Sports Med
DOI 10.1007/s40279-017-0800-5

SYSTEMATIC REVIEW

Post-exercise Ingestion of Carbohydrate, Protein and Water: A Systematic Review and Meta-analysis for Effects on Subsequent Athletic Performance

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Abstract

Background Athletes may complete consecutive exercise sessions with limited recovery time between bouts (e.g. ≤ 4 h). Nutritional strategies that optimise post-exercise recovery in these situations are therefore important.

Objective This two-part review investigated the effect of consuming carbohydrate (CHO) and protein with water (W) following exercise on subsequent athletic (endurance/anaerobic exercise) performance.

Data Sources Studies were identified by searching the online databases SPORTDiscus, PubMed, Web of Science and Scopus.

Study Eligibility Criteria and Interventions Investigations that measured endurance performance (≥ 5 min duration) ≤ 4 h after a standardised exercise bout (any type) under the following control vs. intervention conditions were included: Part 1: W vs. CHO ingested with an equal volume of W (CHO + W); and, Part 2: CHO + W vs. protein (PRO) ingested with CHO and an equal volume of W (PRO + CHO + W), where CHO or energy intake was matched.

Study Appraisal and Synthesis Methods Publications were examined for bias using the Rosendal scale. Random-effects meta-analyses and meta-regression analyses were conducted to evaluate intervention efficacy.

Results The quality assessment yielded a Rosendal score of $63 \pm 9\%$ (mean ± standard deviation). Part 1: 45 trials ($n = 486$) were reviewed. Ingesting CHO + W (102 ± 50 g CHO; 0.8 ± 0.6 g CHO kg^{-1} h^{-1}) improved exercise performance compared with W (1.6 ± 0.7 L); $\%_{\Delta}$ mean power output = 4.0, 95% confidence interval 3.2–4.7 ($I^2 = 43.9$). Improvement was attenuated when participants were 'Fed' (a meal 2–4 h prior to the initial bout) as opposed to 'Fasted' ($p = 0.012$). Part 2: 13 trials ($n = 125$) were reviewed. Ingesting PRO + CHO + W (35 ± 26 g PRO; 0.5 ± 0.4 g PRO kg^{-1}) did not affect exercise performance compared with CHO + W (115 ± 61 g CHO; 0.6 ± 0.3 g CHO·kg body $mass^{-1}$ h^{-1}; 1.2 ± 0.6 L); $\%_{\Delta}$ mean power output = 0.5, 95% confidence interval −0.5 to 1.6 ($I^2 = 72.9$).

Conclusions Athletes with limited time for recovery between consecutive exercise sessions should prioritise CHO and fluid ingestion to enhance subsequent athletic performance.

PROSPERO Registration Number CRD42016046807.

Electronic supplementary material The online version of this article (doi:10.1007/s40279-017-0800-5) contains supplementary material, which is available to authorized users.

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Published online: 02 November 2017

Key Points

Carbohydrate co-ingested with water during and/or following an initial bout of activity improves subsequent endurance exercise performance ($+4.0\%_{\Delta}$ mean power output) compared with water alone.

Whilst the magnitude of carbohydrate-mediated performance-enhancement was significantly diminished when participants were 'Fed' as opposed to 'Fasted', a positive effect of carbohydrate was still detectable under the 'Fed' condition.

No further performance enhancement was observed with the addition of protein to a carbohydrate containing beverage. The performance-enhancing effect of protein demonstrated in some studies appears to be a consequence of the additional energy delivered in the nutrient, rather than an effect of protein ingestion itself.

1 Introduction

Athletes undertaking heavy training or those involved in sporting events with multiple disciplines may be required to complete consecutive exercise sessions with limited recovery time between bouts (e.g. ≤ 4 h). A recent meta-analysis highlighted the importance of consuming fluid (even in volumes inadequate to completely replace sweat losses) to optimise performance during a subsequent exercise session [1]. However, consideration for nutrition interventions that also optimise repletion of endogenous substrate stores (e.g. muscle and liver glycogen) and/or promote the immediate recovery of damaged/inflamed muscle is required. Nutrition recommendations for post-exercise recovery highlight the importance of high carbohydrate (CHO) availability to maximise the rate of muscle glycogen resynthesis, and also indicate that protein may assist in both glycogen restoration (via an insulin-mediated response) and muscle damage repair (via supply of amino acids) [2]. However, trials involving consecutive exercise are needed to determine whether these nutrients can convey meaningful performance enhancements; particularly in a context where limited recovery time exists between exercise bouts (e.g. ≤ 4 h). Under these circumstances, it may not be possible to completely restore substrate losses [3], or promote significant muscle damage repair and attempting to do so may produce negative side effects [e.g. gastrointestinal (GI) discomfort] that hinder athletic performance.

Considerable scientific research has investigated the effect of consuming CHO during and/or following an initial bout of activity on subsequent endurance exercise performance, and some (but not all) studies indicate a performance-enhancing effect [4–6]. Fewer research studies have employed anaerobic performance-based trials. This evidence is yet to be systematically collated in a way that facilitates the exploration of factors that may influence the ergogenic potential of CHO ingestion. For example, overnight fasting has been demonstrated to reduce liver glycogen stores by up to 80% [7], such that CHO availability may already be suboptimal at the onset of the initial exercise bout. Thus, this methodological approach may exaggerate the influence of CHO supplementation on subsequent athletic performance [8]. Hence, the effect of CHO ingestion on subsequent endurance/anaerobic exercise performance requires elucidation.

Whilst protein (alone) contributes minimally to the energetic demands of exercise, other physiological attributes of this nutrient may facilitate performance enhancements on short-term subsequent exercise bouts. For example, when ingested with CHO, dietary protein can potentiate plasma insulin secretion, enhancing muscle glycogen synthase activity and uptake of glucose from the circulation [9]. These actions may accelerate muscle glycogen resynthesis after exercise [10]. Indeed, a previous review [11] concluded that although dietary protein is unlikely to influence glycogen repletion when co-ingested with an 'optimal' dose of CHO (i.e. 1.2 $g \cdot kg^{-1} \cdot h^{-1}$, to maximal glycogen resynthesis), a small quantity of protein (0.2–0.4 $g \cdot kg^{-1} \cdot h^{-1}$) consumed with a 'suboptimal' CHO dose (i.e. < 1.2 $g \cdot kg^{-1} \cdot h^{-1}$) may be of benefit. (Protein ingestion also has the potential to influence skeletal muscle damage repair during recovery from endurance exercise [12]). Therefore, in situations where ingesting large quantities of CHO is not feasible (e.g. between exercise sessions), ingesting protein with CHO may provide an opportunity to enhance substrate recovery.

To date, one systematic review [13] has investigated the effect of protein co-ingested with CHO during and/or following an initial bout of activity on subsequent endurance performance. In keeping with the aforementioned evidence, this review concluded that a significant benefit of dietary protein was frequently observed in studies where CHO was delivered 'sub-optimally'. However, an ergogenic effect was seldom recorded when CHO intake was adequate. The significance of this finding (i.e. from a practical perspective) remains unclear as these conclusions were determined on visual inspection of the available evidence and are not supported by statistical procedures. As such, the magnitude of the performance change was not defined. It is also difficult to determine whether a benefit of protein ingestion exists in the absence of such procedures,

as several methodological inconsistencies (the confounding influence of which may be controlled) are evident across experimental investigations. For instance, the additional energy ingested when protein is added to a CHO-containing fluid may explain the performance benefit reported in some studies, and not others (i.e. where 'isocarbohydrate' vs. 'isoenergetic' beverage treatments are employed) [14]. Hence, the effect of dietary protein intake on subsequent endurance exercise performance requires further clarification.

1.1 Aims

The aim of the present review was to determine, via a two-part investigation, the influence of: (1) CHO co-ingested with water; and (2) protein co-ingested with CHO and water, during and/or following an initial bout of activity on subsequent endurance/anaerobic exercise performance. In addition, the current study sought to clarify the effect of:

(a) CHO (co-ingested with water) on performance when individuals are *not fasted* (i.e. fed) ahead of experimentation, i.e. does fasting exaggerate the benefit of CHO to performance?;
(b) Protein (co-ingested with CHO and water) on performance when CHO intake is 'suboptimal' (i.e. < 1.2 g·kg^{-1}·h^{-1}, as per Beelen et al. [11]); and,
(c) Protein (co-ingested with CHO and water) on performance when the comparator condition is 'isocarbohydrate', rather than 'isoenergetic', i.e. is it the administration of additional energy (i.e. via supplemented protein) that conveys a performance benefit, or the protein itself?

2 Methods

The methodology of this review was devised in accordance with specifications outlined in the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols 2015 Statement* [15] and registered at the International Prospective Register of Systematic Reviews (PROSPERO) (identification code: CRD42016046807) ahead of the formal study selection process.

2.1 Literature Search

Potential research studies were identified by searching the online databases SPORTDiscus (via EBSCOhost), PubMed (MEDLINE), Web of Science (via Thomas Reuters)[1] and Scopus from January 1985 until September 2016 using the terms carbohydrate* OR glucose OR fructose OR lactose OR sucrose OR sugar OR glycogen OR "sport* drink" OR "sport* beverage" OR protein OR "amino acid*" in combination with exercise* OR athletic OR performance OR sport* OR endurance OR sprint OR aerobic OR anaerobic. The star symbol (*) was used to capture the derivatives (by suffixation) of a search term and the enclosed quotation marks were used to search for an exact phrase. Records containing irrelevant terms (obesity, diabetes, rat, mouse, mice, animal, rodent, children, teenagers, adolescents, review, meta-analysis, illness, disease, elderly, older, geriatric, patient and hospital) were excluded from the literature search using the Boolean search operator 'NOT'. (The search was updated in June 2017 to capture recent publications). Two investigators (D.M. and C.I.) independently screened the potential research studies to identify relevant texts. Initially, all irrelevant titles were discarded. The remaining articles were systematically screened for eligibility by abstract and full text, respectively. The decision to include or discard potential research studies was made between two investigators (D.M. and C.I.). Any discrepancies were resolved in consultation with a third investigator (B.D.). The reference lists of all included studies were hand searched for missing publications. Full details of the screening process are displayed in Fig. 1.

2.2 Inclusion and Exclusion Criteria

Research studies that fulfilled the following criteria were eligible for inclusion:

1. Controlled trials (random or non-random participant allocation) employing repeated-measures experimental designs;
2. Human studies on adult (≥ 18 years of age) male and/or female participants devoid of medical conditions and co-morbidities. Studies completed using subjects with paraplegia due to spinal cord injury were accepted for review (where glucose tolerance was normal);
3. Endurance and/or anaerobic exercise performance (refer to Sect. 2.4) was measured under intervention and control conditions (refer to Sect. 2.3);
4. Athletic performance was preceded by an initial bout of physical exercise (any type), during and/or following which, an experimental condition was imposed.

[1] Web of Science (via Thomas Reuters) retrieved a comparatively large number of records [68,347 vs. ≤ 4789 records via each SPORTDiscus (via EBSCOhost), PubMed (MEDLINE) and Scopus] using the search strategy indicated above. To improve the efficiency of the study selection process, only those records categorised within the *Sport Sciences* field (3418 records) were retrieved from Web of Science.

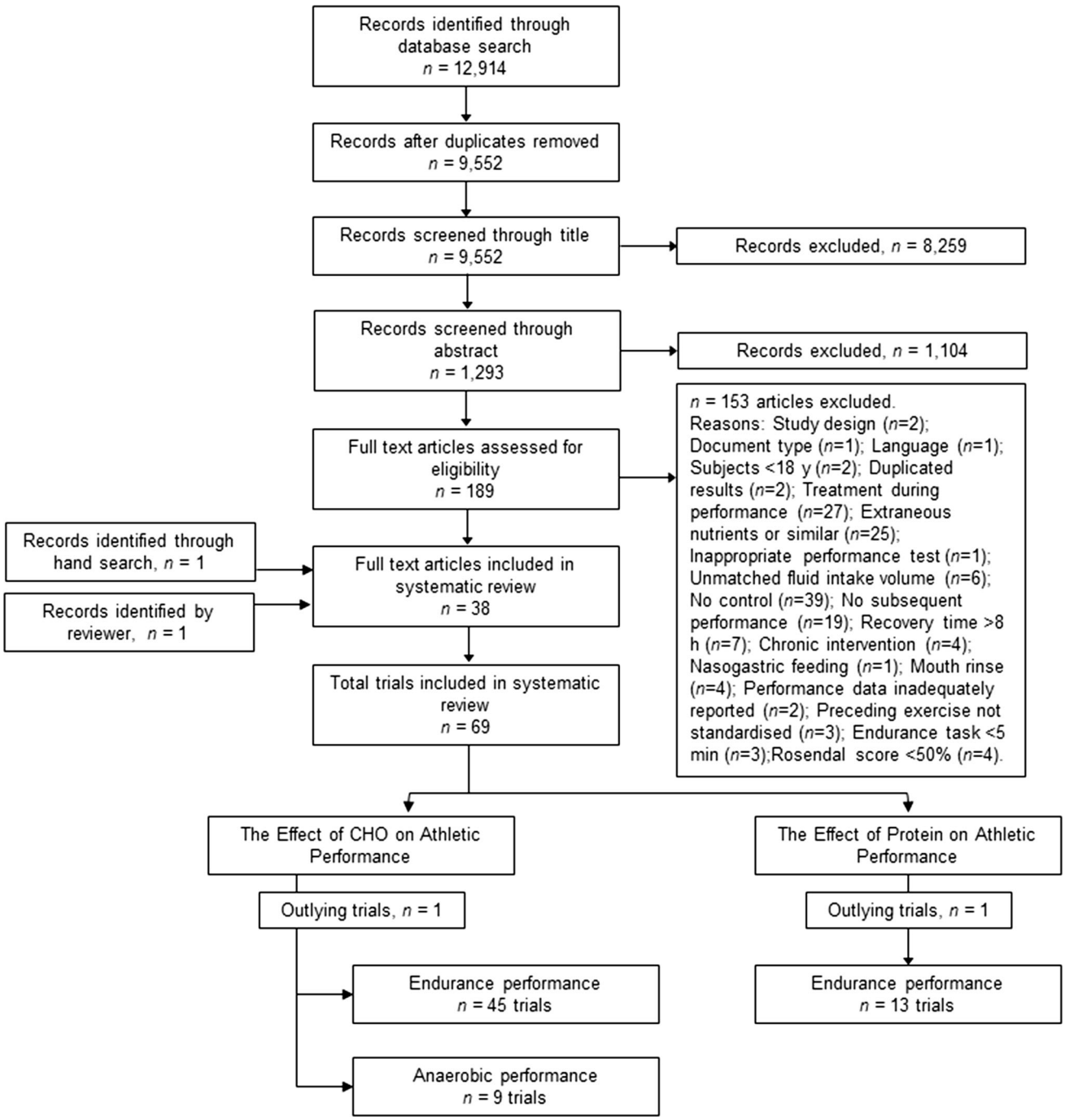

Fig. 1 Preferred reporting items for systematic reviews and meta-analysis flow chart (study selection methodology). Where a study contained more than one intervention arm that was eligible for inclusion (i.e. paired against a suitable control condition), these were treated as separate 'studies' termed '*trials*'. The updated search from September 2016 to June 2017 (not shown) did not identify any eligible studies. *CHO* carbohydrate

