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Journal of Sports Sciences
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713721847

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Online Publication Date: 01 November 2007
To cite this Article: Betts, James, Williams, Clyde, Duffy, Katherine and Gunner, Frances (2007) 'The influence of carbohydrate and protein ingestion during recovery from prolonged exercise on subsequent endurance performance', Journal of Sports Sciences, 25:13, 1449 - 1460
To link to this article: DOI: 10.1080/02640410701213459
URL: http://dx.doi.org/10.1080/02640410701213459

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Journal of Sports Sciences, November 2007; 25(13): 1449 – 1460

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The influence of carbohydrate and protein ingestion during recovery from prolonged exercise on subsequent endurance performance

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(Accepted 11 January 2007)

Abstract
Ingesting carbohydrate plus protein following prolonged exercise may restore exercise capacity more effectively than ingestion of carbohydrate alone. The objective of the present study was to determine whether this potential benefit is a consequence of the protein fraction *per se* or simply due to the additional energy it provides. Six active males participated in three trials, each involving a 90-min treadmill run at 70% maximal oxygen uptake (run 1) followed by a 4-h recovery. At 30-min intervals during recovery, participants ingested solutions containing: (1) 0.8 g carbohydrate · kg body mass $(BM)^{-1} \cdot h^{-1}$ plus 0.3 $g \cdot kg^{-1} \cdot h^{-1}$ of whey protein isolate (CHO-PRO); (2) 0.8 g carbohydrate · kg $BM^{-1} \cdot h^{-1}$ (CHO); or (3) 1.1 g carbohydrate · kg $BM^{-1} \cdot h^{-1}$ (CHO-CHO). The latter two solutions matched the CHO-PRO solution for carbohydrate and for energy, respectively. Following recovery, participants ran to exhaustion at 70% maximal oxygen uptake (run 2). Exercise capacity during run 2 was greater following ingestion of CHO-PRO and CHO-CHO than following ingestion of CHO ($P \leq 0.05$) with no significant difference between the CHO-PRO and CHO-CHO treatments. In conclusion, increasing the energy content of these recovery solutions extended run time to exhaustion, irrespective of whether the additional energy originated from sucrose or whey protein isolate.

Keywords: *Exercise, amino acids, sucrose, insulin, metabolism*

Introduction

The capacity to perform physical exercise of moderate to high intensity is related to muscle glycogen availability at the onset of that exercise (Bergstrom, Hermansen, Hultman, & Saltin, 1967), and fatigue during such activity is often associated with depletion of this substrate (Ahlborg, Bergstrom, Eklund, & Hultman, 1967; Hermansen, Hultman, & Saltin, 1967). Therefore, it is logical to suggest that the rapid replenishment of endogenous carbohydrate stores will constitute a crucial component of recovery. Indeed, many studies have systematically investigated the various nutritional strategies that might facilitate muscle glycogen resynthesis in the hours immediately after prolonged exercise (Ivy, 2001). The culmination of this evidence generally supports the existence of a dose – response relationship between the quantity of carbohydrate consumed following exercise and the rate of muscle glycogen resynthesis (Blom, Hostmark, Vaage, Kardel, & Maehlum, 1987; Jentjens & Jeukendrup, 2003). Nonetheless, the rate of muscle glycogen storage does plateau at higher rates of carbohydrate intake (Ivy, Lee, Brozinick, & Reed, 1988), and ingesting 1.2 g carbohydrate · kg body mass $(BM)^{-1} \cdot h^{-1}$ at 30-min intervals during a 5-h recovery appears to be sufficient to maximize this process (van Loon, Saris, Kruijshoop, & Wagenmakers, 2000).

However, when the amount of carbohydrate ingested is less than that cited above, the rate of muscle glycogen resynthesis can be accelerated through the combined ingestion of protein and carbohydrate (Zawadzki, Yaspelkis, & Ivy, 1992). This is thought to occur via the synergistic influence of protein and carbohydrate on insulin secretion, which may promote both the uptake and storage of glucose by skeletal muscle (Ivy *et al.*, 2002; Zawadzki *et al.*, 1992). However, recent evidence casts doubt over the possibility of increased glucose uptake with protein ingestion (Kaastra *et al.*, 2006). Nonetheless, van Loon and colleagues (2000) have reported increased rates of muscle glycogen resynthesis when protein was added to 0.8 g carbohydrate · kg $BM^{-1} \cdot h^{-1}$, although this study among others suggests that additional protein is no more effective

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ISSN 0264-0414 print/ISSN 1466-447X online © 2007 Taylor & Francis
DOI: 10.1080/02640410701213459

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than either an isoenergetic carbohydrate solution or a quantity of carbohydrate that is sufficient to maximize muscle glycogen storage (Jentjens, van Loon, Mann, Wagenmakers, & Jeukendrup, 2001; Rotman, Slotboom, Kreis, Boesch, Jequier, 2000; Van Hall, Saris, van de Schoor, & Wagenmakers, 2000a; Van Hall, Shirreffs, & Calbet, 2000b; van Loon *et al.*, 2000). Somewhat in contrast, other more recent studies by Ivy *et al.* (2002) and Berardi and colleagues (Berardi, Price, Noreen, & Lemon, 2006) have found that ingestion of carbohydrate–protein mixtures can produce greater rates of muscle glycogen resynthesis even when compared with carbohydrate solutions of matched available energy content.

From a physical performance perspective, exercise capacity is restored more rapidly in the hours following prolonged exercise when carbohydrate is ingested as opposed to a placebo (Fallowfield, Williams, & Singh, 1995). More importantly, Williams and colleagues (Williams, Raven, Fogt, & Ivy, 2003) have demonstrated a further 55% increase in cycle time to exhaustion at 85% maximal oxygen uptake ($\dot{V}O_{2max}$) when a mixture of carbohydrate and protein is ingested during a preceding 4-h recovery rather than a standard 6% carbohydrate solution. However, while this ergogenic benefit is almost certainly due to the 128% greater rate of muscle glycogen storage during recovery in the carbohydrate–protein trial, it is less clear whether the effect can be attributed primarily to the inclusion of protein or simply to the concomitant 167% increase in carbohydrate intake. Interestingly, these same two beverages have been examined in a more recent study in which cycling capacity was impaired by the additional carbohydrate and protein, although a separate contrast against a milk-based carbohydrate–protein mixture did not produce this negative effect (Karp *et al.*, 2006). Regardless of these inconsistencies, the fact remains that neither of the above studies examined the influence of protein on recovery of exercise capacity when all other variables are held constant.

