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Full Title: Carbohydrate-Protein Ingestion Improves Subsequent Running Capacity Towards the End of a Football-Specific Intermittent Exercise.

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Running head: Protein co-ingestion and running capacity.

Abstract:

The majority of football players succumb to fatigue towards the end of the game. The study was designed to examine the influence of protein co-ingestion with CHO versus an isocaloric CHO supplement on subsequent running capacity towards the end of a simulated football match. Six male amateur football players participated in 3 trials applied in a randomized cross-over experimental design. A laboratory based football-specific intermittent exercise was allocated for 75 minutes interspersed with 15 minutes recovery, immediately followed by run time to fatigue at 80% VO_{2peak}. On each trial, participants randomly ingested a placebo, 6.9% carbohydrate (CHO) or 4.8% CHO plus 2.1% protein (CHO-P) supplements matched for color and taste prior to exercise and during half-time. CHO-P resulted in longer run time to fatigue (23.02 ± 5.27 minutes) than CHO (16.49 ± 3.25 minutes) and Plc (11.00 ± 2.80 minutes) ($P < 0.05$). Blood glucose was higher in CHO-P at the point of fatigue (4.68 ± 0.64) compared to CHO and Plc (3.92 ± 0.29 and 3.66 ± 0.36, respectively; $P < 0.05$). RPE were lower in CHO-P trial at the onset of exercise and towards the end of intermittent exercise when compared to Plc and CHO ($P < 0.05$). Subsequent running capacity following limited recovery from intermittent exercise was enhanced when protein was added to a CHO supplement. This improvement may suggest an ergogenic benefit on endurance capacity during intermittent activity with protein co-ingestion.

Keywords: amino acids, glycogen, nutrition, soccer, sports drinks, performance

Introduction

It has been established that the majority of football players succumb to fatigue towards the latter stages of the game (Mohr et al., 2003, Bradley et al., 2009). Furthermore, It has been postulated that the depletion of glycogen stores is a critical factor in the onset of fatigue during the game (Reilly, 1997, Bangsbo et al., 2006). This was suggested to be in relation to the greater reliance on CHO metabolism during match play (Hawley et al., 2006). The ingestion of CHO was shown to influence football-specific intermittent exercise in both field (Kirkendall et al., 1988, Currell et al., 2009), indoor (Balsom et al., 1999b, Welsh et al., 2002, Foskett et al., 2008) and laboratory based (Bangsbo et al., 1992b, Balsom et al., 1999a) investigations. Therefore, it is reasonable to suggest that rapid means of replenishing or sparing these endogenous CHO stores may have a positive influence on performance during the crucial periods of the game, as muscle glycogen depletion closely parallels perception of fatigue (Bergstrom et al., 1967) and consequently lead to the termination of exercise or significant reductions in exercise intensity (Ivy et al., 2003). This may present a means of gaining a competitive edge over rivals through attenuating the decrement in performance shown to be a feature towards the latter stages of the game (Reilly et al., 2008). In addition, the ingestion of CHO was shown to be causally related to rapid restoration of muscle glycogen stores, and a general positive correlation was observed between the amount of CHO ingestion and muscle glycogen resynthesis until it plateaus at CHO intake rates of ~1.2 $g.kg^{-1}.h^{-1}$ (Burke et al., 2004, Jentjens and Jeukendrup, 2003). Indeed, CHO intake was suggested to be the primary nutrient during recovery (Burke et al., 2006). However, it was reported that football players are likely to consume inadequate amounts of CHO

(Maclaren, 2003); considerably below the recommended quantities for maximal glycogen resynthesis (Burke et al., 2006) and therefore would not be likely to achieve such intakes. This suggests sub optimal nutritional strategies for glycogen repletion for players prior to and during a competitive match. Moreover, low muscle glycogen levels before training is often associated with the players' feeling of tiredness and the concomitant negative effects on the intensity of the training session (Bangsbo et al., 2006). Thus, the required optimal adaptations to the training stimuli may also become compromised (Hawley et al. 2006).

