The Effect of Consuming Carbohydrate With and Without Protein on the Rate of Muscle Glycogen Re-synthesis During Short-Term Post-exercise Recovery: a Systematic Review and Meta-analysis

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Abstract

Background: Rapid restoration of muscle glycogen stores is imperative for athletes undertaking consecutive strenuous exercise sessions with limited recovery time (e.g. ≤ 8 h). Strategies to optimise muscle glycogen re-synthesis in this situation are essential. This two-part systematic review and meta-analysis investigated the effect of consuming carbohydrate (CHO) with and without protein (PRO) on the rate of muscle glycogen re-synthesis during short-term post-exercise recovery (≤ 8 h).

Methods: Studies were identified via the online databases Web of Science and Scopus. Investigations that measured muscle glycogen via needle biopsy during recovery (with the first measurement taken ≤ 30 min post-exercise and at least one additional measure taken ≤ 8 h post-exercise) following a standardised exercise bout (any type) under the following control vs. intervention conditions were included in the meta-analysis: part 1, water (or non-nutrient beverage) vs. CHO, and part 2, CHO vs. CHO+PRO. Publications were examined for methodological quality using the Rosendal scale. Random-effects meta-analyses and meta-regression analyses were conducted to evaluate intervention efficacy.

Results: Overall, 29 trials (n = 246 participants) derived from 21 publications were included in this review. The quality assessment yielded a Rosendal score of 61 ± 8% (mean ± standard deviation). Part 1: 10 trials (n = 86) were reviewed. Ingesting CHO during recovery (1.02 ± 0.4 g·kg body mass (BM)−1 h−1) improved the rate of muscle glycogen re-synthesis compared with water; change in muscle glycogen (MGΔ) re-synthesis rate = 23.5 mmol·kg·dm−1 h−1, 95% CI 19.0–27.9, p < 0.001; I² = 66.8%. A significant positive correlation (R² = 0.44, p = 0.027) was observed between interval of CHO administration (≤ hourly vs. > hourly) and the mean difference in rate of re-synthesis between treatments. Part 2: 19 trials (n = 160) were reviewed. Ingesting CHO+PRO (CHO: 0.86 ± 0.2 g·kg BM−1 h−1; PRO: 0.27 ± 0.1 g·kg BM−1 h−1) did not improve the rate of muscle glycogen re-synthesis compared to CHO alone (0.95 ± 0.3 g·kg BM−1 h−1); MGΔ re-synthesis rate = 0.4 mmol·kg·dm−1 h−1, 95% CI −2.7 to 3.4, p = 0.805; I² = 56.4%.

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Conclusions: Athletes with limited time for recovery between consecutive exercise sessions should prioritise regular intake of CHO, while co-ingesting PRO with CHO appears unlikely to enhance (or impede) the rate of muscle glycogen re-synthesis.

Trial Registration: Registered at the International Prospective Register of Systematic Reviews (PROSPERO) (identification code CRD42020156841).

Keywords: Nutrition, Glycogen replenishment, Athletes

Key Points

- Carbohydrate provision enhances muscle glycogen re-synthesis compared to no nutritional provision.
- Co-ingestion of protein with carbohydrate does not enhance muscle glycogen re-synthesis compared to consuming carbohydrate alone.
- The interval of carbohydrate administration was found to be an influential factor on the rate of muscle glycogen re-synthesis.

Introduction

Restoration of both muscle and liver glycogen stores is a critical element of post-exercise recovery [1, 2]. It is particularly important for athletes training multiple times per day, since sessions are often separated by short periods of recovery (i.e. ≤ 8 h) and inadequate muscle and liver glycogen restoration can impair subsequent athletic performance [2, 3]. As such, a considerable amount of research has been directed towards identifying nutritional strategies that facilitate optimal post-exercise glycogen repletion [4–7].

Carbohydrate (CHO) is the main substrate for muscle glycogen re-synthesis [1]. Thus, the impact of various CHO manipulations on restoration of muscle glycogen stores has been a research priority, including the amount of CHO provided [8]; type and combination of mono-saccharides administered [9, 10]; molecular weight of CHO provided [11]; timing of CHO intake relative to the completion of exercise [12, 13]; form of delivery (e.g. liquid vs. solid) [14, 15]; and provision of other nutrients [16–25], most notably protein (PRO) [26–29]. This work has been summarised in a number of narrative reviews [4–7, 30] and has also led to the development of sports nutrition guidelines describing best-practice approaches for athlete recovery [31, 32]. These guidelines suggest that following exercise, athletes should consume between 1.0 and 1.2 g CHO·kg of body mass (BM)⁻¹ h⁻¹ to optimise the rate of muscle glycogen re-synthesis [32]. The addition of PRO (0.3–0.4 g there should be a dot here ‘g·kg’ line 79 kg BM⁻¹ h⁻¹) has also been proposed to increase the rate of muscle glycogen re-synthesis when CHO consumption is sub-optimal (≤ 0.8 g·kg BM⁻¹ h⁻¹) [30]. However, these guidelines have been formulated based on original research and narrative reviews. Synthesising the available literature using meta-analytical techniques will quantify overall effects and assess whether these current guidelines are supported.

Of course, it is important to consider the influence of other contextual factors (other than CHO manipulation and the addition of PRO) on the rate of muscle glycogen re-synthesis during short-term post-exercise recovery. Some noteworthy contextual factors include training status [33], types of muscle contraction during exercise [34], muscle fibre typology [35–38], and the degree of muscle glycogen depletion induced by the initial exercise bout [39, 40]. However, the extent to which these factors moderate the effect of CHO provision on the rate of muscle glycogen re-synthesis during short-term post-exercise recovery has yet to be clarified meta-analytically.

The aim of the present review was to examine the effects of consuming: (1) CHO (in isolation) and (2) CHO+PRO on the rate of muscle glycogen re-synthesis during short-term exercise recovery using meta-analytic techniques. The extent to which other contextual factors (e.g. CHO dose, timing) influence the rate of muscle glycogen re-synthesis are explored.

Methods

The methodology of this review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols 2015 Statement [41] and registered at the International Prospective Register of Systematic Reviews (PROSPERO) (identification code: CRD42020156841) before beginning the formal study selection process.

