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SYSTEMATIC REVIEW

The Influence of Protein on Beverage Hydration Efficacy: A Systematic Review and Meta-analysis

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Abstract

Background Beverage composition can dictate effectiveness of hydration and rehydration by influencing several physiological processes, including gastric emptying rate, intestinal absorption and oncotic pressure. While the benefits of solutes such as carbohydrates and electrolytes are well established in hydration beverages, research into the efficacy of protein inclusion remains sparse.

Objective The aim was to evaluate the effects of protein-containing beverages on hydration and rehydration, in terms of fluid retention, cumulative urine, net fluid balance (NFB) and plasma volume (PV) change, when compared with a control beverage.

Methods The systematic review was pre-registered (Open Science Framework) and conducted in accordance with PRISMA guidelines. PubMed, Embase, the Cochrane Library and Web of Science were searched from inception to February 2026 for human trials (≥ 18 years) researching hydration or rehydration following hypohydration or euhydration. To be eligible, beverages contained ≥ 2 g/L protein and reported at least one hydration outcome. Random-effects meta-analyses was performed using standardised mean differences (SMD).

Results Overall, 16 studies were included in this review (205 participants; 17.1% female, euhydrated: $n = 2$, hypohydrated: $n = 14$). Mean protein content in the studies was 23.7 ± 9.9 g/L. Meta-analysis performed on the rehydration studies revealed greater fluid retention (SMD 0.66, 95% CI 0.39–0.93, $P < 0.00001$, $Z = 4.85$, medium effect), reduced cumulative urine output (SMD − 0.71, 95% CI − 0.99 to − 0.43, $P < 0.00001$, $Z = 4.93$, medium effect), as well as increased NFB (SMD 0.58, 95% CI 0.30–0.85, $P < 0.0001$, $Z = 4.10$, medium effect) and augmented PV change (SMD 0.29, 95% CI 0.03–0.55, $P = 0.03$, $Z = 2.19$, small effect) following ingestion of protein.

Conclusions Protein-containing beverages confer an advantage over a control beverage on hydration metrics. These findings support protein as a potentially beneficial addition to rehydration strategies, however, there was a lack of female participants (17.1%), as well as plant-based beverages, limiting the generalisability of findings to female athletes, as well as those with diets devoid of lactose products.

1 Introduction

Maintaining euhydration, a state of optimal body water balance, is essential for both athletes and the general

Key Points

The addition of protein to a beverage appears to enhance parameters of rehydration.

Further work is required to explore the response in females.

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population, however during bouts of exercise, sweat loss often exceeds fluid intake resulting in a state of total body water deficit greater than normal daily fluctuations known as hypohydration [1]. This makes effective strategies to restore and maintain fluid balance essential. Beverage composition can dictate the efficacy of hydration/rehydration by influencing several physiological processes, including gastric emptying, intestinal absorption and osmotic and oncotic pressure [2, 3]. The use of plain water alone can potentially compromise hydration due to declines in plasma osmolality, [4, 5] and therefore, additional compounds, such as sodium, carbohydrates and protein, can be added to improve hydration/rehydration [2].

Traditionally, sports and rehydration drinks contain sodium and/or carbohydrates and are often combined due to their synergistic properties related to intestinal sodium–glucose co-transporter (SGLT) activity [6, 7]. Independently, sodium creates an osmotic gradient increasing fluid retention in the extracellular space [8], whilst carbohydrate consumption may also promote fluid retention during rehydration by decreasing gastric emptying rate and maintaining blood osmolality [9]. As a component of hydration/rehydration beverages, protein is less common. It can be included in beverages in several forms, including milk, amino acid formulations or concentrated/isolated protein sources (e.g. whey, casein), however it is primarily consumed to aid muscle protein synthesis [10]. There is evidence to suggest that the inclusion of protein in a beverage may influence hydration and rehydration responses beyond those achieved through fluid intake alone, including cumulative urine output, fluid retention, plasma volume and net fluid balance [4, 11–16]. When protein is consumed within a beverage, these potential effects occur concurrently with fluid intake, meaning that any effects on hydration may reflect both the volume of fluid consumed and the physiological effects associated with protein. Consequently, emerging evidence supports the ingestion of protein-containing beverages as a potential means of enhancing hydration efficiency beyond that achieved through fluid intake alone [2, 17]. Protein could influence all three phases (gastric emptying, intestinal fluid absorption and systemic fluid retention) involved in restoring body fluid compartments [15, 18]. Firstly, fluid retention following ingestion of protein-containing solutions may prevent the rapid decrease in blood osmolality and reduce urine output [3], as well as possibly increase plasma albumin synthesis [15]. Secondly, certain amino acids are co-transported out of the intestine, alongside sodium, creating an osmotic gradient to increase water uptake and absorption [19]. Finally, the presence of protein could improve fluid retention by delaying gastric emptying rate [20], which will have downstream effects on intestinal absorption and fluid retention [9].

The aim of this systematic review and meta-analysis was to investigate the efficacy of adding protein to hydration beverages. To our knowledge, this is the first meta-analysis assessing the effect of protein on beverage hydration efficacy. It was hypothesised that the presence of protein would improve hydration outcomes (i.e. a reduction in cumulative urine output, improved net fluid balance and increased drink retention) when compared with a non-protein containing control (i.e. water or a carbohydrate/carbohydrate–electrolyte drink). These outcomes were selected as they are commonly used within the rehydration literature and collectively provide complementary measures of whole-body fluid balance and fluid retention, encompassing both urinary fluid losses and changes in the intravascular fluid compartment. Furthermore, these measures are relatively practical, non-invasive or minimally invasive and feasible to implement across controlled experimental studies.

2 Methods

The protocol and methods for this systematic review and meta-analysis were pre-registered (Open Science Framework February 2026—https://osf.io/kd5m2/overview) and conducted in accordance with the updated Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [21].

2.1 Literature Search

A systematic search using the databases Pubmed, Cochrane Library, Embase and Web of Science plus citation searching was conducted from inception up to September 2026 to identify relevant papers investigating the effect of protein in hydration/rehydration beverages. The search strategy was developed using the Population, Intervention, Comparison and Outcome (PICO) framework; key concepts were identified relating to protein exposure, exercise and hydration status, fluid intake and hydration-related outcomes. For each concept, individual search terms were identified through database-controlled vocabulary, free-text synonyms, terminology used in relevant literature and reference checking of key studies. The search strategy was reviewed against relevant studies identified during preliminary searching to ensure that known eligible studies could be retrieved. Search terms are outlined in Supplementary Fig. 1. Title and abstract keywords were originally screened, subsequently followed by a search with the addition of Medical Subject Headings (MeSH) terms (Supplementary Fig. 2). The search was restricted to original research articles published in English in peer-reviewed journals. There was no restriction on date published. Search results were imported into

Covidence (Veritas Health Innovation, Melbourne, VIC, Australia) for initial screening and removal of duplicates. Articles were independently screened for eligibility by two authors, first by titles and then by abstracts. Full-text articles were then retrieved based on the inclusion/exclusion criteria (Table 1). At each stage if disagreements were not resolved between the two authors, a third author was consulted. The search strategy is outlined in Fig. 1. To isolate the effect of protein, carbohydrate difference between the control and experiment beverage was limited to 40 g/L [22, 46] and sodium difference to 10 mmol/L [2]

2.2 Data Extraction

Eligible studies were coded and data extracted for the following descriptive variables: sample size, participant characteristics, hydration status, method of hydration/rehydration, duration of monitoring period, drink composition, drink retention, net fluid balance and cumulative urine output. Data were extracted into Microsoft Excel independently by two authors. Data were collected directly from text and/or tables and, where possible, by contacting the authors. Data only shown in figures were extracted using graph digitising software (WebPlotDigitizer, automeris.io).

2.3 Study Quality Assessment

Two authors (F.G. and S.M.) independently assessed study bias using the Cochrane Collaborations Risk of Bias 2 tool [23] using pre-designed spreadsheets (available at https://www.riskofbias.info), with disagreements resolved by a third author. A score of > 60% was considered excellent methodological quality. Quality of studies were categorised as low risk of bias, some concern or high risk of bias based on the following categories: (1) randomisation process, (2) risk of bias arising from period and carryover effects, (3) deviations from intended interventions, (4) missing outcome data, (5) measurement of the outcome and (6) selection of the reported result.