For the purpose of this review, athletic performance was considered 'subsequent' to another bout of exercise when: (a) a period of time separated the exercise bouts (i.e. *recovery time*), or (b) there was a change in the demands of the activity [i.e. mode of exercise or intensity, e.g. submaximal exercise followed immediately by a time trial (TT) performance task]. A schematic of the experimental protocol is displayed in Fig. 2;

5. The amount of time separating one exercise bout from another was ≤ 4 h. This cut-off was instated to reflect time restrictions associated with completing

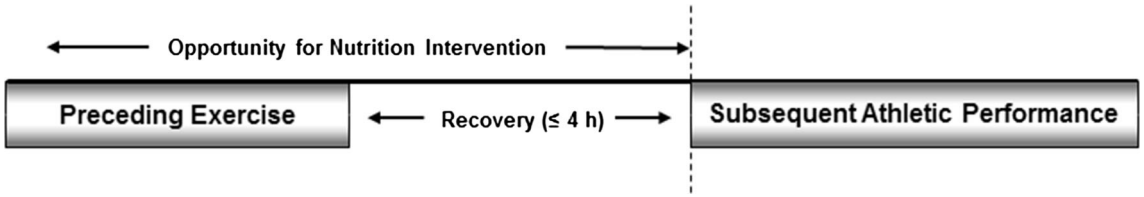

Fig. 2 Schematic of the experimental protocol employed in studies eligible for inclusion in the present review

consecutive exercise sessions. No minimum recovery time was set for inclusion;

6. Accessible full-text research articles (including complete conference proceedings) written in English. Other documents, e.g. review articles, meeting abstracts and research published in non-peer-reviewed sources were discarded.

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

Studies were excluded from the review if: (1) participants' dietary intake and/or exercise behaviour was experimentally altered ahead of testing (e.g. via a CHO loading regime or glycogen depletion diet); (2) the preceding bout of physical exercise was not standardised across experimental conditions [e.g. time to exhaustion (TTE) protocols were employed]; (3) an experimental condition was (a) delivered long term (i.e. a multi-day treatment, e.g. 7-day supplementation period prior to testing); (b) delivered whilst subjects were undertaking the athletic performance; or (c) not administered orally (e.g. via intravenous or nasogastric routes); (4) extraneous dietary and/or pharmacological constituents (e.g. caffeine), including placebo varieties were also administered during exercise and/or recovery; although additional electrolytes, vitamins and small quantities of fat were accepted; or (5) the performance data were not adequately reported, i.e. mean ± standard deviation (SD) was not quantified and could not be calculated. In the event that data were not adequately reported and the study was published within the previous 10 years (2006–2016), the corresponding author was contacted via email in an attempt to retrieve missing data. Potential research studies containing at least one eligible comparison between an intervention and control condition were included in the present review; other ineligible study arms derived from the same investigation were excluded from the current analysis.

2.3 Control and Intervention Conditions

The present systematic review aimed to compare the following experimental conditions (intervention vs. control), via a two-part investigation: (1) CHO co-ingested with water (CHO + W) vs. water (W); and (2) protein co-ingested with CHO and water (PRO + CHO + W) vs. CHO co-ingested with water (CHO + W). All nutrients consumed during the preceding exercise bout and/or recovery period were considered 'co-ingested'. The experimental conditions were defined in accordance with Table 1. Whilst W was accepted as water intake ≥ 200 mL, it was also a requirement that the volume was matched (≤ 5% difference from control) across intervention and control trials, such that the effect of CHO ingestion could be isolated. Similarly, comparison of PRO + CHO + W vs. CHO + W conditions required one of either total CHO content *or* total energy content to be matched across experimental trials (≤ 5% difference from control). Studies administering *whole proteins* were acceptable for review; interventions that contained single amino acids and/or peptides were excluded. Dietary intakes derived from food and/or fluid sources (including 'complex' beverages, e.g. chocolate milk) were accepted, *provided* that all of the aforementioned eligibility criteria were adhered to.

2.4 Primary and Secondary Research Outcomes

The primary research outcomes in this investigation were *endurance* and *anaerobic exercise performance*. Endurance exercise performance was defined as the percent change in mean power output ($\%_{\Delta}$ MPO) on a TT test that involved continuous running (treadmill/road) or cycling (ergometer/road) exercise for ≥ 5 min duration. The common metric (i.e. $\%_{\Delta}$ MPO on a TT test) was selected to facilitate interpretation of the intervention effect in the context of competitive performance [16]. Hopkins [17] suggests a 1% change in endurance power output on a laboratory-based test corresponds to a 1% change in competitive running performance and 0.4% change in competitive cycling performance. To maximise data capture, effects on performance in TTE tests were converted to effects on performance in TT tests, as described below (see Sects. 2.4.1 and 2.4.2). Similarly, where the $\%_{\Delta}$ MPO was not

Table 1 Experimental conditions

Experimental condition	Accepted definition
Part 1: CHO + W vs. W	
W	Total W intake ≥ 200 mL[a]
CHO + W	Digestible CHO (any type) co-ingested with ≥ 200 mL W
Part 2: PRO + CHO + W vs. CHO + W	
CHO + W	Digestible CHO[b] (any type) co-ingested with ≥ 200 mL[a] W
PRO + CHO + W	Whole P (i.e. single amino acids and/or peptides not accepted) co-ingested with digestible CHO (any type) and ≥ 200 mL W

CHO carbohydrate, *P* protein, *W* water

[a]Water intake must be volume matched (≤ 5%) to the corresponding control condition

[b]Either total CHO intake or total energy intake must be matched (≤ 5%) to the corresponding control condition

measured directly, it was derived from other performance outcomes. Anaerobic exercise performance was defined as the percent change in peak power output ($\%_{\Delta}$ PPO) on anaerobic exercise tests (< 60 s duration) that involved running (treadmill/road) or cycling (ergometer/road) exercise (see Sect. 2.4.3). Gastrointestinal tolerance was evaluated as a secondary research outcome. Raw scale ratings were extracted and converted to a 0–100 scale [(mean raw score/highest possible score on a given scale) × 100]. Where the lowest obtainable score was 1 (i.e. rather than *zero*), the raw score was transformed by $x - 1$ and divided by the adjusted maximum score to derive a percentage.

2.4.1 Time Trial Performance

Time trials included all constant work/distance and constant duration performance tests. Where TT performance was reported as mean power output (MPO) (Watts) [18–26], the change in endurance exercise performance was calculated using the following formula:

$$\%\Delta\text{MPO} = \frac{(\text{MPO}_{\text{Intervention}} - \text{MPO}_{\text{Control}})}{\text{MPO}_{\text{Control}}} \times 100,$$

where TT performance was assessed as total work completed on a fixed duration test [27–29], performance scores (J) were divided by test duration (s) to convert to effects on MPO (Watts). Conversely, where performance was assessed as time to complete a fixed amount of work [30, 31], the target work (J) was divided by the performance score (s) to convert to effects on MPO (Watts). (One study [31] expressed the target work in terms of energy expenditure). These values were multiplied by an energy efficiency of 23.2% [31] to approximate the kinetic bicycle energy, before calculating the change in endurance performance. Where TT performance was assessed as the time to complete a fixed distance [4, 5, 32–37], the performance scores(s) were used to determine $\%_{\Delta}$ MPO via the speed–power relationship, as described by Hopkins et al. [16].

Briefly, control scores were divided by intervention scores and raised to the power of x, a constant signifying the coefficient of variation for power output on a given cycle ergometer. (As power output is directly proportional to running speed, x was always equal to 1 on these tests) [38]. Where the Monark [4, 5, 39, 40], VeloTron [19, 22] and Schoberer Rad Messtechnik [36] ergometers were used, x was equal to 1.0 [41], 2.0 [42] and 1.6 [43], respectively. The value of x was not known for the Elite cycle trainer used by Cepero et al. [35]. Therefore, $\%_{\Delta}$ MPO was derived using the power-speed relationship: $P = 9.65S - 86.74$ [44], where S denotes speed ($\text{km}\cdot\text{h}^{-1}$) and P denotes power (Watts). Where TT performance was measured as distance on a constant duration test [39, 40], intervention performance scores were divided by control performance scores and raised to the power of x (as described above). Where studies evaluated TT performance in terms of MPO (Watts) [18–26], the length of time taken to complete the task was also recorded. This outcome was used to generate an 'imputed $\%_{\Delta}$ MPO' (i.e. using the methods indicated previously) for comparison against the reported value. Whilst the majority of the data were comparable, two studies [19, 22] reported a large $\%_{\Delta}$ MPO, with minimal effect on the time taken to complete the performance test (thus, a much smaller imputed $\%_{\Delta}$ MPO, i.e. > 2% points difference). This effect was likely owing to the power output data being non-normally distributed across time, such that the mean value did not accurately reflect the result of the performance test. In these situations, the imputed $\%_{\Delta}$ MPO was used to perform analyses.

2.4.2 Time to Exhaustion Performance

Time to exhaustion performance tests included all constant power/load and incremental exercise tests to fatigue. Prior

research demonstrates that the percent change in the duration of a constant power/load test is approximately equal to the $\%_{\Delta}$ MPO on a TT performance test when it is multiplied by a constant [38]. The constant is calculated as the power/load at which the test was performed [expressed as a percentage of maximal oxygen consumption ($VO_{2\ max}$)] divided by 6.4 [38]. Hence, where TTE was assessed as test duration [6, 14, 45–50], the change in endurance performance was calculated using the following formula [38]:

$$\%\Delta\text{MPO} = \left(\frac{(\text{Mean Test Duration}_{\text{Intervention}} - \text{Mean Test Duration}_{\text{Control}})}{\text{Mean Test Duration}_{\text{Control}}} \times 100\right) \div \left(\frac{\%\text{VO}_{2\text{max}}}{6.4}\right).$$

One study [49] expressed performance as a median and range; presumably because the data were non-normally distributed. Effect estimates for this study were therefore calculated using the *median* test duration. Another study [51] assessed TTE as peak power output (PPO) (Watts) on an incremental test to fatigue. The test commenced at a workload between 180 Watts, and increased by 1 Watts every 2 s, until fatigue. Time to exhaustion was therefore approximated as mean PPO minus 180 Watts, multiplied by 2 s. Scores were used to derive the change in athletic performance using the following formula [17]:

$$\%\Delta\text{MPO} = \left(\frac{(\text{Mean Test Duration}_{\text{Intervention}} - \text{Mean Test Duration}_{\text{Control}})}{\text{Mean Test Duration}_{\text{Control}}} \times 100\right) \times \left(1 - \frac{\%\ \text{PSPO}}{6.4}\right),$$

where %PSPO (Watts) represents the percentage of peak sustainable power output at which the test was commenced (i.e. 180 Watts, in the scenario described previously).

2.4.3 Anaerobic Performance

All anaerobic exercise tests were constant-duration TT performance tests. The change in anaerobic exercise performance was calculated where PPO (Watts) was reported, using the following formula:

$$\%\Delta\text{PPO} = \frac{(\text{PPO}_{\text{Intervention}} - \text{PPO}_{\text{Control}})}{\text{PPO}_{\text{Control}}} \times 100.$$

2.5 Methodological Quality Assessment

Included studies were examined for publication bias using the Rosendal Scale [52], where excellent methodological quality is indicated by a Rosendal score $\geq 60\%$ [53]. Scoring was determined by dividing the number of 'yes' responses by the total number of applicable items. Studies with a Rosendal score $< 50\%$ were excluded from this review owing to an increased risk of experimental bias.

