International Journal of Sport Nutrition and Exercise Metabolism, 2005, 15, 590-609

Recovery of Endurance Running Capacity: Effect of Carbohydrate-Protein Mixtures

James A. Betts, Emma Stevenson, Clyde Williams, Catrin Sheppard, Edwin Grey, and Joe Griffin

Including protein in a carbohydrate solution may accelerate both the rate of glycogen storage and the restoration of exercise capacity following prolonged activity. Two studies were undertaken with nine active men in study A and seven in study B. All participants performed 2 trials, each involving a 90 min run at 70% VO_{2max} followed by a 4 h recovery. During recovery, either a 9.3% carbohydrate solution (CHO) or the same solution plus 1.5% protein (CHO-PRO) was ingested every 30 min in volumes providing either 1.2 g CHO · kg^{-1} · h^{-1} (study A) or 0.8 g CHO · kg^{-1} · h^{-1} (study B). Exercise capacity was then assessed by run time to exhaustion at 85% VO_{2max}. Ingestion of CHO-PRO elicited greater insulinemic responses than CHO ($P \leq 0.05$) but with no differences in run times to exhaustion. Within the context of this experimental design, CHO and CHO-PRO restored running capacity with equal effect.

Key Words: insulin, muscle glycogen, amino acids, exercise

The resynthesis of muscle glycogen following prolonged exercise is an important component of recovery and there is good evidence that consuming carbohydrate in the immediate post-exercise period can enhance this process (11, 14). Further evidence indicates that adding protein to a carbohydrate recovery solution can increase the rate of muscle glycogen resynthesis during short-term recovery (39) and, more recently, it has been reported that this accelerated rate of muscle glycogen resynthesis might result in a more complete recovery of exercise capacity (38).

Carbohydrate-protein solutions have been shown to increase insulin concentrations more effectively than carbohydrate alone (16, 34, 35) and it is possible that increased levels of circulating insulin during recovery could increase the rate of muscle glycogen resynthesis in this period. The results of studies on the influence

The authors are with the School of Sport and Exercise Sciences, Loughborough University, LE11 3TU, U.K.

of such supplements on rates of muscle glycogen resynthesis are inconsistent, however. While some authors have reported increased rates of muscle glycogen resynthesis following the addition of protein to carbohydrate recovery solutions (12, 35, 39), others have reported no such effect (16, 34).

It has been suggested that the inconsistent findings regarding post-exercise ingestion of carbohydrate-protein mixtures might be explained by differences in the quantity of carbohydrate provided (5, 16, 35). According to this explanation, muscle glycogen resynthesis could already be maximal when carbohydrate alone is provided in adequate amounts (i.e., 1 g CHO · kg^{-1} · h^{-1}). Furthermore, Ivy (10) identifies that an appreciation of the specific feeding schedules adopted along with the precise types and quantities of protein provided is essential when interpreting the range of reported findings in this area of investigation.

Given that, in general terms, endurance capacity during moderate to high intensity exercise positively correlates with pre-exercise muscle glycogen content (3), it is possible that consumption of a carbohydrate-protein solution during recovery from such exercise might accelerate the restoration of endurance capacity when recovery time is restricted (i.e., < 8 h). Although research on this topic is limited, there is some evidence suggesting that the addition of protein to a carbohydrate solution could enhance the recovery of endurance capacity (23, 25, 38). The 55% greater time to exhaustion following ingestion of a carbohydrate-protein mixture reported by Williams et al. (38) might be attributable, however, to the amount of carbohydrate included in each supplement. Specifically, in their study the carbohydrate-protein solution provided more carbohydrate than the solution containing carbohydrate alone.

When evaluating the relative merits of either carbohydrate ingestion alone or the coingestion of carbohydrate plus protein, it is perhaps most relevant to match the carbohydrate content between these solutions rather than the total amount of energy provided. A positive correlation appears to exist between the amount of carbohydrate ingested and the rate of muscle glycogen resynthesis and it appears that ≥ 1.2 g CHO · kg^{-1} · h^{-1} is required to maximize this process (11). Recently, synthesis rates slightly in excess of 40 mmol · kg dry $mass^{-1}$ · h^{-1} have been reported following ingestion of 1.2 g CHO · kg^{-1} · h^{-1} at 30 min intervals during a 5 h recovery (35). It has been suggested, however, that a "glycogen synthesis threshold" could occur at this optimal level of carbohydrate intake (14). Given the proposed association between the degree of muscle glycogen synthesis and exercise capacity (3), it would clearly be of interest to the scientific and athletic communities alike to establish the performance benefits of adding more energy in the form of protein both to optimal and sub-optimal quantities of carbohydrate.

Therefore, the main aim of this investigation was to determine if ingestion of a carbohydrate-protein mixture during recovery from prolonged exercise results in a greater recovery of endurance running capacity at 85% VO_{2max} than a solution providing a matched amount of carbohydrate alone. Study A investigated the effects of adding protein to a solution containing optimum amounts of carbohydrate for glycogen resynthesis (i.e., 1.2 g CHO · kg^{-1} · h^{-1}), while study B examined the effects of a carbohydrate-protein mixture that contained a sub-optimal amount of carbohydrate (i.e., 0.8 g CHO · kg^{-1} · h^{-1}).

Methods

Approach to the Problem

In the present study, a comparison was made between a carbohydrate-protein mixture and a solution containing a matched amount of carbohydrate alone. These solutions were consumed during a 4 h recovery from prolonged but not exhaustive exercise and the effectiveness of each solution was assessed by run time to exhaustion at 85% VO_{2max}. The intensity of this capacity test is similar to that previously used to assess the efficacy of carbohydrate-protein on recovery of cycling capacity (38) although the exercise model in the present study involved treadmill running. In addition, we determined whether the response to these solutions varied when the carbohydrate concentration to which the protein was added was either that considered to be optimal for muscle glycogen resynthesis (i.e., 1.2 g CHO · kg^{-1} · h^{-1}) or a more moderate carbohydrate load (i.e., 0.8 g CHO · kg^{-1} · h^{-1}).

