO) was determined as the highest power output (watts) achieved during the last completed 2-min stage.

High-Intensity aerobic interval and time to exhaustion protocol

Participants completed a 6-min warmup that began with an initial workload of 50W for 3 min, 100W for 2 min, and 75W for 1 min[12]. Participants then performed 5 x 4-min high-intensity aerobic intervals (INT) at 70-80% of individual PPO. During the familiarization trial, each participant attempted to complete all five intervals at 80% of individual PPO followed by the time to exhaustion (TTE) trial at 90% of PPO [12]. If a participant was unable to complete the intervals at 80% of individual PPO or was able to complete the intervals but not the TTE trial, then the power was decreased by 5% (to a minimum of 70% PPO) until the participant could perform the entire series of aerobic intervals and TTE protocol. Two minutes of low intensity cycling at 50W separated each aerobic interval. During the experimental protocol, participants completed the 6-min warmup followed by 5 x 4-min high-intensity aerobic intervals (INT) at 70-80% of individual PPO. Immediately following the fifth interval, participants cycled at a work rate (watts) corresponding to 90% PPO until volitional exhaustion or when rpm fell below 60 for 10 seconds[12]. Two minutes of low intensity cycling at 50W separated each aerobic interval. Following completion of the INT and TTE trial, participants performed a self-paced cool down with no resistance[12]. Revolutions per minute were the only performance measure visible to participants; verbal encouragement was provided throughout the aerobic intervals and time to exhaustion. Rating of perceived exertion (RPE) was recorded using the Borg 10 scale during the last 30 seconds of each aerobic and active rest interval. Gas exchange data were collected with a metabolic analyzer (Trueone 2400, Parvo Medics, Utah, USA) throughout the experimental protocol for both bouts of INT and TTE testing.

Supplementation

Participants were randomly assigned via a web-based random assignment generator (Research Randomizer; www.randomizer.org) to receive one of three potential recovery beverages during a 2-hour recovery period[16]. The three treatment conditions were as follows: Placebo (Gatorade® Zero Thirst Quencher Orange, Chicago, IL, USA); Carbohydrate-only (1.2 g/kg bm; Gatorade® Thirst Quencher Orange, Chicago, IL, USA; CHO); Carbohydrate-Protein co-ingestion (0.8 g/kg bm CHO + 0.4 g/kg bm protein; Gatorade® Thirst Quencher Orange, Chicago, IL, USA, biPro Elite French vanilla, Agropur Inc., Appleton, WI, USA; CHO-P). All recovery beverages were standardized to approximately one liter (32 oz.) total fluid volume regardless of treatment group. One 32-ounce bottle of Gatorade® Thirst Quencher contains 58 grams of carbohydrate. When calculating energy needs for the CHO group (i.e., 1.2 g/kg bm), the amount of carbohydrate (i.e., grams) required often exceeded the amount of carbohydrate contained in one 32-ounce bottle of Gatorade®. On a case-by-case basis, Gatorade® (orange) powder was weighed in grams via a digital food scale and added to the existing liter of fluid to reach the total amount of carbohydrate needed for each participant in the CHO group. In order to blind the participants to the treatment, all three solutions contained orange-flavored

Gatorade® and were both mixed and provided in a dark red Nalgene (Nalge Nunc International Corporation, Rochester, NY, USA) water bottle.

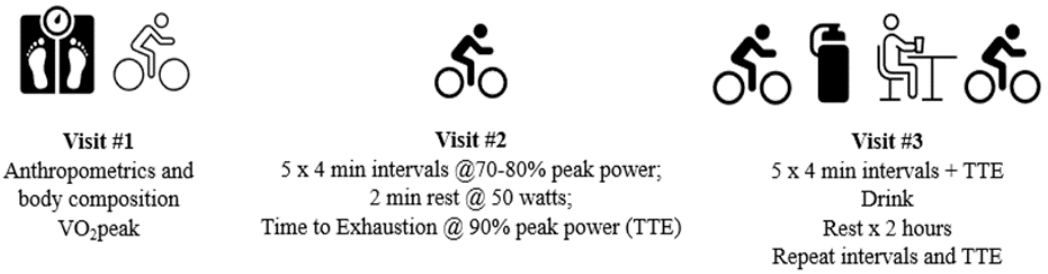

Fig. 1 Experimental design of the study; anthropometrics and body composition, graded exercise test, high-intensity interval and time to exhaustion testing, recovery beverage, 2-hour recovery period