2.6 Data Extraction and Synthesis

Data were extracted from relevant publications following the Cochrane Handbook for Systematic Reviews of Interventions *Checklist of Items to Consider in Data Collection or Data Extraction* [54] and entered into a Microsoft Excel spreadsheet. Extracted information included: (1) standardised pre-trial conditions; (2) participant characteristics (i.e. sample description, sample size, age, weight, height, sex, body fat content, $VO_{2\ max}$, PPO and menstrual phase at performance); (3) characteristics of the preceding exercise bout [i.e. exercise mode, duration, intensity, environmental conditions, fluid loss (calculated as percentage of body mass loss) and recovery time post-exercise]; (4) characteristics of the nutritional intervention [i.e. blinding procedures, nutritional composition of intervention and control treatments (i.e. CHO content, fluid volume, osmolality, temperature, other constituents), time of first intake and time to consume treatment]; (5) characteristics of the subsequent athletic performance [i.e. exercise description (exercise mode, duration, intensity), type of performance test, brand of cycle ergometer/trainer device or treadmill, incentives, environmental conditions and performance], and; (6) subjective ratings of GI discomfort, where these were reported. Where data were presented in graphical form only, high-performance digital calipers (ABSOLUTE Digimatic Caliper 500; Mitutoyo, Kawasaki, Japan) were used to extract numeric values.

2.7 Statistical Analyses

All statistical procedures were performed using IBM SPSS Statistical Software, Version 22.0 (Armonk, NY: IBM Corp) and Comprehensive Meta-Analysis, Version 3.0. Weighted mean effect estimates and meta-regression coefficients are presented as mean ± standard error of the mean. All other data are presented as mean ± SD.

2.7.1 Weighted Mean Effect

Meta-analyses were performed to determine the influence of: (1) CHO + W vs. W, and (2) PRO + CHO + W vs. CHO + W on athletic performance. Individual effect sizes were calculated as the $\%_{\Delta}$ MPO or the $\%_{\Delta}$ PPO (as described in Sect. 2.4), where a positive effect estimate indicates an increase in power output under the intervention condition. As the current review elected to measure the performance change as a *percentage* of the control score (i.e. rather than a *net* difference), the SD of the performance change (SD_{Δ}) could not be determined via standard

methods. Instead, *t*-statistics (or *p* values) derived from paired *t*-tests were used to calculate the SD_{Δ} of the *percent* performance change. Where an exact value was quoted [36, 55], the calculation was performed using the following formula [54]:

$$SD_{\Delta} = \frac{|\%_{\Delta}\text{MPO or PPO}|}{t\text{statistic}} \times \sqrt{n},$$

where the SD_{Δ} is the SD of the *percent* performance change and n is the number of participants. Where $p < x$ ($x \neq 0.05$) was reported [34, 45], p was taken to equal x and used to derive a *t*-statistic. Where only $p > x$ or $p < 0.05$ was reported (and raw performance data could not be retrieved), the missing *t*-statistic was imputed using the correlation coefficient (R). To do this, the SD_{Δ} of the *net* performance change was first calculated using the formula indicated below [54]:

$$\text{SD}_{\Delta} = \sqrt{(\text{SD}^2_{\text{Control}} + \text{SD}^2_{\text{Intervention}}) - (2 \times R \times \text{SD}_{\text{Control}} \times \text{SD}_{\text{Interventions}})},$$

where SD_{Δ} is the SD of the *net* performance change and R is the correlation coefficient. R was approximated as the mean correlation coefficient calculated using *t*-statistics (or *p* values) derived from paired *t*-tests and/or raw performance data, as indicated by Higgins and Green [54]. Sensitivity analyses were performed to test the robustness of the imputed R value. The imputed SD_{Δ} was then used to derive the required *t*-statistic, using the following formula:

$$t\text{statistic} = \frac{\text{Mean Performance Score}_{\text{Intervention}} - \text{Mean Performance Score}_{\text{Control}}}{(\text{SD}_{\Delta} \div \sqrt{n})}.$$

The weighted mean treatment effects were subsequently determined using random-effect models, where trials were weighted by the inverse variance for the performance change. Statistical significance was attained if the 95% confidence interval (CI) did not include zero. Heterogeneity was assessed with 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 [56]. A p value < 0.10 for Cochran's Q was used to indicate significant heterogeneity [54]. Sensitivity analyses were performed to determine the risk of bias due to data dependency (i.e. where multiple trials derived from a single publication bias a result). In this case, meta-analyses were performed using data derived from one trial per publication, only. Results are displayed in Table S1 of the Electronic Supplementary Material (ESM). The practical significance (i.e. under real-world conditions) of the effect of dietary intervention on endurance exercise performance was determined using a spreadsheet developed by Hopkins [57]. The smallest worthwhile $\%_{\Delta}$ MPO was calculated as 1.6% for endurance cyclists and 0.6% for endurance runners. These values were derived by multiplying the coefficient of variation for a particular competitive event [i.e. 1.3% for cycling events (1–40 km) and 1.1% for running events $\leq$ 10 km] by 0.5 [38] and transforming the threshold competition time to an equivalent threshold for cycling/running power output [17]. The effect was interpreted as 'unclear' if there was $> 5\%$ chance of attaining a both a clinically positive and clinically negative influence.

2.7.2 *Meta-regression Analyses*

Restricted maximum likelihood, random-effects meta-regression analyses were performed to determine the effect of: (1) CHO on performance when individuals are *not fasted* (i.e. 2–4 h post-meal) ahead of experimentation; (2) protein (co-ingested with CHO and water) on performance when CHO intake is 'suboptimal' (i.e. < 1.2 g·kg^{-1}·h^{-1}, as per Beelen et al. [11]), and; (3) protein (co-ingested with CHO and water) on performance when the comparator condition is 'isocarbohydrate', rather than 'isoenergetic'. To isolate these effects, it was necessary to control for the potentially cofounding influence of other extraneous variables. Simple meta-regression (i.e. one covariate per analysis) was initially performed to determine the influence of individual covariates on the magnitude of the performance change. If a significant relationship was identified (i.e. $p < 0.05$), each of the covariates were re-examined, this time using multiple meta-regression (i.e. more than one covariate per analysis) to control for the influential factor. All covariates are defined in Table 2. At least ten data points were required for a variable to qualify for meta-regression analysis. Categorical variables were dummy-transformed with $m - 1$, where m is the number of levels of the original variable. Regression analyses were examined for influential cases and outliers (i.e. studentized residuals, Cook's distance and centred leverage values), normality of residuals (Shapiro–Wilk Test) and multicollinearity (variance inflation factor). Statistical significance was accepted as $p < 0.05$.

3 Results

3.1 Overview of Included Studies and Study Quality

The literature search identified 43 eligible investigations. However, one of these studies [59] was removed from the review because the performance data could not be converted to the common metric for endurance exercise performance ($\%_{\Delta}$ MPO on a TT test). Four studies [60–63] scored $< 50\%$ on the Rosendal scale during the

Table 2 Covariates investigated

Covariate	Accepted definition
Study design	
Study blinding	Single- vs. double-blinded protocols. Studies that did not employ a blinded protocol were omitted from the analysis of this variable [32, 47, 48] as there were insufficient data to construct a third 'non-blinded' category
Time since last meal	'Fed' subjects were tested in a post-prandial state (2–4 h post-meal, as defined by Pochmuller et al. [8]) vs. 'Fasted' subjects ($\geq$ 10 h post-meal). When subjects were 4–10 h post-prandial, studies were omitted from the analysis of this variable [27, 28]
Participant population	
$VO_{2\ max}$	Studies that reported $VO_{2\ max}$ in units of $mL \cdot min^{-1}$ were divided by the mean BM of the subject group to convert to $VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), with the exception of Temesi et al. [29] where standardisation against BM was not considered appropriate because of the effects of paraplegia on body mass
Intervention characteristics	
Time from first intake to performance	The length of time (h) between the first intervention exposure and commencement of the athletic performance task
Total fluid intake	The total volume of fluid (L) consumed during the preceding exercise bout and/or subsequent recovery period under the intervention. Studies that administered an unspecified (but controlled) quantity of water alongside the experimental treatment [19, 36, 49, 50] were omitted from the analysis of this variable
Total CHO intake	The total quantity of CHO (g) consumed during the preceding exercise bout and/or subsequent recovery period under the intervention. Values that were reported relative to BM (kg) were multiplied by the mean BM of the subject group to approximate intake
Relative CHO intake	The relative CHO intake ($g \cdot kg^{-1}$) was determined by dividing the total CHO intake by the mean BM of the subject group. Ferguson-Stegall et al. [22] was excluded as values could not be reliably calculated
Rate of CHO delivery	The rate of CHO delivery ($g \cdot kg^{-1} \cdot h^{-1}$) was determined by dividing the relative CHO intake by the time from first intake to performance. Ferguson-Stegall et al. [22] was excluded as values could not be reliably calculated, as intake was stratified by BM
Total protein intake	The total quantity of protein (g) consumed during the preceding exercise bout and/or subsequent recovery period under the intervention. Values that were reported relative to BM (kg) were multiplied by the mean BM of the subject group to approximate intake
Relative protein intake	The relative protein intake ($g \cdot kg^{-1}$) was determined by dividing the total protein intake by the mean BM of the subject group. Ferguson-Stegall et al. [22] was excluded as values could not be reliably calculated, as intake was stratified by BM
Energy difference between beverages	The energy content of the intervention (kJ) minus the energy content of the control (kJ). Where the energy content of a treatment was not reported, it was calculated from the macronutrient composition, assuming an energy density of 16.7, 17.0 and 37.0 $kJ \cdot g^{-1}$ of CHO, protein and fat, respectively [58]
Performance characteristics	
Performance test	TTE vs. TT performance tests, defined as per Sects. 2.4.1 and 2.4.2
Duration of the performance test	The length of time (min) between commencing and concluding the athletic performance task under the control condition. Temesi et al. [29] was excluded as the duration on an arm-crank test may not be comparable to duration on a running or cycling test
Total exercise time	Total exercise time represents the duration of the performance test plus the length (min) of the preceding exercise bout. Temesi et al. [29] was excluded as the duration on an arm-crank test may not be comparable to duration on a running or cycling test
Exercise mode	Running (treadmill/road) vs. cycling (ergometer/road). The arm-crank test used in one study [29] was unable to be included in the analysis of this variable

BM body mass, *CHO* carbohydrate, *TT* time trial, *TTE* time to exhaustion, $VO_{2\ max}$ maximum oxygen consumption

methodological quality assessment and were subsequently ineligible for inclusion. A further two trials were omitted from the analyses as outlying data ($+17.95\%_{\Delta}$ MPO [39]; $+16.22\%_{\Delta}$ MPO [22]), with studentized residuals ≥ 3.3; excluding these trials did not significantly influence the result of the CHO + W ($\%_{\Delta}$ MPO = 4.246, 95% CI 3.413–5.080, $p < 0.001$) [39] or PRO + CHO + W ($\%_{\Delta}$ MPO = 0.848, 95% CI −0.393 to 2.089, $p = 0.180$) [22] meta-analyses. Overall, 67 repeated-measures trials ($n = 745$, 90.4% male) derived from 37 original publications were reviewed. The included studies yielded a Rosendal score of $63 \pm 9\%$ (mean ± SD). The highest Rosendal score of 81% was calculated for Betts et al. [14]. Complete results of the quality assessment are displayed in Table S2 of the ESM. A summary of included investigations is indicated in Table 3.

Table 3 Summary of experimental trials included in the current review

	CHO + W vs. W (endurance performance) 45 trials; $n = 486$ (92.9% male)		CHO + W vs. W (anaerobic performance) 9 trials; $n = 134$ (73.1% male)		PRO + CHO + W vs. CHO + W (endurance performance) 13 trials; $n = 125$ male individuals	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Study characteristics						
Sample size	11.1 ± 3.4	6–20	14.9 ± 12.0	8–36	9.6 ± 3.1	6–15
Double-blinded design	$n = 32$	–	$n = 6$	–	$n = 12$	–
Single-blinded design	$n = 10$	–	$n = 2$	–	$n = 1$	–
Subjects 'Fasted' (≥ 10 h post-prandial)	$n = 25$	–	$n = 6$	–	$n = 12$	–
Subjects 'Fed' (2–4 h post-prandial)	$n = 11$	–	$n = 3$	–	$n = 1$	–
Subject characteristics						
Age (years)	29 ± 4	23–35	24 ± 3	22–30	26 ± 7	21–39
BM (kg)	73.4 ± 4.4	62.2–80.0	69.7 ± 4.7	63.4–78.6	72.3 ± 6.5	61.1–83.5
$VO_{2\ max}$ (mL·kg BM^{-1}·min^{-1})	56.4 ± 6.1	42.8–69.8	56.1 ± 4.5	47.1–61.7	60.8 ± 3.9	51.4–65.6
Intervention characteristics						
Total fluid volume (L)	1.6 ± 0.7	0.2–3.6	0.5 ± 0.3	0.3–1.1	1.2 ± 0.5	0.7–2.6
Time from first intake to performance (min)	124 ± 73	40–375	53 ± 9	36–68	168 ± 61	75–240
CHO concentration (%)	9.4 ± 7.5	1.5–40.0	12.6 ± 7.1	6–20	7.5 ± 1.8	4.8–10.0
Protein concentration (%)	–	–	–	–	2.0 ± 0.7	0.9–3.3
Total CHO intake (g)	102 ± 50	30–247	51 ± 8	36–68	115 ± 61	50–232
Rate of CHO delivery (g·kg^{-1}·h^{-1})	0.8 ± 0.6	0.2–1.3	0.8 ± 0.1	0.8–0.9	0.6 ± 0.3	0.2–1.05
Total protein intake (g)	–	–	–	–	35 ± 26	10–87
Relative protein intake (g·kg^{-1})	–	–	–	–	0.5 ± 0.4	1.2–0.1
Performance test						
TT performance test	$n = 34$	–	$n = 9$	–	$n = 5$	–
TTE performance test	$n = 11$	–	$n = 0$	–	$n = 8$	–
Performance test duration	23.8 ± 16.1 min	6.1–86.1 min	–	30–40 s	38.3 ± 28.8 min	7.2–100 min
Environmental temperature (°C)	21 ± 4	10–32	NS	NS	NS	NS
Mode of exercise cycling	$n = 38$	–	$n = 8$	–	$n = 8$	–
Mode of exercise running	$n = 7$	–	$n = 1$	–	$n = 5$	–

