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Human Kinetics
ORIGINAL RESEARCH

Carbohydrate and Protein Co-Ingestion Postexercise Does Not Improve Next-Day Performance in Trained Cyclists

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Supplementing postexercise carbohydrate (CHO) intake with protein has been suggested to enhance recovery from endurance exercise. The aim of this study was to investigate whether adding protein to the recovery drink can improve 24-hr recovery when CHO intake is suboptimal. In a double-blind crossover design, 12 trained men performed three 2-day trials consisting of constant-load exercise to reduce glycogen on Day 1, followed by ingestion of a CHO drink (1.2 $g \cdot kg^{-1} \cdot 2\ hr^{-1}$) either without or with added whey protein concentrate (CHO + PRO) or whey protein hydrolysate (CHO + PROH) (0.3 $g \cdot kg^{-1} \cdot 2\ hr^{-1}$). Arterialized blood glucose and insulin responses were analyzed for 2 hr postingestion. Time-trial performance was measured the next day after another bout of glycogen-reducing exercise. The 30-min time-trial performance did not differ between the three trials ($M \pm SD$, 401 ± 75, 411 ± 80, 404 ± 58 kJ in CHO, CHO + PRO, and CHO + PROH, respectively, $p = .83$). No significant differences were found in glucose disposal (area under the curve [AUC]) between the postexercise conditions (364 ± 107, 341 ± 76, and 330 ± 147, $mmol \cdot L^{-1} \cdot 2\ hr^{-1}$, respectively). Insulin AUC was lower in CHO (18.1 ± 7.7 $nmol \cdot L^{-1} \cdot 2\ hr^{-1}$) compared with CHO + PRO and CHO + PROH (24.6 ± 12.4 vs. 24.5 ± 10.6, $p = .036$ and .015). No difference in insulin AUC was found between CHO + PRO and CHO + PROH. Despite a higher acute insulin response, adding protein to a CHO-based recovery drink after a prolonged, high-intensity exercise bout did not change next-day exercise capacity when overall 24-hr macronutrient and caloric intake was controlled.

Keywords: endurance, glycogen, recovery

The relationship between postexercise carbohydrate (CHO) intake and subsequent endurance performance is well established, but equivocal evidence exists of the role of protein in the restoration of whole-body glycogen stores (Alghannam et al., 2018). Several studies have suggested that providing protein together with optimal CHO (1.2 $g \cdot kg^{-1} \cdot hr^{-1}$) does not further enhance short-term (<8 hr) glycogen restoration (Margolis et al., 2021). However, in endurance athletes' habitual eating practices, postexercise CHO intake often remains inadequate (Burke et al., 2003; Heikura et al., 2019; Keay et al., 2018); therefore, optimizing recovery meal composition may help to improve glycogen replenishment. Although dietary CHO provides the major source for glycogen resynthesis (Blom et al., 1987; Costill et al., 1981), addition of protein to the postexercise recovery meal has been shown to augment short-term glycogen replenishment when CHO intake is inadequate (Ivy et al., 2002; van Loon et al., 2000). Protein may augment glycogen restoration by stimulating glucose uptake through enhanced membrane permeability both via insulin-dependent (Hirshman et al., 1990; Ivy & Kuo, 1998) and insulin-independent (Nishitani et al., 2002) mechanisms. To the authors' knowledge, no evidence exists on how immediate ingestion of protein compared with delayed protein affects glycogen restoration over a 24-hr period when CHO intake in the acute recovery phase is inadequate for optimal glycogen resynthesis (single serving of 1.2 g/kg for 2 hr, resulting in 0.6 $g \cdot kg^{-1} \cdot hr^{-1}$).

In addition to the timing of protein intake, improving the composition of the postexercise meal may include adding protein hydrolysates, some of which have been shown to possess insulinotropic potential greater than intact proteins and/or to act directly on muscle signaling, enhancing glycogen restoration (Manninen, 2009; van Loon, 2007). To assess whether protein hydrolysates provide additional benefits to intact proteins, they should be compared with their mother protein rather than in isolation, which has generally not been practiced in the context of glycogen resynthesis (Craven et al., 2021).

To apply the results of a postexercise feeding trial to real-world situations, a realistic training load is required to retain ecological validity (Priego Quesada et al., 2018). Amateur endurance athletes training once a day are unlikely to fully deplete and replete their endogenous CHO stores daily; therefore, an ecologically valid study design should simulate typical training of this population rather than extreme depletion protocols followed by short-term performance testing.