Statistical Analysis

All analyses were conducted via the Statistical Package for Social Science (SPSS) software for Windows version 28 (SPSS Inc., Chicago, IL). All data were assessed for normality and homogeneity of variance. While we utilized random assignment for each treatment group (PLA, CHO, CHO-PRO), significant ($F_{2,22}$= 4.548, p= .022) difference in baseline values for TTE still occurred. The data, therefore, was statistically analyzed using separate one-way analysis of covariance (ANCOVA) for time to exhaustion and average respiratory exchange ratio (RER) for all five high-intensity aerobic intervals. The pre-test and the posttest values were used as a covariate and dependent variable, respectively. Differences in total energy intake and macronutrients were assessed with a one-way ANOVA. When appropriate, Fisher's LSD pairwise comparisons were used to examine the differences among the groups. For effect size, the partial eta square (η^2) statistic was calculated, and according to Green et al. [17], η^2 of 0.01, 0.06, and 0.14 represents small, medium, and large effect sizes, respectively. Alpha level was set a priori at $p < 0.05$.

Results

Table 1 contains the mean and standard deviation values for total energy intake (kilocalories), protein, fat, and carbohydrate among the three groups (PLA, CHO, CHO-P) of participants. Due to issues with compliance and technical difficulties related to the dietary intake software, total energy intake, protein, fat, and carbohydrate were reported for 7 of 8 participants in the PLA group; 8 of 9 participants in the CHO group; and 6 of 8 participants in the CHO-P group. Assumptions were met for normality and there was homogeneity of variance for total calories (p = .685), protein (p = .637), fat (p = .588), and carbohydrate (p = .552) as assessed by Levene's test for equality of variances. A one-way ANOVA revealed no significant differences (p > .05) between total energy intake, protein, fat, or carbohydrate.

Table 2 shows the group means (±SD) for the pretest and posttest TTE values. Figure 2 shows the group means (±SEM) for the posttest TTE (sec) values adjusted for the initial differences in the pretest TTE. Assumptions were met for normality and there was homogeneity of variance for the TTE values, as assessed by Levene's test for equality of variances (p = .140). The posttest TTE means were adjusted during the ANCOVA procedure based on the pretest TTE differences for the PLA, CHO, and CHO-P. The ANCOVA indicated a significant difference ($F_{2,21}$= 8.248, p =.002, η^2=.440) among the group means for the posttest TTE values after adjusting for the pretest differences. The strength of the association (i.e., effect size, η^2) was large and indicated that the treatment groups (PLA, CHO, and CHO-P) accounted for 44% of the variance of the posttest TTE values, holding constant the pretest TTE scores. Fisher's LSD pairwise comparisons indicated that the posttest TTE was greater for the CHO-P group than for the PLA group (p = .002) and the posttest TTE was greater for the CHO-P group than for the CHO group (p = .003). There were no differences in posttest TTE between the PLA and CHO group (p = .404).

Table 1. Participant demographics. Values are expressed as mean ± standard deviation. Total energy intake and macronutrient values are reported for 21 of 25 participants.

	Total (n = 25)	**Males (n = 20)**	**Females (n = 5)**
Age (years)	22.0 ± 2.4	22.0 ± 2.6	22.2 ± 1.8
VO_2max ($mL•kg^{-1}•min^{-1}$)	43.8 ± 6.9	45.0 ± 6.7	38.9 ± 5.9
Height (centimeters)	172.6 ± 2.4	174.2 ± 6.8	166.2 ± 5.9
Weight (kilograms)	73.2 ± 10.1	74.4 ± 10.6	68.4 ± 6.4
Body fat (%)	20.5 ± 10.1	14.4 ± 4.8	19.8 ± 5.5
	Total (n = 21)	**Males (n = 18)**	**Females (n = 3)**
Weight (kilograms)	73.9 ± 10.6	74.4 ± 11.2	70.8 ± 5.7
Total energy intake (kcal)	2776.1 ± 783.1	2795.2 ± 799.7	2661.7 ± 821.2
Total energy intake (kcal) (g/kg)	-	38.0 ± 10.8	37.9 ± 13.0
Protein (g)	149.6 ± 46.1	152.2 ± 47.0	134.3 ± 45.1
Protein (g/kg)	-	2.1 ± 0.61	1.89 ± 0.61
Fat (g)	104.7 ± 39.1	104.2 ± 40.4	107.6 ± 37.5
Fat (g/kg)	-	1.4 ± 0.53	1.54 ± 0.61
Carbohydrate (g)	316.3 ± 103.5	319.4 ± 106.4	297.5 ± 101.4
Carbohydrate (g/kg)	-	4.4 ± 1.5	4.3 ± 1.7