Literature Search

Original research studies were identified by searching the online databases Web of Science (via Thomas
Inclusion and Exclusion Criteria
Original research studies that met the following criteria were included in this review:

1. Controlled trials employing repeated-measures experimental designs.
2. Human studies on adult (≥ 18 years of age) men and women with no known medical conditions and co-morbidities.
3. Muscle glycogen concentrations were measured by needle biopsy (the ‘gold standard’ measurement technique) [43] under both control and intervention conditions (refer to the ‘Control and intervention conditions’ section), with the first (i.e. ‘pre-treatment’) measurement taken ≤ 30 min post-exercise and at least one additional (i.e. ‘post-treatment’) measurement taken ≤ 8 h post-exercise; a schematic of the experimental protocol employed in eligible studies is illustrated in Fig. 2.
4. Full-text original research studies were published in English; all other documents were discarded.

Studies were excluded from the review if:

1. A between-subject experimental design was employed.
2. Energy containing dietary constituents other than CHO and PRO (e.g. alcohol, fat) or ergogenic substances (e.g. caffeine, creatine) were administered post-exercise.
3. Exercise was not standardised across trials (or no exercise was performed).
4. Dietary constituents other than water were administered during exercise.
5. Treatments were not administered orally.
6. Muscle glycogen concentrations were not measured by needle biopsy (e.g. nuclear magnetic resonance spectroscopy, ultrasound).
7. Muscle glycogen data were not adequately reported (i.e. mean ± standard deviation (SD) was not reported and could not be calculated).

In the event that data were not adequately reported, the corresponding author was contacted via email in an attempt to retrieve the missing data. Where data were presented in graphical format only, a web-based tool (‘WebPlotDigitizer’, https://apps.automeris.io/wp/) was used to extract numeric values.

Several publications identified via the literature search contained more than one intervention and control comparison that was eligible for inclusion. In these instances, the separate study arms were treated as individual investigations, and termed ‘trials’. Separate trials derived from a single research study are denoted by the addition of a lower-case letter (i.e. a–c) to the citation.

Control and Intervention Conditions
The present systematic review and meta-analysis compared the intervention and control conditions via a two-part investigation: (1) CHO vs. control (i.e. water or a non-nutritive placebo treatment) and (2) PRO (including isolated or mixed amino-acids (AA), e.g. glutamine, leucine) co-ingested with CHO (CHO+PRO) vs. CHO.

Primary and Secondary Research Outcomes
The primary research outcome in this investigation was the rate of muscle glycogen re-synthesis. Values were initially extracted in mmol·kg of dry mass (dm)⁻¹, or in mmol·kg of wet mass⁻¹, and then multiplied by a factor of 4.35 to convert to mmol·kg dm⁻¹, as described by Areta and Hopkins [44]. Where the rate of muscle glycogen re-synthesis was not reported directly (or could not be calculated using raw data supplied by authors) [26, 28, 45–47], but pre- and post-treatment muscle glycogen concentrations were known, the following methods were used to determine the missing values. First, the change in muscle glycogen concentration (MGΔ) (i.e. total amount of glycogen re-synthesised) was calculated for the control and intervention conditions. The SD of this (within-trial) change (SDΔ) was then calculated using the following formula [48]:

\[
\Delta = \frac{\text{post} - \text{pre}}{\text{post} - \text{pre}}
\]

\[
\text{SD}_\Delta = \frac{\left(\sum (x_i - \text{mean})^2\right)^{1/2}}{n - 1}
\]
Fig. 1 PRISMA flow chart (study selection methodology). Where a study contained more than one intervention-arm, the separate arms were treated as discrete 'studies', termed as 'trials'.

- Records identified through database search: n=7433
- Records after duplicates removed: n=5912
- Records screened through title: n=5912
- Records screened through abstract: n=1466
- Records excluded, n=4446
- Records excluded, n=1168
- Full text articles assessed for eligibility: n=298
- Full text articles included for data extraction: n=24
- n=1 included. Recent publication
- Full text included in review: n=21
- Total trials included in review: n=29
- The effect of CHO on muscle glycogen resynthesis: n=10
- The effect of CHO-PRO on muscle glycogen resynthesis: n=19

n=274 articles excluded. Reasons: Study design (n=38); unhealthy/diseased population (n=3); No control condition (n=31); 1st biopsy post-exercise >30-m (n=6); 2nd biopsy post-exercise >8-h (n=49); no CHO treatment (n=1); abstract only (n=8); added additional nutrient during recovery (n=1); consumed CHO during exercise (n=1); consumed FAT during recovery (n=8); delayed feeding (n=1); no exercise performed (n=12); duplicate (n=2); measured glycogen utilisation (n=22); glycogen loading (n=4); in vitro (n=1); glucose infusion (n=6); no biopsy taken (n=17); no change in nutrient intervention (n=27); no glycogen measurement (n=14); non-human (n=5); only 1 post-exercise glycogen measurement (n=7)

n=4 articles excluded. No extractable data available in paper and unable to obtain data on request (n=1); data used in multiple papers (n=1); glycogen data unable to be converted to mmol/kg dm² (n=1); lost a participant for one-arm of trial (n=1)
where $R$ is the mean correlation coefficient calculated using raw data derived from four CHO vs. control trials (CHO: $R = 0.62$; control: $R = 0.64$) [42, 49–51] and 17 CHO+PRO vs. CHO trials (CHO+PRO: $R = 0.79$; CHO: $R = 0.76$) [27, 29, 52–60]. The rate of muscle glycogen re-synthesis under each condition was then determined by dividing the total amount of glycogen re-synthesised (i.e. the mean and SD values) by the length of the recovery period (i.e. time between biopsies).

**Quality Assessment**

Included studies were examined for methodological quality using the Rosendal Scale [61], where excellent methodological quality is indicated by a Rosendal score $\geq 60\%$ [62]. Scoring was determined by dividing the number of ‘yes’ responses by the total number of applicable items. Studies with a Rosendal score $< 50\%$ are typically excluded from reviews owing to their increased risk of experimental bias; however, no study received a score $< 50\%$ in the current analysis.