The primary aim of this double-blind crossover study was to investigate whether providing protein with CHO (1.2 $g \cdot kg^{-1} \cdot 2\ hr^{-1}$,

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inadequate for optimal glycogen repletion; Alghannam et al., 2018) in the early phase of recovery would help to restore next-day exercise capacity. This was achieved by comparing a CHO-only drink with two CHO+protein (one intact whey protein concentrate [CHO + PRO], one hydrolysate [CHO + PROH]) drinks after cycling exercise, designed to deplete most but not all available endogenous glycogen stores when total 24-hr macronutrient and energy intakes were standardized between the conditions. The primary outcome was next-day time-trial (TT) performance, and secondary outcomes were glucose and insulin responses to the postexercise drinks. We hypothesized that immediate protein supplementation postexercise would improve TT performance the next day due to increased glycogen resynthesis in the early phase of recovery, which would be reflected in a smaller glucose and a greater insulin response to the CHO + PRO and CHO + PROH drinks relative to the CHO-only drink.

Methods

Participants

In compliance with the Declaration of Helsinki, all procedures were undertaken following approval of the Faculty of Education and Health Sciences Research Ethics Committee of the University of Limerick (2017_03_19_EHS) and participants' written informed consent. Fourteen experienced male cyclists (age: 37 ± 6 years, body mass [BM]: 75 ± 8 kg, maximal oxygen consumption [$\dot{V}O_2$ max : 62.5 ± 5.9 ml · kg^{-1} · min^{-1}) were recruited from local cycling and triathlon clubs. The criteria for inclusion were at least 2 years of cycling training (≥3 hr/week). The cyclists included competitive road cyclists (categories A4 to A2, $n = 3$; ultra-cycling, $n = 1$), national-level triathletes ($n = 3$), recreational triathletes ($n = 4$), and recreational cyclists ($n = 3$). Two participants withdrew from the study, one due to an unrelated injury and one due to illness. Data from 12 participants who completed all trials were used for analysis.

Study Design

The study was a double-blinded crossover design with each participant performing three 2-day trials in randomized order. Block randomization was performed by automatic random generation of trial order. In each trial, either a CHO (maltodextrin, 1.2 g·kg^{-1}·2 hr^{-1}), CHO + PRO (1.2 g·kg^{-1}· 2hr^{-1}+0.3 g·kg^{-1}· 2 hr^{-1}), or CHO + PROH (1.2 g·kg^{-1}·2 hr^{-1}+0.3 g·kg^{-1}·2 hr^{-1}) drink was provided in a single serving postexercise on Day 1 before 2 hr of postexercise monitoring, resulting in an overall CHO provision of 0.6 kg/hr. Dietary intake was standardized based on subjects' habitual food preferences and scaled to BM for each participant 3 days before and on experimental days. On Day 1 of each trial, after glycogen-lowering exercise, participants received a CHO-based drink, three main meals, and two snacks to consume within the next 24 hr (50 kcal·kg^{-1}·24 hr^{-1} composed of 6 g·kg^{-1}·24 hr^{-1}CHO, 2 g·kg^{-1}·24 hr^{-1} protein, and 2 g·kg^{-1}·24 hr^{-1} fat, including the CHO-based drink). Protein content of meals was adjusted in the CHO-only trial to achieve the same 24-hr protein intake as in CHO + PRO and CHO + PROH (Table 1). The 24-hr standardized diet included breakfast to be consumed 3 hr before the exercise protocol on Day 2 of each trial. A schematic representation of the study is shown in Figure 1.

Standardization of Dietary Intake and Training Before Trials

Prior to the experimental phase, participants were asked to record their habitual dietary intake and training using a 7-day weighed food diary and training log. A registered dietitian and sports and exercise nutritionist prescribed a 3-day meal plan to be followed by participants 3 days before each trial. This standardized meal plan was based on the habitual food record of the participants (Kozior et al., 2019) and provided ($M \pm SD$) 46 ± 10 kcal·kg^{-1}·day^{-1} energy intake composed of 5.6 ± 0.7, 2.0 ± 0.6, and 1.8 ± 0.6 g·kg^{-1}·day^{-1} of CHO, protein, and fat, respectively. In addition, participants were

Table 1 Breakdown and Total Nutrient Intake With Provided Meals During 24-hr Postexercise Phase

Meals	Energy (kcal/kg)	PRO (g/kg)	CHO (g/kg)	Fat (g/kg)
Nutrient intake with eating occasions during 24 hr postexercise for CHO + PRO and CHO + PROH trials				
Postexercise drink	6	0.33	1.2	0
Lunch Day 1	10	0.33	1.2	0.4
Snack 1 Day 1	7	0.33	0.6	0.4
Dinner Day 1	10	0.33	1.2	0.4
Snack 2 Day 1	7	0.33	0.6	0.4
Breakfast Day 2	10	0.33	1.2	0.4
Total	50	2.0	6.0	2.0
Nutrient intake with eating occasions during 24 hr postexercise for CHO trial only				
Postexercise drink	4.8	0	1.2	0
Lunch Day 1	10	0.4	1.2	0.4
Snack 1 Day 1	7.6	0.4	0.6	0.4
Dinner Day 1	10	0.4	1.2	0.4
Snack 2 Day 1	7.6	0.4	0.6	0.4
Breakfast Day 2	10	0.4	1.2	0.4
Total	50	2.0	6.0	2.0

Notes. CHO = carbohydrate; PRO = whey protein concentrate; PROH = whey protein hydrolysate.