BM body mass, *CHO* carbohydrate, *M* male subjects, *NS* not specified (or infrequently specified), *PRO* protein, *SD* standard deviation, *TT* time trial, *TTE* time to exhaustion, $VO_{2\ max}$ maximum oxygen consumption, *W* water

Values are presented as mean ± SD or a proportion (n) of the total number of trials for which the given characteristic is known

Percentage body fat mass, peak sustainable power output and body water loss were reported in too few studies for the data to accurately reflect the reviewed sample and were therefore omitted from the current summary

3.2 Effect of Carbohydrate (CHO) (Co-ingested with Water) on Athletic Performance

3.2.1 Effect of CHO (Co-ingested with Water) on Endurance Exercise Performance

Forty-five trials ($n = 486$; 92.9% male) derived from 25 publications investigated the effect of CHO + W on endurance exercise performance. Characteristics of included studies are summarised in Table 4. The mean correlation coefficient ($R = 0.715$) was imputed using raw performance data from 12 trials [22, 24, 29, 30, 32, 39, 47, 48] and two p values [34, 45]. The weighted mean effect estimate suggests that CHO + W significantly improves endurance exercise performance ($\%_{\Delta}$ MPO = 3.974, 95% CI 3.209–4.739, $p < 0.001$) when it is preceded by an initial bout of activity (Fig. 3). The magnitude and statistical significance of the effect were stable during sensitivity analyses where trials were sequentially removed ($\%_{\Delta}$ MPO range 3.792–4.094, CIs did not include zero). Findings were also comparable across different levels of correlation, suggesting the meta-analysis is robust to the imputed correlation coefficient (Table S3 of the ESM). The magnitude of this effect is such

Table 4 Characteristics of studies that investigated the effect of carbohydrate (CHO) co-ingested with water on endurance exercise performance

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design
Murray et al. [4], USA	12 (7 M)	42.8 ± 6.2	69.2 ± 14.5	DB
Murray et al. [5], USA	10 (8 M)	48.3 ± 2.6	72.9 ± 3.5	DB
Burgess et al. [6], USA	9 M	59.9 ± 5.4	74.1 ± 5.4	SB
	Trained cyclists			
Millard-Stafford et al. [34], USA	8 M	69.8 ± 3.7	70.5 ± 7.2	DB
	Trained long-distance runners			
Cole et al. [27], USA	10 M	59.6 ± 1.3	77.3 ± 1.9	SB
	Trained cyclists			
Below et al. [18], USA	8 M	62.9 ± 3.2	70.6 ± 8.5	DB
	Endurance trained			
El-Sayed et al. [40], UK	9 M	60.7 ± 6.6	69.9 ± 22.1	DB
	Competitive cyclists			
McConell et al. [28], Australia	9 M	68.9 ± 5.6	71.7 ± 4.0	DB
	Trained cyclists/triathletes			
Casey et al. [50], UK	10 M	52.7	76.1 ± 5.7	DB
	Well trained			
Wong et al. [45], UK	9 M	59.5 ± 4.5	71.0 ± .8.1	DB
	Endurance trained			
Ivy et al. [46], USA	9 M	61.3 ± 7.2	69.6 ± 7.5	DB
	Trained cyclists			
Abbiss et al. [19], Australia	10 M	61.7 ± 5.0	77.9 ± 6.6	DB
	Endurance trained cyclists			
Osterberg et al. [20], USA	13 M	56.0 ± 6.9	73.4 ± 9.0	DB
	Trained cyclists/triathletes			
Cox et al. [30], Australia	16 M,	61.7 ± 5.0	75.0 ± 6.7	NB
	Endurance trained cyclists/triathletes			
Smith [21], USA	12 M	55.3 ± 3.6	77.6 ± 6.9	SB
	Recreational cyclists/triathletes			
Temesi et al. [29], Australia	6 (5 M)	62.2 ± 19.7	22.2 ± 7.8	DB
	Tetraplegics/paraplegics			
Alghannam [47], UK	6 M	51.4 ± 5.0	71 ± 5	SB
	Amateur soccer players			
Ferguson-Stegall et al. [22], USA	10 (5 M)	52.6 ± 6.5	67.8 ± 7.3	DB
	Trained cyclists/triathletes			
Lee et al. [48], Singapore	12 M	53.9 ± 8.8	65.2 ± 6.6	(a) DB
	Physically active			(b) NB

Table 4 continued

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design
Robson-Anseley et al. [33]	9 M Trained runners	58 ± 4	75.5 ± 7.4	DB
Bonetti and Hopkins et al. [51], New Zealand	16 (sex NS) Trained cyclists/triathletes	52.4 ± 6.1	82.0 ± 8.8	DB
McGawley et al. [37], Sweden	10 (6 M) Amateur triathletes	62.8 ± 9.3	66.8 ± 9.2	SB
Too et al. [32], USA	11 M Competitive runners	58.2 ± 4.8	72.4 ± 11.1	NB
Heesch et al. [23], USA	8 M Recreational cyclists	56.8 ± 5.2	80.0 ± 6.3	DB
Newell et al. [24], UK	20 M Trained cyclists/triathletes	62 ± 9	74.6 ± 7.9	DB
Greer et al. [39], USA	**9 M** **Untrained**	**36.3 ± 2.2**	**84.2 ± 17.0**	**SB**

Citation, country	Time since last meal (h)	Preceding exercise	Recovery time (min)	Beverage administration
Murray et al. [4], USA	4	Cycle; 60 min; 65% $VO_{2\ max}$	0	2.5 $mL \cdot kg^{-1}$ pre-, and ea. 20 min of P-EX
Murray et al. [5], USA	2–3	Cycle; 120 min; 65–75% $VO_{2\ max}$	0	2.0 $mL \cdot kg\ LBM^{-1}$ ea. 15 min of P-EX
Burgess et al. [6], USA	O/N	Cycle; 165 min; 70% $VO_{2\ max}$	0	3.5 $mL \cdot kg^{-1}$ ea. 20 min of P-EX
Millard-Stafford et al. [34], USA	O/N	Run; 35 km; "moderate" pace	0	400 mL pre-, and 250 mL ea. 5 km of P-EX
Cole et al. [27], USA	6–10	Cycle; 105 min; 70% $VO_{2\ max}$	0	~ 175 mL ea. 15 min of P-EX
Below et al. [18], USA	O/N	Cycle; 50 min; 5% above LT	0	40% pre- and 20% at 15, 25 and 34 min of P-EX
El-Sayed et al. [40], UK	4	Cycle; 60 min; 70% $VO_{2\ max}$	0	3.0 $mL \cdot kg^{-1}$ pre-, and ea. 20 min of P-EX
McConell et al. [28], Australia	6–12	Cycle; 120 min; 70% $VO_{2\ max}$	0	(a) 250 mL pre-, and ea. 15 min of P-EX (b) 250 mL at 90, 105 and 120 min P-EX
Casey et al. [50], UK	O/N	Cycle; 83 ± 25 min; 70% $VO_{2\ max}$	240	1.0 g $CHO \cdot kg^{-1}$ at the onset of REC, only
Wong et al. [45], UK	O/N	Run; 90 min; 70% $VO_{2\ max}$	240	725 mL at 30 min REC and equal dose ea. 30 min of REC
Ivy et al. [46], USA	O/N	Cycle; 190 min; 45–75% $VO_{2\ max}$	0	200 mL pre- and ea. 20 min of P-EX
Abbiss et al. [19], Australia	< 4	Cycle; 90 min; 62% $VO_{2\ max}$	0	0.50 g $CHO \cdot kg^{-1}$ pre-, and 0.25 ea. 15 min of P-EX
Osterberg et al. [20], USA	O/N	Cycle; 120 min; 5% below LT	0	250 mL ea. 15 min of P-EX
Cox et al. [30], Australia	2	Cycle; 100 min; 70% $VO_{2\ max}$	~ 5	5 $mL \cdot kg^{-1}$ ea. 20 min of P-EX
Smith [21], USA	O/N	Cycle; 120 min; 77% $VO_{2\ max}$	0	250 mL pre-, and ea. 15 min of P-EX
Temesi et al. [29], Australia	O/N	Arm-cycle; 60 min; 65% $VO_{2\ max}$	~ 5	125 mL pre-, and 0, 15, 30 min of P-EX

Table 4 continued

Citation, country	Time since last meal (h)	Preceding exercise	Recovery time (min)	Beverage administration
Alghannam [47], UK	O/N	Run; 45 min[c]	0	During P-EX
Ferguson-Stegall et al. [22], USA	O/N	Cycle; 90 min; 75% $VO_{2\ max}$ and 5 × 60 s @ 90% $VO_{2\ max}$	240	50% at 0 and 120 min of REC
Lee et al. [48], Singapore	O/N	Cycle; 75 min; 65% $VO_{2\ max}$	300	Pre-, ea. 15 min of P-EX and at 15, 30, 45 and 60 min REC (dose NS)
Robson-Anseley et al. [33]	O/N	Run; 2 h; 60% $VO_{2\ max}$	0	2.0 mL·kg^{-1} pre-, and ea. 20 min of P-EX
Bonetti and Hopkins et al. [51], New Zealand	NS	Cycle; 120 min; 55–60% PPO	~ 10	250 mL ea. 15 min of P-EX
McGawley et al. [37], Sweden	NS	Swim 1500 m and cycle 40 km (intensity standardised)	0	25% ea. 10 km of P-EX (cycle)
Too et al. [32], USA	O/N	Run; 80 min; 75% $VO_{2\ max}$	0	0.50 g CHO·kg^{-1} pre-, and 0.2 ea. 20 min of P-EX
Heesch et al. [23], USA	O/N	Cycle; 120 min; 62% $VO_{2\ max}$	0	(a) 250 mL pre-, and ea. 15 min of P-EX (b) 250 mL pre-, and ea. 15 min of 1st h P-EX (c) 250 mL ea. 15 min of 2nd h P-EX
Newell et al. [24], UK	O/N	Cycle; 120 min; 59% $VO_{2\ max}$	~ 5	240 mL pre-, and ~ 220 mL ea. 15 min of P-EX
Greer et al. [39], USA	**4**	**Cycle; 90 min; 55% $VO_{2\ max}$**	**0**	**50% pre-, and 50% 60 min into P-EX**

Citation, country	Mean beverage volume (mL)	Intervention beverage CHO content (%)	CHO intake (g)	CHO type(s) (%)
Murray et al. [4], USA	692	(a) 6.0	41.5	S (6.0)
		(b) 8.0	54.3	S (8.0)
		(c) 10.0	69.2	S (10.0)
Murray et al. [5], USA	880	(a) 6.0	52.8	G (6.0)
		(b) 12.0	106	G + GP
		(c) 18.0	158	G + GP
Burgess et al. [6], USA	1900	1.8	34.0	NS
Millard-Stafford et al. [34], USA	1985	7.0	139	F (2.0) + GP (5.0)
Cole et al. [27], USA	1506	(a) 6.0	90.0	G + S
		(b) 8.3	125	[a]G (3.6) + F (4.7)
		(c) 8.3	125	[a]G (2.8) + F (3.5) + GP (2.0)
Below et al. [18], USA	(a) 1330	6.0	79.0	GP (6.0)
	(b) 200	40		GP (40.0)
El-Sayed et al. [40], UK	839	7.5	62.9	G (7.5)

Table 4 continued

Citation, country	Mean beverage volume (mL)	Intervention beverage CHO content (%)	CHO intake (g)	CHO type(s) (%)
McConell et al. [28], Australia	2250	7.0	158	NS
	750 (+ 1.5 L H_2O)	21.0	158	
Casey et al. [50], UK	410 (+ H_2O intake NS)	18.5	76.1	(a) G (18.5)
				(b) S (18.5)
Wong et al. [45], UK	3582	6.9	247	NS
Ivy et al. [46], USA	2000	7.8	155	NS
Abbiss et al. [19], Australia	600 (+ H_2O intake NS)	25.0	150	S (25.0)
Osterberg et al. [20], USA	2000	6.0	120	G + F + S (2.0 ea.)
Cox et al. [30], Australia	1875	10.0	188	G (10.0)
Smith [21], USA	2000	(a) 1.5	30.0	G (1.5)
		(b) 3.0	60.0	G (3.0)
		(c) 6.0	120	G (6.0)
Temesi et al. [29], Australia	500	Variable (0.5 g CHO·kg^{-1})	31.0	GP
Alghannam [47], UK	515	6.9	70.8	GP (6.9)
	(+460 mL H_2O)			
Ferguson-Stegall et al. [22], USA	1200	15.2	181	G (15.2)
	(+ 1.25 L H_2O)			
Lee et al. [48], Singapore	2325	6.8	158	G (4.8) + S (2.0)
Robson-Anseley et al. [33]	1057	8.0	85	NS
Bonetti and Hopkins et al. [51], New Zealand	2000	(a) 3.9	78	NS
		(b) 2.8	56	NS
		(c) 7.6	152	NS
McGawley et al. [37], Sweden	808	14.4	115	GP (9.6) + F (4.8)
Too et al. [32], USA	(a) Raisins (+ 1.23 L H_2O)	N/A	94	NS
	(b) "Chews" (+ 1.23 L H_2O)			
Heesch et al. [23], USA	2000	3.0	60	GP (3.0)
	1000 (+ 1.0 L H_2O)	6.0		GP (6.0)
	1000 (+ 1.0 L H_2O)	6.0		GP (6.0)
Newell et al. [24], UK	2000	(a) 2.0	40.0	NS
		(b) 3.9	78.0	NS
		(c) 6.4	128	NS
Greer et al. [39], USA	**837**	**6.1**	**51.1**	**NS**