2.4 Data Analysis

Meta-analysis was conducted using Review Manager (RevMan) version 7.2.0 (The Cochrane Collaboration, 2024) to aggregate the standardised mean difference (SMD) between drink composition for cumulative urine at the end of the monitoring period. A random-effects model was used for all analyses to account for potential between study variability in effect sizes. Effect sizes were transformed into a uniform scale using SMD summary statistics. These were interpreted in line with Hedges' g criteria (< 0.2 = trivial, 0.2–0.5 = small, 0.5–0.8 = medium, > 0.8 = large [24]). χ^2 and I^2 statistics were used to assess heterogeneity with $P < 0.10$ indicating significant heterogeneity with the following interpretation: < 25% indicates low risk, 25–75%

Table 1 Inclusion and exclusion criteria used for the search

Inclusion criteria	Exclusion criteria
1. Human participants (≥ 18 years old), males or females 2. Participants must be either euhydrated or hypohydrated. Hypohydration must have been induced using any of the following: exercise, heat and/or fluid restriction 3. The beverage had a pre-determined volume (absolute or relative amount) 4. Subjects who were hypohydrated must consume the drink post-exercise (in the 24 h after exercise) 5. The drink must contain ≥ 2 g/L of protein with information provided 6. Sodium content must be < 10 mmol/L difference between conditions 7. Carbohydrate content must be < 40 g/L difference between conditions 8. Volume of fluid consumed is matched between conditions 9. Placebo-controlled trial 10. At least one of the following markers of rehydration was measured and adequate data were provided: total/cumulative urine output, NFB, fraction of drink retention and BHI 11. Controlled trial utilising a repeated-measures experimental design; randomised participant allocation was not required 12. Articles must be in English, peer-reviewed, full-text primary research articles 13. All years of publication	1. Studies containing animals 2. Humans with co-morbidities or medical conditions 3. Beverage was only provided prior to or during exercise and if the fluid was provided intravenously [40] 4. Non-peer-reviewed articles, opinion articles and review articles

NFB, net fluid balance; BHI, beverage hydration index

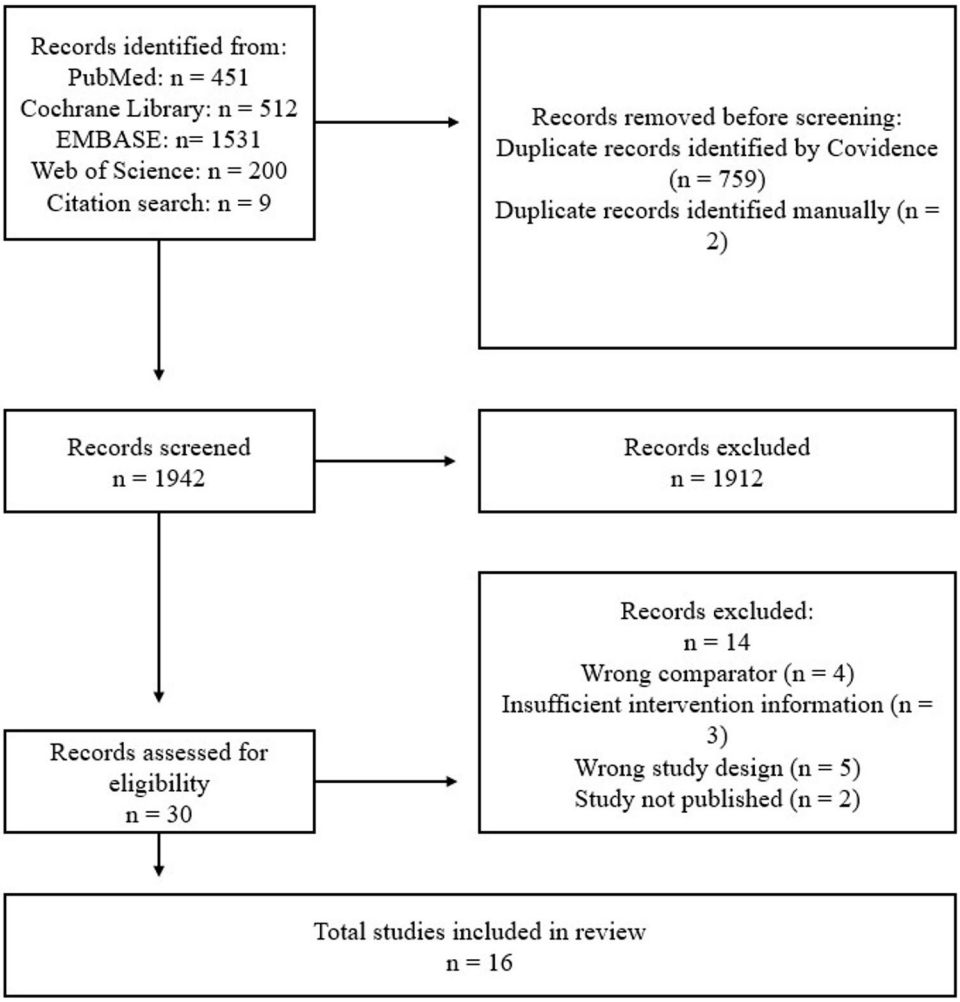


Fig. 1 Overview of selection process for this systematic review

indicates moderate risk and > 75% indicates high risk [23]. Statistical significance was considered at $P < 0.05$. Studies assessing rehydration following a dehydration process were analysed separately from hydration studies (consuming the beverage without a dehydration process), and sensitivity analysis was conducted to determine if studies with multiple arms would affect results.

2.5 Certainty of Evidence

The certainty of evidence for each outcome, fluid retention, cumulative urine output, net fluid balance and plasma volume change, was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. This was assessed via risk of bias, inconsistencies, indirectness and imprecision within the research, as well as an assessment of publication bias.

3 Results

3.1 Search Results

The initial search retrieved 2703 relevant studies (Fig. 1). After removal of 761 duplicates, 1942 studies were screened based on title and abstract, resulting in 1912 studies removed. Of the 30 full-text studies assessed for eligibility, 16 studies were deemed eligible for qualitative synthesis. Of these studies, 14 assessed the effect of protein following dehydration involving exercise and/or heat and the remaining 2 assessed maintenance of euhydration without a period of dehydration. A total of 205 participants (26 ± 10 years) were included (35 female participants, 17.1%). Both male and female participants were included in eight [3, 5, 13, 15, 20, 25–27], while the remaining eight included only male participants. The meta-analysis was performed with the rehydration studies

only, as there were insufficient euhydrated studies ($n = 2$) to perform a separate analysis.

3.2 Study Characteristics

Within the 16 studies, there were 15 (creating 19 comparisons) that compared a protein containing beverage with a carbohydrate-based beverage and 1 study (1 comparison) comparing protein with water (Table 2). A total of 13 studies compared two beverages (protein source versus control), whilst 3 studies compared ≥ 3 beverages. The effect of rehydration from a hypohydrated state was assessed by 14 studies, of which 9 induced dehydration by exercise in the heat (34.1 ± 1.8 °C). One study performed exercise in a temperate environment followed by 15 min intervals in a sauna at 70 °C until the desired body mass loss was reached [27]. The remaining four studies induced dehydration by exercise alone. Average body mass loss at the end of the dehydration phase was 2.1 ± 0.2%. The studies were performed using cycling ($n = 11$, 78.6%) and running ($n = 3$, 21.4%) at varying intensities. Maintenance of euhydration (no prior dehydration) was assessed in the remaining two studies (three comparisons; Table 3).

Within the 20 comparisons, protein sources compared with control drinks included milk ($n = 6$), milk protein ($n = 2$), whey ($n = 7$), casein ($n = 1$), soy ($n = 1$), amino acid blend ($n = 2$) and mixed blend ($n = 1$). Mean protein content in the studies was 23.7 ± 9.9 g/L, while protein content for all comparator beverages was 0 g. The average energy density of the protein containing beverages was 1408 ± 684 kJ/L while the comparator beverages had an average energy density of 908 ± 439 kJ/L. Energy density was matched in 5 of the studies, while the remaining 11 had an average increase of 765 ± 509 kJ/L in the protein-containing beverage when compared with the comparator.

3.3 Methodological Quality

Excellent methodological quality (i.e. a score of ≥ 60% based on criteria detailed in the Methods section) was reached in all 16 studies (Table 4). The order of trials was randomised in 12 studies and non-randomised (or not indicated) in the remaining 4 studies.

3.4 Sensitivity Analysis

Our initial analysis was performed with the inclusion of three studies that had multiple intervention arms per trial [4, 12, 16]. As this could lead to a unit of error analysis and bias the findings towards those studies [31], a sensitivity analysis was conducted to assess how removing studies with more than one intervention arm would affect the outcomes. In removing these studies, fluid retention (SMD 0.62), cumulative urine output (SMD − 0.65) and net fluid balance (SMD 0.52) maintained a medium effect size, while plasma volume change also maintained a small effect size (SMD 0.45). As such, studies with multiple intervention arms from the same study were included as they did not significantly affect the results and increased our statistical power. In all cases, they were treated as separate interventions.

