



# Efficacy and Safety of Whey Protein Supplements on Vital Sign and Physical Performance Among Athletes: A Network Meta-Analysis

Fui-Ching Lam<sup>1</sup>, Allah Bukhsh<sup>1,2</sup>, Habib Rehman<sup>3</sup>, Muhammad Khurram Waqas<sup>2</sup>, Nabeel Shahid<sup>2</sup>, Adil Mohammed Khaliel<sup>4</sup>, Ahlam Elhanish<sup>4</sup>, Mustfa Karoud<sup>5</sup>, Ahmed Telb<sup>6</sup> and Tahir Mehmood Khan<sup>1,2\*</sup>

<sup>1</sup> School of Pharmacy, Monash University Malaysia, Subang Jaya, Malaysia, <sup>2</sup> Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan, <sup>3</sup> Faculty of Biosciences, University of Veterinary and Animal Sciences, Lahore, Pakistan, <sup>4</sup> Department of Urology, Bourn Hall Fertility Clinic Dubai, Jumeriah, United Arab Emirates, <sup>5</sup> Department of Orthopedics, Canadian Specialist Hospital, Abuhail, United Arab Emirates, <sup>6</sup> Department of Radiology, Emirates Hospital, Jumeriah, United Arab Emirates

## OPEN ACCESS

### Edited by:

Deslypere Jean Paul,  
Besins Healthcare, Thailand

### Reviewed by:

Karen Nagel,  
Midwestern University, United States  
Domenico Criscuolo,  
Italian Society of Pharmaceutical  
Medicine, Italy

### \*Correspondence:

Tahir Mehmood Khan  
tahir.khan@uvas.edu.pk

### Specialty section:

This article was submitted to  
Pharmaceutical Medicine and  
Outcomes Research,  
a section of the journal  
Frontiers in Pharmacology

**Received:** 26 September 2018

**Accepted:** 15 March 2019

**Published:** 24 April 2019

### Citation:

Lam F-C, Bukhsh A, Rehman H, Waqas MK, Shahid N, Khaliel AM, Elhanish A, Karoud M, Telb A and Khan TM (2019) Efficacy and Safety of Whey Protein Supplements on Vital Sign and Physical Performance Among Athletes: A Network Meta-Analysis. *Front. Pharmacol.* 10:317. doi: 10.3389/fphar.2019.00317

**Introduction:** Athletes train physically to reach beyond their potential maximum aerobic threshold. Whey protein supplements (WPS) are often used in conjunction with physiotherapy and psychotherapy to regain better vital sign and physical performances. This review aimed to explore the clinical evidence on the efficacy and safety of WPS in sports performance and recovery among athletes.

**Methodology:** A comprehensive literature search was performed to identify relevant randomized control trials (RCTs) that investigated the efficacy and safety of WPS on the vital sign and physical performance among athletes. The Cochrane Risk of Bias (ROB) Assessment tools were used to assess the quality of the studies. Meta-analysis was conducted using the frequentist model with STATA version 14.2<sup>®</sup>.

**Results:** A total of 333,257 research articles were identified out of which 20 RCTs were included for qualitative synthesis and network meta-analysis with 351 participants. Among the studies, 7 had low ROB and 3 RCTs had high ROB. Of these 20 trials, 16 trials were randomized clinical trials which compared whey protein supplements (WPS) with various comparators i.e., L-alanine, bovine colostrum, carbohydrate, casein, leucine, maltodextrin, rice, protein + caffeine were compared with placebo. Analysis from the pairwise meta-analysis revealed that for respiratory exchange ratio (RER) WPS was found to be significantly improving compared to maltodextrin (WMD = 0.012; 95%CI = 0.001, 0.023). Similarity to RPE (Rate Perceived Exertion), slight difference between WPS and the comparators, however, when the estimation was favorable to the comparators, there was moderate-high heterogeneity. For  $VO_{2\max}$ , high heterogeneity appeared when WPS compared to maltodextrin with the  $I^2 = 97.8\%$  (WMD = 4.064; 95% CI = -4.230, 12.359), meanwhile bovine colostrum (WMD = -2.658; 95%CI = -6.180, 0.865) only comparator that was better than WPS. According to the estimated effect of the supplements on physical performance outcome results, maximum power (8 studies, 185 athletes), highest ranked was bovine colostrum (SUCRA = 70.7%) and the lowest ranked was placebo (SUCRA = 17.9%), yet all insignificant. Then again, on

average power (nine studies, 187 athletes), WPS was the highest ranked (SUCRA = 75.4 %) about  $-112.00$  watt ( $-187.91$ ,  $-36.08$ ) and most of the estimations were significant. Body mass was reported in 10 studies (171 athletes), carbohydrate may be at the highest ranked (SUCRA = 66.9%) but it is insignificant. Thought the second highest ranked was WPS (SUCRA = 64.7%) and it is significant (WMD =  $-6.89$  kg; CI =  $-8.24$ ,  $-5.54$ ).

**Conclusion:** The findings of this review support the efficacy and safety of WPS as an ergogenic aid on athletes' sports performance and recovery. The overall quality of clinical evidence was found to be valid and reliable from the comprehensive search strategy and ROB assessment.

**Keywords:** whey protein supplements, network meta-analysis, athletes, vital sign, physical strength, athlete's health and performance

## INTRODUCTION

Athletes train to be skilful and physically fit to compete and ensure success against their opponents. The effect of athletes' stamina, body structure and skill development are essential to able to do so, while an effective while an effective nutrition and diet plan to ensure good health and well-being of athletes. It is support from the supplement as ergogenic aids to maintain their performance and to gain a competitive edge. The availability and consumption of supplements, along with physiotherapy and psychotherapy, have been recognized as ergogenic advantages in sports performance and recovery (Wiese-Bjornstal, 2010; Chan et al., 2011). supplements might have substances that are harmful and life-threatening effects on athlete health such as alcohol, steroid and caffeine (Silver, 2001).

World Anti-doping Agency (WADA) was established to promote coordinate and monitor illicit drugs use in sports internationally. However, dietary and nutritional supplements have become distressing matters. For many countries and manufacturers of supplements have a lack of quality control, some supplements contain substances that were prohibited such as caffeine and alcohol (Willick et al., 2016). Hence, options for supplements are limited for athletes to compete ethically. One of the popular and easy to purchase protein supplement in sports is whey protein supplements (WPS) as it has shown ergogenic aids which absorbed rapidly, includes all the essential amino acids, and has a high proportion of branched-chain amino acids (MacKenzie-Shalders et al., 2015; Frank et al., 2017).

Various systematic review and meta-analysis published that summaries the effect of whey protein (WP) as a dietary supplement (Nissen and Sharp, 2003; Schoenfeld et al., 2013; Miller et al., 2014). However, there is a lack of consensus over the use of WP, yet, some clinical studies concluded consuming other protein sources or supplements are better than WP (Taylor et al., 2016), which is in contrast with some other studies (Kraemer et al., 2015; Hansen et al., 2016) that support WP in comparison to others. Moreover, the quality of studies and risk of bias is another issue that is often neglected while scrutinizing the evidence of other supplements in comparison to WP. The current systematic review and network meta-analysis aim to explore the clinical efficacy and safety of WPS on athletes' vital sign and physical performance.

## METHODS

### Study Design and Selection

A systematic review and network meta-analysis were conducted to identify eligible randomized controlled trials (RCTs). The search strategy used the keyword of "whey\*" combined individually with "athlete\*", "injur\*", "muscle\*", "perform\*", and "recover\*" on databases as well as specific journals: PubMed, EMBASE via Ovid, Scopus, Cochrane, Cumulative Index to Nursing and Allied Health Literature (CINAHL) via EBSCOhost, SPORTDiscus, Health & Medicine Database via ProQuest, Wiley Online Library, Web of Science, ScienceDirect, Taylor & Francis and SAGE. All experimental and observational studies were considered for potential exclusion in this systematic review. No restriction was placed on language. The searched timeframe was from the date of database inception until December 2017. However, studies design on expert opinions, case reports/series, surveys, review articles, editorials, commercial advertisements, magazine articles, unpublished articles were excluded. The protocol of this study was registered in PROSPERO 2016 and the register identification is CRD42016041842 (Lam et al., 2016) and reported the network meta-analysis according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009). Please see **Appendix 1** in the Supplementary Material for detailed PRISMA checklist.

### Population of Interest

The participants included were active athletes who experienced fatigue and had recovered and/or had been hindered in their performance. Also, studies that observed on participations who are resistance-trained, trained and physically active were deem be athletes as these participants undertook overpowering physical activities during the intervention that were equivalent to athletes. Regardless of athletes' age and gender. However, studies that observed on retired athletes, mixed athletes with non-athletes, animals, cells, and gels were excluded.

### Interventions

The intervention was WP or supplements containing WP. The intervention was found in the form of isolate, concentrate, hydrolysate, denature, and protein bars.