Table 2. Mean and SD values for TTE (sec) and RER (VCO_2/VO_2) at pretesting and posttesting for each group.

TTE		**PLA (n = 8)**	**CHO (n = 9)**	**CHO-P (n = 8)**
Pretest	Mean	229.13	134.78	131.38
	SD	78.59	92.48	37.02
Posttest	Mean	158.38	116.44	202.13
	SD	63.15	69.03	92.93
RER				
Pretest	Mean	0.92	0.95	0.91
	SD	0.06	0.05	0.05
Posttest	Mean	0.88	0.94	0.89
	SD	0.06	0.04	0.04

Figure 2. Group mean values (±SEM) for posttest TTE scores adjusted for the initial differences in pretest TTE (covariate). The adjusted pretest value was 163.9 sec. *Posttest TTE was greater for the CHO-P than for the PLA group (p =.002), and greater than the CHO group (p =.003).

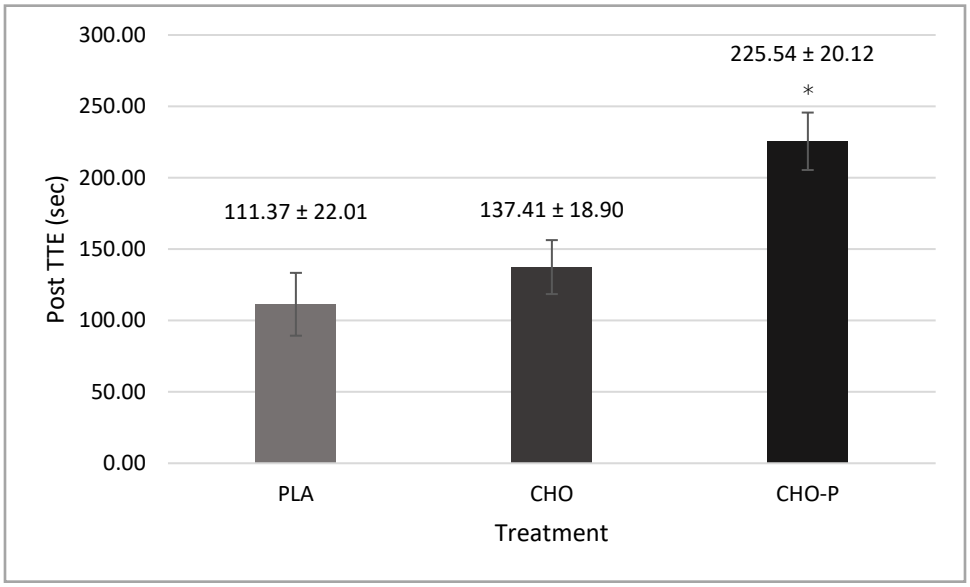

Table 2 shows the group means (±SD) for the pretest and posttest RER values. Assumptions were met for normality and there was homogeneity of variance for the RER values, as assessed by Levene's test for equality of variances (p = .557). The posttest RER means were adjusted during the ANCOVA procedure based on the pretest RER differences for the PLA, CHO, and CHO-P. The adjusted pretest RER value was .926. The adjusted posttest values were PLA = .885, CHO = .920, and CHO-P = .910. The ANCOVA indicated a significant difference ($F_{2,21}$= 6.811, p= .005, η^2 =.393) among the group means for the posttest RER values after adjusting for the pretest differences. The large effect size indicated that the treatment groups (PLA, CHO, and CHO-P) accounted for 39% of the variance of the posttest RER values, holding constant the pretest RER values. Fisher's LSD pairwise comparisons indicated that the posttest RER was greater for the CHO group than for the PLA group (p =.002) and the posttest RER was greater for the CHO-P group than for the PLA group (p =.020). There were no differences between the CHO and CHO-P groups (p =.340).