3.5 The Effect of Protein Consumption During Rehydration on Fluid Retention (FR)

Overall, 13 of the 14 included studies measured fluid retention at the end of a monitoring period (4 ± 1 h) [3, 4, 11–17, 25, 27–29]. Across the 13 studies, 17 between-group comparisons were reported (protein versus water, protein versus carbohydrate–electrolyte, protein versus carbohydrate). Greater fluid retention (FR) values were found following ingestion of protein (SMD 0.66, 95% CI 0.39–0.93, $P < 0.00001$, $Z = 4.85$, medium effect) when compared with a control beverage (Fig. 2). In total, 10 of the 17 comparisons (58.8%) reported one or more conditions to have a significant increase in the volume of fluid retained after consuming the protein beverage when compared with the control, [3, 4, 11, 12, 14, 16, 28, 29], while the remaining 7 found no significant difference between beverages.

When analysing protein formats, whey protein comparisons showed a small effect for increased FR (SMD 0.35; Fig. 3A) whilst whole milk had a large effect for increased FR (SMD 0.98; Fig. 3B).

3.6 The Effect of Protein Consumption During Rehydration on Cumulative Urine Output

Overall, 13 rehydration studies measured cumulative urine output, of which there were 17 between-group comparisons [3, 4, 11–17, 25, 27–29]. In total, 11 of the 17 between-group comparisons found a significant decrease in cumulative urine output following ingestion of protein compared with a control beverage. The time of significant reductions in urine output differed between conditions with differences observed as early as 1 h post-ingestion [3, 25] or as late as 4 h post-ingestion [16]. The remaining six comparisons found no significant differences in cumulative urine output. A meta-analysis showed reduced cumulative urine output after ingestion of beverages containing protein compared with a control (SMD − 0.71, 95% CI − 0.99 to − 0.43, $P < 0.00001$, $Z = 4.93$, medium effect; Fig. 4).

Ingestion of a whey-containing beverage resulted in a small beneficial effect on cumulative urine output (SMD

Table 2 Characteristics of rehydration studies

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Aragon-Vargas et al., 2024 [25]	16	12M/4 F	22.9 ± 2.5	Dehydrated by exercise in heat	Carbohydrate–electrolyte versus protein	Skimmed, lactose-free milk	CHO–E: 6 Milk: 3.2	CHO–E: 18 Milk: 17	CHO–E: 0 Milk: 32	3 h	↔ Fluid retention (milk: 69.8 ± 16.8% versus CHO–E: 55.6 ± 24.4%) ↔ Net fluid balance (milk: − 155 ± 347 g versus CHO–E: − 405 ± 253 g) ↓ Cumulative urine output (milk: 489.9 ± 106.6 mL versus CHO–E: 780.9 ± 329.3 mL)
Desbrow et al., 2014 [4]	15	15M	24.9 ± 5.5	Dehydrated by exercise	Carbohydrate–electrolyte versus protein	Cow's milk, soy milk	CHO–E: 7.3 Cow's milk: 4.9 Soy milk: 5.1	CHO–E: 12 Cow's milk: 18 Soy milk: 20	CHO–E: 0 Cow's milk: 36 Soy milk: 32	4 h	Cow's milk: ↑ Fluid retention (cow: 40 ± 24.9% versus CHO–E: 16.6 ± 16.5%) ↓ Cumulative urine output (cow: 1338.4 ± 578.3 mL versus CHO–E: 1833.8 ± 427.1 mL) ↔ Plasma volume change (cow: − 1.36 ± 8.2% versus CHO–E: − 2.85 ± 7.4%) Soy milk: ↑ Fluid retention (cow: 46.9 ± 19.9% versus CHO–E: 16.6 ± 16.5%) ↓ Cumulative urine output (Soy: 1143.9 ± 446.3 mL versus 1833.8 ± 427.1 mL) ↔ Plasma volume change (soy: − 4.32 ± 6.7% versus CHO–E: − 2.85 ± 7.4%)

Table 2 (continued)

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Hobson et al., 2015 [15]	16	13M/3 F	24 ± 6	Dehydrated by exercise in heat	Carbohydrate versus protein	Whey protein	CHO: 6.2 Protein: 6.2	CHO: 26 Protein: 26	CHO: 0 Protein: 20	4 h	↔ Fluid retention (whey: 55 ± 15% versus CHO: 51 ± 12%) ↔ Net fluid balance (whey: − 307 ± 331 g versus CHO: − 393 ± 272 g) ↔ Cumulative urine output (whey: 970 ± 334 mL versus CHO: 1086 ± 317 mL) ↑ Plasma volume change (whey: 5.3 ± 4.2% versus CHO: 2.5 ± 2.2%)
James et al., 2011 [28]	8	8 M	21 ± 3	Dehydrated by exercise in heat	Carbohydrate versus protein	Milk protein	CHO: 6.5 Protein: 4	CHO: 7 Protein: 7	CHO: 0 Protein: 25	4 h	↑ Fluid retention (milk protein: 55 ± 12% versus CHO: 43 ± 15%) ↑ Net fluid balance (milk protein: −259 ± 266 g versus CHO: −523 ± 297 g) ↓ Cumulative urine output (milk protein: 931 ± 254 mL versus 1212 ± 310 mL)
James et al., 2013 [14]	8	8M	21.9 ± 2.0	Dehydrated by exercise in heat	Carbohydrate versus protein–carbohydrate	Milk protein	CHO: 6 Protein: 4	CHO: 21 Protein: 20	CHO: 0 Protein: 20	4 h	↑ Fluid retention (milk protein: 59 ± 12% versus CHO: 46 ± 9%) ↑ Net fluid balance (milk protein: – 181 ± 280 g versus CHO: − 470 ± 154 g) ↓ Cumulative urine output (milk protein: 857 ± 298 mL versus CHO: 1150 ± 245 mL)

Table 2 (continued)

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
James et al., 2012 [17]	12	12M	21 ± 1	Dehydrated by exercise in heat	Carbohydrate versus protein	Whey protein	CHO:6.5 Protein: 5	CHO: 21 Protein: 22	CHO: 0 Protein: 15	4 h	↔ Fluid retention (whey: 49 ± 11% versus CHO: 50 ± 18%) ↔ Net fluid balance (whey: − 432 ± 302 g versus CHO: − 432 ± 436 g) ↔ Cumulative urine output (whey: 1181 ± 330 mL versus CHO: 1173 ± 481 mL)
James et al., 2014 [13]	10	7M/3F	22 ± 2	Dehydrated by exercise in heat	Water versus protein	Whey protein	Water: 0 Protein: 0	Water: 0.4 Protein: 0.5	Water: 0 Protein: 20	4 h	↔ Fluid retention (whey: 37 ± 14% versus water: 40 ± 14%) ↔ Net fluid balance (whey: − 660 ± 274 g versus water: − 605 ± 318 g) ↔ Cumulative urine output (whey: 1306 ± 268 mL versus water: 1234 ± 358 mL) ↑ Plasma volume change (whey: 2.7 ± 3.2% versus water: − 1.2 ± 3.7%)

Table 2 (continued)

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Li et al., 2015 [12]	10	10M	20.7 ± 1.4	Dehydrated by exercise	Carbohydrate–electrolyte versus carbohydrate + whey protein Carbohydrate–electrolyte versus carbohydrate + casein protein	Whey protein, casein protein	CHO–E: 6.6 Whey protein: 4.4 Casein protein: 4.4%	CHO–E: 14 Whey protein: 14 Casein protein: 14	CHO–E: 0 Whey protein: 22 Casein protein: 22	4 h	Whey protein: ↑ Fluid retention (whey: 54.9 ± 9.2% versus CHO–E: 46.9 ± 16.5%) ↓ Cumulative urine output (whey: 1004 ± 217 mL versus CHO–E: 1184 ± 377 mL) ↔ Plasma volume change (whey: − 5.03 ± 6.71% versus CHO–E: − 6.25 ± 5.49%) Casein protein: ↔ Fluid retention (casein: 45.8 ± 17.3% versus CHO–E: 46.9 ± 16.5%) ↔ Cumulative urine output (whey: 1255 ± 416 mL versus CHO–E: 1184 ± 377 mL) ↔ Plasma volume change (casein: − 7.14 ± 6.98% versus CHO–E: − 6.25 ± 5.49%)

Table 2 (continued)