From a performance perspective, the influence of protein co-ingestion with a CHO sports beverage on subsequent performance was investigated during short-term recovery (≤ 6 hours) following a prior exercise bout in both cycling (Williams et al., 2003, Berardi et al., 2008, Ferguson-Stegall et al., 2011) and running (Betts et al., 2005, Betts et al., 2007) based modes of exercise. Significant improvements were observed by some (Berardi et al., 2008, Ferguson-Stegall et al., 2011), but not all (Toone and Betts, 2010, Breen et al., 2010, van Essen and Gibala, 2006) time trial investigations. Time to exhaustion performance measures, however, seem to elicit more pronounced benefits in cycling (Ivy et al., 2003, Saunders et al., 2004, Saunders et al., 2007, McCleave et al., 2011). A limited number of running-based investigations were instigated, not withstanding the fact that they augmented significant improvements when a CHO-P beverage was compared to CHO matched in their CHO content (Betts et al., 2007) or caloric equivalency (Niles et al., 2001). More recently, protein co-ingestion was indicated to maintain the efficacy of a CHO beverage, even when both CHO and caloric contents were reduced (Martinez-Lagunas et al., 2010, McCleave et al., 2011). Overall, there is a clear evidence of an ergogenic benefit of CHO-P supplementation during exercise (Stearns et al., 2010) and following short-term recovery (Williams et al., 2003, Betts et al., 2007) when time to exhaustion is the performance measure. It remains equivocal, however, whether this benefit is achieved by the protein fraction *per se* or the increased energy content in the CHO-P beverage when compared to CHO. Thus, the efficacy of CHO-P remains ambiguous (Martinez-Lagunas et al., 2010). In light of the uncertainty in the literature regarding the efficacy of CHO-P beverages and the absence of any data regarding CHO-P supplementation during football-specific intermittent exercise, the study was aimed to establish whether the exogenous CHO-P intake prior to exercise and during short-term recovery could induce an ergogenic benefit on subsequent run time to fatigue following a football-specific intermittent exercise when compared to an isocaloric CHO supplement. A secondary aim was to examine whether more glucose would be available at the point of fatigue with protein co-ingestion.

Materials and Methods

Subjects

6 male amateur football players (age 26 ± 2 years, BM 71 ± 5 kg, height 180 ± 7 cm, VO_{2peak} 51.4 ± 5 $ml.kg.min^{-1}$) were randomly recruited from the University of Brighton to participate in the study. The subjects trained for a minimum of 2 sessions/week of endurance exercise, and are regular participants in a minimum of one competitive or recreational match/week. All subjects received a participant information sheet indicating the testing procedure and risks associated. The subjects gave their informed written consent to the study that had been approved by the University of

Brighton Ethical Committee and completed medical questionnaires to ensure the absence of any risk factors related to the nature of study prior to participating.

Experimental design

Each subject was required to attend Welkin laboratories (Chelsea school, Eastbourne) on four separate occasions separated by at least 6 days. The first visit included preliminary measurements for each subject. The subsequent 3 visits included the participants to undertake 3 experimental conditions; placebo (Plc), carbohydrate with added protein (CHO-P) and isocaloric carbohydrate (CHO) beverages ingested 15 minutes prior to the exercise protocol and during the simulated half-time interval on the second, third and fourth visits in a randomized cross-over experimental design applied in a blind manner. Prior to the second visit, the subjects were asked to refrain from strenuous exercise, alcohol and caffeine consumption and to record their dietary intake in the previous 24 hours, which were duplicated on the preceding trials. A dietary and activity record was taken from each subject 24 hours prior to the pilot trial, and was adhered to on subsequent visits. This was aimed to minimize the variability in muscle glycogen concentrations and determine the energy intake of the subjects. The dietary record provided by each subject were analyzed with nutritional assessment software (Microdiet version 2.6, Downlee Systems Ltd, UK). The subjects were instructed to abstain from vigorous exercise on the day preceding the trial and adhere to their normal training and nutritional schedules throughout the experiment. Water intake was permitted *ad libitum* during the second visit and was matched for the subsequent experimental trials (460 ± 130 ml). All experimental beverages (Plc, CHO and CHO-P) were provided in a liquid form (515 ± 33 ml). Both CHO (1 $g.kg^{-1}$ CHO) and CHO-P (0.7 $g.kg^{-1}$ CHO + 0.3 $g.kg^{-1}$ protein) supplements were given to provide 6.9% solutions wt/vol, with equivalent caloric contents (272 ± 19 kcal). This included 6.9% maltodextrin solution in the CHO trial, and 4.8% maltodextrin plus 2.1% whey protein mixture in the CHO-P trial. All test solutions were taste and color matched (apple and blackcurrant). The time taken by each subject to consume the different supplements was recorded. Furthermore, the rating of stomach discomfort following the allocated 2 ingestion points were recorded using adapted Borg scales where the scaled ranged from “no discomfort” to “extreme discomfort”.