**Data Extraction**

Data were extracted in accordance with the Cochrane Handbook for Systematic Reviews of Interventions Checklist of Items to Consider in Data Collection or Data Extraction [48] and entered into a Microsoft Excel spreadsheet. Extracted data included (1) participant characteristics (e.g. athletic population, age, BM, sex, aerobic power ($\dot{V}O_2$peak)); (2) pre-trial standardisation procedures; (3) exercise mode and protocol used to reduce muscle glycogen concentrations; (4) the treatment administration protocol (i.e. the delivery medium, amount, type, timing); (5) timing and number of muscle biopsies; and (6) muscle glycogen concentrations and, where provided, rate of re-synthesis (mmol·kg·dm$^{-1}$·h$^{-1}$).

**Statistical Analyses**

All statistical procedures were performed using SPSS, Version 26.0 (Armonk, NY: IBM Corp) and Comprehensive Meta-Analysis, Version 3.0 (Englewood, NJ: Biostat Inc). Weighted mean effect estimates and metaregression coefficients are presented as mean (95% confidence interval (95% CI) or range). All other data are presented as mean ± SD unless stated otherwise.

**Weighted Mean Effect**

Meta-analyses were performed to determine the influence of (1) CHO vs. control (water or a non-nutritive placebo treatment) and (2) CHO+PRO vs. CHO on the rate of muscle glycogen re-synthesis. Individual effect sizes were calculated as the raw mean difference (i.e. mmol·kg·dm$^{-1}$·h$^{-1}$), where positive effect estimates reflect higher rates of muscle glycogen re-synthesis with the intervention condition. Where the SD of this between-trial change was not reported directly (or was unable to be calculated using raw data supplied by the authors) [8, 26–29, 42, 45–47, 49–51, 57–60], the missing value was imputed using the following formula [48]:

\[
SD_\Delta = \sqrt{(SD_{\text{post-exercise MG}}^2 + SD_{\text{end of recovery MG}}^2) - (2 \times R \times SD_{\text{post-exercise MG}} \times SD_{\text{end of recovery MG}})}
\]

In this case, $R$ was approximated as 0.28 using raw data from eight CHO+PRO vs. CHO trials [52–56]; the same $R$ value of 0.28 was used for the CHO vs. control comparison (as no raw data from these trials could be obtained to determine an independent value). Sensitivity analyses were performed using $R = 0.60$ and 0.90 to test the robustness of the imputed value. In addition, trials were individually excluded to examine the influence of their removal on the overall effect estimate.

Weighted mean treatment effects were calculated using random-effect models, where trials were weighted by the inverse variance for the change in the outcome measure (i.e. rate of muscle glycogen re-synthesis). Statistical significance was attained if the 95% CI did not
include zero. Heterogeneity was assessed using Cochran’s Q and the \( I^2 \) index. Low, moderate, and high heterogeneity was indicated by an \( I^2 \) value of 25, 50, and 75%, respectively [63]. A \( p \) value < 0.10 for Cochran’s Q was used to indicate significant heterogeneity [48].

**Meta-regression Analysis**

Restricted maximum likelihood, random-effects simple meta-regression analyses were performed to determine whether the magnitude of difference in the rate of muscle glycogen re-synthesis between treatments was influenced by: (1) dose of CHO provided (relative and absolute); (2) pre-treatment muscle glycogen concentrations (i.e. ≤ 150 mmol·kg dm\(^{-1}\) vs. > 150 mmol·kg dm\(^{-1}\), as it has been hypothesised that levels ≤ 150 mmol·kg dm\(^{-1}\) can potentially accelerate muscle glycogen re-synthesis [4, 7]); (3) the relative difference in pre-treatment muscle glycogen concentrations (%); (4) interval of CHO administration during recovery (i.e. ≤ hourly vs. > hourly); (5) amount of CHO provided (i.e. met recommended guidelines (≥ 1.0 g·kg BM\(^{-1}\)·h\(^{-1}\)) or not); (6) mode of exercise; (7) difference in the energy content of the CHO+PRO and CHO treatments (i.e. magnitude of energy difference between treatments); (8) PRO source (i.e. whole PRO vs. single (or mixture) AA; (9) providing PRO at a rate ≥ 0.3 g·kg BM\(^{-1}\)·h\(^{-1}\) when the amount of CHO was sub-optimal (≤ 0.8 g·kg BM\(^{-1}\)·h\(^{-1}\)) but matched in control (i.e. CHO only) treatment; and (10) methodological quality (Rosendal score %) of the study. At least 10 data points were required for a variable to qualify for meta-regression analysis. Regression analyses were examined for influential cases and outliers (i.e. studentised residuals, Cook’s distance and centred leverage values) and multicollinearity (variance inflation factor). Statistical significance was accepted as \( p < 0.05 \).

**Results**

**Overview of Included Studies and Study Quality**

The literature search initially identified 25 eligible investigations. However, four of these had to be excluded because the muscle glycogen data (1) could not be extracted (or retrieved) [13]; (2) were the same as those reported in an earlier publication [64] that was already included [50]; (3) incorporated the results of one participant that did not complete both treatments (i.e. was not a within-subject comparison) [65]; and (4) were reported in a metric that could not be reliably converted to mmol·kg dm\(^{-1}\) [66] using the factors outlined by Areta and Hopkins [44]. Overall, 29 trials (\( n = 246 \) participants) derived from 21 publications were included in this review. Methodological quality assessment yielded an average Rosendal score of 61 ± 8%; all trials scored ≥ 50% (range 50–77%). Results of the quality assessment are shown in Supplementary Table S1.

**Effect of CHO vs. Control (Water or Other Non-nutritive Treatment) on Muscle Glycogen Re-synthesis Rate**

Ten trials (\( n = 86; 91\% \) men) derived from nine publications investigated the effect of CHO on the rate of muscle glycogen re-synthesis during post-exercise recovery. Eight [8, 26, 28, 42, 45, 46, 50] used cycling and two [49, 51] used resistance training as the mode of glycogen-depleting exercise. The mean relative CHO intake was 1.02 g·kg BM\(^{-1}\)·h\(^{-1}\) (range 0.50–1.5 g·kg BM\(^{-1}\)·h\(^{-1}\)), and the mean post-exercise recovery time was 2.9 h (range 1.0–5.0 h). On average, participants’ post-exercise (i.e. pre-treatment) muscle glycogen concentrations were 179 mmol·kg dm\(^{-1}\) (range 12–406 mmol·kg dm\(^{-1}\)) and 197 mmol·kg dm\(^{-1}\) (range 30–441 mmol·kg dm\(^{-1}\)) for the CHO and control conditions, respectively. Characteristics of the included trials are summarised in Table 1.