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Li et al., 2018 [16]	10	10M	22.0 ± 0.7	Dehydrated by exercise	High carbohydrate–electrolyte versus CW-L High carbohydrate–electrolyte versus CW-M High carbohydrate–electrolyte versus CW-H	Whey protein	CHO–E: 6.6 CW-L: 5.1 CW-M: 4.4 CW-H: 33	CHO–E: 21 CW-L: 21 CW-M: 21 CW-H: 21	CHO–E: 0 CW-L: 15 CW-M: 22 CW-H: 33	4 h	CW-L: ↔ Fluid retention (CW-L: 43 ± 12% versus CHO–E: 38.4 ± 16.4%) ↔ Cumulative urine output (CW-L: 1141 ± 183 mL versus CHO–E 1295 ± 326 mL) ↔ Plasma volume change (CW-L: − 0.35 ± 7.53% versus CHO–E: − 2.29 ± 6.26%) CW-M: ↑ Fluid retention (CW-M: 51 ± 18% versus CHO–E: 38.4 ± 16.4%) ↓ Cumulative urine output (CW-M: 1049 ± 411 mL versus CHO–E: 1295 ± 326 mL) ↔ Plasma volume change (CW-M: 0.04 ± 6.29% versus − 2.29 ± 6.26%) CW-H: ↑ Fluid retention (CW-H: 55.4 ± 12% versus CHO–E: 38.4 ± 16.4%) ↓ Cumulative urine output (CW-H: 891 ± 231 mL versus CHO–E 1295 ± 326 mL) ↑ Plasma volume change (CW-H: − 0.74 ± 8.76% versus CHO–E − 2.29 ± 6.26%)
Mallari et al., 2019 [20]	11	5M/6F	19.6 ± 1.7	Dehydrated by exercise	Carbohydrate–electrolyte versus protein	Skimmed, lactose-free milk	CHO–E: 6.8 Protein: 5	CHO–E: 15.3 Protein: 17.4	CHO–E: 0 Protein: 36	2 h	↔ Net fluid balance (milk: 410 ± 620 g versus CHO–E: 240 ± 1190 g)

Table 2 (continued)

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Seery et al., 2016 [29]	7	7M	26.2 ± 6.1	Dehydrated by exercise in heat	Carbohydrate–electrolyte versus protein	Milk	CHO–E: 3.9 Protein: 5	CHO–E: 21.7 Protein: 17.9	CHO–E: 0 Protein: 33	5 h	↑ Fluid retention (milk: 71 ± 4% versus CHO–E: 52 ± 16%) ↔ Net fluid balance (milk: 121 ± 16 g versus CHO–E: − 378 ± 499 g) ↓ Cumulative urine output (milk: 659 ± 71 mL versus CHO–E: 1269 ± 340 mL)
Seifert et al., 2006 [3]	13	8M/5F	20–28	Dehydrated by exercise	Carbohydrate–electrolyte versus carbohydrate–electrolyte–protein	Accelerade (Pacific Health Labs, Matawan, NJ)	CHO–E: 5 Protein: 3.9	CHO–E: 20 Protein: 23.1	CHO–E: 0 Protein: 15	3 h	↑ Fluid retention (protein: 88 ± 4.7% versus CHO–E: 74.9 ± 14.6%) ↓ Cumulative urine output (protein: 206.4 ± 99.3 mL versus CHO–E: 395.3 ± 242.2 mL) ↔ Plasma volume change (protein: 7.5 ± 7.3% versus CHO–E: 4.13 ± 13.4%)
Tai et al., 2014 [27]	20	10M/10F	M: 26.7 ± 4.8 F: 27.1 ± 4.7	Dehydrated by exercise	Carbohydrate–electrolyte versus protein	Amino acid blend	CHO–E: 0.26 Protein: 0	CHO–E: 0.02 Protein: 4.64	CHO–E: 0 Protein: 7.8	3 h	↔ Fluid retention (AA: 43.5 ± 17.1% versus CHO–E: 40.8 ± 18.1%) ↔ Cumulative urine output (AA: 1295.5 ± 457.6 mL versus CHO–E: 1348.3 ± 450 mL)
Watson et al., 2008 [11]	7	7M	23 ± 3	Dehydrated by exercise in heat	Carbohydrate–electrolyte versus protein	Skimmed milk	CHO–E: 6 Protein: 5	CHO–E: 23 Protein: 32	CHO–E: 0 Protein: 33	3 h	↑ Fluid retention (milk: 77 ± 6% versus CHO–E: 62 ± 17%) ↑ Net fluid balance (milk: 191 ± 162 g versus CHO–E: − 135 ± 392 g) ↓ Cumulative urine output (milk: 525 ± 118 mL versus CHO–E: 861 ± 396 mL)

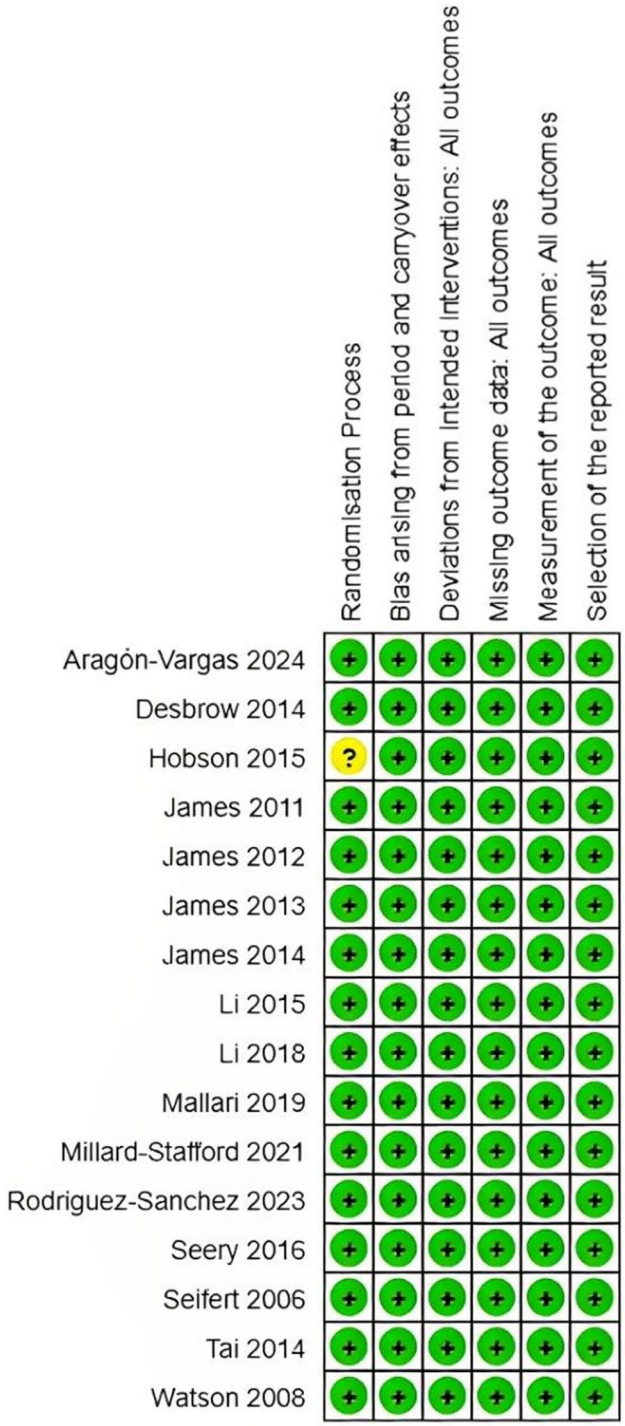
M, male; F, female; ↑, significant increase; ↓, significant decrease; ↔, no difference; CW-L, low protein; CW-M, medium protein; CW-H, high protein; AA, amino acid; CHO, carbohydrate, CHO–E, carbohydrate–electrolyte, SD, standard deviation

Table 3 Characteristics of euhydration studies

Author and year	Sample size	Sex	Mean age ± SD, years	Hydration status	Drink comparisons	Protein source	% CHO	Sodium, mmol/L	Protein quantity, g/L	Monitoring period	Results—effect of protein
Millard-Stafford et al., 2021 [26]	19	15M/4F	24.7 ± 5.6	Euhydrated	Electrolytes versus protein	Alanyl-glutamine	Electrolyte: 0 Protein: 0	Electrolyte: 21.7 Protein: 22.9	Electrolytes: 0 Protein: 2	4 h	↔ Net fluid balance (P: − 272.4 ± 204.7 g versus E: − 294.3 ± 276.4 g) ↓ Cumulative urine output (P: 1178 ± 277 mL versus E: 1182 ± 305 mL)
Rodriguez-Sanchez et al., 2023 [30]	23	12M	Young: 24.5 ± 4.3 Older: 63.8 ± 5.5	Euhydrated	Carbohydrate–electrolyte versus protein	Low fat 1% milk	CHO–E: 6.4 Protein: 5	CHO–E: 22 Protein: 19	CHO–E: 0 Protein: 34	3 h	↔ Net fluid balance (young – milk: 69 ± 210 g versus CHO–E: − 120 ± 311 g, older-milk: 22.5 ± 174 g versus CHO–E: 6.3 ± 199 g) ↔ Cumulative urine output (young-milk: 925.7 ± 338.6 mL versus CHO–E: 1120.3 ± 311.3 mL, older-milk: 977.5 ± 173.6 mL versus CHO–E: 993.6 ± 198.9 mL)

M, male; F, female; ↑, significant increase; ↓, significant decrease; ↔, no difference; CHO, carbohydrate, CHO–E, carbohydrate–electrolyte, SD, standard deviation; P, protein; E, electrolyte

Table 4 Risk of bias summary for all included studies



– 0.41; Fig. 5A), whilst ingestion of whole milk resulted in a large effect on decreasing cumulative urine output (SMD – 1.18; Fig. 5B).