Subjects

Sixteen recreationally active men volunteered for this study (study A: $n = 9$; age 21 ± 1 y, body mass 79.6 ± 11.2 kg, VO_{2max} 59.7 ± 3.8 mL · kg^{-1} · min^{-1}; study B: $n = 7$; age 22 ± 0.5 y, body mass 83.5 ± 11.8 kg, VO_{2max} 55 ± 4 mL · kg^{-1} · min^{-1}; values are means ± standard deviation). All these subjects included running as a substantial component of their habitual training (5 ± 1 h/wk; mean ± standard deviation) and all had taken part in similar investigations on previous occasions. Once fully briefed regarding the nature of the study, each subject provided informed consent in keeping with the requirements of the Loughborough University Ethical Advisory Committee which approved this study.

Preliminary Measurements

Preliminary tests were administered to determine each participant's submaximal and maximal oxygen uptakes (30) on a motorized treadmill (Technogym, Gambettola, Italy). A subsequent test was conducted within 2 wk of trial 1 to familiarize subjects with the procedures and confirm that calculated running speeds were equivalent to 70 and 85% of VO_{2max}. All subjects continued their habitual training throughout the study period but refrained from strenuous physical activity and avoided both alcohol and caffeine consumption during the 48 h prior to main trials.

Experimental Design

Subjects performed two main trials in a randomized, counterbalanced design that were separated by at least 1 wk and applied in a double blind manner. A 2 d dietary record based on participants' habitual diets was completed over the 48 h prior to trial 1 and then subjects adhered to this diet prior to trial 2 (study A: 2458 ± 670 kcal/d, 53 ± 9% CHO, 25 ± 6% fat, 19 ± 5% protein; study B: 3623 ± 1630 kcal/d, 49 ± 8% CHO, 30 ± 8% fat, 21 ± 3% protein; values are means ± standard deviation). Main trials involved a 90 min treadmill run at 70% VO_{2max} (R_1) followed by a 4 h recovery. This first exercise session was designed to be prolonged but not exhaustive in nature and previous findings from our laboratory have demonstrated

that a run of similar intensity and duration without exogenous carbohydrate provision can result in significantly reduced muscle glycogen concentrations, specifically localized to the type I muscle fibers (32). During the recovery period, subjects rested while consuming either carbohydrate alone (CHO trial) or a carbohydrate-protein mixture (CHO-PRO trial). After the recovery period, subjects were required to complete a treadmill run to exhaustion at 85% VO_{2max} (R_2), an intensity previously used by others (38) to investigate the efficacy of carbohydrate-protein solutions during prolonged cycling. A schematic representation of this exercise protocol is illustrated in Figure 1.

Experimental Protocol

All subjects arrived in the laboratory between 8 and 8:30 AM following a 10 h overnight fast. After providing a urine sample, each participant's nude body mass was recorded (Avery Ltd., UK) before a cannula was inserted into an antecubital vein and a 10 mL resting venous blood sample obtained. The cannula was kept patent throughout each trial by frequent flushing with isotonic saline. Prior to exercise, the Douglas bag technique was used to collect a 5 min resting expired gas sample (37). Subjects were required to stand for 15 min prior to the collection of all resting gas and blood samples. A 5 min run at 60% VO_{2max} was used as a standardized warm-up prior to running at a speed equivalent to 70% VO_{2max}

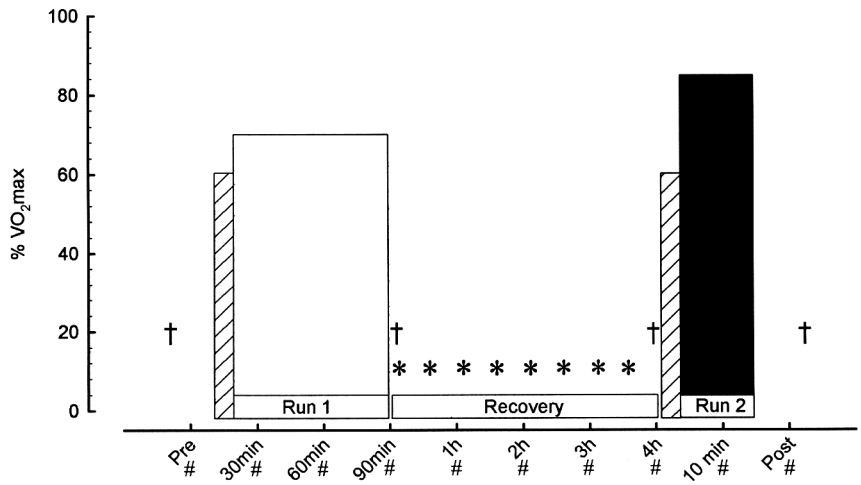

Figure 1 — A schematic representation of the experimental protocol. Key: * = solution provision, # = expired gas sample/blood sample and RPE, † = body mass, hatched area = warm-up (5 min at 60% VO_{2max}), open area = R_1 (90 min at 70% VO_{2max}) and closed area = R_2 (85% VO_{2max} until volitional exhaustion).

for 90 min or until fatigue, whichever came first (R_1). One minute expired gas samples, heart rates (Polar 8810, Kempele, Finland) and RPE (4) followed by 10 mL venous blood samples were taken at 30 min intervals throughout R_1. Water intake was permitted ad libitum during trial 1 and matched in trial 2, nude body mass was recorded immediately following R_1 to assess hydration status through percentage change in mass.