There is clearly some evidence for an ergogenic benefit of combined carbohydrate–protein ingestion during recovery (Williams *et al.*, 2003), yet these results have not yet been verified when using treadmill running and supplements that are matched for either carbohydrate or energy content (Betts *et al.*, 2005; Millard-Stafford *et al.*, 2005). Based upon these findings, we concluded that fatigue during treadmill running at $\geq$85% $\dot{V}O_{2max}$ may not be a direct consequence of compromised carbohydrate availability (Betts *et al.*, 2005). For this reason, the current investigation examined the restoration of treadmill running capacity at a lower relative exercise intensity (i.e. 70% $\dot{V}O_{2max}$) in an attempt to provide a more valid reflection of endogenous carbohydrate availability. Importantly, similar to previous work in this area (van Loon *et al.*, 2000), the carbohydrate–protein supplement in the present study was evaluated both in comparison with a supplement of matched carbohydrate content and a more concentrated carbohydrate solution of matched energy content. We hypothesized that the addition of protein to a carbohydrate recovery solution would improve subsequent exercise capacity relative to the carbohydrate-matched control but no more effectively than an isoenergetic amount of carbohydrate.

Methods

Participants

Six active males participated in this study (mean age 21 years, $s = 3$; body mass 72.6 kg, $s = 8.4$; $\dot{V}O_{2max}$ 61.4 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}, $s = 7.3$). These individuals included a mean 6 h ($s = 2$) per week of endurance running as part of their habitual training. Furthermore, all participants had taken part in similar investigations on previous occasions and therefore were fully familiar with running to the point of volitional exhaustion. Each participant was briefed regarding the nature of the study and provided informed consent in keeping with the requirements of the Loughborough University Ethical Advisory Committee, which approved the study.

Preliminary measurements

Preliminary tests were conducted to determine each participant's sub-maximal and maximal oxygen uptakes (Taylor, Buskirk, & Henschel, 1955) on a motorized treadmill (Technogym, Italy). A subsequent test was then performed 2 weeks before trial 1 to familiarize the participants further with all procedures and also to confirm that calculated running speeds were equivalent to 70% $\dot{V}O_{2max}$. All participants continued their habitual training throughout the study period but refrained from strenuous exercise and avoided both alcohol and caffeine consumption during the 48 h before the main trials.

Experimental design

Participants performed three main trials in a randomized order that were separated by at least one week and applied in a double-blind manner. A dietary record was completed for the 48 h before trial 1 and was then repeated before all subsequent trials [2497 kcal $\cdot$ day^{-1} ($s = 535$); 53% ($s = 9$) carbohydrate, 31% ($s = 9$) fat, 16% ($s = 3$) protein]. The main trials involved a 90-min treadmill run at 70%

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$\dot{V}O_{2max}$ followed by 4 h of recovery and then an exercise capacity test. The 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 ingestion can result in substantially reduced muscle glycogen concentrations within the quadriceps, specifically localized to the type I muscle fibres (Tsintzas, Williams, Boobis, & Greenhaff, 1996). During the recovery period, participants rested in the laboratory while consuming a carbohydrate–protein mixture (CHO-PRO trial), a matched amount of carbohydrate alone (CHO trial) or a solution containing a larger amount of carbohydrate (CHO-CHO trial) that matched the CHO-PRO solution for available energy. After the recovery period, participants were required to complete a treadmill run to exhaustion at 70% $\dot{V}O_{2max}$, an intensity previously used by others to assess the recovery of running capacity following a 4-h recovery (Fallowfield *et al.*, 1995).

Experimental protocol

Each participant arrived at the laboratory between 08:00 and 08:30 h following a 10-h overnight fast. After providing a urine sample, 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. Before exercise, the Douglas bag technique was used to collect a 5-min resting expired gas sample as described previously (Williams & Nute, 1983). Participants were required to stand for 15 min before the collection of all resting gas and blood samples. A 5-min run at 60% $\dot{V}O_{2max}$ was used as a standardized warm-up before running at a speed equivalent to 70% $\dot{V}O_{2max}$ for 90 min (run 1). One-minute expired gas samples, heart rates (Polar 8810, Kempele, Finland), and ratings of perceived exertion (RPE; Borg, 1973) followed by 10-ml venous blood samples were taken at 30-min intervals during run 1. Water intake was permitted *ad libitum* during trial 1 and then matched in subsequent trials (total volume ingested during run $1 = 0.5$ litres, $s = 0.4$ l). Nude body mass was recorded immediately following run 1 to assess fluid loss from pre–post body mass differences (corrected for fluid intake).