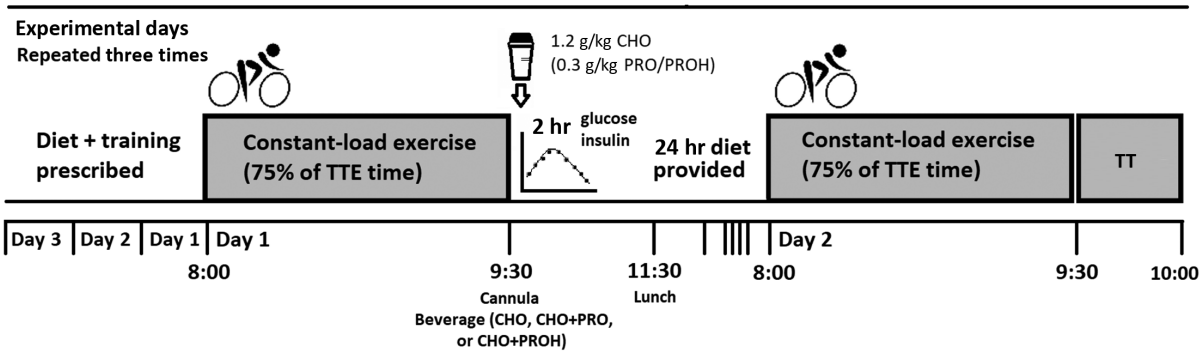

Figure 1 — A schematic representation of the study design. Order of the experimental 2-day visits, each of which included a different postexercise recovery drink (CHO, CHO + PRO, or CHO + PROH), was randomized. CHO = carbohydrate; PRO = whey protein concentrate; PROH = whey protein hydrolysate; TTE = time-to-exhaustion; TT = time trial.

instructed to refrain from alcohol consumption and high-intensity exercise but to maintain their regular activity of daily living for 3 days prior to each trial. Three hours prior to the test on Day 1, participants consumed their prescribed 8 ± 2 kcal/kg BM breakfast composed of 1.1 ± 0.1, 0.3 ± 0.1, and 0.3 ± 0.2 g/kg BM of CHO, protein, and fat, respectively, and refrained from caffeine consumption. Compliance with the dietary standardization protocol pretrials was confirmed before each test by the registered dietitian/registered sports and exercise nutritionist using a short questionnaire.

Laboratory Procedures

Laboratory tests were undertaken in an air-conditioned laboratory maintained at 19–21 °C. SRM cycle ergometers (SRM, Jülich, Germany) measured and controlled cycle power output. Ergometer settings were maintained constant throughout for each subject. Respiratory gas exchange was measured using the Ultima™ CardiO2® breath-by-breath analysis system (MGC Diagnostics, Saint Paul, MN) volume (3.0-L syringe) gas (21% O_2; 15% O_2 + 5% CO_2) calibrated according to the manufacturer's instructions. Gas calibration was repeated every 15 min during the prolonged exercise sessions. Heart rate was measured using a Polar RS800 monitor (Polar Electro, Kempele, Finland).

Submaximal and $\dot{V}O_2$max Test

The $\dot{V}O_2$max test was conducted in two phases. A submaximal step test was performed using 3-min stages and 25 W increments to get an estimation of the highest metabolic steady state. The test was terminated when RER exceeded 1.00 for 60 s or when blood lactate exceeded 4.0 mmol/L. The last 60 s of each stage in the submaximal test (until RER = 1.00) was averaged and a $\dot{V}O_2$-power plot created. After a 7-min recovery and a 5-min warm-up, a step test with 1-min stages and 40 W increments was performed to determine $\dot{V}O_2$max. The $\dot{V}O_2$max was defined as the highest 30-s rolling average of breath-by-breath $\dot{V}O_2$. Maximal aerobic power was extrapolated at $\dot{V}O_2$max from the submaximal step test using the equation obtained from linear regression of the $\dot{V}O_2$-power plot.