3.7 The Effect of Protein Consumption During Rehydration on Net Fluid Balance (NFB)

Net fluid balance (NFB) was measured in ten of the rehydration studies [4, 9, 11, 14, 15, 17, 20, 25, 28, 29] with four finding an increase in NFB following ingestion of the protein-containing beverages 3 h post-rehydration [4, 11, 14, 28]. The remaining six studies found there to be no significant differences in NFB between beverages. Meta-analysis showed an increase in NFB following protein ingestion compared with a control (SMD 0.58, 95% CI 0.30–0.85, $P < 0.0001$, $Z = 4.10$, medium effect) (Fig. 6).

3.8 The Effect of Protein Consumption During Rehydration on Plasma Volume (PV) Change

Six of the rehydration studies measured plasma volume from baseline, providing ten between-group comparisons. All studies used haemoglobin and haematocrit values to estimate changes in plasma volume [32]. There was a positive effect of plasma volume change from baseline to the end of the monitoring phase after ingesting protein (SMD 0.29, 95% CI 0.03–0.55, $P = 0.03$, $Z = 2.19$, small effect; Fig. 7). Of the ten comparisons, seven found there to be no significant difference in plasma volume change from pre-exercise values to the conclusion of the observation period (4 ± 1 h) following ingestion of a protein-containing beverage, when compared with the change in plasma volume following ingestion of the control. The three remaining comparisons found plasma volume changes following protein ingestion returned to pre-exercise values or higher from 3 h in all studies [13, 15, 16], while the control remained below baseline in two of the three comparisons throughout the observation period [13, 16]. The remaining comparison found both the protein-containing beverage (5.3%) and the control (2.5%) increased plasma volume to above pre-exercise values [15].

3.9 The Effect of Protein Consumption in a Euhydrated State

Cumulative urine was measured in both euhydrated studies included in this systematic review [26, 30], of which one found a significant beverage × time interaction effect (reduction at 180- and 240-min post-beverage ingestion [26]. In both studies there was no significant difference in NFB. Only one of the studies measured the beverage hydration index (BHI) [26], which in line with NFB results, showed there was no significant difference between the results of the electrolyte condition or the amino acid blend (alanyl-glutamine dipeptide).

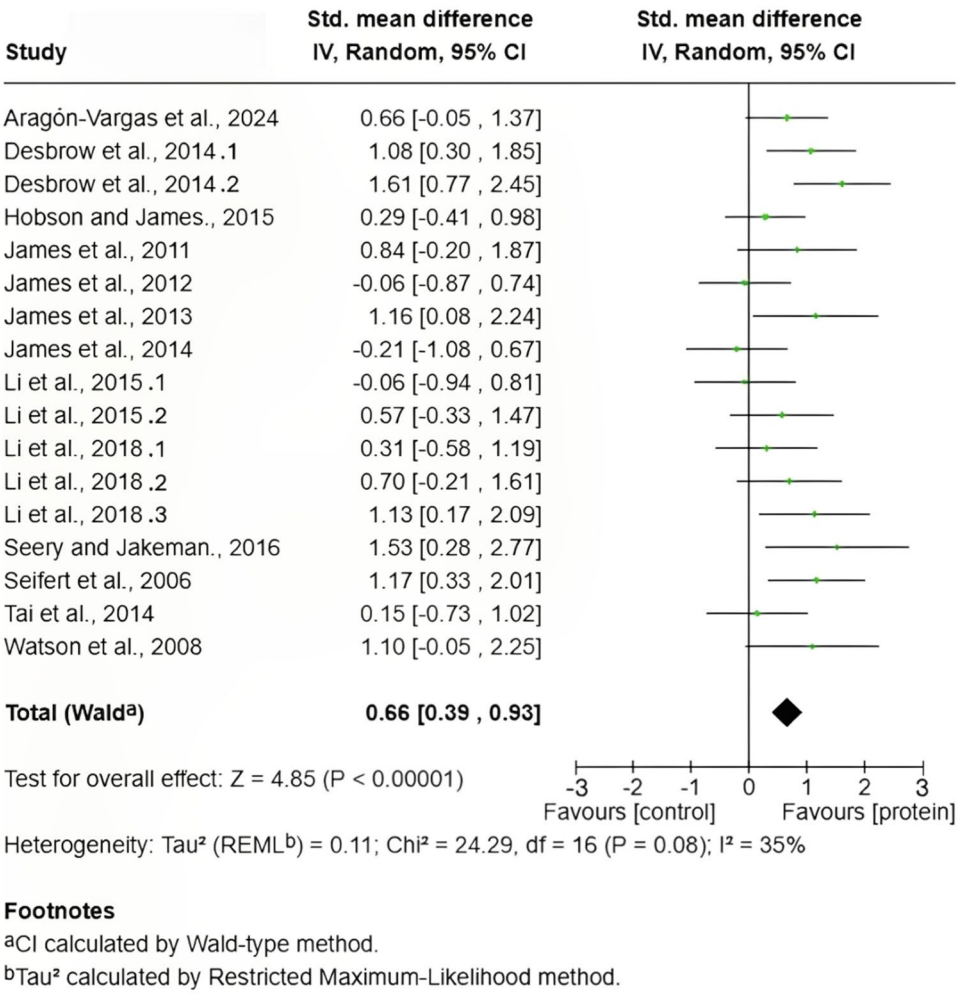


Fig. 2 Comparison of the effects of a protein-containing beverage versus a control on fluid retention. A positive trend shows an increase in fluid retained. Forest plot shows SMDs with 95% CIs. Squares represent the SMD for each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk, Desbrow et al., 2014.2 = soy milk, Li et al., 2015.1 = casein protein, Li et al., 2015.2 = whey protein, Li et al., 2018.1 = low whey protein drink (15 g), Li et al., 2018.2 = medium whey protein drink (22 g) and Li et al., 2018.3 = high whey protein drink (33 g). SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

3.10 Certainty of Evidence

The certainty of evidence for fluid retention, cumulative urine output, net fluid balance and plasma volume change was high. Visual inspection of funnel plots for fluid retention, cumulative urine and net fluid balance (Supplementary Fig. 3) demonstrated no obvious indication of publication bias, while plasma volume change could not be formally assessed as fewer than ten studies were included in the meta-analysis.

4 Discussion

This systematic review with meta-analysis evaluated the effectiveness of protein-containing beverages relative to carbohydrate-containing beverages or water on indices of hydration (fluid retention, cumulative urine output, net fluid balance and plasma volume). The main findings showed that beverages containing protein (23.7 ± 9.9 g/L) improved fluid retention (SMD 0.66, $P < 0.00001$) and reduced cumulative urine output (SMD − 0.71, $P < 0.00001$) following exercise-induced dehydration, as well as increased net fluid balance (SMD 0.58, $P < 0.0001$) and augmented plasma volume change (SMD 0.29, $P = 0.03$) when compared with a control beverage. However, these findings should be interpreted in the context of the composition of the intervention beverages, as the inclusion of protein may also alter other characteristics of the beverage, including energy density and macronutrient composition.