Figure 1. Schematic representation of the experimental protocol. *=Blood sample, † = Heart rate + RPE, U= Urine osmolality, ‡= Fluid provision, TC= Time to consume supplement (minutes), SDS= Stomach discomfort scale, TTE= Time to exhaustion.

Preliminary measurements

A graded exercise test to volitional exhaustion on a motorized treadmill (Ergo ELG 70, Woodway, Germany) was allocated to determine the relative 80% VO_{2peak} to measure exercise capacity following supplement ingestion during RTF. The test commenced with a standardized 10-minute warm-up (jogging at speed of 6 $km.h^{-1}$) for each subject throughout the study. The expired gas samples were obtained via Douglas bag method at the final minute of each 3-minute stage. Heart rate (HR) and rating of perceived exertion (RPE) measurements were collected at similar collection times of the expired gas of each stage. Increments of 1 km.h^{-1} were applied until running at a given speed cannot be maintained. A constant treadmill incline of 1% will be used to reflect the energetic cost of outdoor running at the speeds used in the protocol (Jones and Doust, 1996). The test was terminated when at least two of criteria of the British Association of Sport and Exercise (BASES) were observed to ensure the attainment of VO_{2peak} (Bird and Davison, 1997). Following the incremental $VO2_{peak}$ test, 2 random subjects were recruited to participate in additional testing aimed to ensure the homogeneity of the 3 beverages (Plc, CHO and CHO-P) in color and taste. The subjects consumed 150 ml of each supplement in a random order and separated by 15 minutes between each feeding. Water was provided to the participants between feedings to cleanse their mouth prior to the provision of the subsequent bolus. The 2 participants were unable to distinguish any difference in neither color nor taste between the 3 treatments. At the end of their relative main trials, none of the participants in the study reported any difference in taste between the supplements provided throughout the study during an informal interview where all of which requested to know their relative random order of supplementation. Thus, the 2 random subjects chosen during the preliminary measurements were shown to reflect the group response.

Experimental protocol

Intermittent exercise protocol

The participants were tested between 08:30 and 11:30 following an overnight fast (≥10 hours) to account for the effects of circadian variation (Drust et al., 2005) and to ensure sufficient glycogen depletion before the commencement of the protocol. A laboratory based football-specific intermittent exercise was assigned for the study (Clarke et al., 2008). This protocol was suggested to simulate the work rate and physiological demands of competitive football (Drust et al., 2000). The duration of cycle, speeds and duration of each activity pattern and the proportion of time and corresponding speed were described elsewhere (Clarke et al., 2008). The experimental design comprised of 5 x 15-minute identical intermittent activity cycles, immediately followed by run time to fatigue (RTF) at 80% VO_{2peak}. This mode of exercise was chosen as a measure of performance in the protocol because it was reported that time to exhaustion was directly proportional with elevated muscle glycogen availability (Kirkendall, 1993). The allocated intensity of RTF was chosen because it was shown to be sustained only when sufficient muscle glycogen is available (Coggan and Coyle, 1988). In addition, there is evidence that the reliability of and exercise capacity test is compromised at intensities above 80% VO_{2max} (Krebs and Powers, 1989). The overall duration of the 5 cycles was 75 minutes of intermittent exercise interposed with a 15-minute recovery period. The subjects were instructed to run until the point of volitional exhaustion and could not maintain their relative running speeds. The participants were unaware of their performance in any trial.

Table 1. Nutritional information and volume of fluids provided for the different experimental supplements (mean ± SD).

Physiological measurements

Pre-trial urine samples were obtained to assess the hydration status of the subjects by using a cryoscopic osmometer (Osmocheck, Vitech Scientific Ltd, Japan).