The overall weighted mean effect estimate indicated that CHO administration significantly increased the rate of muscle glycogen re-synthesis during short-term post-exercise recovery (\( MG_{\Delta} \) re-synthesis rate = 23.5 mmol·kg dm\(^{-1}\)·h\(^{-1}\), 95% CI 19.0–27.9, \( p < 0.001 \); \( I^2 = 66.8\% \)) (Fig. 3). The magnitude and statistical significance of the effect were stable during sensitivity analyses where trials were removed (\( MG_{\Delta} \) re-synthesis rate ranged from 21.8 to 26.8 mmol·kg dm\(^{-1}\)·h\(^{-1}\) and 95% CIs did not include zero). Findings were also comparable when alternative correlation coefficients were used (Supplementary Table S2).

Simple meta-regression analyses identified a significant, positive association between the mean difference in muscle glycogen re-synthesis rate and the interval of CHO administration, such that studies providing CHO more frequently (i.e. ≥ hourly) induced a higher rate of muscle glycogen re-synthesis than those providing CHO less frequently (i.e. > hourly) (\( R^2 = 0.44, \ p = 0.027 \)). No significant associations were identified between the mean difference in muscle glycogen re-synthesis rate and any other contextual factors (Table 2).

**Effect of CHO+PRO vs. CHO on Muscle Glycogen Re-synthesis Rate**

Nineteen trials (\( n = 160; 96\% \) men) derived from 13 publications investigated the effect of co-ingesting CHO with PRO on the rate of muscle glycogen re-synthesis during post-exercise recovery. Seventeen [27–29, 47, 54–60] used cycling and two [52, 53] used running as the mode of glycogen-depleting exercise. Mean post-exercise muscle glycogen concentrations were 131 mmol·kg dm\(^{-1}\) (range 85–233 mmol·kg dm\(^{-1}\)) and 129 mmol·kg dm\(^{-1}\) (range 64–235 mmol·kg dm\(^{-1}\)) for the CHO and CHO+PRO trials, respectively. The mean relative intake of CHO was 0.95 g·kg BM\(^{-1}\)·h\(^{-1}\) (range 0.60–1.6 g·kg BM\(^{-1}\)·h\(^{-1}\)) for the CHO trials and 0.86 g·kg BM\(^{-1}\)·h\(^{-1}\) (range 0.50–1.2 g·kg BM\(^{-1}\)·h\(^{-1}\)) for the CHO+
| Study                  | Participants | Mean \(\dot{\text{VO}}_{2\text{peak}}\) (mL kg min \(^{-1}\)) | Fasted vs. Fed | Exercise protocol                                                                 | End of exercise glycogen - control (mmol·kg dm \(^{-1}\)) | End of exercise glycogen - intervention (mmol·kg dm \(^{-1}\)) | Source of CHO                  | Timing of treatment (h) | CHO (g·kg BM \(^{-1}\) h \(^{-1}\)) | Recovery time (h) | Mean difference in re-synthesis rate (mmol·kg dm \(^{-1}\) h \(^{-1}\)) |
|-----------------------|--------------|-------------------------------------------------|----------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------|--------------------------|----------------------|----------------------|----------------------|-------------------------------------------------------------|
| Mathai et al. [46]    | 7 (7 M)      | 48.4                                            | Fed            | Cycled to exh at 65% \(\dot{\text{VO}}_{2\text{peak}}\)                          | 146                                                        | 82                                                          | Maltodextrin + dextrose                                   | 0, 1                  | 1.0                  | 2                    | 59.0                                                             |
| Pascoe et al. [49]    | 8 (6 M)      | N.S                                             | Fasted         | Resistance training: 6 single leg knee ext at 70% 1RM set \(\times\) 9 set with 30 s recovery between set | 441                                                        | 399                                                         | Glucose polymers + sucrose                                | 0, 1                  | 1.5                  | 2                    | 47.8                                                             |
| Cheng et al. [42]     | 8 (6 M)      | 48.5                                            | Fasted         | Cycled for 1 h at 60% \(\dot{\text{VO}}_{2\text{peak}}\) followed by 4 \(\times\) 30 s all-out sprints | 30                                                         | 12                                                          | Glucose + fructose                                        | 0, then every 0.25 (until 2.5) | 1.5                  | 3                    | 36.1                                                             |
| Tarnopolsky et al. [26] | 16 (8 M)    | M: 56.9 F: 51.7                                | Fasted         | Cycled for 1.5 h at 65% \(\dot{\text{VO}}_{2\text{peak}}\)                      | 210                                                        | 163                                                         | Sucrose + glucose polymer                                 | 0, 1                  | 0.5                  | 4                    | 30.6                                                             |
| van Hall et al. [28]a | 5 (N.S)      | 61.0                                            | N.S            | Cycled to exh: 2-min intervals alternating between 50 and 90% \(\dot{\text{VO}}_{2\text{peak}}\) | 78                                                         | 90                                                          | Sucrose                                                 | 0, then every 0.25 (until 3.75) | 1.2                  | 4                    | 28.8                                                             |
| Wilson et al. [30]    | 10 (10 M)    | 54.8                                            | Fed            | Cycled to exh at 65% \(\dot{\text{VO}}_{2\text{peak}}\)                          | 74                                                         | 109                                                         | Dextrose                                                | 0, 1, 2, 3, 4          | 1.0                  | 5                    | 25.1                                                             |
| Ivy et al. [8]b       | 8 (6 M)      | 52.2                                            | Fasted         | Cycled for 2 h: 15-min intervals alternating between 62 and 75% \(\dot{\text{VO}}_{2\text{peak}}\) | 158                                                        | 139                                                         | Glucose polymer                                          | 0                     | 1.5                  | 2                    | 19.1                                                             |
| Roy and Tarnopolsky [51]| 10 (10 M)  | N.S                                             | Fed            | Resistance training: whole-body workout; \(\sim\) 10 rep at \(-\) 80% 1RM set \(\times\) 3 set \(\times\) 9 exercises \(\times\) 20 sit-ups | 248                                                        | 235                                                         | Sucrose + glucose polymer                                 | 0, 1                  | 0.5                  | 4                    | 17.3                                                             |
| Ivy et al. [8]a       | 8 (6 M)      | 52.2                                            | Fasted         | Cycled for 2 h: 15-min intervals alternating between 62 and 75% \(\dot{\text{VO}}_{2\text{peak}}\) | 158                                                        | 156                                                         | Glucose polymer                                          | 0                     | 0.75                 | 2                    | 16.5                                                             |
| Haup et al. [45]      | 6 (6 M)      | 50.8                                            | Fed            | Cycled: 100 kJ test (at 90% peak resistance achieved during GXT)                  | 429                                                        | 406                                                         | Maltodextrin                                             | 0                     | 0.7                  | 1                    | 14.4                                                             |

*All trials tabulated used a randomised, controlled, within-subject experimental design. Fasted vs. Fed: whether participants were fed or fasted prior to exercise. ‘a’ and ‘b’ refer to separate trials derived from a single research study.