The rate of intestinal absorption is directly influenced by the rate of gastric emptying, delaying reductions in plasma osmolality after dehydration, which in turn minimises diuresis, allowing greater drink retention [15]. In the small intestine, amino acid-mediated sodium co-transport represents a more directly protein-related mechanism, with certain amino acids enhancing the co-transport of sodium and increasing osmotic water [20, 33]. The studies showed inclusion of protein within a hydration beverage caused a small effect on plasma volume change from pre-exercise to

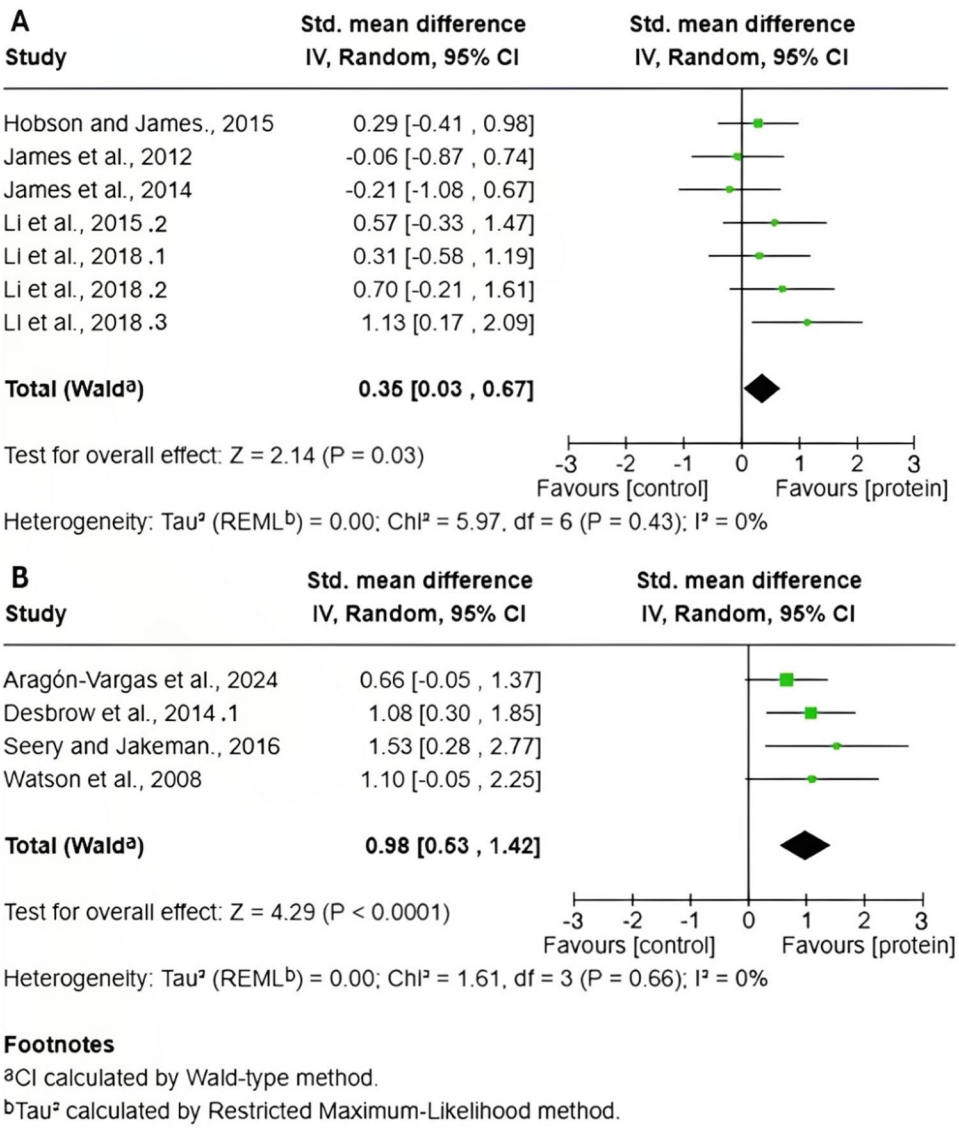


Fig. 3 Comparison of the effects of different protein formats (**A** = whey, **B** = cow's milk) versus a control on fluid retention. A positive trend shows an increase in fluid retained. Forest plot shows SMDs with 95% CIs. Squares represent the SMD for each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk, Li et al., 2015.2 = whey protein, Li et al., 2018.1 = low whey protein drink (15 g), Li et al., 2018.2 = medium whey protein drink (22 g) and Li et al., 2018.3 = high whey protein drink (33 g). SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

post-rehydration. Despite only being measured in a few studies, it is speculated that this effect on plasma volume could be due to increased plasma albumin driven by increased albumin synthetic rate after protein ingestion [34], an effect unique to proteins and amino acids. Increased plasma albumin is suggested to increase oncotic pressure, promoting the retention of fluid in the vascular space and therefore increasing plasma volume change [35, 36]. Collectively, these mechanisms may account for the observed improvements in fluid retention and cumulative urine output within the included studies.

4.1 Energy Density

Increased energy density is suggested to slow gastric emptying [7, 37, 38], which could reduce the rate of water uptake into the circulation. This may in turn attenuate the decline in serum/plasma osmolality observed following ingestion of less energy-dense solutions [7, 9, 28]. Consequently, increased energy density may contribute to greater fluid retention; however, as a proposed mechanism, this remains speculative as it was not directly assessed within the included studies. Five included studies matched energy density between the control and protein-containing beverage [12, 14, 16, 17, 28], of which improvements to fluid retention and cumulative urine output were seen in four [12, 14, 16, 28]. Control beverages with lower energy density would be anticipated to empty from the stomach at a faster rate than the higher density protein-containing beverages owing to the linear relationship between gastric emptying and energy density [38]. However, results of the studies containing energy-matched beverages suggest that improvement to fluid retention and cumulative urine output may be because of

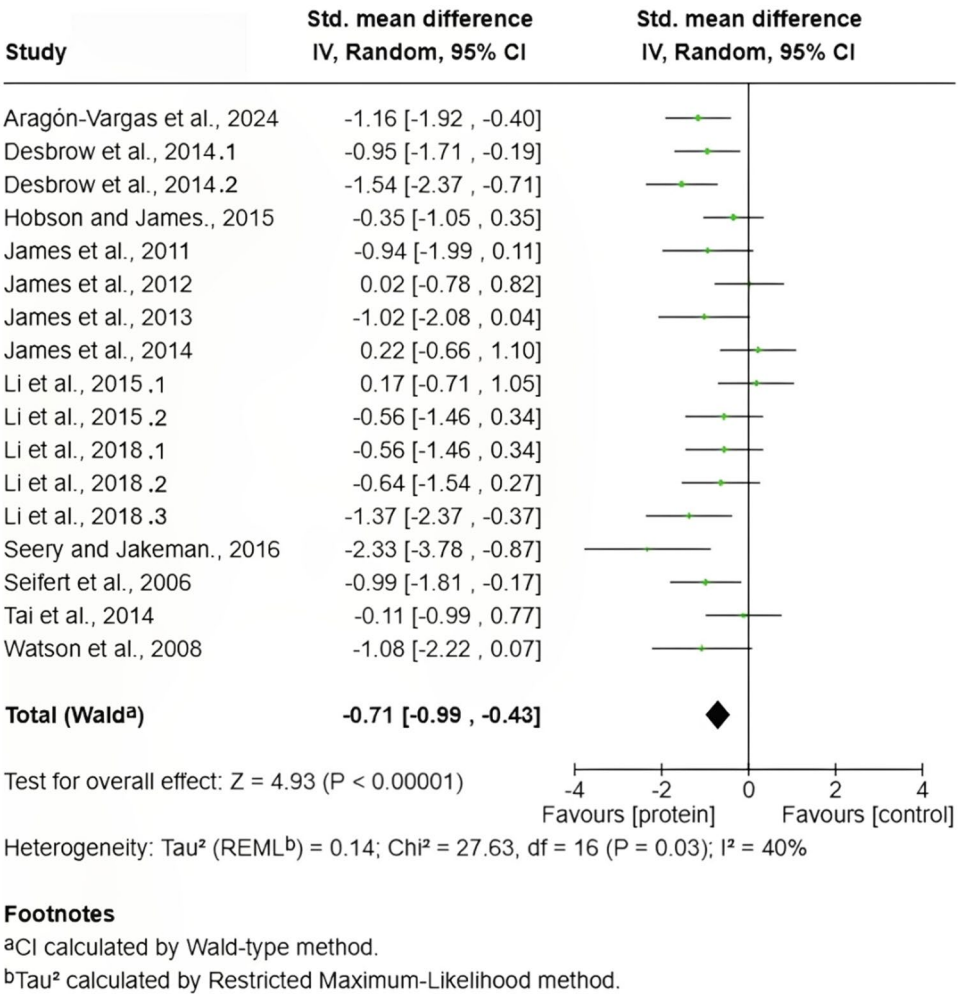


Fig. 4 Comparison of the effects of protein versus a control beverage (water/carbohydrate/carbohydrate–electrolyte) on cumulative urine output. A negative trend shows a decrease in cumulative urine output. Forest plot shows SMDs with 95% CIs. Squares represent the SMD of each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk, Desbrow et al., 2014.2 = soy Milk, Li et al., 2015.1 = casein protein, Li et al., 2015.2 = whey protein, Li et al., 2018.1 = low whey protein drink (15 g), Li et al., 2018.2 = medium whey protein drink (22 g) and Li et al., 2018.3 = high whey protein drink (33 g). SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

more than just an increased energy density alone and supports the role of protein in improving hydration efficacy.