The first volume of the prescribed solution was provided as soon as post run 1 nude body mass had been recorded. The remaining seven volumes of the solution were provided at 30-min intervals during the 4-h recovery because this feeding schedule appears to produce the most rapid rates of muscle glycogen resynthesis (van Loon *et al.*, 2000). Therefore, the final volume was ingested 30 min before run 2, with participants being permitted 15 min to consume each solution. Expired gas samples and venous blood samples were collected during the recovery period every hour prior to feedings. Subjective ratings of gut fullness and thirst were recorded at the same time as gas sampling using adapted Borg scales (Borg, 1973) such that the anchor terms on each 6–20 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, participants began the run to exhaustion at 70% $\dot{V}O_{2max}$ (R_2). As for run 1, water intake was provided *ad libitum* during trial 1 and matched in subsequent trials (total volume ingested during run $2 = 0.8$ litres, $s = 0.6$). During the exercise capacity test, physiological measurements were obtained at regular intervals, background music was standardized between trials (Atkinson, Wilson, & Eubank, 2004), and participants were unaware how much time had elapsed. Participants were verbally encouraged to continue exercise and when they first indicated that they could no longer maintain the required intensity, the treadmill speed was reduced to 4.4 $km \cdot h^{-1}$ for 2 min before the exercise test was resumed at the previous intensity (i.e. 70% $\dot{V}O_{2max}$). This process was repeated on the second occasion that participants could not maintain the required exercise intensity and only on the third occasion was a final expired gas collection made, volitional exhaustion accepted, and run time recorded. This method is similar to that previously employed in our laboratory and was intended to enable participants to gauge more accurately their level of fatigue (Wong & Williams, 2000). The post run 2 blood sample was drawn immediately following the point of volitional fatigue (i.e. within 30 s) and nude body mass was recorded within 5 min, 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: 20.6°C ($s = 0.8$) and 42.5% ($s = 7.4$) in the CHO trial, 21.0°C ($s = 1.0$) and 40.6% ($s = 9.3$) in the CHO-PRO trial, and 19.9°C ($s = 1.5$) and 41.5% ($s = 13.4$) in the CHO-CHO trial.

Solution composition

The rate of carbohydrate (sucrose) ingestion in the CHO and CHO-PRO trials was 0.8 g carbohydrate $\cdot$ kg $BM^{-1} \cdot h^{-1}$, whereas the CHO-CHO solution provided 1.1 $g \cdot kg\ BM^{-1} \cdot h^{-1}$ [total carbohydrate intake 232 g ($s = 27$) and 320 g ($s = 37$), respectively]. The CHO-PRO solution contained 3.3% of whey protein isolate in the carbohydrate

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mixture such that total protein intake was equivalent to 87 g ($s = 10$) (equivalent to an ingestion rate of 0.3 g protein $\cdot$ kg BM$^{-1} \cdot$ h^{-1}). All solutions were provided in equal volumes (581 ml $\cdot$ h^{-1}, $s = 67$) and the higher carbohydrate content of the CHO-CHO solution was therefore achieved by increasing the carbohydrate concentration from 10% to 13.3%. The estimated amount of energy that each solution made available for metabolism was 3.2 kcal $\cdot$ kg BM$^{-1} \cdot$ h^{-1} in the CHO trial and 4.3 kcal $\cdot$ kg BM$^{-1} \cdot$ h^{-1} in both the CHO-PRO and CHO-CHO trials [total energy intake 929 kcal ($s = 107$) and 1278 kcal ($s = 147$), respectively). Pre-testing was conducted to ensure that all three solutions were successfully matched for flavour (orange and passion fruit), consistency, and odour; which was further confirmed via an exit interview in which no participant was able to distinguish between the treatments.

Sampling and analysis

Expired gas samples were collected using a Douglas bag (Williams & Nute, 1983) and the fractions of expired oxygen and carbon dioxide were quantified using paramagnetic and infra-red analysers, 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 analysers were calibrated both before and at 2-h intervals during 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-anticoagulant tube where it was left to clot for 45 min at room temperature and then centrifuged at 2000 *g* for 10 min at 4°C (Beckman-Coulter Allegra X-22R, Germany). The serum fraction was stored at −80°C pending later analysis for insulin by radioimmunoassay (Coat-Count Insulin, MP Biomedicals Ltd., USA) using a gamma counter (Cobra 5000, Packard Instruments, USA). The remaining 5 ml of whole blood was transferred to a tube containing the anti-coagulant ethylene diamine tetra-acetic acid (EDTA), from which triplicate 50-μl and 20-μl samples were taken to determine haematocrit (Hct Centrifuge, Hawksley, UK) and haemoglobin concentration, respectively. The latter was measured using a standard cyanomethaemoglobin method (Boehringer Mannheim, Germany) and a spectrophotometer (Shimadzu 1240, Japan). The equations of Dill and Costill (1974) were applied to these haematocrit and haemoglobin values to assess changes in plasma volume throughout the trials. 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 5415c, Germany), and stored at −80°C for later determination of lactate concentration (Maughan, 1982) using a fluorometer (Locarte 8.9, UK). The remaining whole blood was centrifuged at 2000 *g* for 10 min at 4°C (Beckman-Coulter Allegra X-22R, Germany) before plasma was abstracted, stored at −80°C, and later analysed for glucose (Randox, Ireland), free fatty acids (Wako NEFA C, Germany), glycerol (Randox, Ireland), and urea (Randox, Ireland) using an automatic spectrophotometric analyser (Cobas-Mira plus, Roche). Pre-trial urine osmolality was assessed using a cryoscopic osmometer (Gonometer 030, Gonotec, Germany) and adequate hydration was assumed for osmolality values below 900 mOsmol $\cdot$ kg^{-1} (Shirreffs & Maughan, 1998).

Statistical analyses

The exercise capacity data of Fallowfield and colleagues (1995) were used to estimate that a sample size of six would have a 99.9% power to detect a difference in run times of 22.2 min, assuming a standard deviation of differences (s_{diff}) of approximately 9.9 min, using a paired *t*-test with an alpha level of $P \leq 0.05$. Eight participants were therefore recruited in anticipation of the high participant drop-out rate that often occurs with protocols of this nature. Therefore, while the withdrawal of two participants due to gastrointestinal discomfort did not compromise the statistical power of the study, the loss of these individuals did result in the experimental design not being fully counterbalanced (i.e. five of the possible six trial order combinations were applied). Nonetheless, no trial order effects were observed in relation to any dependent variable and, with regard to the exercise capacity data, the mean difference between participants' first and last trials was 0.6 min ($s_{diff} = 10.6$). A two-way general linear model for repeated measures (treatment $\times$ time) was used to identify differences between experimental conditions with the Greenhouse-Geisser correction being applied for epsilon < 0.75 and the Huynh-Feldt correction adopted for less severe asphericity. When significant *F*-values were found, the Holm-Bonferroni step-wise method was adopted to determine the location of variance (Atkinson, 2002).