N.S not specified, M male, F female, exh exhaustion, Rep repetition, ext extension, RM repetition max, min minute, \(\dot{\text{VO}}_{2\text{peak}}\) peak maximal oxygen uptake, BM body mass, dm dry mass, kJ kilojoule, GXT graded exercise test.

*Estimated last beverage was consumed at 3.75 h.
PRO trials. The mean relative PRO intake was 0.27 g·kg BM$^{-1}$ h$^{-1}$ (range 0.05–0.40 g·kg BM$^{-1}$ h$^{-1}$) for the CHO+PRO trials and the mean energy difference (kJ) between trials (favouring CHO+PRO) was 884 kJ (range 0–2343 kJ). Characteristics of the included trials are summarised in Table 3.

The weighted mean effect estimate indicated that co-ingesting PRO with CHO did not significantly improve muscle glycogen re-synthesis rate (MG$\Delta$ re-synthesis rate = 0.4 mmol·kg dm$^{-1}$ h$^{-1}$, 95% CI −2.7 to 3.4, p = 0.805; $I^2 = 56.4$%) compared to consuming CHO alone (Fig. 4). The magnitude and statistical significance of the effect were stable during sensitivity analyses where trials were individually removed (MG$\Delta$ re-synthesis rate (mmol·kg dm$^{-1}$ h$^{-1}$) range −0.6 to 1.0, 95% CIs did include zero). Findings were also comparable when alternative correlation coefficients were used (Supplementary Table S3).

There were no significant relationships identified between the mean difference in rate of muscle glycogen re-synthesis and any of the contextual factors explored using meta-regression. Results of the meta-regression analyses are summarised in Table 4.

Discussion

The present systematic review and meta-analysis quantified the effects of consuming CHO (in isolation) and CHO+PRO on the rate of muscle glycogen re-synthesis during short-term post-exercise recovery. Overall, a beneficial effect of ingesting CHO (compared to water or non-nutritive placebo treatment) was observed on the rate of muscle glycogen re-synthesis. However, co-ingestion of CHO with PRO conferred no additional benefit compared to CHO ingested alone. Furthermore, the interval of CHO administration was found to be an influential factor on the rate of muscle glycogen re-synthesis.

Effect of CHO on Short-Term Muscle Glycogen Re-synthesis

The current meta-analysis suggests that muscle glycogen re-synthesis rate is enhanced during short-term post-exercise recovery when CHO is consumed compared to control (water or non-nutritive placebo treatment). Except for one trial [45], all individual effect estimates indicated a beneficial effect of CHO. However, the magnitude of these effects was heterogeneous ($I^2 = 66.8$).

Meta-regression analysis identified a significant, positive relationship between the magnitude of CHO-induced improvement in muscle glycogen re-synthesis rate and the interval of CHO administration, which explains some of this heterogeneity ($R^2 = 0.44$). Indeed, the regression model for this relationship predicts a ~11 mmol·kg dm$^{-1}$ h$^{-1}$ increase in the rate of muscle glycogen re-synthesis when trials administer CHO at an interval of ≤ hourly compared to those administering CHO > hourly. It is worth noting that the amount of CHO consumed is not controlled in this comparison. More frequent CHO administration may enhance muscle glycogen re-synthesis rate by prolonging the elevation of plasma glucose and insulin concentrations [7]. However, to the authors’ knowledge, there are currently no studies (employing a within-subject study design) that have directly assessed the effects of providing equal amounts of CHO at varied frequencies during a fixed period of post-exercise recovery. Nonetheless, the results of this meta-analysis suggest that frequent consumption of CHO (i.e. at least hourly) should be a priority for athletes attempting to optimise short-term muscle glycogen replenishment.

No correlation was observed between the dose of CHO (both relative and total) consumed during post-exercise recovery and rate of muscle glycogen re-synthesis (Table 2). Consequently, we were unable to determine the dose of CHO required to optimise the rate of muscle
glycogen re-synthesis. The lack of correlation may be due to the limited number of trials ($n = 10$) available for inclusion in the analysis. As a result, we could not perform multiple meta-regression (due to the limited number of trials) and control for the interval of CHO administration; therefore, this may have prevented the detection of a relationship between CHO dose and muscle glycogen re-synthesis rate. Furthermore, the limited number of trials may have prevented the detection of a relationship between muscle glycogen concentration immediately post-exercise and the rate of muscle glycogen re-synthesis (Table 2). This exploration was of interest because it has previously been hypothesised to have a positive influence (i.e. rate of replenishment is enhanced when post-exercise muscle glycogen concentration is $\leq 150$ mmol·kg $\text{dm}^{-1}$) on the rate of muscle glycogen re-synthesis, via mechanisms that trigger the insulin-independent phase of muscle glycogen re-synthesis [4, 7].

**Effect of CHO+PRO on Short-Term Muscle Glycogen Re-synthesis**

The current meta-analysis suggests that co-ingestion of PRO with CHO during short-term post-exercise recovery provides no additional benefit to (nor does it impair) the rate of muscle glycogen re-synthesis compared to consuming CHO alone. This finding was preserved when contextual factors were explored using meta-regression analysis (Table 4). It is also consistent with results from previous meta-analyses indicating that co-ingestion of PRO with CHO during short-term recovery does not improve short-term muscle glycogen re-synthesis [67] or subsequent exercise performance [68].