Table 4 continued

Citation, country	Time from first intake to performance (min)	Athletic performance	Duration (min)	$\%_{\Delta}$ MPO
Murray et al. [4], USA	70	Cycle, TT (500 revs); 24 °C	13.6	+ 4.53
				+ 2.41
				+ 0.37
Murray et al. [5], USA	105	Cycle, TT (4.8 km); 10 °C	16.1	+ 6.09
				+ 4.55
				+ 6.47
Burgess et al. [6], USA	140	Cycle, TTE (80% $VO_{2\ max}$); 22 °C	16.1	− 0.95
Millard-Stafford et al. [34], USA	180	Run, TT (5 km); 28 °C	24.4	+ 11.42
Cole et al. [27], USA	90	Cycle, TT (15 min); 23 °C	15.0	+ 2.42
				+ 2.19
				+ 2.10
Below et al. [18], USA	50	Cycle, TT[a]; 31 °C	10.9	+ 5.84
				+ 7.49
El-Sayed et al. [40], UK	75	Cycle, TT (10 min); 22 °C	10.0	+ 8.49
McConell et al. [28], Australia	120	Cycle, TT (15 min); 21 °C	15.0	+ 10.74
	45			+ 4.55
Casey et al. [50], UK	240	Cycle, TTE (70% $VO_{2\ max}$)	35	+ 1.31
				+ 2.87
Wong et al. [45], UK	210	Run, TTE (70% $VO_{2\ max}$); 21 °C	45.0	+ 4.94
Ivy et al. [46], USA	180	Cycle, TTE (85% $VO_{2\ max}$); 20 °C	12.7	+ 4.15
Abbiss et al. [19], Australia	90	(a) Cycle, TT (16.1 km); 18 °C	25.4	+ 0.00
		(b) Cycle, TT (16.1 km); 32 °C	27.5	+ 6.88
Osterberg et al. [20], USA	105	Cycle, TT[b]; 23 °C	39.7	+ 6.10
Cox et al. [30], Australia	85	Cycle, TT[c]; 21 °C	31.9	+ 5.87
Smith [21], USA	105	Cycle, TT (20 km); 23 °C	36.4	+ 7.14
				+ 8.10
				+ 10.48
Temesi et al. [29], Australia	75	Cycle, TT (20 km); 21 °C	15	+ 2.73
Alghannam [47], UK	105	Run, TTE (80% $VO_{2\ max}$); 21 °C	11	+ 3.99
Ferguson-Stegall et al. [22], USA	240	Cycle, TT (40 km); 21 °C	86.1	+ 2.11
Lee et al. [48], Singapore	375	Cycle, TTE (65% $VO_{2\ max}$); 32 °C	32.0	+ 2.35
				+ 3.36
Robson-Anseley et al. [33]	120	Run, TT (5 km); 20 °C	24.0	+ 8.70

Table 4 continued

Citation, country	Time from first intake to performance (min)	Athletic performance	Duration (min)	$\%_{\Delta}$ MPO
Bonetti and Hopkins et al. [51], New Zealand	130	Cycle, incremental TTE; 20 °C	6.1	+ 4.15
				+ 1.30
				+ 3.63
McGawley et al. [37], Sweden	40	Run, TT (10 km); 16 °C	40.4	+ 4.39
Too et al. [32], USA	80	Run, TT (5 km); 22 °C	21.6	+ 4.85
				+ 4.35
Heesch et al. [23], USA	120	Cycle, TT (10 km); 22 °C	18.1	+ 5.49
	120			+ 5.06
	60			+ 7.17
Newell et al. [24], UK	125	Cycle, TT[d]; 19 °C	37.0	+ 5.70
				+ 8.00
				+ 9.00
Greer et al. [39], USA	**65**	**Cycle, TT (15 min); 22 °C**	**15**	**+ 17.95**

DB double-blind, *ea.* each, *F* fructose, *G* glucose, *GP* glucose polymers, *LBM* lean body mass, *M* male, $\%_{\Delta}$ *MPO* percent change in mean power output, *NB* non-blinded, *NS* not specified, *O/N* fasted overnight ($\geq$ 10 h), *P-EX* preceding exercise, *REC* recovery time, *S* sucrose, *SB* single-blind, *TT* time trial, *TTE* time to exhaustion, $VO_{2\ max}$ maximum oxygen consumption

The bolded trial was excluded from the meta-analysis

[a]High fructose corn syrup used in this study was presumed to contain 55% fructose (with the remaining CHO as free glucose) [64]

[b]Target work (J) = work rate at VO_2 at 10% above LT × 10 min 1 60 s·min^{-1} (value unpublished)

[c]Target work = 7 kJ·kg^{-1} (value unpublished)

[d]Target work = 0.7 × PPO (Watts) × 800 (value unpublished)

Study name	Difference in means	Standard error	Lower limit	Upper limit	p-Value
Burgess et al. (1991)	-0.95	2.39	-5.63	3.73	0.691
Abbiss et al. (2008a)	0.00	0.78	-1.52	1.52	1.000
Murray et al. (1989c)	0.37	1.70	-2.97	3.71	0.828
Bonetti & Hopkins (2010b)	1.30	2.35	-3.31	5.91	0.581
Casey et al. (2000a)	1.31	1.00	-0.65	3.27	0.191
Cole et al. (1993c)	2.10	2.41	-2.63	6.83	0.384
Ferguson-Stegall et al. (2011a)	2.11	2.20	-2.21	6.43	0.338
Cole et al. (1993b)	2.19	2.42	-2.55	6.93	0.365
Lee et al. (2011a)	2.35	0.79	0.79	3.91	0.003
Murray et al. (1989b)	2.41	1.74	-1.00	5.82	0.166
Cole et al. (1993a)	2.42	2.43	-2.35	7.19	0.320
Casey et al. (2000b)	2.87	1.12	0.68	5.06	0.010
Lee et al. (2011b)	3.36	0.76	1.88	4.84	0.000
Bonetti & Hopkins (2010c)	3.63	2.35	-0.97	8.23	0.122
Alghannam (2011)	3.99	0.68	2.66	5.32	0.000
Bonetti & Hopkins (2010a)	4.15	2.35	-0.46	8.76	0.078
Ivy et al. (2003a)	4.15	1.90	0.42	7.88	0.029
Too et al. (2012b)	4.35	1.37	1.66	7.04	0.002
McGawley et al. (2012)	4.39	0.77	2.88	5.90	0.000
Murray et al. (1989a)	4.53	1.76	1.08	7.98	0.010
Murray et al. (1991b)	4.55	2.71	-0.75	9.85	0.093
McConell et al. (1996b)	4.55	2.83	-0.99	10.09	0.107
Temesi et al. (2010)	4.63	4.79	-4.76	14.02	0.334
Too et al. (2012a)	4.85	1.84	1.24	8.46	0.008
Wong et al. (2000)	4.94	1.47	2.05	7.83	0.001
Heesch et al. (2013b)	5.06	5.27	-5.27	15.39	0.337
Heesch et al. (2013a)	5.49	4.77	-3.87	14.85	0.250
Newell et al. (2015a)	5.70	4.18	-2.50	13.90	0.173
Below et al. (1994a)	5.84	2.09	1.73	9.95	0.005
Cox et al. (2010)	5.87	6.28	-6.45	18.19	0.350
Murray et al. (1991a)	6.09	2.91	0.38	11.80	0.036
Osterberg et al. (2008)	6.10	3.13	-0.03	12.23	0.051
Murray et al. (1991c)	6.47	3.01	0.57	12.37	0.032
Abbiss et al. (2008b)	6.88	3.21	0.58	13.18	0.032
Smith et al. (2010a)	7.14	3.98	-0.65	14.93	0.073
Heesch et al. (2013c)	7.17	5.95	-4.48	18.82	0.228
Below et al. (1994b)	7.49	2.23	3.13	11.85	0.001
Newell et al. (2015b)	8.00	2.70	2.71	13.29	0.003
Smith et al. (2010b)	8.10	3.99	0.29	15.91	0.042
El-Sayed et al. (1995)	8.49	3.99	0.67	16.31	0.033
Robson-Anseley et al. (2011)	8.70	4.39	0.09	17.31	0.048
Newell et al. (2015c)	9.00	2.51	4.07	13.93	0.000
Smith et al. (2010c)	10.48	3.64	3.35	17.61	0.004
McConell et al. (1996a)	10.74	2.56	5.73	15.75	0.000
Millard-Stafford et al. (1992)	11.42	4.21	3.17	19.67	0.007
	3.97	0.39	3.21	4.74	0.000

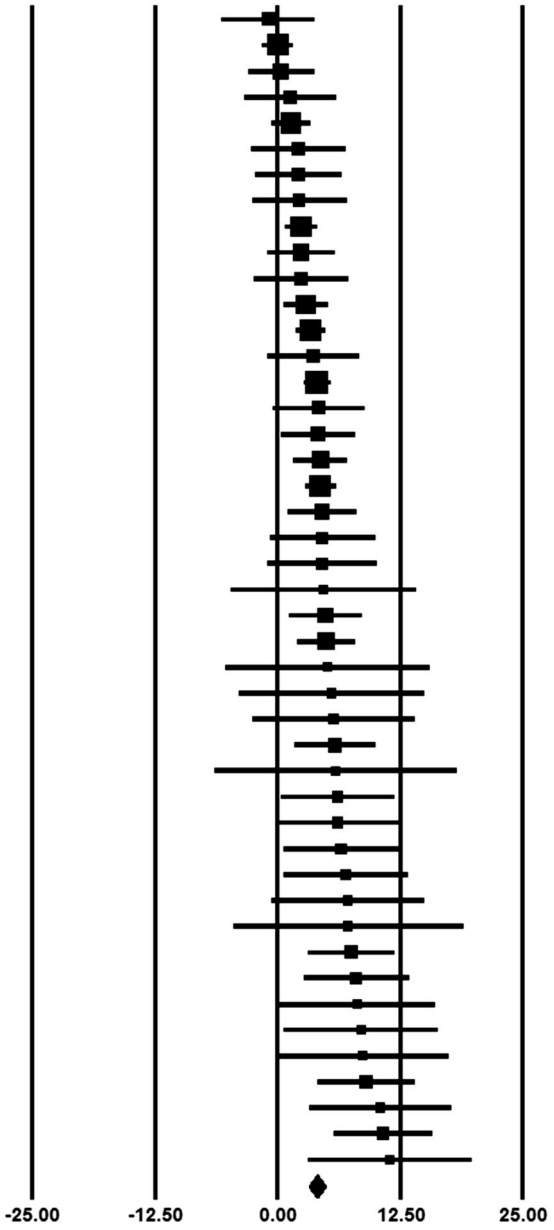

Fig. 3 Forest plot displaying the effect of carbohydrate plus water (CHO + W) vs. water (W) on the percent change in mean power output. The size of the *squares* is proportional to the weight of the study. A positive effect estimate indicates greater power output with CHO + W than W. *CI* confidence interval

that, > 99% of the time, CHO + W (delivered as indicated in Table 3) will *almost certainly* produce a clinically positive effect on endurance exercise performance, i.e. assuming a $+1.6\%_{\Delta}$ in competitive cycling performance or a $+0.6\%_{\Delta}$ in competitive running performance is required to convey a meaningful performance enhancement under real-world conditions. Moderate heterogeneity was present across trials ($I^2 = 43.899$, $p = 0.001$).

Simple meta-regression identified a significant effect of the Performance Test (i.e. 'TTE' $n = 11$ vs. 'TT', $n = 33$) ($p = 0.003$, $R^2 = 0.71$) on the $\%_{\Delta}$ MPO. Hence, the influence of this variable was controlled when modelling the effect of the remaining covariates on the change in endurance exercise performance. These analyses revealed a significant effect of Time Since Last Meal (i.e. 'Fed' $n = 10$ vs. 'Fasted', $n = 25$) ($p = 0.012$), where Time

Fig. 4 Correlation between time since last meal (Fed vs. Fasted) and the percent change in mean power output (%$_\Delta$ MPO) [95% confidence intervals shown], controlling for performance test (time trial vs. time to exhaustion). The *circle diameter* corresponds to the weight of each trial ($n = 35$). %$_\Delta$ MPO = 0.604 [± 0.992] + 2.508 [± 0.949], if Fasted + 2.958 [± 0.708], if time trial. Alternatively, %$_\Delta$ MPO = 3.562 [± 0.765] + 2.508 [± 0.949], if Fasted − 2.958 [± 0.708], if time to exhaustion; or %$_\Delta$ MPO = 6.071 [± 0.628] − 2.508 [± 0.949], if Fed − 2.958 [± 0.708], if time to exhaustion; or %$_\Delta$ MPO = 3.112 [± 0.349] − 2.508 [± 0.949], if Fed + 2.958 [± 0.708], if time trial. *Square brackets* are used to indicate the standard error of the mean of each regression co-efficient in the equation

Since Last Meal was controlled ($p < 0.001$; $R^2 = 1.00$) (Fig. 4). [One trial [19]$_{(a)}$ yielded comparatively large Cook's Distance values in the aforementioned analyses (Cook's $d = 0.50$, all other trials ≤ 0.06; Cook's $d = 1.4$, all other trials ≤ 0.13, respectively) and was therefore omitted owing to potential confounding effects]. These

Table 5 Summary of covariates analysed (via restricted maximum likelihood, multiple meta-regression analyses) for the carbohydrate plus water (CHO + W) treatment