The first volume of the prescribed beverage was ingested as soon as post R_1 nude body mass had been recorded. The remaining 7 volumes of the beverage were ingested at 30 min intervals during the 4 h recovery since the most rapid rates of muscle glycogen resynthesis have been reported using this feeding schedule (35). Therefore, the last volume was ingested 30 min prior to R_2, with subjects being permitted 15 min to consume each solution. Expired gas samples and venous blood samples were recorded during the recovery period every hour prior to feedings. Subjective ratings of gut fullness and thirst were taken coincident with air samples using adapted Borg scales (4) such that the anchor terms on each 6 to 20 scale ranged from "not full" to "very very full" and "not thirsty" to "very very thirsty," respectively. Nude body mass was again recorded and, after the standard warm-up, subjects began the run to exhaustion at 85% VO_{2max} (R_2). As in R_1, water intake was ad libitum during trial 1 and matched in trial 2. Physiological measurements were obtained at regular intervals (10 min) and at the point of volitional fatigue. Nude body mass was recorded immediately after R_2, again to assess hydration status through changes in body mass. Ambient temperature and humidity were recorded at 30 min intervals throughout the trials using a hygrometer (Zeal, UK) and were not different between trials. Average environmental conditions recorded in each study were similar: 22.6 ± 1.9 °C and 42.3 ± 6.7% in study A and 23.2 ± 1.3 °C and 53.1 ± 5.7% in study B. The mean difference between the CHO and CHO-PRO trials of both studies was 0.9 ± 1.8 °C and 1.6 ± 7.2% (values are means ± standard deviation).

Solution Composition

The amount of carbohydrate included in the CHO and CHO-PRO solutions was 1.2 g CHO · kg^{-1} · h^{-1} in study A and 0.8 g CHO · kg^{-1} · h^{-1} in study B. All solutions in both studies were provided in liquid form and were 9.3% CHO solutions composed of 6.2% glucose and 3.1% fructose; the varied carbohydrate content provided between the studies was therefore achieved through reducing the volume of fluid consumed from 1031 ± 144 mL/h (study A) to 722 ± 102 mL/h (study B; values are means ± standard deviation). The CHO-PRO solutions included the addition of 1.5% wheat protein hydrolysate, rich in peptide-bound glutamine, to the CHO mixture. Thus, the addition of protein made approximately 16.7% more energy available for metabolism in both studies (study A: 4.8 kcal · kg^{-1} · h^{-1} versus 5.6 kcal · kg^{-1} · h^{-1}; study B: 3.2 kcal · kg^{-1} · h^{-1} versus 3.7 kcal · kg^{-1} · h^{-1}; values are means ± standard deviation). The carbohydrate-protein ratio of approximately 6:1 was maintained for the CHO-PRO solutions in both studies; thus, reducing the volume consumed in study B resulted in equal reductions in both carbohydrate and protein provision. Both test solutions were taste matched (orange and passion fruit flavor).

Sampling and Analysis

Expired gas samples were collected using a Douglas bag (37) and the fractions of expired O_2 and CO_2 were analyzed using paramagnetic and infra-red analyzers respectively (Servomex 1440, UK). Total volumes expired were determined using a dry gas meter (Harvard Apparatus, UK) and the temperatures of expired gases measured with a digital thermometer (model C, Edale Instruments, UK). These analyzers were calibrated prior to each test with gases of known composition and volume within the physiological range.

From each 10 mL whole blood sample, 5 mL was dispensed into a non-anti-coagulant tube where it was left to clot for ~ 45 min at room temperature and centrifuged at 2000 × g for 10 min at 4 °C (model Allegra X-22R, Beckman-Coulter, Germany) before the serum fraction was stored at –80 °C pending later analysis for insulin and cortisol by radioimmunoassay (Coat-Count Insulin, Corti-Cote, MP Biomedicals Ltd., Irvine, CA) using a gamma counter (model Cobra 5000, Packard Instruments, Meriden, CT). The remaining 5 mL was transferred into a tube containing the anti-coagulant ethylenediaminetetraacetic acid (EDTA) and was used to determine hematocrit (Hct Centrifuge, Hawksley, UK) and hemoglobin concentration. Hemoglobin concentration was measured using a standard cyanomet-hemoglobin method (Boehringer Mannheim, Germany) and a spectrophotometer (Shimadzu model1240, Japan). The equations of Dill and Costill (8) were applied to these hematocrit and hemoglobin values to assess changes in plasma volume. Two further 20 µl samples of whole blood were deproteinized using 2.5% perchloric acid (200 µl), centrifuged at 7000 × g for 3 min (Eppendorf Centrifuge, model 5415c, Germany) and stored at –80 °C for later determination of lactate concentration (19) using a fluorometer (Locarte 8.9, UK). The remaining whole blood was centrifuged at 2000 × g for 10 min at 4°C (model Allegra X-22R, Beckman-Coulter, Germany) before plasma was abstracted, stored at –80 °C and later analyzed for glucose (Randox, Ireland), free fatty acids (Wako NEFA C, Germany), glycerol (Randox, Ireland) and urea (Randox, Ireland) using an automatic spectrophotometric analyzer (Cobas-Mira plus, Roche Diagnostics). Pre-trial urine samples were analyzed for osmolality using a cryoscopic osmometer (Gonometer 030, Gonotec, Germany) and adequate hydration was assumed for osmolality values below 900 mosmol/ × kg^1 (26).

Statistical Analyses

The endurance capacity data of Williams et al. (38) were used to estimate that a sample size of 7 has an 80% power to detect a difference in run times of 11.1 min, assuming a standard deviation of differences of approximately 9.4 min, using a paired *t*-test with a one-sided significance level. Variables were tested for normal distribution and a Wilcoxon test was applied to compare median run times between trials, while a *t*-test was applied to the incremental area under the curve (IAUC) data that was used to assess the glycemic and insulinemic responses to each treatment during recovery. The IAUC was calculated as the cumulative area above baseline (i.e., 90 min R_1) but under the concentration curve solely for the 4 h recovery period. A two-way general linear model for repeated measures (drink × time) was used to identify differences between experimental conditions. The Greenhouse-Geisser correction was used for epsilon < 0.75, while the Huynh-Feldt correction

was adopted for less severe asphericity. When significant *F* values were found, the Holm-Bonferroni step-wise method was used to determine the location of the variance (2). Statistical analyses were performed using the SPSS for Windows version 11.0 software (SPSS, Inc., Chicago, IL) and significance was accepted at the $P \leq 0.05$ level for each set of variables. Unless otherwise stated, all results are expressed in text as means ± standard error of the mean or median (range). The error variance bars displayed on the figures are confidence intervals (CI), corrected for between-subject variation (18). The magnitude of these error bars directly infers the difference between means (i.e., statistical significance) and not the variance of individual values around the mean.