Adequate hydration was assumed for osmolality values below 900 mOsmol.kg-1 (Shirreffs and Maughan, 1998). During the football-specific protocol, HR measurements were monitored via short-range radio telemetry (Polar Sports Tester, Polar Electro, Kempele, Finland) during the 2 static pauses in each exercise block. Thereafter, HR was obtained at 1-minute intervals during RTF until volitional exhaustion. RPE were also collected at the same designated points as HR measurements during the intermittent protocol and RTF using Borg's 6-20 scale (Borg, 1970). Ambient temperature and humidity were recorded at 45-minute intervals throughout the trials using a hygrometer (BAR688HGA, Oregon Scientific, UK) and were not different between trials: 20.6 ± 0.06 C°; 42 ± 0.76% respectively.

Sampling and analysis

All the equipment were calibrated prior to testing. Expired gas samples were collected via Douglas bag method and were analyzed by paramagnetic and Infrared Analyzers, respectively (Servomex, Crowborough, UK). The total volume of expired gas within the Douglas bags was measured by a dry gas meter (Cubix U6, Sensus, Raleigh, USA) and the temperatures of expired gases was determined with a digital thermometer. Blood samples were collected from each participant at rest, during the second static pause of each block, the simulated half-time interval and upon cessation of RTF to analyze blood glucose and lactate concentrations. These were obtained via fingertip capillary method through a 3 mm puncture (Accu-check Softclix Pro, Roche dignostics GmbH, Germany) and were dispensed into microvettes (~25 µl; CB300, Sarstedt, Germany) containing lithium heparin that acts as an anticoagulant and subsequently were placed for analysis (YSI 2300 STAT plus, YSI Limited, UK).

Statistical analysis

Statistical procedures were conducted using IBM SPSS statistics version 18.0 (SPSS Inc., Chicago, IL). A two-way ANOVA with repeated measures (beverage x time) was employed to identify the significant effects on the physiological parameters (heart rate, blood glucose and blood lactate) at designated points throughout the study. The difference in RTF times, distance covered during RTF, the time consumed to ingest the supplements and the stomach discomfort ratings were analyzed via one-way ANOVA with repeated measures between the three different treatment conditions. Mauchly's test was used for sphericity; where asphericity was assumed, the Greenhouse-Geisser correction was used for epsilon < 0.75; if not, the Huynh-Feldt was adopted for less severe asphericity. Where significant F values were found a Bonferroni post hoc test was used to determine the location of the variance (Atkinson, 2002). Significance was set at $P < 0.05$ and all results were reported as the mean ± standard deviation (SD) of the mean. Despite the achievement of significance with only 6 participants during the time to exhaustion and in the absence of any comparable data regarding CHO vs. CHO-P for intermittent running based studies, a post hoc power analysis was applied to explore the adequacy of the sample size. From this it was determined that the applied sample of 6 provided ~60% power to detect the observed difference between CHO and CHO-P of 6.53 minutes with a pooled SD of 4.46 minutes using a 2-tailed t-test with a Bonferroni correction at α level 0.05 (i.e. future similar investigations would require a sample size of ~8 participants to achieve 80% power to detect such a difference statistically).

Results

The one-way ANOVA showed significant effects on the distances covered during RTF between the different drinks $F_{(2,10)} = 22.47$ ($P < 0.01$) effect size= 0.82. The mean distance covered during the 5 blocks of intermittent exercise protocol was 11.1 ± 0.01 km. The distance covered by the participants during the subsequent RTF was 2.28 ± 0.7; 3.40 ± 0.8; 4.70 ± 1.2 km in Plc, CHO and CHO-P treatments, respectively. The covered distance during RTF was significantly greater ($P < 0.05$) in the CHO-P treatment when compared to CHO and Plc. Moreover, The distance during the CHO treatment was significantly greater ($P < 0.05$) than Plc.

Table 2. Heart rate and blood lactate responses to the intermittent football-specific exercise and RTF (mean ± SD).

Significant differences were found in mean time to fatigue between the experimental trials $F_{(2,10)} = 22.71$ ($P < 0.01$) effect size= 0.82. The participants were able to run longer when CHO-P was ingested (23.02 ± 5.27 minutes) as opposed to CHO (16.49 ± 3.25 minutes) and Plc (11.00 ± 2.80 minutes) treatments. Thus, a 49% improvement in time to exhaustion was observed when CHO was compared to a placebo. In the CHO-P trial, 39% and 107% improvements were observed when compared with CHO and Plc, respectively. The Bonferroni post hoc test revealed that times to exhaustion were significantly greater ($P < 0.05$) in CHO-P and CHO trials when compared to a placebo. Significantly greater times to exhaustion ($P < 0.05$) were also observed in the CHO-P treatment versus CHO.