## Comparators

Comparators were carbohydrate supplements, protein-containing foods from animal sources (e.g., meat, fish, dairy products, and eggs), protein-containing vegetarian sources (e.g., tofu, legumes, and soy protein), vitamins (e.g., multivitamin, vitamin B, beta-carotene, and folic acid), minerals (e.g., calcium, iron and zinc) and placebos (include no treatment and treatment as usual).

## Outcomes Measure

This systematic review and meta-analysis have measured two outcomes which associated with aims: -

- a. Vital signs of heart rate, respiratory exchange ratio (RER), rate perceived exertion (RPE), and maximum volume of oxygen ( $VO_{2\max}$ );
- b. Strength and body composition which were maximum power, average power, and body mass.

## Data Extraction

The extracted data were entered into Microsoft Excel 2016, namely (Boutron et al., 2008):

1. General information (first author surname, title, year of publication, journal name).
2. The article study methods and characteristic (study design).
3. Participants (age, gender, weight, heights, and sporting activity).
4. Intervention (dose of WP consumed and number times consumed).
5. Comparators (type, dose, and number times consumed).
6. Outcomes: -
  - a. Outcomes that contributed to vital sign and physical performances.
  - b. The data obtained after the participants consumed the intervention or control.
  - c. Most of the data located within the text of the articles and presented in tabular form or graphs
  - d. When data was in standard error or standard error mean, it was transformed into a standard deviation (Higgins et al., 2011b).

## Assessment of Risk of Bias for Included Studies

The included studies were assessed for their ROB by two reviewers independently. Both assessment results were compared and verified for accuracy. A Cochrane ROB criteria were used to assess the quality of the RCTs (Higgins et al., 2011a) (Appendix 2.1 in Supplementary Material).

## Statistical Analysis

Two types of meta-analysis were conducted with STATA version 14.2<sup>®</sup>. Firstly, standard pairwise meta-analysis with a random-effects model (Borenstein et al., 2009) was performed and assessed I-squared ( $I^2$ ) metrics for heterogeneity. Thus, appearance of heterogeneous when the  $I^2$  appeared to have 50% and above (Higgins et al., 2003). Secondly, random-effect network meta-analysis was performed using frequentist

model to compare different interventions with each other. For a common heterogeneity variable for all comparisons was assessed by studies tau-square ( $\tau^2$ ) test (Turner et al., 2012). The type of data for the meta-analysis was continuous data, which contained mean, standard deviation and sample size (Saez de Villarreal et al., 2012). The results pooled estimates of weighted mean difference (WMD) at 95% confidence interval (95% CI).

For indirect and mixed comparisons, network meta-analysis was performed to compare different strategies and the meta-analysis assumes transitivity (i.e., learn about supplement A (vs.) supplement B via supplement B) (Salanti, 2012). The transitivity embraced when the direct comparison between supplements do not differ with respect to the distribution of effect modifiers. For example, studies comparing WPS with placebo were similar to studies comparing carbohydrate with placebo in heart rate parameter. The potential effect modifiers for trials in this setting were the duration of intervention, physical activities during intervention duration and dosage of supplements. On the other hand, disagreement between direct and indirect evidence suggests that the transitivity assumption might not be embraced.

Firstly, investigate a loop-specific approach for consistency within every triangular or quadratic loop as the difference between direct and indirect estimates for a specific comparison in the loop (inconsistency factor) (Salanti, 2012) (Veroniki et al., 2013). Secondly, performed the design-by-treatment interaction model and examined chi-square ( $\chi^2$ ) test for a single inference about the plausibility of assuming consistency throughout the entire network (Higgins et al., 2012).

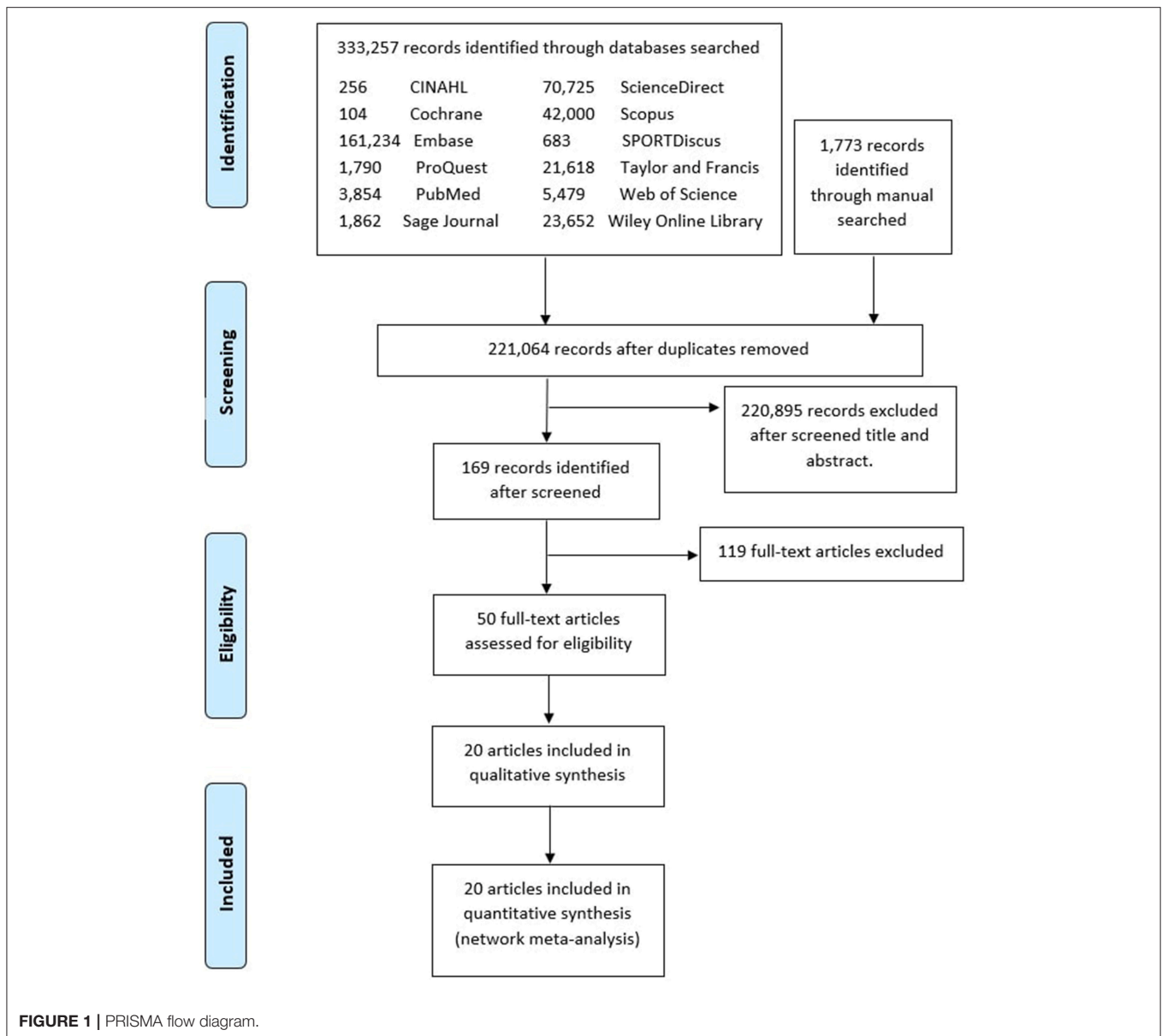
For a better understanding of intervention, surface under the cumulative ranking curve (SUCRA) probabilities conducted to rank the supplements for an outcome. The larger the SUCRA value (express in percentage) was, the better the rank of the intervention would be (Chaimani et al., 2013).

## RESULT

### Study Characteristics

The PRISMA flowchart (Figure 1) shows electronic searching processes. Of 169 potentially relevant articles initially screened, 20 articles with 351 participant athletes met the inclusion criteria. The descriptive study characteristics are presented in (Table 1). All the included articles are 16 RCTs are blinding while four articles are non-blinding (Hoffman et al., 2009; Gunnarsson et al., 2013; Impey et al., 2015; Al-Nawaiseh et al., 2016). The most studies demographic were from United States (6 articles) (Hoffman et al., 2009; Smith et al., 2010; Joy et al., 2013; Schroer et al., 2014; Al-Nawaiseh et al., 2016; Taylor et al., 2016). One study each from China (Li and Zhao, 2007), Finland (Breen et al., 2011), New Zealand (Macdermid and Stannard, 2006), Norway (Vegge et al., 2012), and South Africa (Oosthuysen and Millen, 2016).

Additionally, the researchers who were consistent in publishing the most regarding WPS for athletes were Lollo and colleagues, had two publications (Lollo et al., 2011, 2014). Two articles have both males and female athletes (Schroer



et al., 2014; Al-Nawaiseh et al., 2016), an article has only female athletes (Taylor et al., 2016) while an article was not taken account regarding their gender (Fukuda et al., 2010). In total 351 athletes participated: 298 males, 29 females and 24 athletes did not categories. Furthermore, the minimum number of participants in a study among the included for this review was  $n = 7$  (Macdermid and Stannard, 2006) and the maximum was  $n = 33$  participants (Hoffman et al., 2009).