Results

Run Time to Fatigue

In studies A and B, recovery of running capacity was not significantly improved through ingestion of the CHO-PRO solution in comparison with CHO ingestion alone. During study A, median run times to exhaustion at 85% VO_{2max} (R_2) were 14.5 (7 to 57) min during the CHO trial and 18 (7 to 72) min during the CHO-PRO trial (effect size: 0.3). Median run times observed during R_2 in study B were also similar between trials: 18 (12 to 52) min in the CHO trial and 19.5 (15 to 35) min in the CHO-PRO trial; (effect size: 0.1). A relatively large degree of between-subject variation was noted in terms of time to fatigue; Figure 2 illustrates the individual

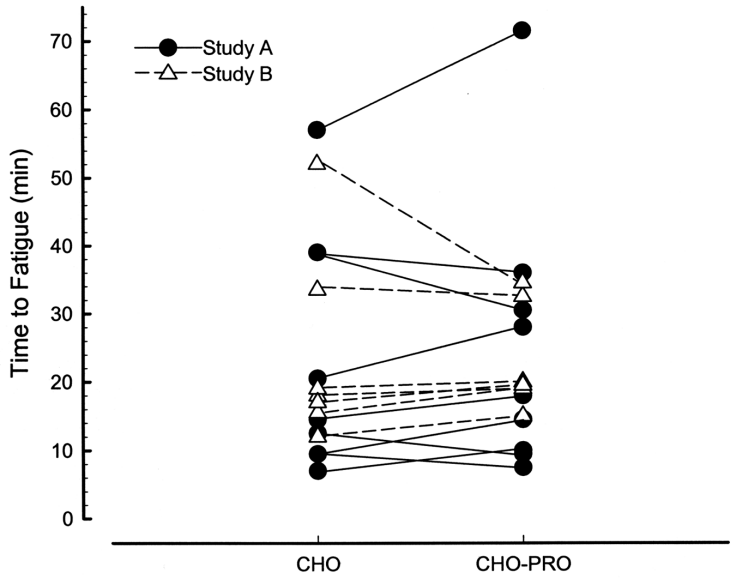

Figure 2 — Run times to exhaustion recorded for each individual during R_2 following ingestion of either CHO or CHO-PRO supplements during recovery.

data recorded during both studies A and B. It is apparent from this figure that, apart from the 2 subjects who ran for longer than 40 min, the difference in time to fatigue between treatments was minimal for most individuals.

Serum Insulin

During study A, serum insulin concentrations were higher during recovery in the CHO-PRO trial than in the CHO trial ($P \leq 0.05$; Figure 3A). When these study A insulin concentrations were converted into an insulinemic response for the

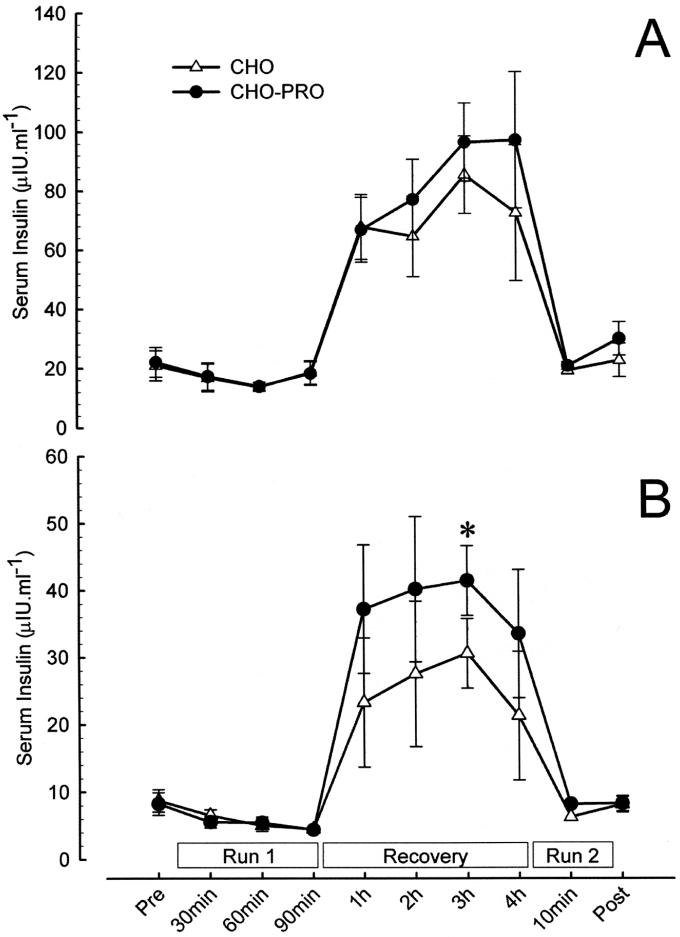

Figure 3 — Serum insulin concentrations during R_1, recovery, and R_2 for studies A and B. Subjects received either CHO or CHO-PRO supplements during recovery. Values are means ± confidence interval. Study A: main effect ($P \leq 0.05$) and study B: *denotes values different between trials ($P \leq 0.05$).

4 h recovery, expressed as incremental area under the curve, this was also higher in the CHO-PRO trial (13.5 ± 1.1 mIU · 240 min^{-1} · mL^{-1}) than in the CHO trial (11.4 ± 0.9 mIU · 240 min^{-1} · mL^{-1}; $P \leq 0.05$). During study B, this insulinemic response for the 4 h recovery period was again higher following the ingestion of CHO-PRO (4.6 ± 0.4 mIU · 240 min^{-1} · mL^{-1}) rather than CHO alone (3.1 ± 0.2 mIU · 240 min^{-1} · mL^{-1}; $P \leq 0.01$) and post-hoc statistical analysis of the insulin concentration data revealed that serum insulin concentrations were significantly higher in the CHO-PRO trial than in the CHO trial after 3 h of recovery ($P \leq 0.05$; Figure 3B).