Effect estimate	Mean difference (%$_\Delta$ MPO)	
Covariate	Coefficient (95% CI)	*p* value
Study blinding (SB vs. DB)	1.128 (− 0.371 to 2.626)	0.134
$VO_{2\ max}$	0.126 (− 0.025 to 0.276)	0.100
Time from first intake to performance	− 0.004 (− 0.010 to 0.003)	0.231
Total fluid intake	0.001 (− 0.001 to 0.001)	0.885
Total CHO intake	− 0.637 (− 2.627 to 1.353)	0.518
Relative CHO intake	0.031 (− 0.779 to 0.840)	0.939
Rate of CHO delivery	− 0.637 (− 2.627 to 1.353)	0.518
Duration of performance test	− 0.027 (− 0.061 to 0.008)	0.127
Total exercise duration	− 0.002 (− 0.018 to 0.014)	0.787
Exercise mode (run vs. cycle)	0.821 (− 0.512 to 2.153)	0.218

The influence of the performance test and time since last meal was controlled in each model

CI confidence interval, *DB* double-blind, %$_\Delta$ *MPO* percent change in mean power output, *SD* single-blind, $VO_{2\ max}$ maximum oxygen consumption

Table 6 Characteristics of studies that investigated the effect of carbohydrate (CHO) co-ingested with fluid on anaerobic exercise performance

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design	Time since last meal (h)	Preceding exercise	Recovery time (min)	Beverage administration	Mean beverage volume (mL)	Intervention beverage CHO content (%)	CHO intake (g)	CHO type(s) (%)	Time from first intake to performance (min)	Athletic performance	$\%_{\Delta}$ PPO
Ball et al. [55], USA	8 M Competitive cyclists	61.7 ± 5.2	78.6 ± 8.2	SB	O/N	Cycle; 50 min; 80% $VO_{2\ max}$	0	2 $mL \cdot kg^{-1}$ at 10, 20, 30 and 40 min P-EX	629	8.0	50.3	NS	40	30 s Wingate Test	+6.21
Sugiura et al. [67], Japan	8 M Competitive cyclists/triathlete	56.1 ± 3.8	66.9 ± 4.5	DB	O/N	(a, b) Cycle; 2 × 45 min blocks (with 15 min REC); 75% $VO_{2\ max}$ (c, d) Cycle; 2 × 45 min blocks (with 15 min REC); 65–100% $VO_{2\ max}$	0	250 mL during 15 min REC	250	20.0	50.0	(a) GP (20.0) (b) F (20.0) (c) GP (20.0) (d) F (20.0)	60	40 s Wingate Test	+3.81 +1.79 + 2.78 + 2.03
Jarvis et al. [68], USA	10 (0 M) Trained cyclists	47.1 ± 3.8	63.4 ± 7.3	DB	O/N	Cycle; 50 min; 80% $VO_{2\ max}$	0	2.0 $mL \cdot kg^{-1}$ at 0, 20, 30 and 40 min P-EX	507	7.0	35.5	GP (7.0)	40	40 s Wingate Test	+ 3.40
Clarke et al. [66], UK	12 M University soccer players	59.4 ± 6.0	74.5 ± 6.0	DB	3–4	Run, 45 min[a]	15	7 $mL \cdot kg^{-1}$ before P-EX and 7 $mL \cdot kg^{-1}$ during 15 min REC	1,065	6.4	67.7	NS	60	Run, 9 × 3.3 s sprints (30 s total)	− 0.67

Table 6 continued

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design	Time since last meal (h)	Preceding exercise	Recovery time (min)	Beverage administration	Mean beverage volume (mL)	Intervention beverage CHO content (%)	CHO intake (g)	CHO type(s) (%)	Time from first intake to performance (min)	Athletic performance	% Δ PPO
O'Neil et al. [69], USA	36 (23 M)	NS	71.4 ± 12.1	(a) SB (b) NB	2–4	Cycle; 5 × 10 min blocks (with 2 min REC); 60–65% HR_{max}	0	25% at 0, 20, 40 and 60 min P-EX	847	6.0	50.8	NS	50	30 s Wingate Test	+ 2.36 + 1.25

DB double-blind, *F* fructose, *G* glucose, *GP* glucose polymers, HR_{max} age predicted maximum heart rate, *M* male, *NB* non-blinded, *NS* not specified, *O/N* fasted overnight (≥ 10 h), *P-EX* preceding exercise, $\%_{\Delta}$ *PPO* percent change in peak power output, *REC* recovery time, *SB* single-blind, $VO_{2\ max}$ maximum oxygen consumption

[a]Soccer-specific exercise protocol consisting of various exercise intensities often observed during competitive soccer matches (e.g. walking, jogging and sprinting)

data suggest that the effect of CHO + W to enhance endurance exercise performance may be attenuated in individuals who have consumed food 2–4 h prior to testing ($\%_{\Delta}$ MPO = 0.605, if TTE; $\%_{\Delta}$ MPO = 3.562, if TT) in comparison to individuals who are fasted ≥ 10 h ahead of experimentation ($\%_{\Delta}$ MPO = 3.112, if TTE; $\%_{\Delta}$ MPO = 6.070, if TT). No other covariates significantly affected the magnitude of the performance change ($p > 0.05$) (Table 5).

3.2.2 *Effect of CHO (Co-ingested with Water) on Anaerobic Exercise Performance*

Nine trials ($n = 134$; 73.1% male) derived from five publications investigated the effect of CHO + W on anaerobic exercise performance. Characteristics of included studies are summarised in Table 6. The mean correlation coefficient ($R = 0.905$) was imputed using raw performance data from one trial [66] and one p-value [55]. The weighted mean treatment effect (Fig. 5) suggests that CHO + W significantly improves anaerobic exercise performance ($\%_{\Delta}$ PPO = 2.548, 95% CI 1.114–3.982, $p < 0.001$), when it is preceded by an initial bout of physical exercise. Low heterogeneity was present across trials ($I^2 = 0.000$, $p = 0.679$). The magnitude and statistical significance of the weighted mean effect were stable during sensitivity analyses ($\%_{\Delta}$ PPO range 2.026–2.845, CIs did not include zero). Findings were also comparable across different levels of correlation (Table S4 of the ESM).

3.2.3 *Effect of Protein (Co-ingested with CHO and Water) on Endurance Exercise Performance*

Thirteen trials ($n = 125$ male individuals) derived from nine publications investigated the effect of PRO + CHO + W on subsequent endurance exercise performance. Characteristics of included studies are summarised in Tables 7 and 8. The mean correlation coefficient ($R = 0.752$) was imputed using raw performance data from four trials [22, 31, 47] and one p value derived from a paired t test [36]. The weighted mean treatment effect indicates no difference in endurance exercise performance between PRO + CHO + W and CHO + W ($\%_{\Delta}$ MPO = 0.547, 95% CI −0.523 to 1.616, $p = 0.316$) (Fig. 6), despite the CHO dose being 'suboptimal' (< 1.2 g·kg body mass^{-1}·h^{-1}) on all trials. The magnitude and statistical significance of the effect were stable during sensitivity analyses ($\%_{\Delta}$ MPO range 0.188–0.866, 95% CIs included zero). Findings are also comparable across different levels of correlation (Table S5 of the ESM). The magnitude of this effect is such that, 97% of the time, PRO + CHO + W (delivered as indicated in Table 3) will *very likely* produce a clinically trivial effect on cycling

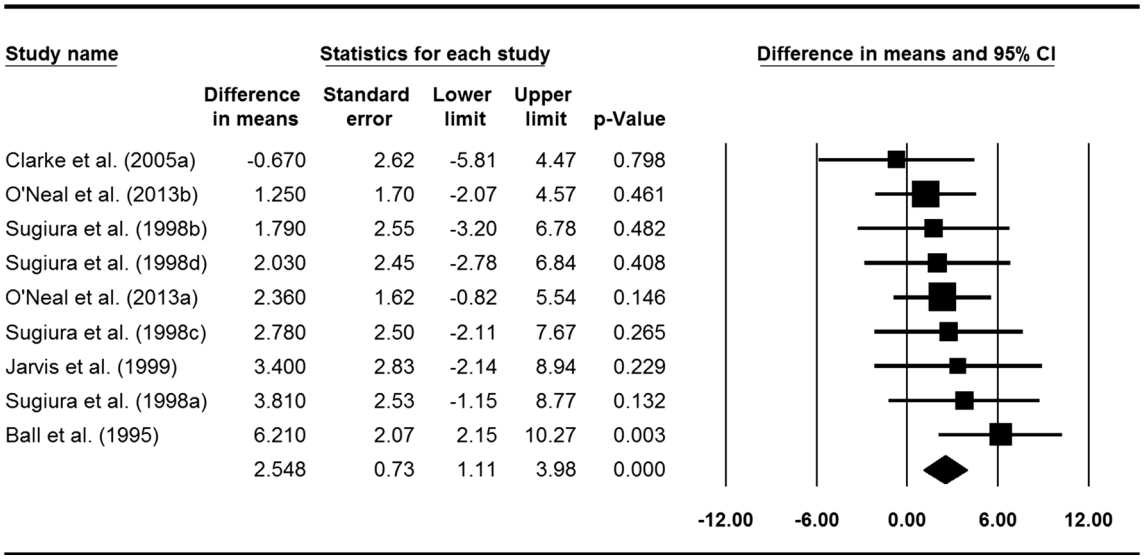

Fig. 5 Forest plot displaying the effect of carbohydrate with water (CHO + W) vs. water (W) on the percent change in peak power output. The size of the *squares* is proportional to the weight of the study. A positive effect estimate indicates greater power output with CHO + W than W. *CI* confidence interval

performance; and 51% of the time will *possibly* produce a clinically trivial effect on running performance, i.e. assuming a $+1.6\%_{\Delta}$ in competitive cycling performance or a $+0.6\%_{\Delta}$ in competitive running performance is required to convey a meaningful performance enhancement under real-world conditions. Moderate-to-high heterogeneity was observed amongst trials ($\%_{\Delta}$ MPO $I^2 = 72.92$, $p < 0.001$).

Initially, none of the proposed moderators were able to account for the between-trial variability observed (all simple meta-regression analyses, $p > 0.10$). However, on removing the study that received the lowest Rosendal score (53%) (and the only investigation that did not employ a double-blinded experimental design) [47], a significant effect of the energy difference between beverages was observed ($p = 0.015$, $R^2 = 1.00$) (Fig. 7). [One trial [14]$_{(b)}$ yielded a very large Cook's Distance (Cook's $d = 8.12$, all other trials ≤ 0.25) and was therefore omitted from this analysis owing to potential confounding effects]. These data suggest that the $\%_{\Delta}$ MPO may be increased in trials that administered an intervention beverage that contained more energy than the control beverage (i.e. those that matched beverage CHO content). Whilst it important to acknowledge that the two trials omitted from this analysis observed a large benefit of protein ingestion using isoenergetic beverages, a trend for a significant effect of this covariate on the $\%_{\Delta}$ MPO ($p = 0.098$, $R^2 = 1.00$) remained detectable when the outlying study [22] was reintroduced to the analysis. The remaining covariates were investigated using simple meta-regression analyses, given that the small cohort of trials ($n = 11$) was not appropriate for multiple meta-regression. These covariates did not significantly affect the magnitude of the performance change ($p > 0.05$) (Table 9).

3.3 Subjective Gastrointestinal Tolerance

Twelve trials derived from six publications measured GI symptomology following dietary intervention [4, 5, 32, 37, 45, 47]. These data are summarised in Table S6 of the ESM. The median CHO intake (at the time symptomology was assessed) was 49.6 g (range 10.4–247 g), whereas fluid intake was 522 mL (range 174–3582 mL) [excluding baseline values]; only one trial [47] assessed GI discomfort following protein ingestion (21.2 g). The majority of trials observed negligible/mild GI distress (e.g. scores 0–25), irrespective of the dietary treatment imposed (i.e. W, CHO + W and PRO + CHO + W); no treatment elicited a score > 50. That said, one trial [14]$_{(a)}$ (which did not present GI symptomology data graphically or numerically) commented that two participants experienced such severe GI distress on the CHO + W treatment that the performance test had to be terminated. This trial delivered the largest quantity of CHO in the present review (320 g). Only Wong et al. [45] assessed GI tolerance *during* the athletic performance. The collective data do not appear to indicate a trend for increased GI discomfort on intervention vs. control trials.