Due to COVID-19 related disruptions, blinding results were only available for 19 of 25 total participants with 42.1% (n=8) correctly identifying which treatment beverage they consumed.

Discussion

The purpose of this study was to determine the effectiveness of three different beverages (carbohydrate, carbohydrate-protein coingestion, placebo) on recovery from consecutive bouts of exhaustive exercise within a 2-hour period. It was hypothesized that both carbohydrate and carbohydrate-protein coingestion would attenuate performance decrements to a similar degree during the second bout of exercise. The results indicate that carbohydrate-protein co-ingestion (0.8 g/kg bm CHO + 0.4 g/kg bm PRO) was more effective than both carbohydrates alone (1.2 g/kg bm CHO) and placebo for promoting an increase in time to exhaustion (TTE) performance following a 2-hour recovery period. The other main finding of this study was that the respiratory exchange ratio (RER) was significantly lower for the PLA condition as compared to the CHO and CHO-P treatment groups.

In the current study, the absence of a performance benefit following CHO ingestion in the amount of 1.2 g/kg bm is similar to a recently published investigation[12]. McCarthy and Spriet (2020) examined the effect of CHO (1.2 g/kg bm) versus PLA on endurance capacity when either drink was consumed during the first 15 minutes of a 2-hour recovery period between bouts of high-intensity aerobic intervals. Results of the McCarthy and Spriet (2020) publication indicated a decrement to performance (i.e., cycling to fatigue at ~90% VO_2peak) for both CHO and PLA conditions. However, CHO consumption during the 2-hour recovery period attenuated the performance decrement that occurred

during the subsequent endurance trial. Endurance trial performance in the second bout of exercise declined by approximately 50% for PLA but only about 27% for the CHO group [12]. Similarly, in the current study, TTE performance in the second bout of exercise declined by approximately 31% for the PLA and 14% for the CHO group.

The heterogeneous sample and individual differences may partly explain the lack of significant difference in TTE performance between the CHO and PLA groups in the present study. Five of nine participants (56%) in the CHO group performed better (19 ± 16.23 sec) as a result of consuming 1.2 g/kg bm CHO within 90 min of a 2-hour rest period, whereas a decrement in TTE performance of -62.5 ± 49.6 sec occurred for the other four participants in that group. This is in comparison to the CHO-P group where seven of eight (~88%) participants had an average increase of 84 ± 63.6 sec in TTE performance, with an 18-sec decrement in performance for one participant. Water and electrolytes were not sufficient for promoting recovery in the PLA group as the average decrement to TTE performance was 70.8 ± 50.5 sec for 100% of the participants in that condition. Taken together results from the present study and others [7,12,18] indicate that adequate caloric intake in the short-term is essential for promoting recovery from exhaustive exercise.

According to McKay et al. (2022) and their participant classification framework, the participants in our study were identified as "Tier 1" recreationally active individuals who perform at least 150-300 min moderate-intensity or 75-150 min of vigorous intensity activity per week[13]. It is possible that within a heterogenous sample such as ours that TTE performance may not be as reliable as in a group of trained athletes (i.e., Tier 2 or above) [9,13,19]. Four of the eight participants within the CHO-P condition and one participant in the CHO group were routinely training at the club or collegiate level (i.e., soccer, cycling, wrestling), twelve of the remaining study participants within the CHO and CHO-P conditions were either former high school athletes now training non-systematically (i.e., weightlifting, casual cycling, running) or primarily weightlifting, marathon running, or participating in organized activity such as CrossFit. Therefore, while meeting the classification of (Tier 1) recreationally active, the level of training and fitness between participants may be a confounding variable and thus may not provide an accurate reflection of TTE performance (i.e., endurance capacity), particularly using a protocol completed on a cycle ergometer [7,19].