Plasma Glucose and Urea

Glycemic responses were not different between the CHO and CHO-PRO trials in study A but statistically significant differences between trials were observed during study B ($P \leq 0.05$). In study B, the glycemic response was lower during recovery in the CHO-PRO trial (76.4 ± 25 mmol · 240 min^{-1} · mL^{-1}) than in the CHO trial (237.8 ± 70 mmol · 240 min^{-1} · mL^{-1}). Plasma glucose concentrations after 10 min of running (R_2) in both studies were often below 3.5 mmol/L, suggesting that most subjects were hypoglycemic at this point ($P \leq 0.05$ versus resting values; Figure 4A & B). In study A, plasma urea concentrations were greater during R_2 following CHO-PRO ingestion than following ingestion of CHO ($P \leq 0.05$; Figure 5); there were no differences in urea concentrations between trials in study B.

Plasma FFA and Glycerol

Concentrations of free fatty acids and glycerol were not influenced any differently by the ingestion of CHO-PRO or CHO solutions in either study. Both plasma FFA and glycerol concentrations gradually increased during R_1 in all trials (peaking at approximately 1 mmol/L and 0.5 mmol/L, respectively) before decreasing sharply upon ingestion of the first solution, thereafter remaining close to baseline throughout the recovery period.

Expired Air Data

During study A, the respiratory exchange ratio (RER) tended to be lower during the CHO-PRO trial, although this difference could not be detected statistically ($P = 0.06$; Table 1). Nevertheless, the estimated contributions of fat and carbohydrate oxidation towards overall energy expenditure were not different between the CHO and CHO-PRO trials in either study A or B (Table 1).

Control and Subjective Data

Pre-exercise urine osmolality, changes in body mass, plasma volume, and participants' subjective ratings of thirst were not different between trials in either study. During study A but not during study B, however, subjective ratings of gut fullness were higher following CHO-PRO rather than after CHO ingestion ($P \leq 0.05$). All other relevant variables were not different between the CHO and CHO-PRO trials in either study (Table 2).

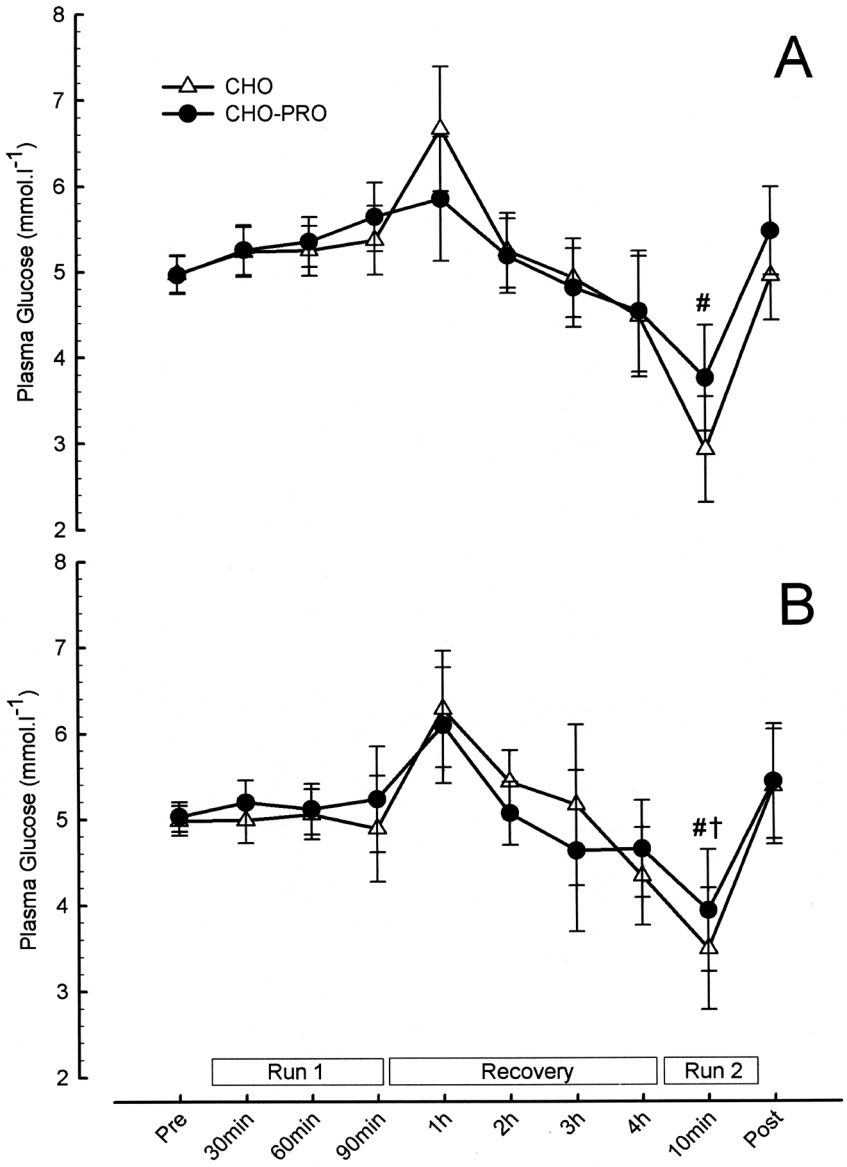

Figure 4 — Plasma glucose concentrations during R_1, recovery, and R_2 for studies A and B. Subjects received either CHO or CHO-PRO supplements during recovery. Values are means ± confidence interval. # denotes values different from baseline CHO trial; † denotes values different from baseline CHO-PRO trial ($P \leq 0.05$).