Statistical analyses were performed using the SPSS for Windows version 14.0 software (Chicago, USA) and all data in the text are reported as means and standard deviations (*s*). The variance bars shown on figures are confidence intervals (CI) that have been corrected for between-participant variation (Masson & Loftus, 2003). The magnitude of these confidence intervals therefore directly infers the difference

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between means at each time point (i.e. statistical significance) and not the variance of individual values around the mean.

Results

Mean run times to exhaustion at 70% $\dot{V}O_{2max}$ (run 2) were 83.7 min ($s = 16.9$) in the CHO trial, 91.2 min ($s = 15.8$) in the CHO-PRO trial, and 99.9 min ($s = 19.9$) in the CHO-CHO trial (treatment: $F = 6.1$, $P = 0.05$). Ingestion of the CHO-PRO solution resulted in a significantly improved restoration of exercise capacity compared with a matched quantity of carbohydrate alone ($P = 0.02$; effect size = 1.5). However, there was no significant difference in time to exhaustion between the CHO-PRO solution and the other carbohydrate solution that was matched for available energy content (effect size = 0.7). Furthermore, the increase in carbohydrate concentration from 10% to 13.3% therefore also significantly extended exercise time ($P = 0.05$; effect size = 1.1). Figure 1 illustrates that this pattern between treatments was consistent for every participant in the present study except for one individual who produced his shortest run time following ingestion of the CHO-CHO solution.

Serum insulin concentrations increased significantly in all three trials at the onset of recovery (Figure 2A). However, the magnitude of this increase varied between trials such that statistical analyses revealed an overall effect of treatment irrespective of time (treatment: $F = 9.8$, $P = 0.05$). Conversion of this insulin concentration data into an insulinaemic response for the entire 4-h recovery, expressed as incremental area under the curve, revealed differences between the CHO and CHO-PRO trials ($P = 0.03$). The mean insulinaemic responses were 28.9 nmol · 240 min^{-1} · l^{-1} ($s = 12.4$) in the CHO trial, 47.0 nmol · 240 min^{-1} · l^{-1} ($s = 18.6$) in the CHO-PRO trial, and 43.9 nmol · 240 min^{-1} · l^{-1} ($s = 22.9$) in the CHO-CHO trial.

Plasma glucose concentrations peaked after 1 h of recovery in all trials but were lower at this point with CHO-PRO ingestion than with CHO-CHO ingestion ($P = 0.01$; Figure 2B). Glucose concentrations then decreased in both the CHO and CHO-CHO trials, while the CHO-PRO trial displayed more stable glucose concentrations throughout the remainder of recovery (treatment × time: $F = 3.7$, $P = 0.03$). Upon starting run 2, blood glucose concentrations decreased markedly in all three trials. However, this hypoglycaemia was more severe in the CHO trial than in the CHO-PRO trial ($P \leq 0.03$). Shortly after ingesting the first aliquot of the CHO-PRO solution, concentrations of plasma urea began to rise (treatment × time: $F = 24.6$, $P = 0.001$) and became significantly higher than in the CHO-CHO trial after 2 h of recovery ($P \leq 0.04$) and higher than the CHO trial after 4 h of recovery ($P \leq 0.01$; Figure 3).

Plasma free fatty acids and glycerol concentrations showed similar responses to all three treatments during run 1 and recovery (Figure 4). Estimations of lipid and carbohydrate oxidation rates via indirect calorimetry were also not significantly different

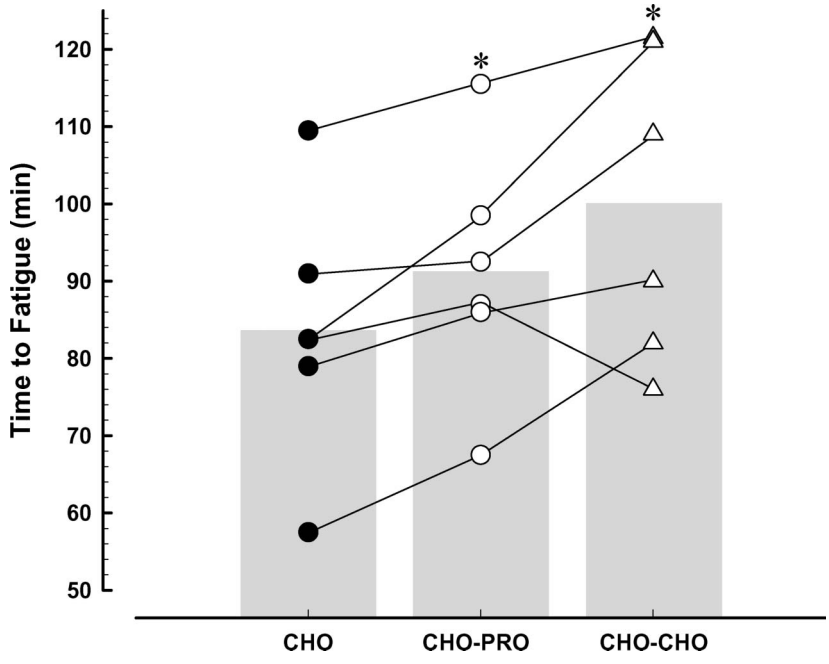

Figure 1. Mean and individual run times to exhaustion during run 2 following ingestion of CHO, CHO-PRO or CHO-CHO supplements during recovery. *Mean values greater than CHO trial ($P \leq 0.05$).