TTE Test

Participants undertook a time-to-exhaustion (TTE) test at a constant power output corresponding to 70–80% $\dot{V}O_2$max designed to induce volitional exhaustion between 1 and 2.5 hr of exercise pedaling at their preferred cadence (93 ± 7 revolutions/minute). Gas exchange data were collected during Minutes 6–10 inclusive of each 15-min epoch. RPE values (Borg, 1982) and capillary blood glucose and lactate samples were obtained during the last 30 s of every 30 min. About 150 ml of water with added electrolytes (Elete Electrolyte Add-In®, Mineral Resources International, Ogden, UT) was provided every 15 min. The test ended when the participant was unable to maintain the required power output despite verbal encouragement.

TT Familiarization

Two 30-min TT baseline trials were performed to familiarize the subjects with the feeling of giving a maximal effort in the isokinetic mode of the SRM ergometer, to establish the baseline work capacity in the nondepleted state, and to get an estimate of TT reliability. The cadence was fixed based on individual preference and repeated in the second trial. The familiarization trials were completed a minimum of 72 hr before the beginning of the experimental trials.

Day 1: Glycogen Depleting Exercise

Each glycogen depletion–repletion trial was conducted as follows: on Day 1, the participants completed a glycogen-reducing exercise session in which they cycled for 75% of achieved time in the TTE trial at the same fixed power output. Gas exchange data were collected as per the TTE test. RPE values and fingerprick blood samples for glucose and lactate were obtained every 30 min. After exercise, an antegrade cannula was inserted into a dorsal hand vein and the hand inserted to a heating box kept at 55 °C to arterialize the venous drainage of the hand 5 min before the first blood sample (Zello et al., 1990). A baseline blood sample was drawn before drinks were provided. Arterialized venous blood samples were drawn every 15 min up to 120 min after consuming the test drink. A standardized meal was provided at 120 min. After the meal, participants left the laboratory with the remaining prescribed meals provided for the next 24 hr. Participants were informed about the recommended time for meal consumption and required minimum water intake before the visit on Day 2.

Day 2: Glycogen Depleting Exercise and TT

The athletes returned to the laboratory for Day 2 at the same time as Day 1. The test consisted of the same glycogen-reducing exercise session (75% TTE) as on Day 1, followed immediately by a 30-min

set TT in which the subjects were instructed to attempt to achieve the highest possible average power output. RPE values were recorded at 5, 15, 25, and 30 min. Encouragement was given in a standardized manner for each participant, and they were informed only about remaining time. A 3-min gas exchange sample was recorded between Minutes 17 and 20.

Biochemical Assays

Plasma glucose was analyzed using a glucose assay colorimetric kit (STA-60; Cell Biolabs, Inc., San Diego, CA) and a high throughput microplate reader (Biotek Synergy HT, Winooski, VT) following the providers' instructions. Samples were determined by duplicate, and results are expressed as the $M \pm$ SEM. The intraassay and interassay coefficients of variation (CVs) were 2.7% and 3.3%, respectively.

Plasma levels of insulin were determined using Milliplex maximal aerobic power human metabolic hormone magnetic bead panel kit (Merck Millipore, Cork, Ireland) following the manufacturer's instructions. Bead readings were performed using a MAGPIXTM Multiplex reader (Luminex Corporation, Austin, TX). Intraassay and interassay CVs were 3.6% and 7.8%, respectively. Samples were determined by duplicate, and results are expressed as the $M \pm$ SEM.

Calculations

Rate and cumulative total substrate oxidation in the constant-load exercise tests were calculated from the breath-by-breath gas exchange data using the following equations (Jeukendrup & Wallis, 2005) with the assumption that energy from protein was negligible (nonprotein RER):

$$\text{Energy expenditure (in kilocalorie per minute)} = 0.550 \cdot \dot{V}CO_2 + 4.471 \cdot \dot{V}O_2. \quad (1)$$

$$\text{CHO oxidation (in gram per minute)} = 4.210 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2 - 0.4\ \text{UN}. \quad (2)$$

$$\text{fat oxidation (in gram per minute)} = 1.695 \cdot \dot{V}CO_2 - 1.701 \cdot \dot{V}O_2. \quad (3)$$

UN represents urinary nitrogen and is neglected in our calculations.

Cumulative total energy expenditure (tEE), CHO (tCHO), and fat (tFAT) oxidation was calculated as the mean multiplied by time (in minutes).

The %CHO oxidized was calculated from tEE assuming that 80% of the CHO oxidized was glycogen (4.15 per gram) and 20% was glucose (3.74 kcal/g), thus an average CHO energy density of 4.068 kcal/g (Jeukendrup & Wallis, 2005).

Data Analysis

Data were assessed for normality using the Shapiro–Wilk test. A repeated-measures analysis of variance was conducted to detect effects between the trials. The alpha level was set to .05 with the Bonferroni correction applied. Sphericity was assessed by Mauchly's sphericity test, and if violated, the Greenhouse–Geisser correction was applied.