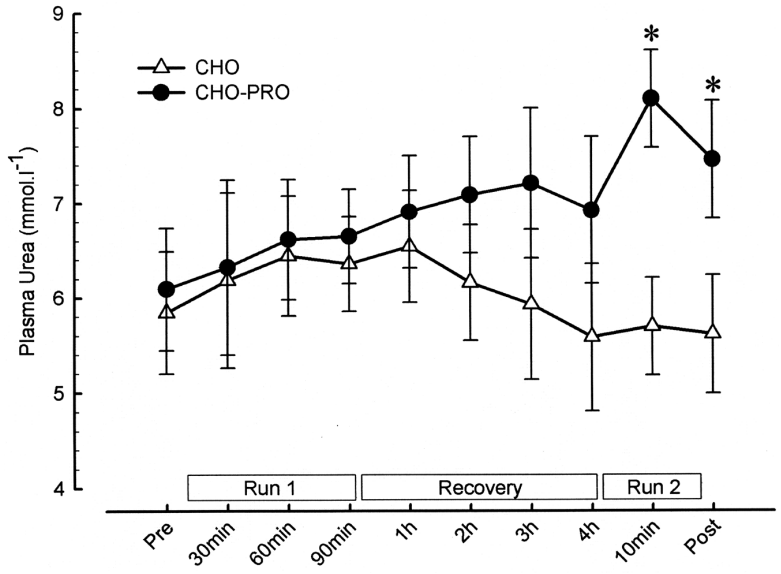

Figure 5 — Plasma urea concentrations during R_1, recovery, and R_2 for study A. Subjects received either CHO or CHO-PRO supplements during recovery. Values are means ± confidence interval. *denotes values different between trials ($P \leq 0.05$).

Discussion

The main finding of both these studies was that there were no differences in recovery of running capacity following ingestion of a carbohydrate-protein mixture or a solution containing carbohydrate alone, regardless of whether that solution provided carbohydrate in moderate or larger amounts. In both studies, however, the insulinemic response was higher during recovery in the CHO-PRO trial than in the CHO trial.

A higher rate of muscle glycogen resynthesis during recovery will result in a larger pre-exercise muscle glycogen store, therefore it is reasonable to assume that this might be reflected in a greater capacity to perform subsequent exercise (3). Previous research has attributed the improved cycling capacity following CHO-PRO rather than CHO ingestion both to an increased rate of muscle glycogen storage during recovery (38) and to an attenuation of exercise-induced muscle damage (25). Given the similarity of run times between trials in the present study, we might conclude that glycogen accumulation occurred at a similar rate in both the CHO and CHO-PRO trials. Since muscle glycogen was not directly measured in these studies, however, it cannot be ruled out that factors other than carbohydrate availability could have contributed to eventual fatigue. Thus, pre-exercise carbohydrate availability might not have dictated time to exhaustion during R_2. Recent evidence using ^{13}C MRS to quantify muscle glycogen has indicated that, when individuals

perform repeated exercise bouts with limited recovery and following an overnight fast, the depletion of liver glycogen stores could contribute to the onset of fatigue (6). In the present studies, however, the blood glucose concentrations following R_1 were maintained at around 5 mmol/L, suggesting that this first run did not severely reduce liver glycogen stores. Furthermore, it is likely that a large quantity of the carbohydrate ingested during recovery would be stored as liver glycogen prior to R_2 (6). Therefore, it is doubtful that fatigue during R_2 occurred as a direct result of decreased availability of liver glycogen. Likewise, while some protein-mediated mechanism could potentially have enhanced the repair of muscle tissue during recovery (25), this is also unlikely given the present design since many of the inflammatory responses to the initial exercise bout would not be expected to occur within just 4 h of recovery (27). Overall, the fact that the first exercise session was not exhaustive might explain why no differences in subsequent exercise capacity were identified in the present studies, especially given that the glycogen remaining in type II muscle fibers following R_1 would undoubtedly contribute to metabolism substantially during the second exercise bout at 85% VO_{2max}. Indeed, had total depletion of endogenous carbohydrate occurred during R_1 this would certainly have increased the reliance on exogenous supplementation for recovery and possibly reveal any beneficial effect of the added protein more clearly.

It also remains a possibility that an accumulation of metabolic by-products within muscle fibers could have contributed to fatigue. The blood lactate values recorded immediately following R_2 were 4.7 ± 0.3 mmol/L in study A and 6.9 ± 0.6 mmol/L for study B (see Table 2 for treatment differences) which might suggest a significant intramuscular accumulation of hydrogen ions, along with other metabolic by-products that are associated with high-intensity activity (e.g., inorganic phosphate and ADP). These metabolites can potentially induce fatigue both through an inhibition of glycolytic enzymes (7, 31) and through interference with excitation-contraction coupling (1, 22). Our results could, therefore, suggest that factors other than carbohydrate availability contributed to fatigue during the treadmill run to exhaustion at 85% VO_{2max}. If fatigue during R_2 was primarily the result of increased anaerobic metabolism rather than compromised substrate availability, then this might question the validity of a capacity test at 85% VO_{2max} as a proxy measure for muscle glycogen availability. There is evidence, however, that an increased pre-exercise muscle glycogen availability does have the potential to improve exercise capacity even at maximal and supramaximal workloads (20, 24) possibly due to an enhancing effect of muscle glycogen either on H^+ buffering capacity or fiber excitability (28). Nonetheless, this evidence arises from protocols in which chronic dietary manipulation was used to induce large differences in muscle glycogen content between trials and the effects of such regimens on exercise capacity at higher intensities have typically been small (20, 24). It has therefore been suggested that a more prolonged capacity test at a lower relative exercise intensity could provide a more valid reflection of pre-exercise muscle glycogen concentrations since fatigue would be more likely to coincide with depletion of these stores (9). In addition, there is evidence that the reliability of an exercise capacity test is compromised at intensities in excess of 80% VO_{2max} (17), which could explain the relatively large variability of run times recorded in the present study while also providing further support for the suggestion that a lower intensity running capacity test might have identified a positive effect of the carbohydrate-protein mixture.