The mean HR during the intermittent exercise blocks and RTF during the 3 experimental conditions were 157 ± 6 and 175 ± 1 bpm, respectively. The two-way ANOVA showed no significant effects of type of drink consumed on HR $F_{(1.001,\ 5.007)} = 0.002$ ($P > 0.05$). The ANOVA revealed a significant main effect of time on HR $F_{(7,35)} = 828.42$ ($P < 0.01$). However, no interaction between time and trial were identified $F_{(14,70)} = 0.486$ ($P > 0.05$).

Figure 2. Mean run time to fatigue following the ingestion of Plc, CHO and CHO-P beverages before exercise and during half time. *= Significantly greater than placebo ($P < 0.05$), † = Significantly greater than CHO ($P < 0.05$).

Ratings of perceived exertion were shown to be significantly different between the different beverages $F_{(2,10)} = 12.34$ ($P < 0.05$). The time of exercise showed a significant effect on RPE ($F_{(4,20)} = 38.74$; $P < 0.01$). The repeated measure ANOVA indicated an interaction between time and trial on RPE ($F_{(8,40)} = 3.49$; $P < 0.05$). Significantly lower ratings of perceived exertion were observed in the CHO-P trial at the first block of exercise following the first feeding when compared to CHO and Plc trials ($P < 0.05$). RPE was also shown to be significantly lower during the fourth block of exercise following the second feeding when compared to the CHO trial ($P < 0.05$). The final intermittent exercise block revealed lower RPE when CHO-P was ingested versus CHO and Plc treatments ($P < 0.05$).

Pre-trial blood glucose concentrations were similar for all three trials. There was a significant effect of trial on blood glucose levels $F_{(2,10)} = 86.84$ ($P < 0.01$). A significant effect of time was observed on blood glucose levels $F_{(7,35)} = 20.82$ ($P < 0.01$). The repeated measures ANOVA also identified a significant interaction between time and trial $F_{(14,70)} = 13.12$ ($P < 0.01$). Blood glucose concentrations were significantly higher at 15 minutes in the CHO trial when compared to Plc ($P < 0.05$). At the end of half-time and following the second bolus, blood glucose concentrations increased markedly ($P < 0.01$) in CHO and CHO-P treatments compared with Plc treatment. The increase at the end of half-time in the CHO trial was also significantly greater than CHO-P ($P < 0.05$). During the subsequent 2 intermittent exercise blocks, no significant differences in glucose concentrations were observed. By the end of time to exhaustion, 19% and 28% greater blood glucose was available in CHO-P trial when compared to CHO and Plc ($P < 0.05$).

Table 3. Time to consume supplements and ratings of stomach discomfort with the different experimental supplements ingested before exercise and during half-time (mean ± SD). *= Significantly greater than placebo ($P < 0.05$).

A significant effect of exercise time was shown on blood lactate concentrations $F_{(7,35)} = 29.10$ ($P < 0.01$). No significant differences were identified by the two-way ANOVA between trials $F_{(2,10)} = 0.071$ ($P > 0.05$). The interaction between the type of drink consumed and time did not show any significant effects $F_{(14,70)} = 0.471$ ($P > 0.05$). Pre-trial blood lactate concentrations were similar between trials. A marked increase was shown in the first exercise block in all trials, reaching the highest point during the protocol. Thereafter, blood lactate underwent a gradual decline during the first-half until reaching near resting levels during half-time. During the second-half and RTF, blood lactate increased higher than half-time values. However, values did not reach peak levels observed at the beginning of exercise.

Discussion

The primary purpose of this investigation was to determine whether a CHO-P beverage induced an enhanced subsequent running capacity versus an isocaloric CHO beverage ingested before exercise and during half-time. The current study revealed that subsequent running capacity following football-specific intermitted exercise can be restored more completely when a mixture of CHO and whey protein is ingested compared with CHO fraction alone matched in caloric equivalency. A secondary aim of the study was to determine whether there was more glucose available at the point of fatigue in the CHO-P trial when compared with CHO. As hypothesized, greater RTF and blood glucose at the point of fatigue were observed in CHO-P as opposed to CHO ($P < 0.05$).