WPS and the comparators (L-alanine, bovine colostrum, carbohydrate, casein, leucine, maltodextrin, rice, protein + caffeine) were compared with placebo. The number of participants based on the analyses indicated that a total of 351 athletes consumed WPS, followed by carbohydrate (154 athletes), and placebo (137 athletes), respectively. While protein + caffeine (9 athletes) as well as leucine (9 athletes) had the smallest number

of participants. The results of network meta-analysis showed in terms of efficacy in **Table 2** on the respective outcomes.

The shortest intervention duration, on average, was 2 days (Gunnarsson et al., 2013) and the longest duration was 180 days (Lollo et al., 2014). Participants consumed supplements, in extreme cases; one study has participants taking supplements every 15 min (Schroer et al., 2014). Participants consumed supplements before, during and/or after physical activities.

## Risk of Bias

A total of 20 RCTs were assessed using the Cochrane ROB Tools assessment (**Appendix 2.2** in Supplementary Material). The summary of Cochrane ROB for RCTs (**Figure 2**) shows 7 studies (35%) have overall low ROB, 10 studies

**TABLE 1 |** Characteristics of the included studies.

| First Author surname and Year | Country of study | Study Design                                      | Participates                                       |                          |                                  | Supplement                       |      |        | Protocol |   |   |  |  |                             |  |  |
|-------------------------------|------------------|---|--|--------------------------|----------------------------------|----------------------------------|------|--------|----------|---|---|--|--|-----------------------------|--|--|
|                               |                  |   | Categories of athletes                             | Average age <sup>1</sup> | Average weight (kg) <sup>1</sup> | Average height (cm) <sup>1</sup> | Male | Female | Total    | Intervention group (WP)   | Number of times to consume WP supplement in a day | Control group  | Number of times to consume supplement in a day | Intervention duration (day) | Physical activity during intervention duration   | Consume the supplementation during intervention duration |
| Al-Nawaiseh et al., 2016      | United States    | Random, crossover, counterbalanced                | College athletes, college-club athletes            | 21.5                     | 76.5                             | NA                               | 11   | 11     | 22       | 23g with 10.6g EAA, 7.3g of conditionally EAA, and 5.6g of non-EAA ON was mixed with 200ml of skimmed milk to form a protein shake. 2 oral doses of 1,000 mg of vitamin C (ascorbic acid with citrus bioflavonoids) and 400 IU of vitamin E soft gel capsules | 3   | Placebo (non-treatment)  | 3  | 17                          | Stretch and cycle  | Before, during, after physical activity                  |
| Breen et al., 2011            | Finland          | Random, counterbalanced, single-blinding          | Cyclists   | 29                       | 77.2                             | NA                               | 10   | -      | 10       | (1) 10.2 g with 25.4 g carbohydrate dissolved in 250 ml of cold water; (2) 20.4 g with 50.8 g carbohydrate dissolved in 250 ml of cold water  | 2   | (1) 25.2 g of carbohydrate dissolved in 250 ml of cold water, (2) 50.4 g CHO dissolved in 250 ml of cold water | 2  | 28                          | Cycle  | After physical activity                                  |
| Coomes et al., 2002           | Australia        | Random, placebo-controlled study, double-blinding | Cyclists   | 30                       | 74                               | NA                               | 28   | -      | 28       | 60 g (pure)   | 2   | 60 g bovine colostrum  | 2  | 56                          | Warm up, stretching exercises, cycle   | Before and after physical activity                       |
| Gummarsson et al., 2013       | Denmark          | Random  | Soccer players                                     | 24                       | 80.5                             | 182                              | 16   | -      | 16       | HPC with carbohydrates  | 1   | Placebo (normal diet)  | 1  | 2                           | Soccer game  | After physical activity                                  |
| Hansen et al., 2016           | Denmark          | Random, block, single-blinding                    | Elite orienteers                                   | 19.5                     | 71.9                             | 183                              | 18   | -      | 18       | 0.2 g/kg/h with 1 g/kg/h of carbohydrate  | 4   | 1.2 g/kg/h of carbohydrate   | 4  | 7                           | Cycle, mix of distance training, interval training, mountain climb   | After physical activity                                  |
| Highton et al., 2013          | United Kingdom   | Random, crossover, double-blinding                | Soccer, rugby union                                | 23.4                     | 75.3                             | 177.5                            | 9    | -      | 9        | 2% with 6% carbohydrate   | 5   | 8% carbohydrate  | 5  | 14                          | Walk and sprint  | During physical activity                                 |
| Hoffman et al., 2009          | United States    | Random  | Resistance-trained (30/33 were college's football) | 20.1                     | 99.2                             | 182.1                            | 33   | -      | 33       | 42 g of a proprietary blend of protein enzymatically hydrolyzed collagen protein isolate, WPI, and casein protein isolate) with 2 g of carbohydrate   | 2   | Placebo (normal diet)  | 2  | 70                          | High pull, bench press, seated shoulder press, dumbbell shoulder press/behind-the-neck, triceps push-downs, partner neck exercise etc. | Before and after physical activity                       |
| Impey et al., 2015            | United Kingdom   | Random, counterbalanced (Latin Squares approach)  | Cyclists and triathletes                           | 29                       | 79.4                             | 179.7                            | 9    | -      | 9        | 22 g with 2.1 g leucine, 4.9 g BCAA, 9.3 EAA, 500 ml water  | 1   | 5 g/kg carbohydrate 2 g/kg protein, 1 g/kg fat   | 1  | 7                           | Cycle  | Before physical activity                                 |

(Continued)



TABLE 1 | Continued

| First Author surname and Year | Country of study | Study Design   | Participates                   |                          |                                  | Supplement                       |      |              | Protocol                |  |               |  |                             |  |   |   |
|-------------------------------|------------------|--|--------------------------------|--------------------------|----------------------------------|----------------------------------|------|--------------|-------------------------|--|---------------|--|-----------------------------|--|---|---|
|                               |                  |  | Categories of athletes         | Average age <sup>1</sup> | Average weight (kg) <sup>1</sup> | Average height (cm) <sup>1</sup> | Male | Female Total | Intervention group (WP) | Number of times to consume WP supplement in a day  | Control group | Number of times to consume supplement in a day                     | Intervention duration (day) | Physical activity during intervention duration | Consume the supplementation during intervention duration  |   |
| Joy et al., 2013              | United States    | Random, double-blinding                                      | Resistance training experience | 21.3                     | 76.1                             | 177.8                            | 24   | -            | 24                      | 48g  | 1             | 48 grams of rice   | 1                           | 56   | Resistance training, cycle test   | Before physical activity                |
| Li and Zhao, 2007             | China            | Random, blinding   | Amateur football players       | 21                       | 64.8                             | 172.4                            | 16   | -            | 16                      | 25g with 800ml   | 2             | 25g carbohydrate 800ml   | 2                           | 72   | Cycle, jump, push up, run   | After physical activity                 |
| Lollo et al., 2011            | Brazil           | Random, double-blinding                                      | Soccer players                 | 19                       | 74.4                             | 181.5                            | 24   | -            | 24                      | 91.40%   | 1             | 88.6% casein   | 1                           | 56   | Cycle, soccer   | After physical activity                 |
| Lollo et al., 2014            | Brazil           | Random, double-blinding                                      | Soccer players                 | 18                       | 74                               | 178.5                            | 24   | -            | 24                      | 0.5 g/kg concentrate   | 2             | Maltodextrin   | 2                           | 180  | Soccer training   | Before and after physical activity      |
| Macdermid and Stannard, 2006  | New Zealand      | Random, balanced order, blinding                             | Cyclists                       | 33.6                     | 68.6                             | 175.4                            | 7    | -            | 7                       | 1.2-1.4 g/kg/d with carbohydrate intake of 7-10 g/kg   | 1             | Protein intake of 3-4 g/kg/d and a carbohydrate intake of ≤ 5 g/kg | 1                           | 16   | Cycle   | During physical activity                |
| Naciero et al., 2015          | United Kingdom   | Random, counterbalanced, double-blinding                     | Amateur soccer players         | 24                       | 77.5                             | 181                              | 16   | -            | 16                      | 14.5g with multi-ingredient (MTN; carbohydrate (63g), L-glutamine (5g), and L-carnitine L-tartrate (1.5g). | 4             | 69.5g carbohydrate   | 4                           | 13   | Run, jog, run   | Before, during, after physical activity |
| Oosthuysen and Millen, 2016   | South Africa     | Random, four way crossover, double-blinding                  | Cyclists                       | 38.9                     | 78.5                             | 179.8                            | 8    | -            | 8                       | Carbohydrate-whey hydrolysate  | 3             | Carbohydrate   | 3                           | 30   | Cycle   | Before and during physical activity     |
| Schoer et al., 2014           | United States    | Random, counterbalanced, placebo-controlled, double-blinding | Cyclists                       | 22.3                     | 70                               | 167                              | 4    | 4            | 8                       | 45 g/L   | Every 15 min  | 15 g/ of L-alanine   | Every 15 minutes            | 16   | Cycle   | Before and after physical activity      |
| Shing et al., 2006            | Australia        | Random, placebo controlled, double-blinding                  | Road cyclists                  | 28                       | 76.4                             | 179.9                            | 29   | -            | 29                      | 10g with 50ml water and 100ml skim milk  | 1             | 10g intact bovine CPC, 50ml water and 100ml skim milk              | 1                           | 70   | Cycle   | Unknown                                 |
| Smith et al., 2010            | United States    | Random, placebo controlled parallel, single-blinding         | Moderately-trained             | 21.1                     | 66.2                             | 173.4                            | 24   | 24           | 24                      | 8g with cholesterol, sodium carbohydrates, sugar, vitamin A, C B12, B6                                     | 1             | Maltodextrin: 17g  | 1                           | 21   | Run   | Before physical activity                |
| Taylor et al., 2016           | United States    | Random, double-blinding                                      | Basketball players             | 20.5                     | 67.1                             | 169.5                            | 14   | 14           | 14                      | 24g with in water  | 2             | 24g of maltodextrin  | 2                           | 56   | Lower body resistance, "explosive" exercises such as squat jumps, push jerks, and hang cleans), training drills | Before and after physical activity      |
| Vegge et al., 2012            | Norway           | Random, crossover, double-blinding                           | Cyclists                       | 22                       | NA                               | NA                               | 12   | -            | 12                      | 15.3 g/h with maltodextrin   | 1             | 60 g/h maltodextrin  | 1                           | 60   | Cycle   | During physical activity                |