Table 1 Substrate Metabolism and Respiratory Exchange Ratios During Run 1, Recovery, and Run 2

	Run 1				Recovery				Run 2	
	Pre	30 min	60 min	90 min	1 h	2 h	3 h	4 h	10 min	Post
Study A										
Carbohydrate oxidation (g/min)										
CHO	0.3 ± 0.07	3.0 ± 0.36	2.7 ± 0.35	2.8 ± 0.34	0.4 ± 0.05	0.4 ± 0.05	0.5 ± 0.04	0.5 ± 0.04	4.3 ± 0.31	4.8 ± 0.38
CHO-PRO	0.3 ± 0.05	2.9 ± 0.29	2.6 ± 0.27	2.5 ± 0.30	0.4 ± 0.05	0.4 ± 0.05	0.5 ± 0.07	0.5 ± 0.07	4.2 ± 0.56	4.6 ± 0.36
Fat oxidation (g/min)										
CHO	0.1 ± 0.02	0.4 ± 0.09	0.6 ± 0.12	0.6 ± 0.11	0.1 ± 0.02	0.1 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.1 ± 0.03
CHO-PRO	0.1 ± 0.02	0.5 ± 0.07	0.6 ± 0.09	0.7 ± 0.10	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.04	0.2 ± 0.06
RER										
CHO	0.88 ± 0.04	0.90 ± 0.01	0.89 ± 0.02	0.89 ± 0.01	0.94 ± 0.03	0.95 ± 0.03	0.97 ± 0.02	0.98 ± 0.02	1.0 ± 0.02	1.0 ± 0.01
CHO-PRO	0.87 ± 0.03	0.90 ± 0.01	0.89 ± 0.01	0.88 ± 0.01	0.90 ± 0.02	0.90 ± 0.02	0.93 ± 0.02	0.95 ± 0.02	0.99 ± 0.02	0.98 ± 0.01

Study B

Carbohydrate oxidation (g/min)										
CHO	0.3 ± 0.07	3.2 ± 0.17	2.8 ± 0.19	2.5 ± 0.20	3 ± 0.04	0.3 ± 0.04	0.4 ± 0.05	0.5 ± 0.04	4.4 ± 0.29	4.5 ± 0.29
CHO-PRO	0.3 ± 0.03	3.0 ± 0.17	2.8 ± 0.14	2.8 ± 0.21	0.3 ± 0.07	0.4 ± 0.05	0.4 ± 0.04	0.5 ± 0.06	4.7 ± 0.26	4.7 ± 0.28
Fat oxidation (g/min)										
CHO	0.1 ± 0.02	0.3 ± 0.09	0.5 ± 0.06	0.6 ± 0.09	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.04 ± 0.01	0.1 ± 0.07	0.1 ± 0.09
CHO-PRO	0.1 ± 0.02	0.3 ± 0.04	0.5 ± 0.09	0.5 ± 0.04	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.02	0.04 ± 0.02	0.04 ± 0.03	0.03 ± 0.03
RER										
CHO	0.86 ± 0.03	0.95 ± 0.01	0.92 ± 0.01	0.90 ± 0.01	0.86 ± 0.02	0.88 ± 0.01	0.91 ± 0.02	0.96 ± 0.01	1.0 ± 0.02	1.0 ± 0.02
CHO-PRO	0.91 ± 0.04	0.94 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.86 ± 0.03	0.89 ± 0.02	0.91 ± 0.02	0.97 ± 0.02	1.0 ± 0.02	1.0 ± 0.02

Note. Values are means ± standard error of the mean.

Table 2 Percent VO_{2max}, RPE, Heart Rate, and Blood Lactate Responses to Run 1, Recovery, and Run 2

	Run 1				Recovery				Run 2	
	Pre	30 min	60 min	90 min	1 h	2 h	3 h	4 h	10 min	Post
Study A										
%VO_{2max}										
CHO	8.9 ± 0.6	69.9 ± 1.3	70.9 ± 1.4	72.1 ± 1.3	10.3 ± 0.5	9.9 ± 0.6	10.1 ± 0.6	10.2 ± 0.5	80.9 ± 0.9	85.8 ± 2.1
CHO-PRO	9 ± 0.7	71.5 ± 1	71.2 ± 0.7	71.3 ± 1.3	10.5 ± 0.7	10.7 ± 0.5	10.7 ± 0.5	11.5 ± 0.7	85 ± 1.8	85.9 ± 0.8
RPE (6-20)										
CHO	—	12 ± 1	13 ± 1	15 ± 1	—	—	—	—	15 ± 1	19 ± 1
CHO-PRO	—	12 ± 0.5	14 ± 1	15 ± 1	—	—	—	—	15.5 ± 1	18 ± 1
Heart rate (beats/min)										
CHO	67 ± 6	166 ± 4	171 ± 4	172 ± 4	—	—	—	—	178 ± 3	183 ± 2
CHO-PRO	68 ± 7	167 ± 3	169 ± 3	170 ± 4	—	—	—	—	178 ± 2	183 ± 2
Blood lactate (mmol/L)										
CHO	0.6 ± 0.1	1.6 ± 0.3	1.7 ± 0.3	1.6 ± 0.3	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	3.7 ± 0.3	5 ± 0.3
CHO-PRO	0.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.04	3.7 ± 0.7	4.4 ± 0.4

Study B

$\%VO_{2max}$										
CHO	9.4 ± 0.5	70.7 ± 0.9	70.5 ± 0.8	72 ± 0.7	10.5 ± 0.9	10 ± 0.2	10.3 ± 0.5	11 ± 0.2	84.2 ± 1.1	85.3 ± 1.1
CHO-PRO	9.3 ± 0.6	69.9 ± 1.3	71.4 ± 1.4	70.1 ± 0.8	10.5 ± 0.5	10.6 ± 0.6	10.4 ± 0.6	11.8 ± 0.5	85.9 ± 1.7	87.2 ± 1.8
RPE										
CHO	—	11 ± 1	14 ± 0.4	14 ± 1	—	—	—	—	15 ± 1	17 ± 0.3
CHO-PRO	—	13 ± 1	13 ± 1	14 ± 1	—	—	—	—	15 ± 0.3	17 ± 0.4
Heart rate (beats/min)										
CHO	64 ± 4	160 ± 4	164 ± 5	168 ± 5	—	—	—	—	175 ± 4	178 ± 3
CHO-PRO	65 ± 4	159 ± 4	164 ± 3	167 ± 3	—	—	—	—	176 ± 3	181 ± 3
Blood lactate (mmol/L)										
CHO	0.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.2	2.1 ± 0.3	1.9 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	5.6 ± 0.7	6.6 ± 1
CHO-PRO	0.9 ± 0.1	1.9 ± 0.3	2.0 ± 0.4	2.5 ± 0.6	1.8 ± 0.3	1.6 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	6.3 ± 1.1	7.1 ± 0.9

Note. Values are means ± standard error of the mean.