Table 7 Characteristics of studies that investigated the effect of protein co-ingested with carbohydrate (CHO) and fluid on endurance exercise performance (see Table 8 for beverage characteristics)

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design	Time since last meal (h)	Preceding exercise	Recovery time (h)	Beverage administration	Time from first intake to performance (min)	Athletic performance	Duration (min)	% $_{\Delta}$ MPO
Ivy et al. [46], USA	9 M Trained cyclists	61.3 ± 7.2	69.6 ± 7.5	DB	O/N	Cycle; 180 min; 45/75% $VO_{2\ max}$	0	200 mL pre-, and ea. 20 min of P-EX	180	Cycle, TTE (85% $VO_{2\ max}$); 20 °C	19.7	+ 2.75[a]
Betts et al. [49], UK	(a) 9 M Recreationally active	59.7 ± 11.4	79.6 ± 11.2	DB	O/N	Run, 90 min; 70% $VO_{2\ max}$	4	12.5% ea. 30 min of REC	240	Run, TTE (85% $VO_{2\ max}$); 23 °C	23.4	+ 1.82
	(b) 7 M Recreationally active	55 ± 11	83.5 ± 11.8								25.0	+ 0.63
Betts et al. [14], UK	6 M Recreationally active	61.4 ± 7.3	72.6 ± 8.4	DB	O/N	Run, 90 min; 70% $VO_{2\ max}$	4	12.5% ea. 30 min of REC	240	Run, TTE (70% $VO_{2\ max}$); 21 °C	(a) 99.9 (b) 83.7	(a) − 0.80 (b) + 0.82
Breen et al. [25], UK	12 M Trained cyclists	62.7 ± 6.3	70.5 ± 5.0	DB	NS	Cycle, 120 min; 50% PPO	0	270 mL ea. 15 min of P-EX	105	Cycle, TT (880 ± 94 kJ)	60.2	− 1.04
Cepero et al. [35], Spain	15 M Cyclists	65.6 ± 10.3	74.4 ± 7.2	DB	O/N	Cycle, 60 min; 75% $VO_{2\ max}$	2	1 L during 2 h REC	120	Cycle, TT (20 km)	29.5	(a) − 7.14 (b) − 8.38
Toone and Betts [36], UK	12 M Highly trained cyclists	64.3 ± 6.4	72.5 ± 5.2	DB	O/N	Cycle, 45 min; 60–90% $VO_{2\ max}$	0	7.0 $mL \cdot kg^{-1}$ pre-, and 2.5 ea. 15 min of P-EX	195	Cycle, TT (6 km)	7.2	− 1.82[a]
Alghannam et al. [47], UK	6 M Amateur footballers	51.4 ± 5.0	71 ± 5	SB	O/N	Run, 75 min[b]	0	During P-EX	75	Run, TTE (80% $VO_{2\ max}$)	16.5	+ 3.17[a]
Morifuji et al. [31], Japan	8 M Trained	60.1 ± 8.8	61.1 ± 5.6	DB	2	Cycle, 70 min; 68/88% $VO_{2\ max}$	2	350 mL at 0, 30, 60, 90 and 120 min of REC	120	Cycle, TT (365 ± 40 kJ); 21 °C	36.8	(a) − 1.19 (b) + 4.17
Siegler et al. [26], Australia	12 M	52.5 ± 5.2	76.0 ± 8.3	DB	O/N	Cycle, 90 min; 50% $VO_{2\ max}$	0	180 mL ea. 15 min of P-EX	90	Cycle, TT (5 km); 21 °C	7.6	+ 1.24

Table 7 continued

Citation, country	Participants	$VO_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Weight (kg)	Study design	Time since last meal (h)	Preceding exercise	Recovery time (h)	Beverage administration	Time from first intake to performance (min)	Athletic performance	Duration (min)	% $_{\Delta}$ MPO
Ferguson-Stegall et al. [22], USA	**10 (5 M) Trained cyclists/triathletes**	**52.6 ± 6.5**	**67.8 ± 7.3**	**DB**	**O/N**	**Cycle, 100 min; 70% $VO_{2\ max}$**	**4**	**50% at 0 and 120 min of REC**	**240**	**Cycle, TT (40 km)**	**86.1**	**+ 16.22**

DB double-blind, *ea.* each, *M* male subjects, *%*$_{\Delta}$ *MPO* percent change in mean power output, *NS* not specified, *O/N* fasted overnight (≥ 10 h), *P-EX* preceding exercise, *REC* recovery, *TT* time trial, *TTE* time to exhaustion, $VO_{2\ max}$ maximum oxygen consumption

[a]Significant difference between performances undertaken with and without protein ($p < 0.05$)

Bolded trial was excluded from the meta-analysis

[b]A soccer-specific exercise protocol consisting of various exercise intensities that are often observed during competitive soccer matches (e.g. walking, jogging and sprinting) [65]

4 Discussion

The present systematic review and meta-analysis summarise evidence for the effect of: (1) CHO co-ingested with water; and (2) protein co-ingested with CHO (and water), during and/or following an initial bout of exercise on subsequent athletic (endurance/anaerobic exercise) performance. Results indicate a beneficial effect of CHO on subsequent endurance exercise performance. Whilst the magnitude of improvement was significantly diminished when participants were 'Fed' (i.e. a meal 2–4 h prior to the initial bout) as opposed to 'Fasted' on commencing the initial exercise bout, a positive effect of CHO was still detectable under the 'Fed' condition. No further benefit was derived with the addition of protein to a CHO-containing beverage. Indeed, the performance-enhancing effect of protein demonstrated in some studies appears to be a consequence of the additional energy delivered, rather than an isolated effect of protein ingestion itself. A significant improvement in anaerobic exercise performance was also observed with CHO ingestion. Collectively, findings from the present investigation indicate that athletes with limited time for nutritional intake between consecutive exercise sessions should prioritise CHO ingestion (with fluid) to enhance subsequent athletic performance.

4.1 Effect of CHO (Co-ingested with Water) on Athletic Performance

The weighted mean effect estimate indicates that CHO co-ingested with water during and/or following an initial bout of activity improves subsequent endurance exercise performance, compared with control conditions (i.e. water only). More specifically, CHO administration (102 ± 50 g; 0.8 ± 0.6 $g \cdot kg^{-1} \cdot h^{-1}$) was demonstrated to increase MPO on a TT test by $\sim$ 4.0%, such that > 99% of the time, the magnitude of the performance enhancement (i.e. during competitive endurance cycling or running) is almost certain to be meaningful. Whilst the precise mechanisms underpinning these effects were not assessed in this review, accelerated muscle glycogen resynthesis [3], sparing of endogenous substrate stores [70], maintenance of blood glucose levels and CHO oxidation rates in the latter stages of exercise [71], and activation of central mechanisms [72] may be contributing factors. It is important to acknowledge that the inferences in this investigation are based on calculations of the smallest change required to enhance performance in a competitive endurance event (i.e. a *single* maximum effort) [17]. A performance test that is conducted after an initial exercise bout (and a period of recovery) may demonstrate greater test-retest variability;

Table 8 Characteristics of beverages used in studies that investigated the effect of protein (PRO) co-ingested with carbohydrate (CHO) and fluid on endurance exercise performance

Citation, country	Mean beverage volume (mL)	Control beverage				Intervention beverage								Energy difference from control beverage (kJ)
		CHO content (%)	CHO intake (g)	CHO intake ($g \cdot kg \cdot h^{-1}$)	CHO type(s) (%)	CHO content (%)	CHO intake (g)	CHO intake ($g \cdot kg \cdot h^{-1}$)	CHO type(s) (%)	PRO content (%)	PRO intake (g)	PRO intake ($g \cdot kg^{-1}$)	PRO type(s) (%)	Mean energy intake (kJ)	
Ivy et al. [46], USA	2000	7.8	157	0.75	NS	7.8	157	0.75	NS	1.9	38.8	0.56	NS	3282	+ 660
Betts et al. [49], UK	(a) 1031 (+ H_2O intake NS)	9.3	95.9	0.30	G (6.2) + F (3.1)	9.3	95.9	0.30	G (6.2) + F (3.1)	1.5	15.5	0.19	WP(H)	1867	+ 264
	(b) 722 (+ H_2O intake NS)	9.3	67.1	0.20		9.3	67.1	0.20		1.5	10.8	0.13		1303	+ 184
Betts et al. [14], UK	581	(a) 13.3	320	1.10	S (13.0)	10.0	232	0.80	S (10.0)	3.3	87.0	1.20	WP	5342	0
	(+ 1.3 L H_2O)	(b) 10.0	232	0.80	S (10.0)	10.0	232	0.80	S (10.0)	3.3	87.0	1.20		5342	+ 1459
Breen et al. [25], UK	2160 (+ 430 mL H_2O)	6.0	130	1.05	GP (6.0)	6.0	130	1.05	GP (6.0)	1.8	39.0	0.55	Protein (H)	2834	+ 663
Cepero et al. [35], Spain	1000	9.0	90.0	0.60	NS	7.0	70.0	0.47	NS	2.0	20.0	0.27	(a) WP (H)	1505	0
													(b) CP (H)		0
Toone and Betts [36], UK	1053 (+ H_2O intake NS)	9.0	94.8	0.40	S (9.0)	6.8	71.6	0.30	S (6.8)	2.2	23.0	0.32	WP (I)	1586	0
Alghannam et al. [47], UK	515 (+ 460 mL H_2O)	6.9	70.8	0.80	GP (6.9)	4.8	49.6	0.56	GP (4.8)	2.1	21.2	0.30	WP	1189	0
Morifuji et al. [31], Japan	1750 (+ 300 mL H_2O)	5.0	87.5	0.72	GP (5.0)	5.0	87.5	0.72	GP (5.0)	(a) 0.9	15.0	0.25	WP (H)	1725	+ 254
										(b) 2.3	40.0	0.65		2150	+ 680
Siegler et al. [26], Australia	1260	8.3	105	0.92	GP (8.3)	6.3	79.4	0.70	GP (6.3)	1.6	20.2	0.27	WP (I)	1680	− 74
Ferguson-Stegall et al. [22], USA	**1200**	**15.2**	**181**	**0.67**	**NS**	**11.5**	**138**	**0.51**	**NS**	**3.7**	**44.0**	**0.65**	**Milk-protein**	**3965**	**0**

CP casein protein, *F* fructose, *G* glucose, *GP* glucose polymers, *H* hydrolysate, *I* isolate, *NS* not specified, *S* sucrose, *WP* whey protein

Bolded trial was excluded from the meta-analysis

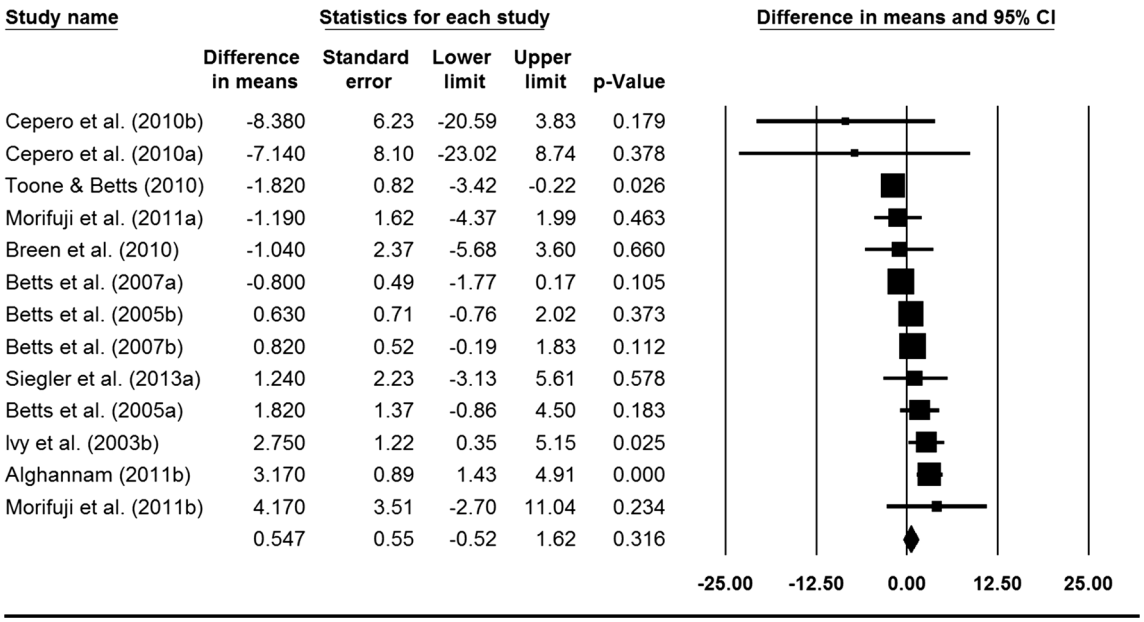

Fig. 6 Forest plot displaying the effect of protein plus carbohydrate plus water (PRO + CHO + W) vs. carbohydrate plus water (CHO + W) on the percent change in mean power output. The size of the *squares* is proportional to the weight of the study. A positive effect estimate indicates greater power output with PRO + CHO + W than CHO + W. *CI* confidence interval

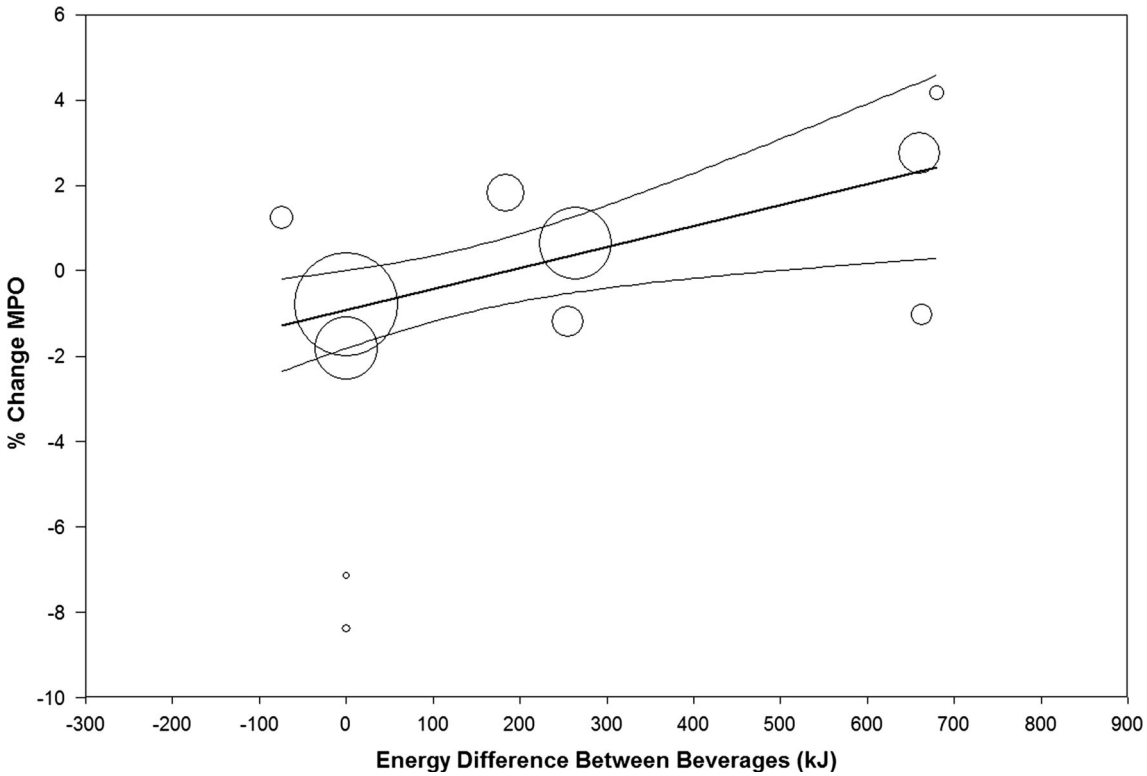

Fig. 7 Correlation between energy difference between beverages (kJ) and $\%_{\Delta}$ MPO (95% CIs). Circle diameter corresponds to the weight of each trial ($n = 11$). $\%_{\Delta}$ MPO $= -0.909[\pm 0.402] + 0.005$ $[\pm 0.002] \times$ Energy Difference Between Beverages (kJ). *Square brackets* are used to indicate the SEM of each regression co-efficient in the equation