All data are reported as the $M \pm SD$ unless stated otherwise. The coefficient of variation (CV) as a percentage was used to report intraindividual variability in measurement of tEE, tCHO, and tFAT oxidation across the first day experiments of the three trials. Overall variability of the measure was calculated as the mean of the within-participant CVs.

Effect sizes were calculated as Cohen's d_z for correlated samples by dividing the mean of difference scores by the *SD* of the difference scores (Lakens, 2013).

Sample Size Calculation

A required sample size of 12 was calculated a priori by using work completed in the TT as the main outcome and a large effect size (1.0; Rustad et al., 2016) with $\alpha = .05$ and $1 - \beta = 0.8$.

Results

Participant Characteristics

Participant characteristics are reported in Table 2.

Participants consumed all provided meals and did not consume additional food products within 24 hr postexercise on Day 1. Self-reported adherence to the prescribed 3-day diet was very precise (26%), mostly precise (72%), mostly not precise (2%), and not at all (0%). Prescribed 3-day training was followed very precisely (28%), mostly precisely (63%), mostly not precisely (9%), and not at all (0%).

TT Performance

Work completed in the 30-min TT on Day 2 after glycogen-lowering exercise was not different between the conditions (401 ± 75, 411 ± 80, and 404 ± 58 kJ in CHO, CHO + PRO, and CHO + PROH, respectively, $p = .83$; $d_z = 0.11$ and 0.07 for CHO + PRO and CHO + PROH, respectively). Mean RPE over the TT as well as blood glucose and lactate at the end of the TT were also similar between trials. Furthermore, gas exchange variables and CHO and fat oxidation rates during Minutes 17–20 in the TT did not differ between trials (Table 3). TT performance in the non-depleted pretrials was 489 ± 72 kJ with an intraindividual CV of 1.7%. Performance in the depleted TTs was lower than in pretrials ($p < .001$ for all conditions; $d_z = 1.43$, 1.46, and 1.64 in CHO, CHO + PRO, and CHO + PROH, respectively).

Substrate Oxidation During Exercise on Days 1 and 2

There was no difference in tEE, tCHO, and tFAT oxidized during the Day 1 and Day 2 75% TTE exercise bout between conditions. However, tCHO was higher and tFAT lower on Day 1 compared with Day 2 in all trials ($p < .01$ for all; Figure 2).

Table 2 Participant Characteristics ($n = 12$)

Variables	$M \pm SD$	Range
Age (years)	37.5 ± 6.1	23–44
BM (kg)	74.9 ± 8.3	60.4–88.3
$\dot{V}O_2$max (L/min)	$4{,}660 \pm 500$	3,690–5,700
$\dot{V}O_2$max ($ml \cdot min^{-1} \cdot kg^{-1}$)	62.5 ± 5.9	50.1–72.4
MAP (W)	333 ± 42	246–408

Notes. BM = body mass; $\dot{V}O_2$ max = maximal oxygen consumption; MAP = maximal aerobic power.

Table 3 Performance and Physiological Parameters in the 30-min TT Following Heavy Constant-Load Exercise on Day 2 (*M* ± *SD*)

Outcome variables	CHO	CHO + PRO	CHO + PROH
Work (kJ)	401 ± 75	411 ± 58	404 ± 80
$\dot{V}O_2$ (L/min)	3.53 ± 0.54	3.61 ± 0.39	3.66 ± 0.34
CHO oxidation (g/min)	2.0 ± 0.6	2.1 ± 0.6	2.2 ± 0.5
Fat oxidation (g/min)	1.0 ± 0.2	1.0 ± 0.3	0.9 ± 0.1
Blood glucose$_{30min}$ (mmol/L)	3.7 ± 0.8	3.5 ± 1.1	3.4 ± 0.5
Blood lactate$_{30min}$ (mmol/L)	6.5 ± 3.5	6.4 ± 2.9	7.6 ± 3.5
HR_{avg}	154 ± 12	157 ± 8	157 ± 8
RPE_{avg}	17.1 ± 1.7	17.3 ± 1.3	17.3 ± 1.1

Notes. CHO = carbohydrate, PRO = whey protein concentrate; PROH = whey protein hydrolysate; HR = heart rate; RPE = rating of perceived exertion; Avg = average; TT = time trial.