The primary finding in this study of CHO-P co-ingestion and enhanced performance following short-term recovery contrasts to other publications showing no additional benefit of carbohydrate-protein co-ingestion when recovery beverages are isocaloric (matched for energy content). A recent meta-analysis examining CHO-P co-ingestion versus CHO only on muscle glycogen synthesis reported that when isocaloric beverages (i.e., matched for energy content) are consumed during the recovery period there is no significantly greater effect on glycogen synthesis [20]. Carbohydrate-protein co-ingestion, compared to a carbohydrate-only beverage, was found to enhance glycogen synthesis in the post-exercise recovery period only when the caloric content was increased beyond that of CHO alone by the addition of protein to the recovery beverage [20].

Results vary in terms of the amount of muscle glycogen synthesized following isocaloric carbohydrate-protein and carbohydrate-only consumption within the first 90-120 min of an extended recovery period, with one study showing an increase in glycogen synthesis following high-intensity exercise but no change in performance in a subsequent cycling time trial [21]. Alghannam et al. (2016) demonstrated an equivalent increase in glycogen resynthesis with isocaloric carbohydrate-protein and carbohydrate-only beverages but there was no significant difference in performance when participating in a second run to exhaustion [22]. Rustad et al. (2016) reported an increase in cycling time to exhaustion in favor of carbohydrate-protein co-ingestion (63.5 ± 4.4 min) as compared to an isocaloric carbohydrate (49.8 ± 5.4 min) beverage. Young (24 ± 0.4 years) endurance-trained cyclists in that study [9] consumed each of three recovery beverages in a crossover fashion during the first two hours of an 18-hour recovery period. Consumption of the placebo resulted in the shortest time to exhaustion (42.8 ± 5.1 min), which was significantly less than carbohydrate ($p<0.05$), and CHO-P ingestion resulted in a longer time to exhaustion for each participant under that condition, as compared to CHO and PLA [9]. The results of Rustad et al. (2016) are similar to the present study where consumption of the CHO-P beverage resulted in an increase in performance for seven of eight participants.

More recently studies have shown a performance improvement following high-intensity exercise with CHO-P co-ingestion and have suggested a positive nitrogen balance and/or enhanced muscle protein synthesis as possible mechanisms underlying the increase in performance. In support, Dahl et al. (2020) provided a group of young endurance-trained (cycling, triathlon) male athletes with isoenergetic (i.e., energy matched) CHO-P and carbohydrate-only drinks following a bout of glycogen depleting exercise. The recovery beverages were consumed in the first 90 minutes of a 5- hour recovery period followed by a second bout of cycling to exhaustion [10]. Participants were able to

cycle on average 8.4 ± 1.8 min longer after consuming the CHO-P beverage (54.6 ± 11.0 min), as compared to CHO only (46.1 ± 9.8 min), with no significant difference in the rate of glycogen synthesis between the two conditions [10]. Positive nitrogen balance as a result of CHO-P co-ingestion was a primary outcome for both the Rustad et al. (2016) and Dahl et al. (2020) investigations. In contrast, both studies reported a negative nitrogen balance for the CHO group [9,10]. Levenhagen et al. (2001) reported a positive leg muscle protein balance when CHO-P supplementation was provided immediately following a bout of moderate-intensity exercise, as compared to three hours later, which resulted in a net negative leg muscle protein balance [23]. While it has been proposed the enhanced performance was related to a positive protein/nitrogen balance, the exact mechanism of the ergogenic effect is unknown.

In a different study, TTE performance was enhanced by 23% following consumption of 16 ounces of fat-free chocolate milk and a 3-hour recovery period, as compared to an isocaloric CHO beverage (250 ± 43 sec vs 203 ± 31 s, respectively) [24]. Lunn et al. (2012) reported mixed muscle protein fractional synthetic rate was significantly enhanced (i.e., 38% greater) for chocolate milk as compared to the carbohydrate-only drink, with no difference in muscle glycogen content at 30- or 60-min post beverage consumption [24]. Thus, the nutritional benefit of post-exercise CHO-P intake in the short term recovery period may extend beyond the provision of substrate for muscle glycogen synthesis [7,24].