The elevated insulin concentrations following CHO-PRO ingestion compared to CHO ingestion found in the present study are in agreement with some (16, 33-35, 39), but not all (5, 12, 13, 29), studies in this area. It is possible that these inconsistent findings might be due to the differing amounts of protein included in the CHO-PRO mixtures in these studies (10). All those studies reporting an increased insulin concentration following the addition of protein to a carbohydrate solution have included in excess of approximately 0.3 g PRO · kg^{-1} · h^{-1} (16, 33-35, 39), while those studies reporting similar insulin concentrations following ingestion of CHO and CHO-PRO solutions have provided protein in quantities closer to 0.1 g PRO · kg^{-1} · h^{-1} (5, 12, 13, 29). The present results, however, are not consistent with this explanation because increased insulin concentrations were achieved through the addition of only 0.2 g PRO · kg^{-1} · h^{-1} in study A and 0.1 g PRO · kg^{-1} · h^{-1} in study B. Despite this effect on insulin secretion, previous evidence supporting the efficacy of CHO-PRO supplements has involved a far higher dose of protein than that provided in the current investigation. It is therefore entirely possible that ergogenic benefits can only be achieved with these supplements when the carbohydrate-protein ratio is closer to 4:1 (38). In addition, the specific composition of the ingested protein is known to influence the magnitude of the resultant insulin response (36). This could further explain the disparity between the current findings and those of Williams et al. (38) since these authors provided whey protein isolate to their subjects during recovery while in the present study the protein fraction was composed of wheat protein hydrolysate.

Although the insulinemic response during recovery was found to be higher following CHO-PRO ingestion than ingestion of CHO in both studies A and B, the glycemic response during this same period was only different between experimental conditions (i.e., lower with CHO-PRO) in study B. Other investigators have also reported lower blood glucose concentrations following CHO-PRO ingestion compared with the ingestion of CHO alone (12, 33-35, 39). While it is appealing to conclude that, during study B, the lower glycemic response during the CHO-PRO trial was a product of increased glucose uptake, it should be recognized that plasma glucose concentration actually reflects whole body glucose turnover rather than glucose uptake per se. In support of this, Van Hall et al. (34) have reported that the reduced glucose concentrations they observed following ingestion of a CHO-PRO mixture were not the product of an increased rate of leg glucose uptake. Therefore we cannot draw any firm conclusions from the present data regarding glucose uptake following either CHO or CHO-PRO ingestion. The suggestion from other investigators (5, 16, 35) that glucose transport might already be maximal following ingestion of 1.2 g CHO · kg^{-1} · h^{-1} without additional protein cannot be refuted, however, and could explain why differences in glycemic response were observed between the CHO and CHO-PRO trials during recovery in study B (0.8 g CHO · kg^{-1} · h^{-1}) but not in study A (1.2 g CHO · kg^{-1} · h^{-1}).

Another important consideration when evaluating the contrasting findings of studies A and B was the apparent hypoglycemia (nadir of 2.9 mmol/L) experienced by most subjects during R_2 in both studies (Figure 4A and B). These low plasma glucose concentrations tended to be most severe during the CHO trial in study A and might be a product of "rebound" hypoglycemia in response to the combination of high carbohydrate intake and the commencement of exercise. The available literature generally supports the notion that rebound hypoglycemia during the early

stages of exercise is quickly compensated for and, therefore, does not ultimately contribute to fatigue (15, 21). It cannot be ruled out, however, that the metabolic disturbances caused by ingesting the final volume of each solution within 30 min of the exercise capacity test could have masked any potential differences between the two solutions. The improved maintenance of blood glucose in the early minutes of R_2 during the CHO-PRO trial might have been due to an increased hepatic glucose output. In support of this, the higher plasma urea concentrations during R_2 in the CHO-PRO trial of study A could indicate an increased deamination of the exogenously provided amino acids in the liver, thus supplying carbon for synthesis of glucose through gluconeogenesis.

In conclusion, the consumption of CHO-PRO solutions resulted in greater serum insulin concentrations than consumption of a matched amount of CHO alone. This finding was consistent regardless of whether CHO was provided in high (1.2 g CHO $\cdot$ $kg^{-1} \cdot h^{-1}$) or moderate (0.8 g CHO $\cdot$ $kg^{-1} \cdot h^{-1}$) amounts. The glycemic response was only reduced following ingestion of CHO-PRO when moderate amounts of CHO were consumed, possibly due to an increased rate of glucose uptake. In contrast to the findings of Williams et al. (38), who found the addition of protein to a carbohydrate solution ingested during a 4 h recovery to increase cycle time to exhaustion at 85% VO_{2max}, the present results reveal no such improvement in the recovery of endurance running capacity. There are a number of factors which might account for the inconsistent findings between our own study and that of Williams et al. (38), not least are the differences in protein content between the two studies and the frequency of supplement ingestion during recovery. In addition, the enhancing effect of the additional protein might only be manifested when subjects have previously been exercised to fatigue. Overall, these results could suggest that factors other than carbohydrate availability contributed to fatigue during the treadmill run to exhaustion at 85% VO_{2max}.

Acknowledgments

This study received financial support from GlaxoSmithKline.

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