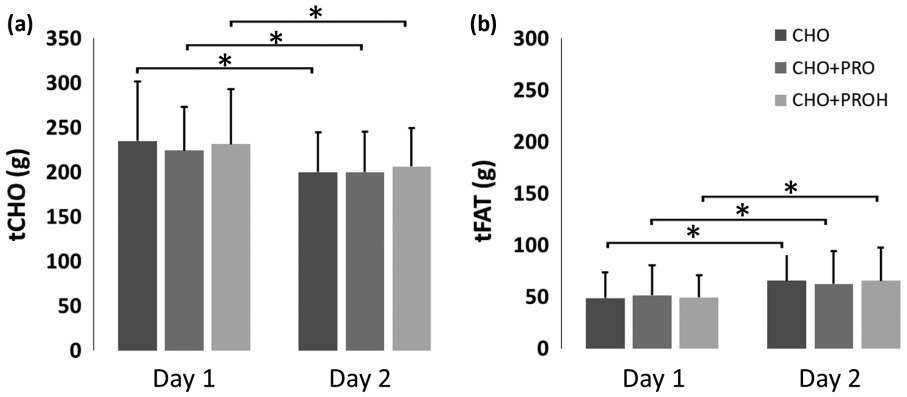

Figure 2 — Total CHO (a) and total fat (b) oxidized on Day 1 and Day 2 in each 75% TTE trial (CHO, CHO + PRO, and CHO + PROH). Bars represent *M* + *SD*. CHO = carbohydrate; PRO = whey protein concentrate; PROH = whey protein hydrolysate; TTE = time-to-exhaustion; tCHO = total CHO; tFAT = total fat. $^*p < .05$.

Glucose and Insulin Responses

No significant differences in 2-hr glucose AUC between CHO and CHO + PRO (364 ± 107 vs. 341 ± 76 mmol·L^{-1}·2 hr^{-1}, $p = .38$), CHO and CHO + PROH (364 ± 107 vs. 330 ± 147, $p = .38$), or CHO + PRO and CHO + PROH (341 ± 76 vs. 330 ± 147, $p = .81$) were found.

The insulin response measured by AUC following the post-exercise recovery drink was lower in CHO (18.1 ± 7.7 nmol·L^{-1}·2 hr^{-1}) compared with CHO + PRO and CHO + PROH (24.6 ± 12.4 vs. 24.5 ± 10.6, $p = .036$ and .015, $d_z = 0.98$ and 1.12, respectively). No difference in insulin AUC was found between CHO + PRO and CHO + PROH ($p = 1.00$). Glucose and insulin responses are illustrated in Figure 3. As an index of the effect of insulin, glucose responses were also expressed in relation to insulin responses (Figure 3e–f). ΔGlucose AUC·Δinsulin AUC^{-1} was not significantly different in CHO compared with CHO + PRO and CHO + PROH ($p = .44$ and .35, $d_z = 0.59$ and 0.76, respectively).

Substrate Oxidation During TTE

Mean power output for the TTE test was 233 ± 29 W (78 ± 6% $\dot{V}O_2$max) and TTE was 106 ± 31 min. Due to a drift in oxygen uptake over time, $\dot{V}O_2$ was 75 ± 5% $\dot{V}O_2$max during the first 15 min and 81 ± 6% $\dot{V}O_2$max during the last 15 min before exhaustion. Mean tEE was 1906 ± 629 kcal, and mean tCHO and tFAT oxidation were 297 ± 97 and 72 ± 37 g, respectively. A significant correlation was found between tCHO and time in TTE ($r = .76$, $p = .004$). The mean rate of CHO oxidation of 3.2 ± 0.6 g·min^{-1} during the first 15 min of exercise decreased to 2.6 ± 0.6 g/min at 75–90 min and to a low of 2.1 ± 0.5 g/min at the 105- to 120-min epoch. Capillary mean blood glucose was 4.3 ± 0.6 mmol/L at the beginning of exercise, decreasing to 3.3 ± 0.6 mmol/L at exhaustion.

Discussion

This study revealed no effect of the addition of PRO or PROH to a CHO recovery drink on next-day TT performance undertaken after glycogen-lowering exercise, nor did these supplements affect the blood glucose response to an iso-CHO, nonprotein recovery drink. Lack of these effects was observed despite both protein drinks inducing a higher insulin response than the CHO-only drink. CHO oxidation on Day 2 at submaximal workload was reduced compared with Day 1 independent of condition, possibly indicating incomplete glycogen restoration in 24 hr regardless of recovery drink composition.