Table 9 Summary of covariates analysed (via restricted maximum likelihood simple meta-regression) for protein plus carbohydrate plus water (PRO + CHO + W) [excluding Alghannam [47], $n = 12$]

Effect estimate	Mean difference ($\%_{\Delta}$ MPO)	
covariate	Coefficient (95% CI)	p value
$VO_{2\ max}$	− 0.223 (− 0.569 to 0.122)	0.181
Time from first intake to performance	0.004 (− 0.020 to 0.028)	0.717
Total CHO intake	0.002 (− 0.016 to 0.020)	0.778
Relative CHO intake	0.173 (− 0.010 to 1.445)	0.769
Rate of CHO delivery	0.414 (− 4.295 to 5.122)	0.859
Total protein intake	− 0.001 (− 0.040 to 0.040)	0.994
Relative protein intake	0.024 (− 2.824 to 2.873)	0.985
Performance test (TT vs. TTE)	− 1.761 (− 3.972 to 0.450)	0.106
Duration of performance test	− 0.006 (− 0.042 to 0.031)	0.738
Total exercise duration	0.012 (− 0.011 to 0.035)	0.270
Exercise mode (run vs. cycle)	0.566 (− 1.886 to 3.018)	0.618

TTE time to exhaustion, *TT* time trial, $VO_{2\ max}$ maximum oxygen consumption

Analysis of study blinding (single vs. double blind), Time Since Last Meal (fed vs. fasted) and total fluid intake could not be completed owing to insufficient trials

such that the magnitude of improvement required to convey a performance enhancement may be increased. However, the authors are not aware of calculated coefficients of variation that would facilitate this assessment. In any case, the smallest worthwhile change would need to increase considerably to alter the outcome of the present analysis.

Except for one trial [6], all individual effect estimates indicated a beneficial effect of CHO ingestion on endurance exercise performance. However, the magnitude of improvement was heterogeneous ($I^2 = 43.9$). The meta-regression analysis determined that differences in Time Since Last Meal ('Fed' vs. 'Fasted') and Performance Test (TT vs. TTE) could explain a large proportion of this heterogeneity ($R^2 = 1.00$). In regard to the influence of Time Since Last Meal, results suggest that the CHO-mediated performance effect may be exaggerated in 'Fasted', compared with 'Fed', individuals. This may be owing to a larger contrast in substrate availability under W vs. CHO + W treatments, i.e. resulting from lower glycogen levels post-exercise, and subsequently, accelerated glycogen resynthesis on exposure to CHO [3]. In most circumstances, athletes are recommended to avoid commencing exercise in a fasted state [2]. The current data indicate greater variability in the effect of CHO within the 'Fed' sub-group (Fig. 4). This may be partly owing to the smaller number of 'Fed' trials analysed. However, it could also reflect differences in the nutritional composition of the pre-exercise diet. Indeed, where the CHO content of the pre-exercise diet was specified, it ranged between 1.0 and 2.1 $g{\cdot}kg^{-1}$ [5, 19, 30]. This, along with other food-related factors (e.g. glycaemic load, other macronutrients/constituents and timing of intake) [2], could potentially influence the response to CHO during exercise. A detailed description of participants' pre-exercise diets was not always indicated in the manuscripts reviewed; hence, it was not possible to explore the influence of these factors on subsequent performance further. Despite the observed variability, a significant benefit of CHO ingestion was still detectable in the presence of a pre-exercise meal.

The current results also suggest that the CHO-mediated performance effect may be accentuated on TT compared with TTE performance tests. This observation is consistent with evidence from Vandenbogaerde and Hopkins [73], who detected a small difference in the magnitude of the effect of CHO supplementation across different performance tests in a meta-analytic investigation.

The weighted treatment effect demonstrates that CHO (53 ± 9 g; 0.8 ± 0.1 $g{\cdot}kg^{-1}{\cdot}h^{-1}$) co-ingested with water during and/or following an initial bout of exercise significantly increases PPO on a subsequent anaerobic performance test, compared with control conditions (i.e. water only). Endogenous CHO availability is not usually a limiting factor in anaerobic exercise performance. Furthermore, pre-exercise muscle glycogen levels do not generally influence PPO on short-duration performance tests [74, 75]. One factor that might explain the observed effect of CHO is enhanced central drive and/or motivation owing to the presence of CHO in the oral cavity (i.e. oral CHO receptor-mediated effects) [76]. Indeed, CHO mouth rinsing (i.e. *repeating* CHO exposures *during* exercise) has been shown to enhance exercise performance [72]. In the reviewed studies, the time between the final CHO exposure and the onset of performance was typically ≥ 10 min (up to 45 min [67]). At present, it is unclear how long CHO receptor-

mediated effects persist. In addition, given that the CHO in the current studies was ingested, gut-mediated responses (not just via the oral cavity) may be involved in influencing performance results [72]. The capacity for nutrient-sensitive receptors within the GI tract to modulate exercise performance is not well understood [72].

4.2 Effect of Protein (Co-ingested with CHO and Water) on Endurance Exercise Performance

Protein (35 ± 26 g; 0.5 ± 0.4 g·kg^{-1}) co-ingested with CHO (115 ± 61 g; 0.6 ± 0.3 g·kg^{-1}·h^{-1}) [and water] during and/or following an initial bout of activity does not appear to influence subsequent endurance exercise performance, compared with control conditions (i.e. CHO + W). Indeed, the present analyses indicated only a $\sim$ 0.5% increase in MPO on a TT test, such that 97% of the time, the effect of PRO + CHO + W on real-world endurance cycling performance is *very likely* to be trivial (i.e. no practical benefit or harm). Similarly, PRO + CHO + W will *possibly* produce a trivial effect on real-world endurance running, 51% of the time. Again, it is important to acknowledge that these inferences are based on the smallest change required to enhance performance in a competitive endurance event (i.e. a *single* maximum effort), which may not reflect the performance variability observed under the conditions of a subsequent exercise task.

While prior research suggests that protein is unlikely to influence muscle glycogen resynthesis when co-ingested with an 'optimal' dose of CHO (i.e. ≥ 1.2 g·kg^{-1}·h^{-1}, to maximise muscle glycogen repletion), protein consumed with a 'suboptimal' CHO dose (i.e. < 1.2 g·kg^{-1}·h^{-1}) may accelerate this process [11]. (Protein ingestion also has the potential to influence skeletal muscle damage repair during recovery from endurance exercise [12]; however, the amount of protein synthesis that occurs within ≤ 4 h is probably small). No studies in the current review administered protein with an 'optimal' CHO dose. Rather, the rate of CHO delivery ranged between 0.2 and 1.05 g·kg^{-1}·h^{-1}. Even with 'suboptimal' CHO intake, endurance performance was unaffected by PRO + CHO + W. Furthermore, subsequent regression analyses failed to indicate a significant effect of Relative CHO Intake (g kg^{-1}) on $\%_{\Delta}$ MPO, i.e. suggesting that the effect of dietary protein may be unrelated to CHO availability. These data are inconsistent with findings from a previous review [13], which reported that protein ingestion could improve subsequent endurance performance, provided CHO delivery was inadequate. However, this investigation defined 'optimal' and 'suboptimal' based on the rate of *nutrient* delivery (i.e. ≥ 1.0 g CHO/PRO kg^{-1}·h^{-1} was 'optimal'), as opposed to the rate of CHO delivery. Furthermore, the conclusions of the review were determined via visual inspection of the available data and were unsupported by statistical methods. One possible explanation for the lack of effect of dietary protein is that the difference in muscle glycogen levels under the PRO + CHO + W vs. CHO + W conditions ≤ 4 h post-treatment are too small to convey a practical benefit.

The magnitude and direction of the individual effect estimates in the PRO + CHO + W analysis were heterogeneous ($I^2 = 72.92$). Initially, none of the proposed moderator variables were able to account for the inconsistencies observed. However, a significant effect of the Energy Difference Between Control and Intervention Beverages ($R^2 = 1.00$) did become apparent on removing one study. This study [47] received the lowest Rosendal score (53%) and was the only investigation in the analysis that did not employ a double-blind experimental design. Clearly, blinding of investigators is an important consideration in experimental trials. This may be particularly true of *performance-based trials* where conscious or unconscious actions of an investigator (e.g. differences in verbal or non-verbal encouragement) have the potential to impact physical performance [77]. During Part 1 of the this investigation (W vs. CHO + W), regression analyses were performed to evaluate the influence of blinding on the performance result observed. However, this was not possible in the current analysis where only one study failed to employ a double-blind experimental design. Therefore, we determined that the most conservative approach was to conduct the analyses whilst both including, and excluding, this investigation. The significant influence of the Energy Difference Between Beverages (detected where Alghannam [47] was omitted) suggests that the magnitude of the performance effect may be related to the quantity of additional energy administered under the PRO + CHO + W condition, such that the benefit of protein demonstrated in some studies appears to be a consequence of the energy delivered in this nutrient, rather than an isolated effect of protein itself. This observation is consistent with experimental data by Betts et al. [14], who demonstrated a benefit of protein ingestion in comparison to an 'isocarbohydrate' control (+1400 kJ); where no effect was observed against an 'isoenergetic' control.

4.3 Gastrointestinal Tolerance

A subgroup of 12 trials evaluated GI symptomology following dietary intervention. Collectively, these data indicate similar mild levels of GI distress following either CHO + W or W ingestion (only one trial [47] assessed GI discomfort following PRO + CHO + W). Thus, ingestion of CHO with fluid provides a performance benefit without exacerbating GI intolerance. However, there are several

limitations to the current evidence. First, the quantity of CHO and fluid ingested at the time of performance assessment was relatively low ($\sim$ 50 g and 500 mL, respectively). Current guidelines [2] recommend individuals ingest fluid in volumes equivalent to 1.25–1.50 L·kg body mass lost^{-1} and consume 1–1.2 g CHO·kg^{-1}·h^{-1} (for 4 h) to restore fluid losses and optimise glycogen resynthesis, where the length of time separating one bout of exercise from another is <8 h. Thus, nutrients ingested in amounts/rates as per the guidelines may elicit different GI responses. Second, only Wong et al. [45] assessed GI tolerance during the athletic performance task. Gastrointestinal symptomology may be exacerbated during high-intensity exercise [78]; therefore, ratings obtained at rest or during submaximal intensity exercise may not provide a true indication of tolerance. Nevertheless, it appears that CHO ingested with fluid in amounts likely to benefit athletic performance does not augment GI distress any more than water alone. However, the extent to which CHO, protein and fluid are tolerated when ingested between consecutive exercise sessions in amounts corresponding with current nutrition recommendations requires further consideration.

4.4 Limitations

This review does contain several limitations. First, only studies with accessible full-text articles written in English were included. Second, it is likely that differences in the preceding exercise bout (i.e. duration/intensity) affected the level of glycogen depletion incurred across trials. Whilst these differences may moderate the effect of dietary intervention on the magnitude of the performance change, it was not possible to reliably estimate the severity of substrate depletion (based on a description of the exercise task) and subsequently control for this influence. Third, the practical relevance of the effect of CHO ingestion on endurance exercise performance in a 'Fed' state could not be calculated whilst simultaneously controlling for the influence of the Performance Test (TT vs. TTE). The practical relevance of the effect of CHO ingestion on $\%_{\Delta}$ PPO is also unknown, as the significance of this outcome in a real-life context is yet to be fully characterised. Finally, whilst pre-loaded exercise protocols were accepted in this review, these may not precisely reflect the demands of consecutive exercise sessions, owing to the limited amount of time separating the pre-load task from the performance test.

5 Conclusions

Results of the present review suggest that individuals who have limited opportunity for nutritional recovery between exercise bouts (e.g. ≤ 4 h) should prioritise CHO ingestion (with fluid) during and/or following the initial exercise session to enhance performance on subsequent tasks involving endurance and/or anaerobic activity. Protein ingestion is unlikely to benefit or harm subsequent endurance exercise performance and should be consumed as recommended to facilitate muscle protein synthesis [2].

Acknowledgements We thank all of the authors of the reviewed studies that provided raw experimental data for this investigation.

Author Contributions All authors were involved in the conception and design of this review. DM and CI were responsible for collating manuscripts and retrieving data. DM conducted the analysis of the data. All authors contributed to the drafting and revising of the article, and the final approval of the published version of the manuscript.

Compliance with Ethical Standards

Funding No sources of funding were received for the preparation of this article.

Conflict of interest Danielle McCartney, Ben Desbrow and Christopher Irwin have no conflicts of interest directly relevant to the content of this article.

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