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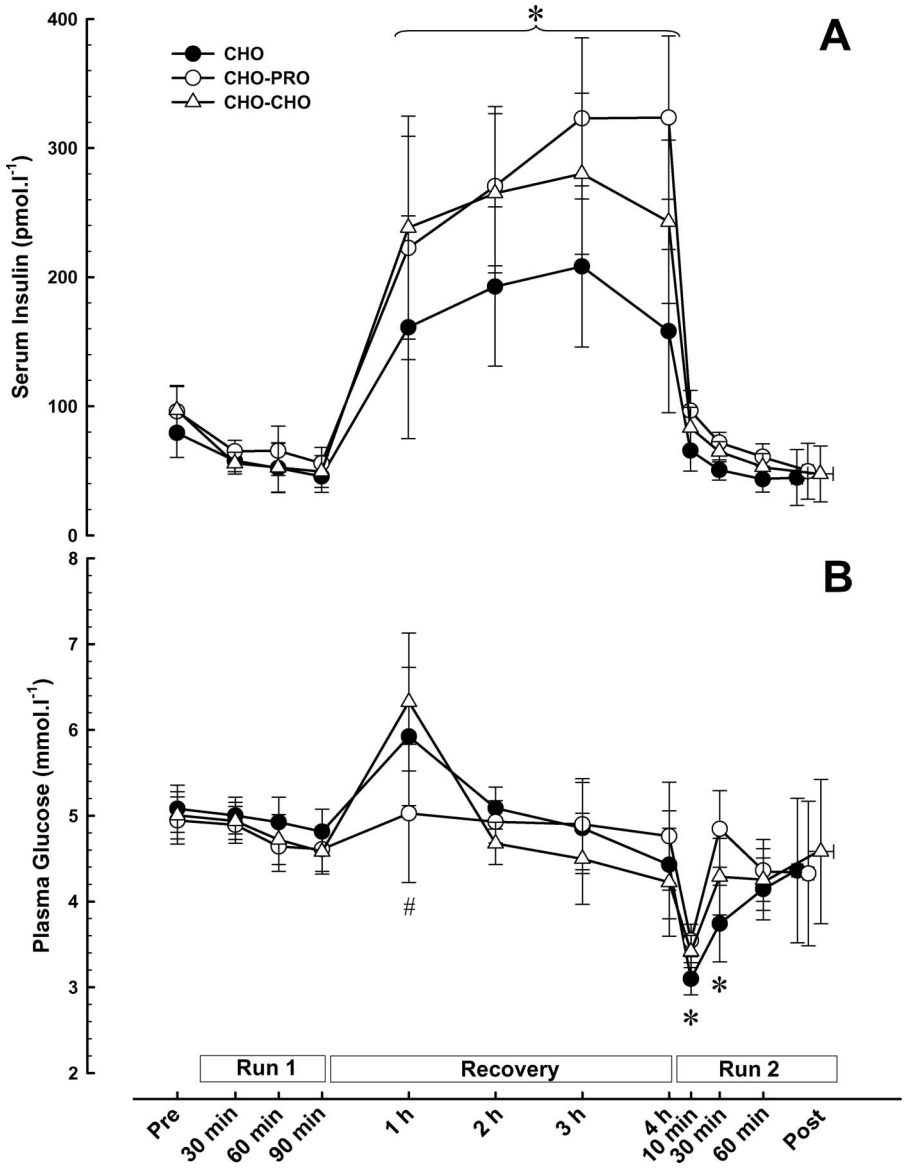

Figure 2. Serum insulin (A) and plasma glucose (B) concentrations during run 1, recovery, and run 2. Participants received CHO, CHO-PRO or CHO-CHO supplements during recovery. Values are means ± confidence intervals. *Difference between CHO and CHO-PRO ($P \leq 0.03$). #Difference between CHO-PRO and CHO-CHO ($P = 0.01$).

between trials, although the respiratory exchange ratio (RER) was lower with CHO-PRO ingestion than with CHO-CHO ingestion at 2, 3, and 4 h of recovery ($P \leq 0.05$; Figure 5).

All measurements used to assess hydration status (i.e. pre-exercise urine osmolality, changes in body mass, plasma volume, and subjective ratings of thirst) were similar across main trials and indicated a moderate degree of dehydration during each exercise session (change in body mass: run $1 = -1.7\%$, $s = 0.9$; run $2 = -1.8\%$, $s = 1.3$). Other variables reflecting the intensity of the exercise sessions are included in Table I; the relative exercise intensity of both run 1 and run 2 was successfully standardized at 70% ($s = 3$) $\dot{V}O_{2max}$ between trials, as reflected by the similar ratings of perceived exertion.

Participants' subjective ratings of gut fullness were similar after ingesting each of the three recovery solutions. However, three of the six participants who completed this investigation reported severe gastrointestinal discomfort during run 2 following ingestion of the CHO-CHO solution. In addition, the data collected from two other participants who were initially recruited was disregarded because they experienced such severe gastrointestinal distress that the exercise capacity test was terminated before a metabolic endpoint could be established. Notably, no such ill effects were reported by any participant in response to either the CHO or CHO-PRO solutions.

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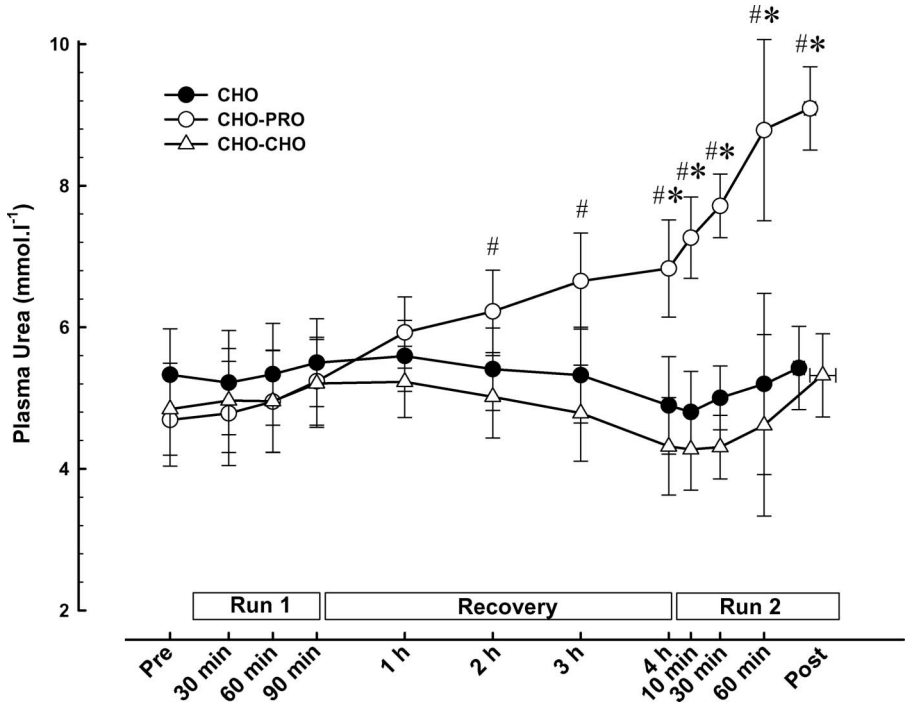