Testing protocols implemented in sport nutrition research are often far removed from the reality of most athletes who consume recovery supplements. The novelty of our study is that it provides

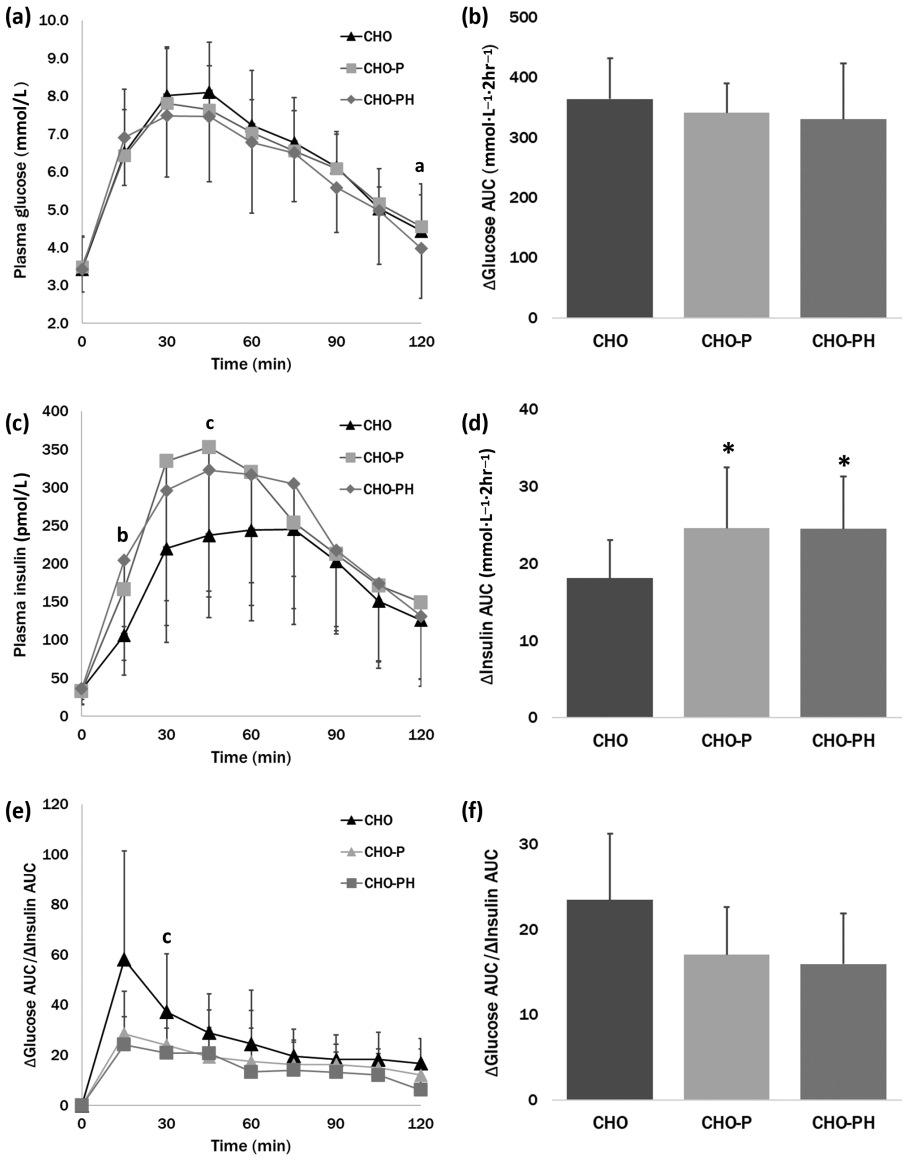

Figure 3 — Glucose and insulin responses measured at baseline and every 15 min after ingestion of recovery drink (*M* + *SD*). (a) Plasma glucose response, (b) plasma glucose AUC above baseline, (c) plasma insulin response, (d) plasma insulin AUC above baseline, (e) change in glucose AUC over change in insulin AUC at 15–120 min, (f) overall change in glucose AUC over change in insulin AUC. CHO = carbohydrate; PRO = whey protein concentrate; PROH = whey protein hydrolysate. [a]Different from CHO, [b]CHO + PRO different from CHO + PROH, [c]CHO different from CHO + PROH. *Different from CHO.

this point of view by employing a more ecologically valid design, lacking in most investigations on CHO versus CHO+protein. In contrast to previous original investigations, we specifically asked whether athletes could benefit from acute addition of PRO or PROH to CHO after 24 hr (rather than 4–8 hr) of recovery if CHO intake is suboptimal. The results of three recent meta-analyses (Craven et al., 2021; Kloby Nielsen et al., 2020; Margolis et al., 2021) demonstrate that CHO+protein is effective in the short term (<8 hr) when energy intake is higher than CHO as a result of additional protein but not in isocaloric experiments. Suboptimal CHO may be where the positive effect of protein manifests itself, but the question remains whether this short-term benefit has relevance to real-world training of amateur athletes. The present data make us question the next-day performance benefits of immediate protein supplementation, even with inadequate CHO, if overall caloric intake is fixed.