Of the 19 trials included in the analysis, only two [29, 58] indicated a significant positive effect favouring CHO+PRO on the rate of muscle glycogen re-synthesis. This result may be due to the co-ingestion of PRO in the context of sub-optimal CHO intake (i.e. $\leq 0.8$ g·kg $\text{BM}^{-1}$ $\text{h}^{-1}$), which has been previously reported as being beneficial [30]. It was suspected this result was due to a large insulinemic response by PRO in combination with CHO, despite inadequate ingestion of the latter. Some research reports a greater insulinemic response when PRO (specifically, containing the AA leucine and phenylalanine) is co-ingested with CHO [4, 7, 30, 69], which has made this an area of interest. This strategy may allow a total reduction in the amount of nutrition needed to stimulate an equivalent insulin response, thus, potentially permitting lower caloric intake while maintaining adequate glycogen re-synthesis. This may be an effective strategy in athletes who are trying to reduce energy consumption (e.g. to make a specific weight division), but need rapid glycogen recovery to maintain subsequent training performance, as well as promote muscle growth and development. However, this strategy is not supported in other trials [27, 53]. The difference amongst trials may be attributed to methodological factors, such as the timing and type of PRO provided (e.g. insulinemic- vs. non-insulinemic-stimulating AA), the mode of exercise performed (e.g. cycling vs. running), and the interval in which muscle tissue was collected between trials (i.e. the length of recovery).

Only one trial [56] showed a significant effect favouring CHO over CHO+PRO on the rate of muscle glycogen re-synthesis. In this trial [56], a mixture of AA were provided, although only in a relatively small dose (0.09 g·kg $\text{BM}^{-1}$ $\text{h}^{-1}$). The authors hypothesised that the lower rate of muscle glycogen re-synthesis observed in the PRO trial may be due to AA triggering protein synthesis, resulting in glucose being oxidised to support the energy requirement for this process in place of glycogen storage [56]. Nonetheless, while the overall effect of our analysis suggests that co-ingesting PRO with CHO does not provide any benefit beyond that of CHO alone to muscle glycogen restoration (even when CHO intake is

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**Table 2** The influence of contextual factors on the mean difference in rate of muscle glycogen re-synthesis (analysed via restricted maximum likelihood, simple meta-regression) for CHO vs. control treatments

| Effect estimate | Mean difference (re-synthesis rate mmol·kg $\text{dm}^{-1}$ $\text{h}^{-1}$) |
|----------------|-----------------------------------------------|
| **Covariate**  | **Coefficient (95% CI) $p$ value**              |
| Glycogen post-exercise ($\leq 150$ mmol·kg $\text{dm}^{-1}$ vs. $> 150$ mmol·kg $\text{dm}^{-1}$) | 4.32 (−7.78 to 16.4) 0.484 |
| Interval of CHO administration ≤ hourly (yes vs. no) | 11.0 (1.24 to 20.9) 0.027 |
| Total CHO (g) intake | −0.00 (−0.06 to 0.04) 0.665 |
| Relative CHO (g·kg $\text{BM}^{-1}$ $\text{h}^{-1}$) intake | 5.00 (−11.5 to 20.5) 0.553 |
| Met CHO guidelines (yes vs. no) | 7.05 (−5.26 to 19.4) 0.262 |
| Length of recovery (h) | 2.09 (−1.98 to 6.17) 0.314 |
| Relative difference in muscle glycogen immediately post-exercise | −0.12 (−0.35 to 0.11) 0.317 |
| Mode of exercise (cycling vs. resistance) | 1.84 (−15.3 to 19.0) 0.833 |
| Study quality (Rosendal score %) | 0.92 (−0.10 to 1.94) 0.076 |

**CHO** carbohydrate, **BM** body mass, **dm** dry mass
| Study            | Participants | Study Characteristics | Mean VO\(_2\) peak (mL·kg·min\(^{-1}\)) | Exercise protocol | Post-exercise glycogen (mmol·kg dm\(^{-1}\)) | Post-exercise glycogen control | Post-exercise glycogen intervention | Type of CHO+PRO | Timing of treatment (h) | CHO (g·kg BM\(^{-1}\) h\(^{-1}\)) intervention | CHO (g·kg BM\(^{-1}\) h\(^{-1}\)) control | PRO (g·kg BM\(^{-1}\) h\(^{-1}\)) intervention | Recovery time (h) | Mean difference in re-synthesis rate (mmol·kg dm\(^{-1}\) h\(^{-1}\)) |
|-----------------|--------------|-----------------------|----------------------------------------|-------------------|---------------------------------------------|---------------------------------|-------------------------------|-----------------|------------------------|----------------------------------------|------------------------------------------|---------------------------------------------|-----------------|-----------------------------------------------|
| van Loon et al. [29]a | 8 (8M) N.S | Cycled to exh: 2-min intervals alternating between 50 and 90% VO\(_2\) peak | 8 (8M) N.S | Cycled to exh: 2-min intervals alternating between 50 and 90% VO\(_2\) peak | 190 | 174 | Glucose + maltodextrin + wheat protein hydrolysate + leucine + phenylalanine | 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 | 0.8 | 0.8 | 0.4 | 5 | 18.7 |
| Zawadzki et al. [58] | 9 (9M) 666 | Cycled for 2 h alternating between 65 and 80% VO\(_2\) peak | 144 | 134 | Dextrose + maltodextrin + milk + WPI | 0, 2 | 0.76 | 0.76 | 0.27 | 4 | 10.2 |
| Yaspelkis and Ivy [57] | 12 (12M) 672 | Cycled for 2 h alternating between 60 and 80% VO\(_2\) peak | 107 | 74 | Maltodextrin + arginine | 0, 1, 2, 3 | 1.0 | 1.0 | 0.08 | 4 | 9.5 |
| van Hall et al. [27]a | 8 (8M) N.S | Cycled to exh: 2-min intervals alternating between 50 and 90% VO\(_2\) peak | 107 | 108 | Glucose + wheat protein hydrolysate | 0.25, 1, 2 | 0.8 | 0.8 | 0.3 | 3 | 5.8 |
| van Hall et al. [27]b | 8 (8M) N.S | Cycled to exh: 2-min intervals alternating between 50 and 90% VO\(_2\) peak | 107 | 108 | Glucose + WPH | 0.25, 1, 2 | 0.8 | 0.8 | 0.3 | 3 | 4.9 |
| Alghannam et al. [52] | 6 (5M) 640 | Ran to exh at 70% VO\(_2\) peak | 103 | 100 | Sucrose + WPH | 0, 1, 2, 3 | 1.2 | 1.2 | 0.4 | 4 | 4.3 |
| Beelen et al. [59] | 14 (14M) 61.5 | Cycled to exh: 2-min intervals alternating between 50 and 90% VO\(_2\) peak | 172 | 184 | Maltodextrin + glucose + casein hydrolysate + leucine | Every 0.5 | 1.2 | 1.2 | 0.3 | 6 | 2.1 |
| Howarth et al. [55]a | 6 (6M) 493 | Cycled for 2 h 10-min intervals alternating between 50 and 80% VO\(_2\) peak | 97 | 64 | Maltodextrin + WPC | 0, then at 0.25 until 3 | 1.2 | 1.2 | 0.4 | 4 | 1.9 |
| Betts et al. [53] | 6 (6M) 610 | Ran for 1.5 h at 70% VO\(_2\) peak | 203 | 235 | Sucrose + WPI | 0, 5, 1.5, 2, 2.5, 3, 3.5 | 0.8 | 0.8 | 0.3 | 4 | −0.2 |
| Howarth et al. [55]b | 6 (6M) 493 | Cycled for 2 h 10-min intervals alternating between 50 and 80% VO\(_2\) peak | 85 | 64 | Maltodextrin + WPC | 0, then at 0.25 until 3 | 1.6 | 1.2 | 0.4 | 4 | −0.4 |
| Carrithers et al. [47] | 8 (8M) 55.7 | Cycled for 1.25 h at 70% VO\(_2\) peak, followed by 6x | 108 | 89 | Sucrose + fructose + dextrose + mixed AA | 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 | 1.0 | 0.86 | 0.14 | 4 | −1.3 |
Table 3: Characteristics of included trials evaluating post-exercise muscle glycogen re-synthesis during short-term recovery (≤ 8 h) with provision of CHO + PRO vs. CHO (Continued)