Figure 3. Plasma urea concentrations during run 1, recovery, and run 2. Participants received CHO, CHO-PRO or CHO-CHO supplements during recovery. Values are means ± confidence intervals. *Difference between CHO and CHO-PRO ($P \leq 0.01$). #Difference between CHO-PRO and CHO-CHO ($P \leq 0.04$).

Discussion

The primary finding of this investigation is that running capacity can be restored more completely within 4 h of prior exercise when a mixture of carbohydrate and protein is ingested during recovery rather than the carbohydrate fraction alone. However, there appeared to be no ergogenic benefit of the additional protein *per se* when compared with a more concentrated carbohydrate solution of equal energy content. These findings are consistent with our original hypothesis and, to our knowledge, represent the first evidence of an enhanced restoration of running capacity over a short-term recovery (i.e. <8 h) when such solutions have been matched for carbohydrate content.

Although carbohydrate content was controlled for in the present study, our finding of an increased capacity to perform exercise following carbohydrate–protein ingestion rather than ingestion of carbohydrate alone is also consistent with the findings of Williams and colleagues (2003). In combination with other research regarding muscle glycogen storage following prolonged cycling (Berardi *et al.*, 2006; Ivy *et al.*, 2002; van Loon *et al.*, 2000; Zawadzki *et al.*, 1992), it might at first seem reasonable to suggest that the effect of CHO-PRO on exercise capacity in the present study was the result of differences in muscle glycogen availability before run 2. However, recent evidence does not support any effect of CHO-PRO solutions on plasma glucose disposal during recovery (Kaastra *et al.*, 2006). Furthermore, our observations from a subsequent biopsy study have not revealed any beneficial effects of the additional protein on muscle glycogen availability during a protocol similar to the present one (Betts, Williams, Boobis, & Tsintzas, 2006). This could be due to the fact that the CHO and CHO-PRO solutions were matched for carbohydrate content whereas this was not the case in the study of Williams and colleagues (2003), a factor which is likely to account, at least in part, for the more modest improvement in performance reported in the present study in comparison with that reported previously (Williams *et al.*, 2003). Alternatively, the mechanism through which CHO-PRO facilitates the restoration of exercise capacity may differ according to the precise mode of exercise (i.e. running vs. cycling) that is performed prior to recovery.

Importantly, the differences in exercise capacity between the CHO and CHO-CHO solutions clearly demonstrate that any bias towards a carbohydrate-protein mixture in terms of carbohydrate content would be expected to prolong exercise capacity irrespective of the additional protein. It should be noted, however, that not all studies support the suggestion that an increase in carbohydrate intake during recovery will facilitate the restoration of exercise capacity (Fallowfield & Williams, 1997; Wong & Williams, 2000). The finding of similar

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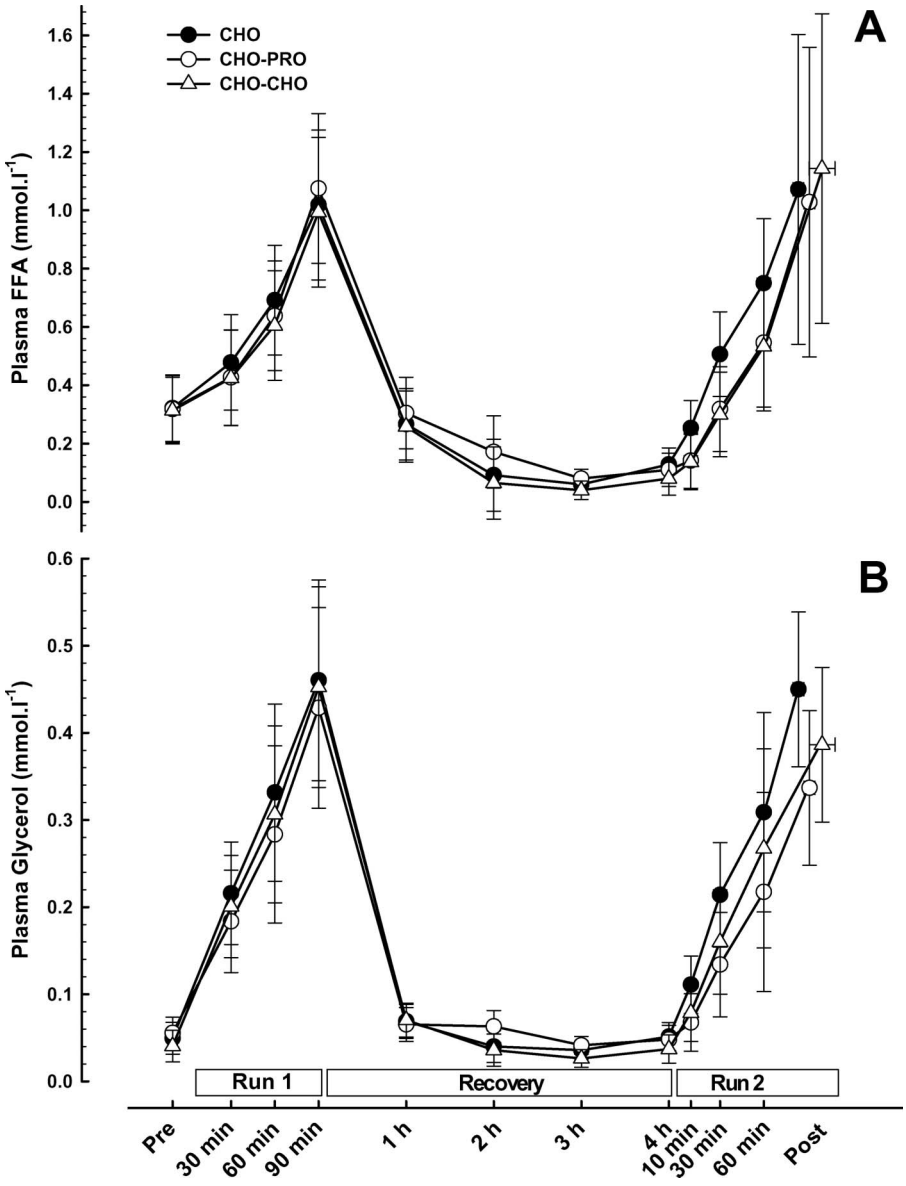