<sup>1</sup> Mean ± Standard Deviation.  
NA, not available.

TABLE 2 | Results of network meta-analyses.

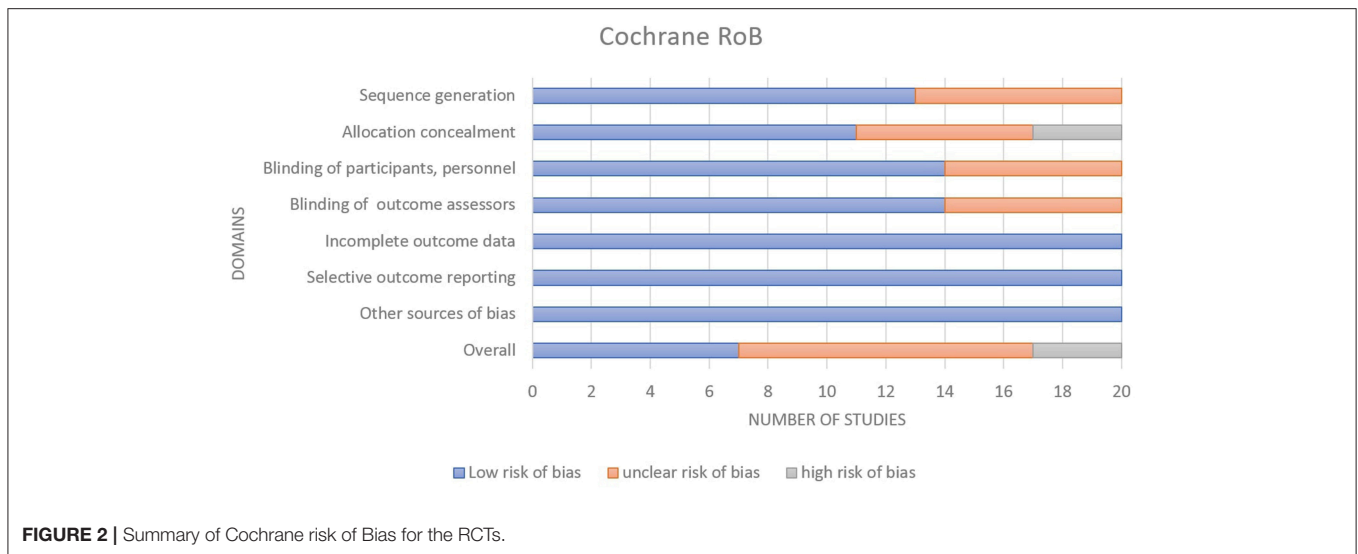
| a) HEART RATE (bpm)                     |                                  |                           |                         |                       |                               |  |                           |
|---|----------------------------------|---------------------------|-------------------------|-----------------------|-------------------------------|--|---------------------------|
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 0.88 (–14.82, 16.58)                    | <b>L-alanine</b>                 |                           | <b>Carbohydrate</b>     |                       | <b>Casein</b>                 |  | <b>Leucine</b>            |
| 4.94 (–5.19, 15.07)                     | 3.06 (–7.07, 13.19)              |                           |                         |                       |                               |  |                           |
| 1.88 (–15.48, 19.24)                    | –2.79 (–20.20, 14.62)            | –3.06 (–18.86, 12.74)     |                         |                       |                               |  |                           |
| 0.88 (–16.24, 18.00)                    | –0.00 (–12.12, 12.12)            | –4.06 (–19.60, 11.48)     | –1.00 (–9.34, 7.34)     |                       |                               |  |                           |
| –1.00 (–2.09, 0.09)                     | –1.00 (–12.78, 10.78)            | –5.94 (–16.13, 4.25)      | –2.88 (–20.27, 14.51)   | –1.88 (–19.03, 15.28) |                               |  |                           |
| 1.00 (–8.60, 10.60)                     | –2.88 (–15.35, 9.59)             | –3.94 (–17.90, 10.02)     | –0.88 (–20.72, 18.96)   | 0.12 (–19.51, 19.75)  | 2.00 (–7.66, 11.66)           |  | <b>Placebo</b>            |
| b) RESPIRATORY EXCHANGE RATION          |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 0.01 (–0.01, 0.03)                      | <b>L-alanine</b>                 |                           | <b>Carbohydrate</b>     |                       | <b>Maltodextrin</b>           |  | <b>Placebo</b>            |
| –0.00 (–0.03, 0.03)                     | –0.01 (–0.03, 0.01)              |                           |                         |                       |                               |  |                           |
| 0.00 (–0.00, 0.00)                      | –0.01 (–0.04, 0.02)              |                           | 0.00 (–0.03, 0.03)      |                       |                               |  |                           |
| 0.02 (–0.01, 0.05)                      | 0.01 (–0.03, 0.05)               |                           | 0.02 (–0.02, 0.06)      |                       | 0.02 (–0.01, 0.05)            |  |                           |
| c) RATE PERCEIVED EXERTION              |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| <b>–2.00 (–3.54, –0.46)</b>             | <b>L-alanine</b>                 |                           | <b>Carbohydrate</b>     |                       | <b>Placebo</b>                |  | <b>Protein + Caffeine</b> |
| 0.00 (–1.97, 1.97)                      | 0.10 (–1.84, 2.04)               |                           |                         |                       |                               |  |                           |
| 0.60 (–2.10, 3.31)                      | –0.12 (–3.07, 2.83)              |                           | 0.60 (–2.11, 3.31)      |                       |                               |  |                           |
| 1.90 (–0.56, 4.37)                      | 0.70 (–1.20, 2.60)               |                           | 1.90 (–0.57, 4.37)      |                       | 1.30 (–1.14, 3.74)            |  |                           |
| d) MAXIMUM VOLUME OF OXYGEN (ml/kg/min) |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 1.56 (–30.75, 33.87)                    | <b>L-alanine</b>                 |                           | <b>Bovine colostrum</b> |                       | <b>Carbohydrate</b>           |  | <b>Maltodextrin</b>       |
| 44.84 (–210.49, 300.16)                 | 43.27 (–211.92, 298.46)          |                           |                         |                       |                               |  |                           |
| –3.99 (–18.53, 10.55)                   | –5.55 (–40.95, 29.84)            | –48.83 (–304.56, 206.91)  |                         |                       |                               |  |                           |
| 0.07 (–13.63, 13.77)                    | –1.50 (–35.30, 32.30)            | –44.77 (–300.37, 210.83)  | 4.06 (–15.92, 24.03)    |                       |                               |  |                           |
| 5.72 (–4.08, 15.52)                     | 4.16 (–28.88, 37.19)             | –39.12 (–294.58, 216.35)  | 9.71 (–7.82, 27.24)     | 5.65 (–11.13, 22.44)  |                               |  | <b>Placebo</b>            |
| e) MAXIMUM POWER (WATT)                 |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 66.64 (–135.37, 268.65)                 | <b>Bovine colostrum</b>          |                           | <b>Carbohydrate</b>     |                       | <b>Placebo</b>                |  | <b>Rice</b>               |
| 14.38 (–249.13, 277.89)                 | –52.25 (–383.51, 279.00)         |                           |                         |                       |                               |  |                           |
| –91.78 (–250.97, 67.41)                 | –158.41 (–414.78, 97.95)         | –106.16 (–413.99, 201.67) |                         |                       |                               |  |                           |
| 31.14 (–245.32, 307.60)                 | –35.49 (–377.79, 306.81)         | 16.76 (–365.17, 398.69)   |                         |                       | 122.92 (–196.09, 441.93)      |  |                           |
| f) AVERAGE POWER (WATT)                 |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 0.44 (–22.32, 23.20)                    | <b>Bovine colostrum</b>          |                           | <b>Carbohydrate</b>     |                       | <b>Placebo</b>                |  | <b>Placebo</b>            |
| <b>–112.00 (–187.91, –36.08)</b>        | <b>–112.43 (–191.69, –33.18)</b> |                           |                         |                       |                               |  |                           |
| –2.03 (–7.00, 2.93)                     | –2.47 (–25.34, 20.39)            |                           |                         |                       | <b>109.96 (33.88, 186.04)</b> |  |                           |
| g) BODY MASS (kg)                       |                                  |                           |                         |                       |                               |  |                           |
| <b>WPS</b>                              |                                  |                           |                         |                       |                               |  |                           |
| 1.50 (–20.39, 23.40)                    | <b>Bovine colostrum</b>          |                           | <b>Carbohydrate</b>     |                       | <b>Casein</b>                 |  | <b>Maltodextrin</b>       |
| 2.00 (–10.40, 14.40)                    | 0.50 (–24.66, 25.66)             |                           |                         |                       |                               |  |                           |
| 0.20 (–26.06, 26.46)                    | –1.30 (–35.48, 32.88)            | –1.80 (–30.84, 27.24)     |                         |                       |                               |  |                           |
| <b>–6.89 (–8.24, –5.54)</b>             | –8.39 (–30.32, 13.54)            | –8.89 (–21.36, 3.58)      | –7.09 (–33.38, 19.20)   |                       |                               |  |                           |
| –1.96 (–4.76, 0.84)                     | –3.46 (–25.53, 18.61)            | –3.96 (–16.67, 8.75)      | –2.16 (–28.57, 24.25)   |                       | <b>4.93 (1.82, 8.04)</b>      |  | <b>Placebo</b>            |