Figure 3. Mean blood glucose concentrations following the ingestion of the 3 experimental beverages before exercise and during half-time. *= Significantly greater than placebo ($P < 0.05$), † = Significantly greater than CHO ($P < 0.05$), ‡= Significantly greater than CHO-P ($P < 0.05$).

The culmination of the results from numerous studies indicate that protein co-ingestion with CHO increases the efficiency of muscle glycogen storage when supplementation feedings are greater than 1 hour intervals, or when the amount of CHO is below the threshold of maximal glycogen resynthesis (Zawadzki et al., 1992, Ivy et al., 2002, Williams et al., 2003, Berardi et al., 2006). Post-exercise CHO-P ingestion was reported to be twice as fast during the initial 40 minutes of recovery than following isocarbohydrate or isocaloric CHO ingestion, and therefore demonstrates a distinct advantage in rapid glycogen restoration during limited recovery periods (Ivy et al., 2002). It would be pertinent to suggest that higher rates of glycogen synthesis may have occurred more rapidly with a CHO-P supplement. Specifically, a preferential fiber type glycogen resynthesis may have occurred in the exercising muscle. It was shown by means of intermittent shuttle running that the amount of glycogen utilized was greater in fast-twitch (FT) than slow-twitch (ST) muscle fibers, indicating a greater reliance on FT fibers during intermittent activity (Nicholas et al., 1999). Indeed, this was shown during a football game, where 71% of FT fibers were completely or almost empty of glycogen compared with 54% in ST fibers (Krustrup et al., 2006). Interestingly, glycogen depletion in FT fibers to a critical level where maximal glycolytic rate cannot be maintained (Bangsbo et al., 1992a) was shown to determine the point of fatigue during a simulation of football (Nicholas et al., 1999) and actual match play (Krustrup et al., 2006). Thus, the observed elevated blood glucose concentration late in exercise in CHO-P trial may have contributed to enhanced glycogen synthesis during the low-intensity periods (standing, walking and jogging), as has been reported with CHO ingestion versus a placebo (Yaspelkis et al., 1993), and could provided tentative explanations for the observed ergogenic benefit with CHO-P supplementation.

It has been suggested that CHO ingestion attenuates fatigue during steady state moderate intensity exercise by preventing hypoglycemia and maintaining CHO oxidation (Coyle et al., 1986). In concurrence, it was shown that CHO provision before exercise and during half-time of a simulated football match elicited significantly greater ($P < 0.05$) CHO oxidation at 45 minutes and towards the end of the game (Clarke et al., 2008). Moreover, it was demonstrated that whole-body CHO oxidation during subsequent performance and following recovery was significantly greater ($P < 0.01$) in the CHO-P treatment than with CHO (48.4 ± 2.2 and 41.7 ± 2.6 $mg.kg^{-1}.min^{-1}$, respectively); even when CHO oxidation and storage were similar during recovery between both trials (Betts et al., 2008). Blood glucose oxidation was suggested to be dictated primarily by its availability in circulation (Weltan et al., 1998). Therefore, given that higher blood glucose levels were observed in CHO-P trial in the present study, it is likely that performance enhancements in CHO-P could be attributed to an increase in extramuscular CHO oxidation with protein co-ingestion, as recently observed (Betts et al., 2008). In the current study, improvements in performance were apparent with protein added to a CHO supplement at an exercise intensity of 80% VO_{2peak}. Indeed, this comes in agreement with another study (Martinez-Lagunas et al., 2010) and suggests that the maintenance of euglycemia observed in the current study is, at least in part, related to the enhanced endurance capacity towards the latter stages of exercise. However, it is noteworthy that blood glucose cannot fully reinforce the CHO requirements for exercise intensities over 75% VO_{2max} (Coyle et al., 1986) Thus, enhancements in exercise capacity my occur independent of changes in whole-body oxidation and thus may become dissociated with prevention of hypoglycemia (Claassen et al., 2005).

Figure 4. Mean RPE during the intermittent exercise blocks when placebo, CHO and CHO-P were ingested. *= Significantly lower than placebo ($P < 0.05$) † = Significantly lower than CHO ($P < 0.05$).