Figure 4. Plasma free fatty acids (A) and glycerol (B) concentrations during run 1, recovery, and run 2. Participants received CHO, CHO-PRO or CHO-CHO supplements during recovery. Values are means ± confidence intervals.

run times to exhaustion by Wong and Williams (2000) following ingestion of either 0.2 or 0.5 g carbohydrate · kg $BM^{-1} \cdot h^{-1}$ is of particular interest in view of a subsequent investigation by Tsintzas and colleagues (2003). This latter study confirmed that an increase in carbohydrate intake of the magnitude stated above would indeed increase muscle glycogen storage during recovery but without increasing the glycogen degradation rate during subsequent exercise. While this finding clearly provides a mechanism for the ergogenic effect of the CHO-CHO supplement in the present study, it also makes it difficult to explain why this dose-dependent effect of carbohydrate intake on exercise capacity was not identified in earlier investigations (Fallowfield & Williams, 1997; Wong & Williams, 2000). However, one potential explanation might be related to the characteristics of the participants that were recruited for these studies. Specifically, in both the investigations cited above, the blood lactate concentrations during the exercise test at 70% $\dot{V}O_{2max}$ were approximately twice as high as those reported in the present study. When combined with the fact that participants in the present study were younger and had higher $\dot{V}O_{2max}$ values, these data appear to be consistent with the interpretation that more aerobically trained individuals who are fully familiar with exercise capacity testing may be required to detect small but worthwhile intervention effects (Hopkins, Schabort, & Hawley, 2001).

Further to the above discussion regarding the proposed relationships between carbohydrate

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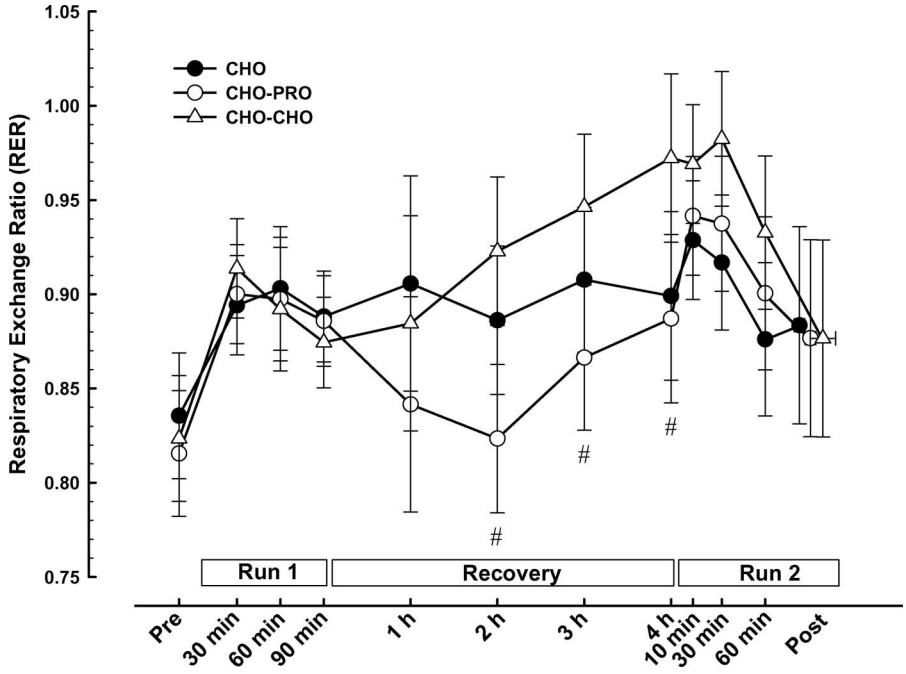

Figure 5. Respiratory exchange ratios during run 1, recovery, and run 2. Participants received CHO, CHO-PRO or CHO-CHO supplements during recovery. Values are means ± confidence intervals. #Difference between CHO-PRO and CHO-CHO ($P \leq 0.05$).

Table I. % $\dot{V}O_{2max}$, RPE, heart rate & blood lactate responses to run 1 and run 2 (mean ± *s*).