The presence of protein in a recovery beverage promotes a rise in circulating amino acids which fosters an anabolic environment and promotes skeletal muscle protein synthesis while suppressing breakdown post exhaustive exercise [7,9,10,24–26]. In support, Breen and colleagues (2011) reported an approximate 35% increase in myofibrillar protein synthesis following CHO-P co-ingestion (50.8 g CHO and 20.4 g PRO) as compared to CHO alone (50.4 g) when consumed immediately and 30-min post high-intensity cycling in an equally divided dose [26]. Adding protein to a carbohydrate-based beverage immediately after high-intensity exercise may enhance muscle protein synthesis and thus promote the repair and remodeling of acutely damaged proteins to recover muscle functionality [26,27].

Results from the present study demonstrate a significantly lower RER for the placebo condition compared to both the CHO and CHO-P groups. In support, Wallis et al. (2006) reported a significantly lower RER when participants consumed water during 120 min of moderate-to-high intensity exercise (~67% VO_2max), as compared to CHO in the amount of 90 g/h [28]. In a separate study, RER significantly decreased during 145 min of high-intensity cycling (70% VO_2max) in a group of trained endurance athletes who began exercise with low muscle glycogen but received an infusion of glucose to maintain euglycemia [29]. Additionally, CHO oxidation was significantly lower and fat oxidation higher throughout the 145 min of cycling for the low glycogen euglycemic group as compared to athletes who started exercise with sufficient muscle glycogen (euglycemic during exercise) [29]. Although not measured, it is plausible that based on the high-intensity protocol, participants in the present study began the second bout of exercise in a markedly glycogen depleted state [12]. The lack of energy intake during the 2-hour recovery period could have yielded a shift in substrate oxidation from carbohydrate to fat, in turn negatively affecting the participant's ability to maximally perform high intensity exercise (i.e., a reduced endurance capacity during TTE testing).

There are several limitations to this study, including a sample with relatively fewer female participants as compared to males. While we did not control for menstrual cycle length, stage of cycle, or use of oral contraceptives, studies have demonstrated inconsistent findings related to CHO and fat metabolism with varying exercise intensity and phases of the menstrual cycle, and no difference between males and females in the resynthesis of muscle glycogen post exercise [2,28,30]. Dietary intake was not standardized among participants prior to the experimental protocol; however, statistical analysis revealed no significant differences in energy intake or macronutrient distribution between the three treatment groups for the day prior to testing. We did not quantify or restrict caffeine intake 24 hours prior to testing. It has been suggested that caffeine consumption following an acute period of withdrawal functions to relieve the negative symptoms of caffeine abstinence, thus positively influencing performance [31]. Therefore, it can be argued that restricting caffeine intake in a habitual user may unnecessarily interfere with acute testing outcomes and may lack application in an applied setting. A between subject design was used to minimize any potential learning effect that would result from performing a novel stimulus repetitively such as with the high-intensity aerobic interval and TTE protocol (i.e., familiarization, pre-post testing x three treatments vs. familiarization, pre-post testing x one treatment). Future research investigations may consider focusing on a more homogenous population familiar with endurance capacity testing.

This is the first study to examine the effect of an isocaloric carbohydrate-protein vs carbohydrate-only drink on performance during a repeated bout of exhaustive exercise following a 2-hour recovery period. Carbohydrate in the amount of 1.2 g/kg bm has been shown to more effectively attenuate performance decrements during a subsequent

bout of intense aerobic intervals, as compared to a placebo. The primary novel outcome of this study is that CHO-P co-ingestion (0.8 g/kg bm CHO + 0.4 g/kg bm PRO) is effective for enhancing performance in a subsequent bout of high-intensity exercise (i.e., series of aerobic intervals with time to exhaustion testing) with a limited amount of time to recover (i.e., 2 hours) in college-aged recreationally trained males and females. Along with prior research findings consistently demonstrating the importance of calorie intake immediately post-exercise to support glycogen and muscle protein synthesis, practitioners and athletes can use the current data to inform their post-exercise recovery practices.

Conclusions

A CHO-P beverage appears to be effective for promoting recovery and enhancing performance when performing repeated bouts of high-intensity exercise with limited time to recover.

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