With regards to the current investigation, it cannot be ruled out that an enhanced central drive to exercise was induced as a result of protein co-ingestion. Thereby, fatigue during RTF may have coincided with an increase perception of fatigue originating from the central nervous system. It was indicated that the free fatty acid concentration rise progressively during a football match and a more pronounced increase is evident during the second half (Krustrup et al., 2006). Furthermore, during prolonged exercise, fatty acid mobilization exceeds that of muscle uptake and consequently elevate blood fatty acid concentration (Newsholme and Blomstrand, 2006), and may influence the onset of fatigue during prolonged endurance exercise (Fernstrom and Fernstrom, 2006). It was postulated that the ingestion of branched-chain amino acids (BCAA) with CHO could mediate significant improvements in performance via interactions that attenuate the development of central fatigue (Meeusen et al., 2006). While there is some evidence in support of improved performance (Mittleman et al., 1998), this is not universal (Davis et al., 1999). However, the ingestion of BCAA was shown to influence ratings of perceived exertion (Blomstrand, 2001). This comes in concurrence with the current study where mean RPE in the CHO-P trial were lower throughout the protocol than both Plc and CHO treatments. This provides further support of an improvement in the central drive for exercise may be an explanation for the enhanced endurance capacity observed in the CHO-P treatment, as previously speculated (Betts et al., 2007).

It is acknowledged that the inclusion of a number of metabolic data in the current investigation would allow for a more informative discussion regarding the potential ergogenic mechanism(s) related to protein co-ingestion. Nonetheless, a myriad of studies were aimed to investigate the mechanistic effects of CHO-P and CHO supplementation on human metabolism (Cermak et al., 2009, Howarth et al., 2009, Betts et al., 2008, Ivy et al., 2003, Saunders et al., 2004). However, these investigations failed to measure the effects on subsequent endurance capacity where few studies were instigated (Betts et al., 2007, Thomas et al., 2009) and none of which measured endurance performance during intermittent exercise. Therefore, in the current study, the primary aim was to determine whether CHO-P supplementation may elicit an ergogenic benefit on subsequent running capacity following short-term recovery. Correspondingly, the approach adopted in the current study was aimed to maintain the ecological validity of the experimental design that could allow for comparisons between investigations of subsequent endurance capacity with the majority of the available literature.

The findings in the current study suggest important implications in sports that encompass multiple training sessions and/or competitive situations with limited recovery such as football (Burke et al., 2004). This could mediate practical nutritional interventions in team sports, given that the quantities in the CHO supplements ingested (≥ 1.2 g.kg^{-1}.h^{-1}) in many of the studies (van Loon et al., 2000, Jentjens et al., 2001) were shown to exceed that of voluntary intakes consumed by athletes (Noakes, 1993) and thus would limit *in situ* application. Furthermore, the levels of fluid (>1 L.h^{-1}) and nutrient intake similar to the aforementioned studies were shown to evoke severe gastrointestinal discomfort *in vitro* (Betts et al., 2007). Thus, it would be reasonable to suggest that a mixed nutrient diet would avoid such complications and would be advantageous, given they elicited similar recovery rates with isocaloric CHO (Berardi et al., 2006) and was shown to equal (Betts et al., 2005) or improve (Niles et al., 2001) endurance capacity.

In conclusion, the current investigation exhibited an improvement of running capacity following short-term recovery from intermittent football-specific exercise when ~2% wt/vol of protein was added to a CHO supplement (~6-8% wt/vol). This comes in agreement with some of the available literature that has investigated protein co-ingestion during endurance exercise (Ivy et al., 2003, Saunders et al., 2007) and subsequent endurance capacity following short-term recovery (Betts et al., 2007, Williams et al., 2003, Thomas et al., 2009). The precise mechanism behind the ergogenic benefit on endurance capacity with CHO-P ingestion remains unclear and may be related to an enhanced central drive to exercise induced by the improved extramuscular glucose oxidation late in exercise. A novel finding from the current investigation is that performance towards the final stages of the simulated game was enhanced following 75 minutes of intermittent exercise when CHO-P was ingested prior to exercise and during half-time when compared to an isocaloric CHO beverage.

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