Contrary to this, skimmed milk was the most common protein beverage included and contains ~ 1.5× the energy density of typical carbohydrate–electrolyte sports beverages [29]. The overall review data concluded that milk has a large effect on both increasing fluid retention, as well as decreasing cumulative urine output when compared with a carbohydrate–electrolyte/carbohydrate/water beverage. For example, fluid retention was ~ 31% greater following milk ingestion compared with a carbohydrate–electrolyte beverage [3]. It has been suggested that substantial increases in energy density may be required to meaningfully influence fluid retention with, for example, > 10–12% carbohydrate (400–480 kcal/L) needed to increase hydration responses compared with energy-free beverages [7, 39]. Within this review the largest difference in energy density was 375 kcal/L [4]. Importantly, energy density represents a property of the beverage rather than an independent effect of protein and should therefore be distinguished from both the quantity of fluid consumed and the presence of protein within that fluid. Overall, this review is unable to conclude the extent to which energy density influences hydration metrics and the potential mechanisms influencing gastric emptying following ingestion of protein may be of interest to further research to measure how different protein formats and, in turn, varying energy densities could influence hydration outcomes.

4.2 The Effect of Different Protein Formats on Hydration/Rehydration Metrics

Formats of protein used in this study varied with the inclusion of whey protein ($n = 7$), casein protein ($n = 1$), skimmed milk ($n = 6$), milk protein ($n = 2$), soy protein ($n = 1$), amino acid blends ($n = 2$) and mixed blends ($n = 1$). The mechanisms augmenting hydration status may differ between formats of protein. Sub-group analysis found milk to have a large effect on hydration metrics, both increasing fluid retention and decreasing cumulative urine. Due to

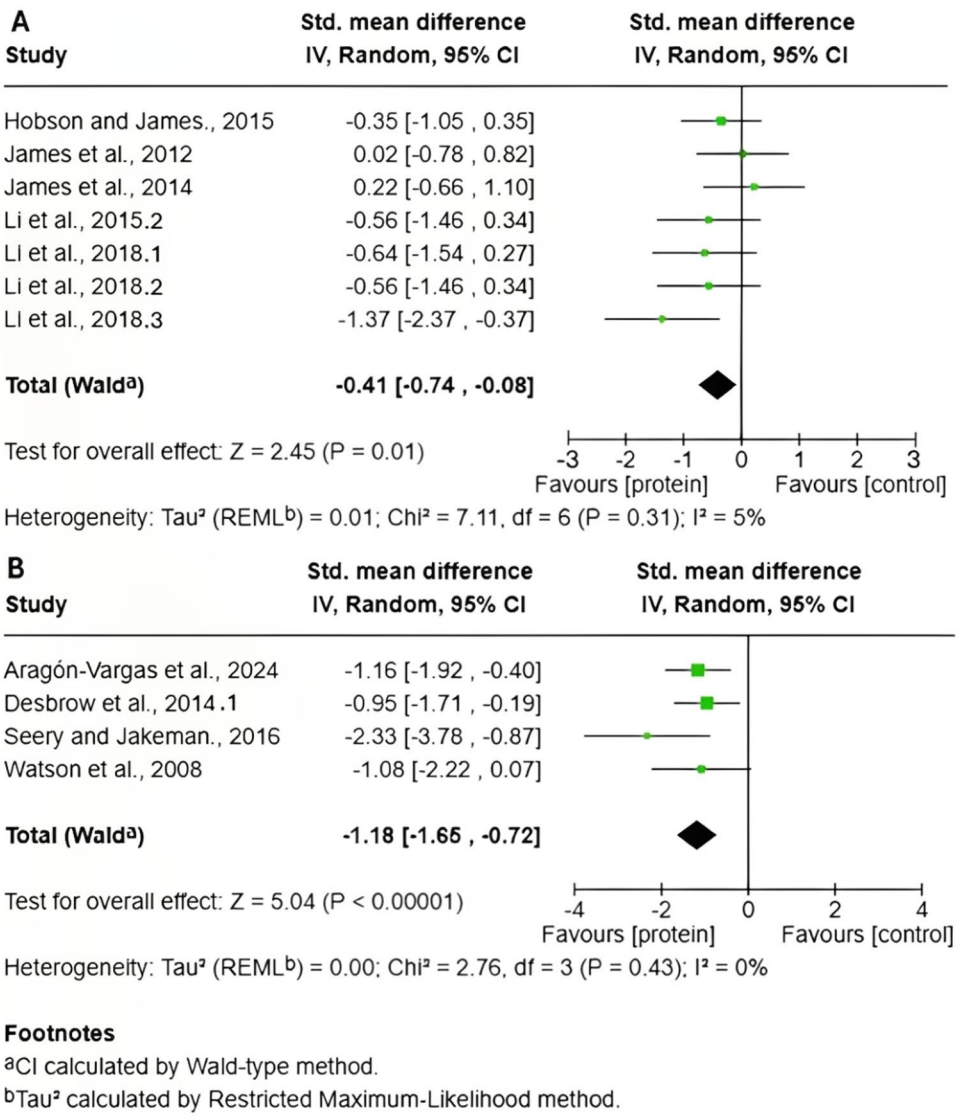


Fig. 5 Comparison of different protein formats (**A** = whey, **B** = cow's milk) versus a control beverage on cumulative urine output. A negative trend shows a decrease in cumulative urine output. Forest plot shows SMDs with 95% CIs. Squares represent the SMD of each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk, Li et al., 2015.2 = whey protein, Li et al., 2018.1 = low whey protein drink (15 g), Li et al., 2018.2 = medium whey protein drink (22 g) and Li et al., 2018.3 = high whey protein drink (33 g). SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

the composition of milk, it is reasonable to assume these benefits could be attributed to the presence of lactose and/or, as previously mentioned, the higher energy density [40]. Desbrow et al. [4] found similar changes to fluid retention following ingestion of ~ 36 g/L cow's milk versus ~ 32 g/L soy milk where energy density, macronutrient distribution and sodium levels were similar in both conditions. Milk approximately contains a 1:4 ratio of whey to casein protein. Casein protein is suggested to digest at a slower rate and, in the presence of gastric acid, forms clots in the stomach [41, 42], which may influence fluid retention. However, the only study directly assessing casein and whey protein independently against a control [12] found increased fluid retention and reduced urine output following whey protein ingestion, but not with the same amount of casein (22 g). This suggests that either changes in gastric emptying are not important for hydration effects of protein or that the mechanisms influencing hydration may differ between sources. Whey protein is suggested to have a much faster rate of gastric emptying and intestinal absorption than casein protein [26, 42]. Sub-group analysis showed that whey protein had a small effect on fluid retention and cumulative urine output [12, 43], while milk showed a large effect. Only two studies directly compared protein sources [4, 12] with each other and the same control, and none of the studies compared more than two protein sources within the same research. Therefore, this highlights a clear gap in the literature in which testing of multiple protein sources is needed in order to decipher the optimal format for hydration.

4.3 Practical Implications

Protein is commonly ingested post-exercise to stimulate muscle protein synthesis and/or to enhance other areas of recovery [43]. The addition of > 20 g/L of protein to hydration beverages may therefore provide a practical

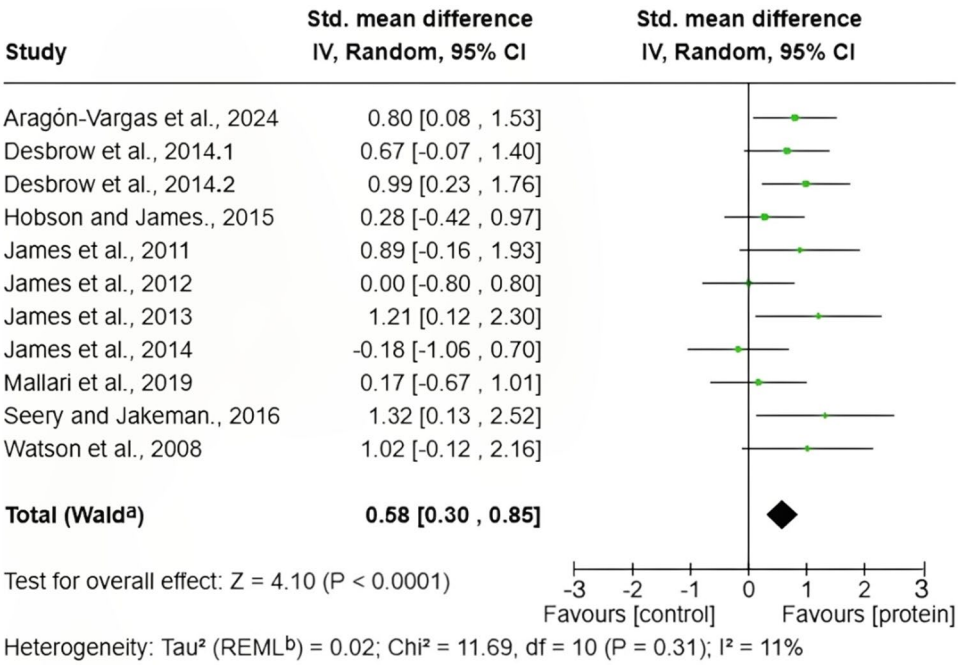


Fig. 6 Comparison of protein versus a control beverage (water/carbohydrate/carbohydrate–electrolyte) on net fluid balance. A positive trend shows an increase in net fluid balance. Forest plot shows SMDs with 95% CIs. Squares represent the SMD of each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk and Desbrow et al., 2014.2 = soy milk. SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