Estimates are presented as WMD and 95% confidence intervals. Comparisons between supplements should be read from column to row. The estimate is in a cell between the column-defining supplement and the row-defining supplement. When WMD lower than 0, it is favor to the column-defining supplement. Statistically significant values have been bold and underlined.

(50%) have overall unclear ROB and 3 studies (15%) have overall high ROB (Smith et al., 2010; Breen et al., 2011; Hansen et al., 2016).

Moreover, the high ROB distributed only at that allocation concealment shows on **Figure 3** of the individual studies

ROB. The cause of the high ROB was single blinding conducted in the 4 studies, thus, either participants or investigators could possibly foresee assignments and impact on participants' behavior and participation and outcome assessment. While, 20 studies (100%) low ROB for incomplete



outcome data, selective outcome reporting and other sources of bias domains.

## Meta-Analysis

The pairwise comparisons of the efficacy of WPS as compared to other supplements on vital sign and physical performances among athletes (**Appendix 3** in Supplementary Material). In terms of the efficacy on vital sign outcome, the analysis result shows heart rate (bpm) slightly increases and decreases. The highest heart rate is 5 bpm (95%CI =  $-5.231, 15.231$ ) favorable to L-alanine, and lowest heart rate is  $-1$  bpm (95%CI =  $-2.089, 0.089$ ) favorable to protein + caffeine, yet not significant. For RER, all slightly favorable to WPS compared to the comparators, and it is significant when WPS compared to maltodextrin (WMD = 0.012; 95%CI = 0.001, 0.023). Similarity to RPE, slight difference between WPS and the comparators, however, when the estimation was favorable to the comparators, there was moderate-high heterogeneity. For  $VO_{2\max}$ , high heterogeneity appeared when WPS compared to maltodextrin with the  $I^2 = 97.8\%$  (WMD = 4.064; 95%CI =  $-4.230, 12.359$ ), meanwhile bovine colostrum (WMD =  $-2.658$ ; 95%CI =  $-6.180, 0.865$ ) only comparator that was better than WPS. Apart from RPE and  $VO_{2\max}$  on WPS against maltodextrin have heterogeneity, no evidence of heterogeneity was seen in general.

In terms of the efficacy on physical performances outcome, maximum, and average power (watt) results favorable to WPS and show no heterogeneity but favorable to the comparators show moderate-high heterogeneity, yet not significant. Body mass (kg) parameter has slightly different but no significant between WPS and the comparators; the highest body mass was 0.585 kg (95%CI =  $-6.122, 7.292$ ) compare to bovine colostrum while the lowest body mass was casein of  $-5.593$  kg (95%CI =  $-8.131, -3.054$ ;  $I^2 = 86.0\%$ ) with high heterogeneity. Detailed on the results of pairwise meta-analyses are given in the **Appendix 3** (Supplementary Material).

In the results of network meta-analysis, statistical heterogeneity found moderate only in the network of RPE on a triangular loop of evidence including supplementation

comparison between carbohydrate, combined placebo and WPS (carbohydrate—placebo—WPS) (For Network meta-analysis plots please see **Appendix 4** in Supplementary Material). Besides that, no appearance of statistical heterogeneity was seen throughout (**Appendixes 5** and **6** in Supplementary Material). The estimates from inconsistency factor (IF) did not show evidence of statistical inconsistency. Moreover, the physical performance outcome shows no triangular or quadratic loops found.

The direct comparisons and network estimates for both vital sign and physical performances outcomes are shown in the league table (**Table 2**). The comparative efficacy of all supplements ranked with SUCRA probabilities (**Appendix 7** in Supplementary Material). For the vital sign outcome result, heart rate was reported in 12 studies (216 athletes), the highest SUCRA ranked was carbohydrate of 74.9% (WMD =  $-5.94$  bpm; 95%CI =  $-16.13, 4.25$ ) and the lowest ranked was WPS of 33.1% (WMD = 4.94 bpm; 95%CI =  $-5.19, 15.07$ ) were not significant. RER was reported in five studies (100 athletes), although L-alanine had the highest (SUCRA = 71.1%) ranked while placebo had the lowest (SUCRA = 42.8%) ranked, the estimations of supplements were generally similar to placebo and non-significant (**Table 2b**). RPE (eight studies, 170 athletes) was superior to protein + caffeine (SUCRA = 93%) yet insignificant, whereas WPS may be at the low ranked (32.7%) but it was significantly lower (WMD =  $-2.00$ ; 95%CI  $-3.54, -0.46$ ) of RPE level compared to L-alanine (**Table 2c**). For  $VO_{2\max}$  (nine studies, 190 athletes), the highest ranked was placebo (SUCRA = 69.4%) and the lowest ranked was carbohydrate (SUCRA = 29.8%). However, it was revealed from the results of NMA that bovine colostrum had the highest rate of oxygen consumption attainable during the incremental or intensity of physical activities, yet not significant (**Table 2d**).

According to the estimated effect of the supplements on physical performance outcome results (showed in **Tables 2e-g**), maximum power (8 studies, 185 athletes), highest ranked was bovine colostrum (SUCRA = 70.7%) and the lowest ranked was placebo (SUCRA = 17.9%), yet all insignificant. Then again, on average power (nine studies, 187 athletes), WPS was the





highest ranked (SUCRA = 75.4 %) about -112.00 watt (-187.91, -36.08) and most of the estimations were significant. While, the lowest ranked was carbohydrate (SUCRA = 0.2%). Body mass was reported in 10 studies (171 athletes), carbohydrate may be at the highest ranked (SUCRA = 66.9%) but it is insignificant. Though the second highest ranked was WPS (SUCRA = 64.7%) and it is significant (WMD = -6.89 kg; CI= -8.24, -5.54). While, the lowest ranked was maltodextrin (12.4%).

## DISCUSSION

### Quality of the Studies

The search strategy was robust and unlikely to have missed eligible studies. Majority of the studies had low and unclear ROB. This could be due to the methodological difference (may know as methodological heterogeneity), such as binding allocation, a

washout period of time and data analysis strategy. For instance, Hoffman et al. (2009) had 70 days of intervention period that was without blinding and had washout period while Breen et al. (2011) on 28 days of intervention period that was single-blinding and no washout period.

### Vital Signs Outcome Heart Rate

Heart rate for athletes is an instrument to determine and monitor their daily effort for every training and how hard their body is being trained. A slower increase in heart rate while training sessions act as proof that athletes are physically fit (Aubert et al., 2003; Li and Kim, 2017). Although a slower heart rate is preferable, the small differences between the comparators have indicated that WPS is capable and comparable to the comparators.