Limited data from experiments similar to the present study exist in the literature. Of the studies that have compared additional protein or protein hydrolysate to a CHO-only recovery drink, few have also measured performance after a long (overnight) recovery period. The most promising study by Rustad et al. (2016) reported an improvement in a TTE test 18 hr after an initial exhaustive cycling exercise session when the subjects ingested a protein-CHO recovery drink compared with an isocaloric CHO drink in the early phase of recovery. Their study used cycling to exhaustion as the glycogen-depleting exercise, presumably leaving the participants with lower levels of glycogen compared with the present study. Thus, added protein might have proven effective after exhaustive exercise. A positive effect was also found in a study by Saunders et al. (2004) where performance at 85% of $\dot{V}O_2$max was significantly better 12–15 hr after an initial exhaustive exercise session

when protein was provided in the recovery drink. However, in their study, only the CHO, not total energy intake, was matched between the trials, resulting in lower energy availability in the CHO-only condition. Consistent with our results, a study by Romano-Ely et al. (2006) revealed that TTE performance 24 hr after an exhaustive bout of cycling was not different between isocaloric CHO and CHO+protein (+antioxidants). It, thus, seems likely that if protein enhances early glycogen restoration, this acute benefit diminishes after 18 hr at the latest.

The rationale for the importance of acute protein intake postexercise is the proposed existence of a critical metabolic window during which glycogen resynthesis is greatly enhanced (Ivy et al., 1988) and during which protein may help to stimulate glycogen replenishment in the absence of optimal glucose (Hara et al., 2011; Ivy et al., 2002). The time course of this metabolic window is not clearly described in the literature; and therefore, the effect of the first versus second hour is uncertain. The 2-hr time point is commonly used for glycogen assessment after postexercise (Jentjens & Jeukendrup, 2003), but the effect of protein may vanish sooner. However, the glucose and insulin responses were remarkably different between Hours 0–2 and 2–4 in the study by Ivy et al. (2002) in response to a single feeding at 0 and 2 hr, justifying our feeding protocol. We could not replicate this large effect in the first 2 hr in the present design despite similar macronutrient composition, possibly due to a lower degree of depletion (low glycogen strongly stimulates its resynthesis).

Although biopsies are typically taken to analyze glycogen content, whole-body CHO oxidation can give equally valuable information on the extent of glycogen depletion. Early data from Bergstrom et al. (1967) revealed a strong correlation between initial glycogen stores and TTE at 75% of $\dot{V}O_2$max (Bergstrom et al., 1967). Also, more recent work by Murgatroyd et al. (1993) demonstrated that the accumulated amount of CHO oxidized during prolonged steady-state test to exhaustion agreed well with endogenous glycogen availability; therefore, indirect calorimetry can be used as a noninvasive method for estimating and achieving desired levels of glycogen depletion. We chose this approach to benefit from a crossover design while avoiding multiple skeletal muscle biopsies typical to glycogen depletion–repletion studies, using a TTE test with frequent breath-by-breath measurements to estimate the total whole-body glycogen availability.

A limitation of the present study is that it was powered to detect a large effect size, thus a smaller effect on performance could potentially not be detected. However, the physiological, metabolic, and perceptional data collected align with the TT power output in that PRO or PROH had no apparent effect on next-day exercise capacity. It is also possible that an effect, negligible in a single 2-day experiment, could be amplified during several days or weeks of high-volume endurance training. Previous studies with chronic protein supplementation have revealed positive impacts on either indices of recovery (D'Lugos et al., 2016; Hansen et al., 2015; Witard et al., 2011) or exercise performance (Ferguson-Stegall et al., 2011; Hansen et al., 2015; Knuiman et al., 2019), although others do not report benefits (Hansen et al., 2016; Roberson et al., 2018). Whereas most studies have added the protein supplement to the normal diet, the study by Hansen et al. (2016) controlled for total protein and caloric intake and, yet, failed to demonstrate any benefits for elite cyclists during a 1-week intensified training camp. We also acknowledge the lack of a placebo control and/or a positive control with adequate CHO as a limitation. Including these conditions would have allowed us to assess whether the acute 2-hr postexercise window is at all important for restoration of next-day exercise capacity. However, as existing evidence suggests that the 2-hr metabolic window is important (Ivy et al., 1988) and the effect carries over to the next day (Rustad et al., 2016), we focused specifically on the role of the composition of the recovery drink: the CHO-only drink was the control against which we compared additional supplementation.

To conclude, these findings demonstrate that acute protein supplementation after heavy but nonexhaustive cycling exercise does not affect next-day endurance performance in trained male athletes following an isocaloric overall 24-hr diet. The greater insulin response following CHO + PRO relative to CHO does not significantly reduce blood glucose AUC or improve markers of glycogen-dependent exercise capacity over 24 hr. Accordingly, immediate protein supplementation after nonexhaustive training is not beneficial for restoration of next-day endurance capacity.

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