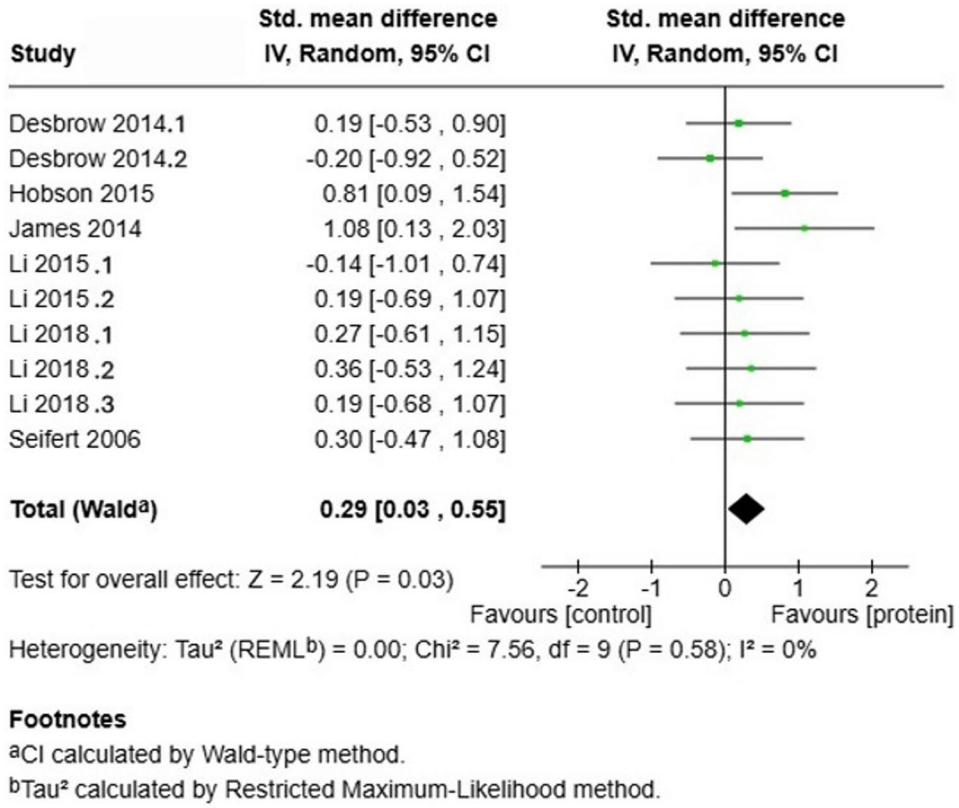


Fig. 7 Comparison of protein versus a control beverage (water/carbohydrate/carbohydrate–electrolyte) on plasma volume. A positive trend shows an increase in plasma volume. Forest plot shows SMDs with 95% CIs. Squares represent the SMD of each study, with square size proportional to the statistical weight of the study, and the diamond represents the pooled SMD for all studies overall. Studies with multiple intervention arms: Desbrow et al., 2014.1 = cow's milk, Desbrow et al., 2014.2 = soy milk, Li et al., 2015.1 = casein protein, Li et al., 2015.2 = whey protein, Li et al., 2018.1 = low whey protein drink (15 g), Li et al., 2018.2 = medium whey protein drink (22 g) and Li et al., 2018.3 = high whey protein drink (33 g). SMD, standardised mean difference; CI, confidence interval; df, degree of freedom; std, standard; IV, inverse variance; REML, restricted maximum likelihood

strategy to support both fluid balance and recovery, readily achievable through commercially available protein-containing beverages. Fluid retention, cumulative urine output, net fluid balance and plasma volume provide complementary measures of rehydration, capturing the fluid retained within the body. Effective rehydration is particularly important following exercise-induced sweat losses, especially when recovery time between sessions is limited. Greater fluid retention and reduced urinary losses may therefore support more efficient rehydration between training or competition. Many athletes already consume a protein containing beverage post-exercise, in the form of protein shakes or recovery drinks; therefore, it is an attractive proposition to benefit both fluid balance and muscle protein synthesis in a single drink, providing practicality for athletes recovering after exercise.

Factors such as palatability may influence fluid intake [11]. Although studies in this meta-analysis prescribed fluid volumes, in a real-world situation this would likely be consumed ad libitum or in smaller commonly consumed commercial volumes (e.g. a 500 mL bottle). Traditional carbohydrate–electrolyte sports drinks are often perceived as more palatable [4, 5, 11], which when ingesting the recommended 125–150% of volume of fluid lost to offset urine losses [2, 44], may be an easier option than beverages such as milk. Practical considerations also include storage requirements, as some beverages require refrigeration to keep the product fresh and therefore may not be appropriate if training takes place in an area lacking the facilities to keep beverages cold.

4.4 Limitations and Further Research

Female individuals were under-represented in this review; of the 205 participants included, 35 were female (17.1%). This limits the generalisability of findings and does not account for sex-related differences in hormonal regulation and fluid balance, which may influence hydration responses to protein ingestion [45]. It should also be noted that there was some methodological variability in the measurement of nude body mass between studies, with reported values rounded to differing levels of precision, ranging from 10 to 100 g; although unlikely to materially affect the overall findings, this methodological variation may have contributed to between-study variability. Only one beverage was plant-based (soy; [4]) so application/relevance to these types of proteins is limited, which is of particular importance to athletes following a plant-based/vegan diet. Additionally, beverages such as milk contain lactose, a common food intolerance, which could be a barrier to use for some athletes. Therefore, further work is needed into the effect of plant-based proteins and milk alternatives, which are being more frequently consumed. Protein was compared with carbohydrate (CHO) in 15 of the 16 studies, and therefore the effect of protein compared with a non-energy control (such as water) is not clear and may under-represent the independent effect of protein on hydration. The average age of participants was 26 ± 10 years; however, Rodriguez-Sanchez [30] included participants up to the age of 64 years. The inclusion of an older participant population may result in differing hydration responses compared with the predominantly younger population represented across the included studies.

5 Conclusions

In summary, this systematic review and meta-analysis show that protein-containing hydration/rehydration beverages confer an advantage over a control beverage (i.e. water and carbohydrate-based beverages) for improving fluid retention and reducing cumulative urine output. Further research is warranted to evaluate the optimal protein format and concentration to elicit the greatest benefit on hydration status, including plant-based options which are currently underrepresented throughout the current literature. Additionally, understanding if responses differ between male and female individuals would be important to understand given the limited data on female-only cohorts and the potential for hydration differences between sexes. In addition, it would be useful to understand how the current findings could translate into a benefit for real-life health and performance outcomes.

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Declarations

Conflict of Interest T.C. has received research funding from Rousselot BV, VDF Futraceuticals Inc and Made By Brave Ltd. S.A.M. and S.J.B. have current/previous funding from Herbalife Europe Ltd. S.A.M. has had previous funding from Entrinsic Beverage Company LLP. H.Z.M. is an employee of the Gatorade Sports Science Institute, a division of PepsiCo, Inc. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc. L.J.J. has current/previous funding from the Defence Science and Technology Laboratory, National Institute for Health and Care Research, Mars Inc., Bunge Loders Croklaan B.V., Bridge Farm Nurseries Ltd, Entrinsic Beverage Company LLP, Decathlon SA, PepsiCo, Inc. and Volac International, has performed consultancy for the British Horseracing Authority, Lucozade Ribena Suntory and PepsiCo, Inc. and has received conference fees from PepsiCo, Inc. and Danone Nutricia. L.J.J. is part of the National Institute for Health Research's Leicester Biomedical Research Centre, which is a partnership between University Hospitals of Leicester National Health Service (NHS) Trust, Loughborough University and the University of Leicester.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Availability of Data and Material All data relevant to the study are included in the article.

Code Availability Not applicable.

Authors' Contributions F.R.G., H.N., T.C., S.J.B., L.J.J. and S.A.M. designed research. F.R.G., L.M., H.N., H.Z.M., L.J.J. and S.A.M. conducted research. F.R.G., L.M., L.J.J. and S.A.M. analysed data. F.R.G. wrote the paper, and F.R.G., L.J.J. and S.A.M. had the main responsibility for final content. All authors read and approved the final manuscript.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process The author(s) declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing of this manuscript.

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