Rapid absorption of fluids and nutrition assist in better cardiovascular performance in athletes (Oosthuysen and Millen, 2016). These twelve studies have individually shown that WPS and comparators were comparably absorbed rapidly. For WPS, it is known to be absorbed more rapidly than most of the other protein sources, thus it appears to resist coagulation in the stomach and surpass intestines relatively fast (Frank et al., 2017). Whereby, Breen et al. (2011), Li and Zhao (2007) and Impey et al. (2015) studies have individually examined slower heart rate in WPS as compared to carbohydrate supplements.

Moreover, Oosthuysen and Millen (2016) studied specifically the effect of supplements (WPS and comparators) and placebo. This study had the carbohydrate-casein only supplement that intended to maintain all measures of systolic function, yet, these supplements during the intervention period were parallel consistently ingestion. Thus, the benefits associated with consumption of WPS in the context of heart rate may not be significant in contrast to athletes who consumed comparators (carbohydrate, casein, L-alanine, maltodextrin supplements and placebo).

Based on these findings on examined all supplements had similar heart rate results, therefore, WPS is capable to act as ergogenic aids in athletes' heart rate. Nevertheless, athletes must be mindful about continuous of having low heart rates as their heart enlarged over a prolonged period of time (Dixon et al., 1992; Imai et al., 1994). This may lead to suffering from athletic heart syndrome and they may need pacemaker later in their lives.

### Respiratory Exchange Ratio (RER)

Respiratory exchange ratio is one of the most metabolic measurements that indicates fuel (mainly carbohydrate or lipid) is being metabolized to supply the body with energy. When RER value is high, carbohydrates are being utilized, whereas when RER value is low, lipid oxidation is being enhanced (Bergman and Brooks, 1999). Furthermore, the individual studies of RER source data are between 0.8 and 0.9 which corresponds to 50% fat and 50% carbohydrate metabolism (Nelson et al., 2015). Vegge et al. (2012) reported that WPS and maltodextrin supplements were associated RER and had similar RER values throughout the prolonged submaximal exercise, while Schroer et al. (2014) found that WPS did not influence RER or performance. Surprisingly, Breen et al. (2011) found that RER value was not extraordinary high with carbohydrate containing supplements, though the study has high ROB (Figure 3). Therefore, athletes consumed WPS has contributed to a higher RER value for better generation of energy.

### Rate Perceived Exertion (RPE)

Rate perceived exertion is a method to quantify internal training load or intensity of exercise for athletes. Normally, it is a scale measurement that runs from 0 to 10 rating. Whereby, 0 is no training done and 10 extremely heavy training that athletes are able to cope (Ekblom and Golobarg, 1971; Amtmann et al., 2008; Iellamo et al., 2014). There is a slight difference between the supplements and a study has high ROB (Breen et al., 2011) (Figure 3). Moreover, Highton et al. (2013) reported that athletes who consumed WPS were exercising at a higher exercise intensity

compare to carbohydrate, yet for both groups, RPE value had no great difference. Additionally, Naclerio et al. (2015) examined that WPS provided lower RPE values at the beginning and toward the end of soccer compared to carbohydrates alone or a low calorie placebo. The low RPE, especially at the end of exercising sessions, suggested that availability of glycogen would attenuate the rise in fatigue (Naclerio et al., 2015). This indicates that WPS group had lower RPE compared to comparators with the similar workload done. Hence, athletes who consumed WPS were able to have lower RPE and better coping with the intensity of physical exercise.

### Maximum Volume of Oxygen ( $VO_{2max}$ )

Maximum volume of oxygen is defined as the highest rate of oxygen consumption attainable during the incremental or intensity of physical activities (Dlugosz et al., 2013). It also reflects the cardiorespiratory fitness associated with endurance capacity during the prolonged physical activities (Ross et al., 2016). In general, if athletes are performing more intensely, higher will the  $VO_{2max}$  consumption (Dlugosz et al., 2013). Although the network meta-analysis showed bovine colostrum had better efficacy among all the supplements, Coombes et al. (2002) studied that WPS had similar performance benefits with bovine colostrum alone. Similar to Shing et al. (2006) and Schroer et al. (2014) examined that at the beginning of intensity, there may variance in the intake  $VO_{2max}$ , but, at longer duration, there was no difference in improving intake of oxygen and performance. Thus, this might be one of causes to the huge amount of 95% CI in the network meta-analysis. On the other hand, Smith et al. (2010) studied that 90–115% of  $VO_{2max}$  for higher-intensity exercise, while consuming caffeine supplementation. Although, the study may have increase the performance, caffeine is an illegal substance that prohibited by WADA (World Anti-Doping Agency, 2017). With these findings, WPS has better ergogenic effect in  $VO_{2max}$  that allows athletes to have cardiorespiratory fitness while performing intensively.

## Physical Performance

### Maximum and Average Power

To perform in sport, strength is key performance measurement and one of the main interest that athletes seek for ergogenic aids (Lemon et al., 1992; Tarnopolsky et al., 1992; Al-Nawaiseh et al., 2016). The ergogenic effect in maximum and average power were higher in placebo and carbohydrates, respectively, as compare bovine colostrum. According to the included studies, WPS is comparable as ergogenic aids in strength for athletes as there are only slight differences between WPS and the comparators (Coombes et al., 2002; Hoffman et al., 2009; Joy et al., 2013; Hansen et al., 2016). Moreover, Shing et al. (2006) examined that athletes who consumed WPS experienced a decrease in strength in the beginning, but they recovered from any residual fatigue and remained unchanged at following the 5–6 days. Moreover, Highton et al. (2013) discovered that WPS ingestion enabled a small increase in exercise intensity in the latter stages of the sports exercise compared to carbohydrate. Al-Nawaiseh et al. (2016) also investigated that average power recovered better and managed about 4 times higher for athletes who consumed WPS

than placebo. Hence, WPS would assist athletes in strength at a longer period of consumption with the physical activities.

### Body Mass

The key objectives of athletes' development and well-being are body composition. One of the important body composition measurement is body mass (Anding and Oliver, 2015). The analysis had illustrated that WPS improved athletes' body mass by lowering their body mass better than the competitors, though with a marginal difference. Additionally, the individual studies explained that WPS is an ergogenic aid to the body composition as a whole. The relationship of WPS with body mass is well studied and elaborated by Lollo et al. (2011), Lollo et al. (2014). Additionally, Lollo et al. (2011) have examined that WPS provided an additional benefit for maintaining and gaining muscle mass in athletes, while Lollo et al. (2014) further assessed that WPS has a net effect on muscle mass gain over prolonged exercise. Moreover, Taylor et al. (2016) reported in particular for female athletes who improved lean body mass and reduction in fat mass. Thus, the results suggested that athletes who need to have achieved Ideal weight by losing their body mass for the sports performance are encouraged to consumed WPS while maintaining or gaining muscle mass (Brukner and Khan, 2009).

### Safety

There was no relevant data available on the safety, and no side effect was reported in all of the included studies. Therefore, this systematic review and network meta-analysis study are not in the position to discuss it. Nonetheless, WP is recognized as safe supplements for athletes (Tipton et al., 2004; Bolster et al., 2005), concern arises from WADA insight whereby illegal substances can be found in the interventions from the included studies which are Smith et al. (2010) contained caffeine. Hence, athletes shall be cautious while taking supplements in the content of not violating WADA rule and regulation (World Anti-Doping Agency, 2017).

### Limitation

Several limitations of this systematic review and meta-analysis are worth considering. Foremost, the study did not overcome problems that were inherent in the primary studies. Also, the review did not correct the biases of the primary studies (Garg et al., 2008). Besides, there would have imprecision related to the impossibility of generalizing diverse characteristics from study to study such as age, gender or geographic factors (Higgins and Green, 2011). On top of that, the discussion and conclusion draw from this systematic review and meta-analysis upon the sports performance and recovery among athletes are at the time they were measured. Therefore, this review cannot establish the

causation between the parameters and long-term performances and recovery progress for athletes.

### Recommendations

Future directions for research and conducting research that includes larger sample sizes, the inclusion of both gender (especially on female athletes), ages, geographical, type of sport and categories of athletes. Interventions that are consumed before, during and/or after sports performances and recovery process also deserve further considering the effectiveness of improving athletes' vital signs and physical performances. Additionally, follow-up studies could establish effectiveness for the relation between interventions and long-term on vital signs and physical performances progress for athletes. Importantly, it is highly recommended for athletes and their providers are well-inform and updated on WADA guidelines that updated annually before consuming any WPS. These findings are worthy of further inquiry and investigation.

### CONCLUSION

The systematic review and network meta-analysis study has attempted on the clinical evidence efficacy and safety of WPS on performance and recovery among athletes are promising. First of all, the quality of studies has delivered assure validity and reliability of the clinical evidence. Whereby, all the studies were RCTs, thus, many sources of biases have omitted. Therefore, athletes and multidisciplinary team that manages athlete's health and performance can have a clear directive on the evidence with regards WPS as compared to other protein supplements for a vital sign and physical performance. Besides, the included studies examined as close as possible to real life conditions of sports performances and competition for athletes. Therefore, the study can be used as a guide for better decision-making especially when working with a multidisciplinary approach.