		Run 1			Run 2			
	Pre	30 min	60 min	90 min	10 min	30 min	60 min	Last minute
%$\dot{V}O_{2max}$								
CHO	8.7 ± 1.2	69.6 ± 2.6	70.5 ± 2.6	69.9 ± 1.6	69.6 ± 1.7	70.7 ± 1.4	71.9 ± 2.9	74.9 ± 2.4
CHO-PRO	8.7 ± 1.0	69.7 ± 3.2	69.9 ± 3.9	71.0 ± 2.2	70.6 ± 2.6	72.3 ± 1.9	73.1 ± 4.1	73.6 ± 6.2
CHO-CHO	8.3 ± 0.5	69.4 ± 3.4	69.2 ± 3.5	69.1 ± 2.9	68.3 ± 3.1	69.6 ± 2.7	69.2 ± 3.8	69.9 ± 6.0
RPE (6–20)								
CHO	–	11 ± 1	12 ± 1	13 ± 2	11 ± 1	13 ± 2	14 ± 2	18 ± 2
CHO-PRO	–	11 ± 2	12 ± 1	13 ± 1	11 ± 2	13 ± 2	14 ± 2	17 ± 1
CHO-CHO	–	11 ± 1	12 ± 1	13 ± 2	11 ± 1	12 ± 1	14 ± 2	18 ± 3
Heart rate (beats · min^{-1})								
CHO	66 ± 13	166 ± 6	172 ± 6	172 ± 5	168 ± 9	172 ± 6	175 ± 5	177 ± 8
CHO-PRO	67 ± 16	166 ± 7	170 ± 10	172 ± 11	169 ± 11	173 ± 8	174 ± 9	174 ± 10
CHO-CHO	67 ± 14	164 ± 8	167 ± 9	168 ± 9	168 ± 9	171 ± 9	173 ± 12	173 ± 10
Blood lactate concentration (mmol · l^{-1})								Post
CHO	0.8 ± 0.3	1.6 ± 0.6	1.5 ± 0.5	1.6 ± 0.7	1.6 ± 0.9	2 ± 0.8	1.5 ± 0.5	1.8 ± 0.5
CHO-PRO	0.9 ± 0.3	1.7 ± 0.6	1.6 ± 0.6	1.5 ± 0.6	1.5 ± 0.6	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.4
CHO-CHO	0.8 ± 0.3	1.5 ± 0.5	1.4 ± 0.4	1.3 ± 0.3	1.6 ± 0.7	1.6 ± 0.4	1.4 ± 0.4	1.5 ± 0.4

ingestion, muscle glycogen availability, and exercise capacity, our observation of higher plasma glucose concentrations during run 2 following CHO-PRO ingestion may reflect an increased appearance of glucose either from the gastrointestinal tract or from the liver. In support of the former suggestion, the additional energy in the form of protein may have delayed the rate of gastric emptying (Maughan, Leiper, & Vist, 2004) such that exogenous glucose was still appearing into the circulation at the start of run 2. If this were the case, then it is possible that the final CHO-PRO feeding during recovery may have served as a pre-exercise dose of carbohydrate that was available to support metabolism during subsequent exercise.

With regard to the possibility of increased hepatic glucose output during run 2, the elevated concentrations of plasma urea in the CHO-PRO trial could indicate an increased availability of α-keto acids for gluconeogenesis. This explanation would certainly

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be consistent with the treatment differences in RER during recovery despite unchanged plasma free fatty acids and glycerol concentrations. This is because catabolism of the ingested amino acids would be expected to artificially reduce the RER, especially when newly synthesized glucose is stored rather than oxidized (Jequier, Acheson, & Schutz, 1987). Indeed, it is likely that a substantial quantity of glucose would have been retained by the liver during recovery in all three trials, particularly given both the relative hyperinsulinaemia during this period (Kaastra *et al.*, 2006; Pencek *et al.*, 2004) and the fact that participants had fasted overnight before each trial. Evidence from rodent studies certainly supports the preferential resynthesis of liver glycogen following prolonged exercise (Terjung, Baldwin, Winder, & Holloszy, 1974) and it appears that ingestion of whey protein may stimulate this process (Morifuji, Sakai, Sanbongi, & Sugiura, 2005a; Morifuji, Sakai, & Sugiura, 2005b). It therefore remains a possibility that the differences in exercise capacity observed in the present study may have been due to an increased availability of blood glucose during run 2. However, recent evidence indicates that such a mechanism can operate independently of changes in total carbohydrate oxidation rate and need not be associated simply with the avoidance of hypoglycaemia (Claassen *et al.*, 2005).

In addition to the various peripheral mechanisms of fatigue that have been discussed, it cannot be ruled out that fatigue during run 2 may have coincided not only with compromised substrate availability but also with an increased perception of fatigue originating from the central nervous system (CNS). There is some evidence to suggest that a solution containing protein might exert an ergogenic benefit via some interaction of the ingested amino acids with the CNS (Mittleman, Ricci, & Bailey, 1998) and such an explanation would certainly be in line with reports from other authors showing that the addition of only $\approx$0.15 g amino acids $\cdot$ kg $BM^{-1} \cdot h^{-1}$ to a carbohydrate supplement can enhance performance above carbohydrate alone even when ingested *during* exercise (Ivy, Res, Sprague, & Widzer, 2003; Saunders, Kane, & Todd, 2004). However, a more recent study in which $\approx$0.26 g protein $\cdot$ kg $BM^{-1} \cdot h^{-1}$ was ingested along with carbohydrate did not replicate the above findings (van Essen & Gibala, 2006). It therefore remains debatable whether such effects can be attributed entirely to the protein fraction *per se*, particularly given that ingesting $\approx$0.09 g amino acids $\cdot$ kg $BM^{-1} \cdot h^{-1}$ without carbohydrate has not been found to consistently produce comparable effects on exercise capacity (Watson, Shirreffs, & Maughan, 2004). Nonetheless, it should be noted that a large inter-individual variation was reported in this latter study and the possibility therefore remains that carbohydrate–protein ingestion might improve the central drive for exercise, at least in certain individuals. Should this be the case, then additional research will be necessary to establish whether fatigue could be further postponed through the inclusion of protein in the CHO-CHO solution, even though muscle glycogen storage would not be expected to vary between these treatments (Jentjens *et al.*, 2001; Rotman *et al.*, 2000; Van Hall *et al.*, 2000a, 2000b; van Loon *et al.*, 2000).

In conclusion, these results provide support for the previously unconfirmed hypothesis that running capacity can be restored more effectively following prolonged exercise when a mixture of carbohydrate and protein is ingested during recovery rather than when ingesting the carbohydrate fraction alone. However, the inclusion of protein in the solution was no more beneficial than when ingesting a more concentrated carbohydrate solution of equivalent energy content. This study provides further insight into the practical benefits of varying the energy and macronutrient content of recovery supplements, although the primary mechanism through which fatigue was postponed remains unclear and therefore warrants further investigation.

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