| Study                        | Participants | Mean VO₂peak (mL·kg·min⁻¹) | Exercise protocol | Post-exercise glycogen (mmol·kg dm⁻¹) control | Post-exercise glycogen (mmol·kg dm⁻¹) intervention | Type of CHO+PRO | Timing of treatment (h) | CHO (g·kg BM⁻¹ h⁻¹) control | CHO (g·kg BM⁻¹ h⁻¹) intervention | PRO (g·kg BM⁻¹ h⁻¹) intervention | Recovery time (h) | Mean difference in re-synthesis rate (mmol·kg dm⁻¹ h⁻¹) |
|------------------------------|--------------|------------------------------|-------------------|-----------------------------------------------|--------------------------------------------------|---------------|------------------------|--------------------------------|---------------------------------|---------------------------------|-----------------|---------------------------------------------------|
| van Hall et al. [27]c        | 8 (8M)       | N.S                          | 1 min sprint at 12.5% VO₂peak | 107                                            | 80                                               | Glucose + glutamine | 0.25, 1, 2 | 0.8                             | 0.8                          | 0.3                             | 3                | −2.0                                              |
| Cogan et al. [54]a           | 11 (11M)     | 61.2                         | Cycled for 2 h at 70% VO₂peak | 136                                            | 134                                              | Glucose + maltodextrin + sodium caseinate          | 0, 2                         | 0.6                             | 0.5                          | 0.08                           | 4                | −2.7                                              |
| Wang et al. [56]b            | 10 (7M)      | 50.2                         | Cycled for 2 h at 70%VO₂peak, followed by 5 x 1 min sprint at 85%VO₂peak | 114                                           | 146                                              | Dextrose + mixed AA | 0, 2                        | 0.6                             | 0.6                          | 0.045                           | 4                | −3.7                                              |
| van Hall et al. [28]b        | 5 (NS)       | 61.0                         | Cycled to exh: 2 min intervals alternating between 50 and 90%VO₂peak | 90                                            | 69                                               | Sucrose + WPH | 0, and then every 0.25 (until 3.75h) | 1.2                          | 1.2                             | 0.36                           | 4                | −4.5                                              |
| Wang et al. [56]a            | 10 (7M)      | 50.2                         | Cycled for 2 h at 70%VO₂peak, followed by 5 x 1 min sprint at 85%VO₂peak | 114                                           | 124                                              | Dextrose + mixed AA | 0, 2                        | 0.6                             | 0.6                          | 0.09                           | 4                | −6.6                                              |
| Cogan et al. [54]b           | 11 (11M)     | 61.2                         | Cycled for 2 h at 70%VO₂peak | 136                                            | 107                                              | Glucose + maltodextrin + sodium caseinate hydrolysate | 0, 2                        | 0.6                             | 0.5                          | 0.08                           | 4                | −7.1                                              |
| van Loon et al. [29]b        | 8 (8M)       | N.S                          | Cycled to exh: 2 min intervals alternating between 50 and 90%VO₂peak | 138                                           | 174                                              | Glucose + maltodextrin + wheat protein hydrolysate + leucine + phenylalanine | 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 | 1.2                         | 0.8                          | 0.4                           | 5                | −9.6                                              |
| Jentjens et al. [60]         | 8 (8M)       | 63.3                         | Cycled to exh: 2 min intervals alternating between 50 and 90%VO₂peak | 106                                           | 176                                              | Glucose + maltodextrin + wheat protein hydrolysate + leucine + phenylalanine | 0, 0.5, 1, 1.5, 2, 2.5 | 1.2                        | 1.2                          | 0.4                           | 3                | −14.4                                             |

All trials tabulated used a randomised, controlled, within-subject experimental design. ‘a’, ‘b’, and ‘c’ refer to separate trials derived from a single research study.

NS not specified, M male, min minute, exh exhaustion, VO₂peak peak maximal oxygen uptake, BM body mass, dm dry mass, CHO carbohydrate, PRO protein, AA amino acids, WPI whey protein isolate, WPC whey protein concentrate; WPH: whey protein hydrolysate, mix mixture

*Estimated last beverage was consumed at 3.75h
suboptimal), it is important to recognise that PRO remains critical for many physiological recovery processes (e.g. muscle repair). As such, a combined CHO+PRO approach is likely to remain an important consideration for nutrition recovery strategies more broadly.