### AUTHOR CONTRIBUTIONS

TK performed Statistical analysis and interpretation of results. F-CL wrote the initial draft. AB finalized the paper. HR, MKW, NS, AMK, AE, MK, and AT helped in making revisions and finalization of the manuscript.

### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2019.00317/full#supplementary-material>

### REFERENCES

- Al-Nawaiseh, A. M., Pritchett, R. C., and Bishop, P. A. (2016). Enhancing short-term recovery after high-intensity anaerobic exercise. *Journal of Strength and Conditioning Research* 30, 320–325. doi: 10.1519/JSC.0000000000001060
- Amtmann, J. A., Amtmann, K. A., and Spath, W. K. (2008). Lactate and rate of perceived exertion responses of athletes training for and competing in a mixed martial arts event. *J. Strength Conditioning Res.* 22, 645–647. doi: 10.1519/JSC.0b013e318166018e
- Anding, R., and Oliver, J. M. (2015). Football player body composition: importance of monitoring for performance and health. *Sports Sci. Exchang.* 28, 1–8.
- Aubert, A. E., Seps, B., and Beckers, F. (2003). Heart rate variability in athletes. *Sports Med.* 33, 889–919. doi: 10.2165/00007256-200333120-00003



- Bergman, B. C., and Brooks, G. A. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J. Appl. Physiol.* 86, 479–487. doi: 10.1152/jappl.1999.86.2.479
- Bolster, D. R., Pikosky, M. A., Gaine, P. C., Martin, W., Wolfe, R. R., Tipton, K. D., et al. (2005). Dietary protein intake impacts human skeletal muscle protein fractional synthetic rates after endurance exercise. *Am. J. Physiol. Endocrinol. Metab.* 289, E678–E683. doi: 10.1152/ajpendo.00060.2005
- Borenstein, M., Hedges, L. V., Higgins, J. P. T., and Rothstein, H. R. (2009). “Fixed-effect versus random-effects models,” in *Introduction to Meta-Analysis*, ed M. Borenstein (Chichester: John Wiley & Sons, Ltd), 77–85.
- Boutron, I., Moher, D., Altman, D. G., Schulz, K. F., and Ravau, P. (2008). Extending the CONSORT statement to randomized trials of nonpharmacologic treatment: explanation and elaboration. *Ann. Intern. Med.* 148, 295–309. doi: 10.7326/0003-4819-148-4-200802190-00008
- Breen, L., Philp, A., Witard, O. C., Jackman, S. R., Selby, A., Smith, K., et al. (2011). The influence of carbohydrate-protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *J. Physiol.* 589, 4011–4025. doi: 10.1113/jphysiol.2011.211888
- Brukner, P., and Khan, K. (2009). *Clinical Sports Medicine*. North Ryde, NSW: McGraw-Hill
- Chaimani, A., Higgins, J. P. T., Mavridis, D., Spyridonos, P., and Salanti, G. (2013). Graphical tools for network meta-analysis in STATA. *PLoS ONE* 8:e76654. doi: 10.1371/journal.pone.0076654
- Chan, D. K.-C., Hagger, M. S., and Spray, C. M. (2011). Treatment motivation for rehabilitation after a sport injury: application of the trans-contextual model. *Psychol. Sport Exerc.* 12, 83–92. doi: 10.1016/j.psychsport.2010.08.005
- Coombes, J. S., Conacher, M., Austen, S. K., and Marshall, P. A. (2002). Dose effects of oral bovine colostrum on physical work capacity in cyclists. *Med. Sci. Sports Exerc.* 34, 1184–1188. doi: 10.1097/00005768-200207000-00020
- Dixon, E. M., Kamath, M. V., McCartney, N., and Fallen, E. L. (1992). Neural regulation of heart rate variability in endurance athletes and sedentary controls. *Cardiovasc. Res.* 26, 713–719. doi: 10.1093/cvr/26.7.713
- Dlugosz, E. M., Chappell, M. A., Meek, T. H., Szafranska, P. A., Zub, K., Konarzewski, M., et al. (2013). Phylogenetic analysis of mammalian maximal oxygen consumption during exercise. *J. Exp. Biol.* 216, 4712–4721. doi: 10.1242/jeb.088914
- Eklblom, B., and Golobarg, A. N. (1971). The influence of physical training and other factors on the subjective rating of perceived exertion. *Acta Physiol. Scand.* 83, 399–406. doi: 10.1111/j.1748-1716.1971.tb05093.x
- Frank, K., Patel, K., Lopez, G., and Willis, B. (2017). *Whey Protein Research Analysis*. Available online at: <https://examine.com/supplements/whey-protein/> (accessed September 15, 2018).
- Fukuda, D. H., Smith, A. E., Kendall, K. L., and Stout, J. R. (2010). The possible combinatory effects of acute consumption of caffeine, creatine, and amino acids on the improvement of anaerobic running performance in humans. *Nutr. Res.* 30, 607–614. doi: 10.1016/j.nutres.2010.09.004
- Garg, A. X., Hackam, D., and Tonelli, M. (2008). Systematic review and meta-analysis: when one study is just not enough. *Clin. J. Am. Soc. Nephrol.* 3, 253–260. doi: 10.2215/CJN.01430307
- Gunnarsson, T. P., Bendiksen, M., Bischoff, R., Christensen, P. M., Lesivig, B., Madsen, K., et al. (2013). Effect of whey protein- and carbohydrate-enriched diet on glycogen resynthesis during the first 48 h after a soccer game. *Scand. J. Med. Sci. Sports* 23, 508–515. doi: 10.1111/j.1600-0838.2011.01418.x
- Hansen, M., Bangsbo, J., Jensen, J., Bibby, B. M., Madsen, K., Krause-Jensen, M., et al. (2016). Protein intake during training sessions has no effect on performance and recovery during a strenuous training camp for elite cyclists. *J. Int. Soc. Sports Nutr.* 13:9. doi: 10.1186/s12970-016-0120-4
- Higgins, J. P., Jackson, D., Barrett, J. K., Lu, G., Ades, A. E., and White, I. R. (2012). Consistency and inconsistency in network meta-analysis: concepts and models for multi-arm studies. *Res. Synth. Methods* 3, 98–110. doi: 10.1002/jrsm.1044
- Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011a). The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. *BMJ* 343:d5928. doi: 10.1136/bmj.d5928
- Higgins, J. P. T., and Green, S. (eds.) (2011). *Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0. The Cochrane Collaboration*. Chichester: John Wiley & Sons Ltd.
- Higgins, J. P. T., Thompson, S. G., Deeks, J. J., and Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ* 327, 557–560. doi: 10.1136/bmj.327.7414.557
- Higgins, J. P. T., Thompson, S. G., Deeks, J. J., and Altman, D. G. (2011b). “Selecting studies and collecting data,” in *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*, eds J. P. T. Higgins and J. J. Deeks (Chichester: John Wiley & Sons Ltd.), 151–187.
- Highton, J., Twist, C., Lamb, K., and Nicholas, C. (2013). Carbohydrate-protein coingestion improves multiple-sprint running performance. *J. Sports Sci.* 31, 361–369. doi: 10.1080/02640414.2012.735370
- Hoffman, J. R., Ratamess, N. A., Tranchina, C. P., Rashti, S. L., Kang, J., and Faigenbaum, A. D. (2009). Effect of protein-supplement timing on strength, power, and body-composition changes in resistance-trained men. *Int. J. Sport Nutr. Exerc. Metab.* 19, 172–185. doi: 10.1123/ijsem.19.2.172
- Iellamo, F., Manzi, V., Caminiti, G., Vitale, C., Massaro, M., Cerrito, A., et al. (2014). Validation of rate of perceived exertion-based exercise training in patients with heart failure: insights from autonomic nervous system adaptations. *Int. J. Cardiol.* 176, 394–398. doi: 10.1016/j.ijcard.2014.07.076
- Imai, K., Sato, H., Hori, M., Kusuoka, H., Ozaki, H., Yokoyama, H., et al. (1994). Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 24, 1529–1535. doi: 10.1016/0735-1097(94)90150-3
- Impey, S. G., Smith, D., Robinson, A. L., Owens, D. J., Bartlett, J. D., Smith, K., et al. (2015). Leucine-enriched protein feeding does not impair exercise-induced free fatty acid availability and lipid oxidation: beneficial implications for training in carbohydrate-restricted states. *Amino Acids* 47, 407–416. doi: 10.1007/s00726-014-1876-y
- Joy, J. M., Lowery, R. P., Wilson, J. M., Purpura, M., De Souza, E. O., Wilson, S. M., et al. (2013). The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr. J.* 12, 86–93. doi: 10.1186/1475-2891-12-86
- Kraemer, W., Hooper, D., Szivak, T., Kupchak, B., Dunn-Lewis, C., Comstock, B., et al. (2015). The addition of beta-hydroxy-beta-methylbutyrate and isomaltulose to whey protein improves recovery from highly demanding resistance exercise. *J. Am. Coll. Nutr.* 34, 91–99. doi: 10.1080/07315724.2014.938790
- Lam, F.-C., Khan, T. M., and Quek, K.-F. (2016). *Efficacy and Safety of Whey Protein Supplements on Performance and Recovery Among Athletes: a Systematic Review and Meta-Analysis*. University of York. Available online at: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.asp?ID=CRD42016041842](http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016041842) (accessed September 15, 2018).
- Lemon, P. W., Tarnopolsky, M. A., MacDougall, J. D., and Atkinson, S. A. (1992). Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *J. Appl. Physiol.* 73, 767–775. doi: 10.1152/jappl.1992.73.2.767
- Li, M., and Kim, Y. T. (2017). Design of a wireless sensor system with the algorithms of heart rate and agility index for athlete evaluation. *Sensors* 17, 2373–2387. doi: 10.3390/s17102373
- Li, S. C., and Zhao, Y. F. (2007). Effects of carbohydrate and whey protein supplement at appropriate time on physical performance during football game. *J. Clin. Rehabil. Tissue Eng. Res.* 11, 10304–10307.
- Lollo, P. C., Amaya-Farfan, J., and de Carvalho-Silva, L. B. (2011). Physiological and physical effects of different milk protein supplements in elite soccer players. *J. Human Kinet.* 30, 49–57. doi: 10.2478/v10078-011-0072-3
- Lollo, P. C., Amaya-Farfan, J., Faria, I. C., Salgado, J. V. V., Chacon-Mikahil, M. P. T., Cruz, A. G., et al. (2014). Hydrolysed whey protein reduces muscle damage markers in Brazilian elite soccer players compared with whey protein and maltodextrin. A twelve-week in-championship intervention. *Int. Dairy J.* 34, 19–24. doi: 10.1016/j.idairyj.2013.07.001
- Macdermid, P. W., and Stannard, S. R. (2006). A whey-supplemented, high-protein diet versus a high-carbohydrate diet: effects of endurance cycling performance. *Int. J. Sport Nutr. Exerc. Metab.* 16, 65–77. doi: 10.1123/ijsem.16.1.65
- MacKenzie-Shalders, K. L., Byrne, N. M., Slater, G. J., and King, N. A. (2015). The effect of a whey protein supplement dose on satiety and food intake in resistance training athletes. *Appetite* 92, 178–184. doi: 10.1016/j.appet.2015.05.007
- Miller, P. E., Alexander, D. D., and Perez, V. (2014). Effects of whey protein and resistance exercise on body composition: a meta-analysis

- of randomized controlled trials. *J. Am. Coll. Nutr.* 33, 163–175. doi: 10.1080/07315724.2013.875365
- Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann. Intern. Med.* 151, 264–269. doi: 10.7326/0003-4819-151-4-200908180-00135
- Naclerio, F., Larumbe-Zabala, E., Cooper, R., Allgrove, J., and Earnest, C. P. (2015). A multi-ingredient containing carbohydrate, proteins L-glutamine and L-carnitine attenuates fatigue perception with no effect on performance, muscle damage or immunity in soccer players. *PLoS ONE* 10: e0125188. doi: 10.1371/journal.pone.0125188
- Nelson, M. T., Biltz, G. R., and Dengel, D. R. (2015). Repeatability of respiratory exchange ratio time series analysis. *J. Strength Conditioning Res.* 29, 2550–2558. doi: 10.1519/JSC.0000000000000924
- Nissen, S. L., and Sharp, R. L. (2003). Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. *J. Appl. Physiol.* 94, 651–659. doi: 10.1152/jappphysiol.00755.2002
- Oosthuyse, T., and Millen, A. M. (2016). Comparison of energy supplements during prolonged exercise for maintenance of cardiac function: carbohydrate only versus carbohydrate plus whey or casein hydrolysate. *Appl. Physiol. Nutr. Metab.* 41, 674–683. doi: 10.1139/apnm-2015-0491
- Ross, R., Blair, S. N., Arena, R., Church, T. S., Després, J.-P., Franklin, B. A., et al. (2016). Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American heart association. *Circulation* 134:E653. doi: 10.1161/CIR.0000000000000461
- Saez de Villarreal, E., Requena, B., and Cronin, J. (2012). The effects of plyometric training on sprint performance: a meta-analysis. *J. Strength Conditioning Res.* 26, 575–584. doi: 10.1519/JSC.0b013e318220fd03
- Salanti, G. (2012). Indirect and mixed-treatment comparison, network, or multiple-treatments meta-analysis: many names, many benefits, many concerns for the next generation evidence synthesis tool. *Res. Synth. Methods* 3, 80–97. doi: 10.1002/jrsm.1037
- Schoenfeld, B. J., Aragon, A. A., and Krieger, J. W. (2013). The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *J. Int. Soc. Sports Nutr.* 10, 53–66. doi: 10.1186/1550-2783-10-53
- Schroer, A. B., Saunders, M. J., Baur, D. A., Womack, C. J., and Luden, N. D. (2014). Cycling time trial performance may be impaired by whey protein and L-alanine intake during prolonged exercise. *Int. J. Sport Nutr. Exerc. Metab.* 24, 507–515. doi: 10.1123/ijsnem.2013-0173
- Shing, C. M., Jenkins, D. G., Stevenson, L., and Coombes, J. S. (2006). The influence of bovine colostrum supplementation on exercise performance in highly trained cyclists. *Br. J. Sports Med.* 40, 797–801. doi: 10.1136/bjism.2006.027946
- Silver, M. D. (2001). Use of ergogenic aids by athletes. *J. Am. Acad. Orthopaed. Surg.* 9, 61–70. doi: 10.5435/00124635-200101000-00007
- Smith, A. E., Fukuda, D. H., Kendall, K. L., and Stout, J. R. (2010). The effects of a pre-workout supplement containing caffeine, creatine, and amino acids during three weeks of high-intensity exercise on aerobic and anaerobic performance. *J. Int. Soc. Sports Nutr.* 7, 10–22. doi: 10.1186/1550-2783-7-10
- Tarnopolsky, M. A., Atkinson, S. A., MacDougall, J. D., Chesley, A., Phillips, S., and Swarcz, H. P. (1992). Evaluation of protein requirements for trained strength athletes. *J. Appl. Physiol.* 73, 1986–1995. doi: 10.1152/jappphysiol.1992.73.5.1986
- Taylor, L. W., Wilborn, C., Roberts, M. D., White, A., and Dugan, K. (2016). Eight weeks of pre- and postexercise whey protein supplementation increases lean body mass and improves performance in Division III collegiate female basketball players. *Appl. Physiol. Nutr. Metab.* 41, 249–254. doi: 10.1139/apnm-2015-0463
- Tipton, K. D., Elliott, T. A., Elliott, T. A., Cree, M. G., Wolf, S. E., Sanford, A. P., et al. (2004). Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Med. Sci. Sports Exerc.* 36, 2073–2081. doi: 10.1249/01.MSS.0000147582.99810.C5
- Turner, R. M., Davey, J., Clarke, M. J., Thompson, S. G., and Higgins, J. P. (2012). Predicting the extent of heterogeneity in meta-analysis, using empirical data from the Cochrane database of systematic reviews. *Int. J. Epidemiol.* 41, 818–827. doi: 10.1093/ije/dys041
- Vegge, G., Rønnestad, B. R., and Ellefsen, S. (2012). Improved cycling performance with ingestion of hydrolyzed marine protein depends on performance level. *J. Int. Soc. Sports Nutr.* 9, 14–24. doi: 10.1186/1550-2783-9-14
- Veroniki, A. A., Vasiladis, H. S., Higgins, J. P., and Salanti, G. (2013). Evaluation of inconsistency in networks of interventions. *Int. J. Epidemiol.* 42, 332–345. doi: 10.1093/ije/dys222
- Wiese-Bjornstal, D. M. (2010). Psychology and socioculture affect injury risk, response, and recovery in high-intensity athletes: a consensus statement. *Scand. J. Med. Sci. Sports* 20, 103–111. doi: 10.1111/j.1600-0838.2010.01195.x
- Willick, S. E., Miller, G. D., and Eichner, D. (2016). The anti-doping movement. *PMandR* 8(3, Suppl), S125–32. doi: 10.1016/j.pmrj.2015.12.001
- World Anti-Doping Agency (2017). *Index of Prohibited Substances and Methods*. Available online at: <https://www.wada-ama.org/en/what-we-do/prohibited-list/index-prohibited-substances-and-methods> (accessed September 15, 2018).

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Lam, Bukhsh, Rehman, Waqas, Shahid, Khaliel, Elhanish, Karoud, Telb and Khan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.