A recent meta-analysis [67] similar to the present study reported a significant main effect (favouring CHO+PRO over CHO) on muscle glycogen re-synthesis rate when the energy intake was not matched between treatments (non-isocaloric). This finding contrasts the results of the present study (Table 4). The discrepancy between findings may be due to a number of factors. Firstly, different effect estimates were used between studies; we reported the mean difference for ease of interpretation [70], whereas Margolis et al. [67] reported Hedges’ $g$. Secondly, studies employing $^{13}$C-MRS techniques to determine muscle glycogen concentration were included in the previous study, while our results are based on studies using muscle tissue samples for glycogen analysis (as a means of reducing methodological heterogeneity). Thirdly, the previous meta-analysis included studies that provided CHO+PRO in combination with fat, and in some circumstances, the fat content was not matched between treatments [26, 47, 51, 71, 72]. Finally, one study [56] was omitted from the previous meta-analysis without clear explanation and a number of trials [27, 47, 54] that were part of a parallel design were also excluded; in contrast, we included trials from a single study that provided PRO from different sources. As a result, direct comparison of findings between the two meta-analyses is difficult and each should be interpreted on their individual merits.

![Fig. 4 Forest plot displaying the effect of CHO+PRO vs. CHO on rate of muscle glycogen re-synthesis during short-term recovery. The size of the squares is proportional to the weight of the study. A positive effect estimate indicates greater rate of muscle glycogen replenishment with CHO+PRO than CHO. Note: 'a', 'b', and 'c' refer to separate trials derived from a single research study](image)

| Study                  | Difference in means (rate of muscle glycogen re-synthesis mmol·kg dm$^{-1}$ h$^{-1}$) | p-value |
|------------------------|---------------------------------------------------------------------------------|---------|
| Jenčíns et al. [60]    | -14.4                                                                            | 0.378   |
| van Looij et al. [29]a | -9.6                                                                            | 0.179   |
| Cogen et al. [54]b     | -7.1                                                                            | 0.095   |
| Wang et al. [56]a      | -6.6                                                                            | 0.001   |
| van Hall et al. [27]b  | -4.5                                                                            | 0.315   |
| Wang et al. [56]a      | -3.7                                                                            | 0.150   |
| Cogen et al. [54]a     | -2.7                                                                            | 0.536   |
| van Hall et al. [27]b  | -2.0                                                                            | 0.764   |
| Carrithers et al. 47   | -1.3                                                                            | 0.866   |
| Howarth et al. [50]b   | -0.4                                                                            | 0.953   |
| Siets et al. [53]      | -0.2                                                                            | 0.951   |
| Howarth et al. [55]a   | 1.9                                                                             | 0.582   |
| Beeelen et al. [59]    | 2.1                                                                             | 0.642   |
| Alghamam et al. [52]   | 4.3                                                                             | 0.428   |
| van Hall et al. [27]a  | 4.9                                                                             | 0.373   |
| van Hall et al. [27]b  | 5.8                                                                             | 0.258   |
| Yaspeklis and Iry [57] | 9.5                                                                             | 0.086   |
| Zawadzki et al. [58]   | 10.2                                                                            | 0.002   |
| van Looij et al. [29]a | 18.7                                                                            | 0.018   |
| Overall                | 0.4                                                                             | 0.805   |

**Table 4** The influence of contextual factors on the mean difference in rate of muscle glycogen re-synthesis (analysed via restricted maximum likelihood, simple meta-regression) for CHO+PRO vs. CHO treatments

| Effect estimate                              | Mean difference (re-synthesis rate mmol·kg dm$^{-1}$ h$^{-1}$) | Coefficient (95% CI) | p value |
|----------------------------------------------|-----------------------------------------------------------------|----------------------|---------|
| Relative difference in muscle glycogen       | $-0.10\text{ (95% CI: }-0.25\text{ to }-0.06\text{)}$          | 0.216                |
| immediately post-exercise                    |                                                                  |                      |
| Magnitude of energy (kJ) difference between  | $0.01\text{ (95% CI: }-0.00\text{ to }0.01\text{)}$            | 0.084                |
| treatments                                   |                                                                  |                      |
| Whole PRO vs. single/mixed AA                | $3.46\text{ (95% CI: }-2.84\text{ to }9.77\text{)}$            | 0.282                |
| Recovery duration (h)                        | $0.28\text{ (95% CI: }-4.39\text{ to }4.95\text{)}$            | 0.906                |
| Mode of exercise (running vs. cycling)       | $1.33\text{ (95% CI: }-8.12\text{ to }10.8\text{)}$            | 0.783                |
| CHO provided is sub-optimal (yes vs. no)     | $0.73\text{ (95% CI: }-5.79\text{ to }7.26\text{)}$            | 0.826                |
| Study quality (Rosendal score %)             | $-0.19\text{ (95% CI: }-0.49\text{ to }0.11\text{)}$            | 0.211                |

PRO: protein, CHO: carbohydrate, AA: amino-acids, kJ: kilojoule
Limitations
This review does contain several limitations. Firstly, only studies with accessible full-text articles written in English were included. Secondly, the relatively limited number of trials included in the present meta-analysis prevented a comprehensive exploration of other factors (e.g. CHO type/combinations, dose of CHO/PRO, training status, muscle typology) that can potentially influence the rate of muscle glycogen re-synthesis. The low number of female participants included in original investigations (9.3 and 4.4 % for CHO vs. control and CHO+PRO vs. CHO, respectively) also precluded the exploration of sex as an influential factor on the rate of muscle glycogen re-synthesis. Thus, despite the plethora of research investigating the effect of CHO intake on muscle glycogen re-synthesis, opportunities for further research remain. Studies exploring the influence of sex, muscle fibre typology, training status, CHO type/composition, and the dose of CHO/PRO, on the short-term muscle glycogen re-synthesis, are warranted to enhance our understanding of CHO and CHO+PRO provision during short-term recovery.

Conclusion
Results of the present review suggest that individuals with limited opportunity for nutritional recovery between consecutive bouts of exercise (e.g. ≤ 8 h) should prioritise CHO ingestion to enhance the rate of muscle glycogen re-synthesis. Co-ingesting PRO with CHO does not appear to enhance the rate of muscle glycogen re-synthesis, nor is it detrimental. The interval of CHO administration appears to be an important factor that may influence the magnitude of effect CHO has on the rate of muscle glycogen re-synthesis. Hence, athletes should be encouraged to consume CHO at least hourly (or more frequently) over the longest period of short-term recovery (≤ 8 h) feasible.

Supplementary Information
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