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Review

Nutritional review of the methodology applied in sports supplementation research

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Abstract. Both high-performance athletes and amateurs resort to taking supplements in order to improve their performance and/or their health. Many of them do not know the effects of these and take them without a professional supervision. The aim is to know the population who is studied by other different studies related to supplementation and improvement of sports performance and the nutritional method used in these cases. Systematic bibliographic research (March-May2021) was carried out in MEDLINE, with Boolean operators (((((caffeine[MeSH Major Topic]) OR (creatine[MeSH Major Topic])) OR (nitrate[MeSH Major Topic])) OR (sodium bicarbonate[MeSH Major Topic])) AND (dietary supplement[MeSH Terms])) AND (athletic performance[MeSH Terms]). 17 articles were included. Mean participants of these studies were 16.8subjects, which corresponds to a small sample. Only two studies were conducted to women. Several others were based on people who are not considered high-performance. In most of the selected studies, there is no a nutritional assessment, dietary record or follow-up of the subjects' diet before and during the intervention, and this is a limitation. In order to reach accurate conclusions about supplementation and optimization of sports performance, it is necessary to know the nutritional and hydration status, supplementation habits and lifestyle of the participants in these trials are.

Key words: Review, supplementation, methodology, nutrition, sample, surveys

Introduction

Nowadays, for an athlete, nutrition and hydration are the two main factors that influence their health and sports performance. It is important that the athlete can perform frequent and high-intensity training and competitions without affecting his health. The type of diet and foods that it includes, the number of nutrients and energy, water and adaptation to training and competition schedules, are factors to consider (1). Sometimes, both amateur and high-performance athletes resort to supplementation to improve their athletic performance, although many of them do not know their function or are supervised by a health professional (1,2).

There must be a balance between the positive aspects of using sports supplements or sports foods and their risks, including anti-doping rule violations (3). To evaluate the use of a supplement as an ergogenic aid, attention should be paid to factors such as the population in which the study is carried out, previous research that shows benefits on sports performance in athletes or people who exercise regularly, type of control of the investigations, the statistical significance of the study, as well as the repetition of results in different studies of different workgroups (1).

The ABCD classification system (Australian Institute of Sport-AIS, 2021), organize sports supplements and their ingredients into four groups, according to scientific evidence and other practical considerations to determine whether a product is safe, permitted and effective. In group A, is formed by supplements with solid scientific evidence for use in specific situations in sport, guided by evidence-based protocols. Within this group, there is the subgroup "performance supplements". This subgroup includes supplements or ingredients such as caffeine, beet juice, creatine, and baking soda, among others (7).

Caffeine is an alkaloid in the xanthine family. Its use as an ergogenic aid is due to the improvement of the athlete's physical performance. Caffeine supplementation can optimize athletic performance thanks to different effects on the central and peripheral nervous systems (1). Dietary nitrate is used to improve the availability of nitric oxide (NO) in the body. Supplementation with NO improves performance in situations where its production is compromised. Beet juice is a rich source of nitrate in the diet. Supplementation with it can reduce exercise oxygen cost, exercise capacity, and performance (4). Creatine is a nitrogenous organic compound that belongs to the non-essential amines group, present in the diet. Creatine monohydrate supplementation is safe and effective in increasing athletic performance according to various studies, especially in sports that involve repeated high-intensity, short-duration series with short recovery intervals (1). Bicarbonate is an endogenously produced extracellular anion, a component of the body's pH buffering system. Sodium bicarbonate helps to mitigate the increase in intramuscular [H +]. Supplementation in high-intensity exercise can improve performance during single repeated high-intensity bouts (1-10 minutes) (1,5). All these results on the different supplements described above come from various research but, do we know the population under study, the total number of study subjects, if they are really athletes or the nutritional method used in these to draw conclusions and be extrapolated?. The main goal of this systematic review is to identify the population under study, verify whether they are really athletes, to know the total number of subjects under study, and as well as the nutritional method used in these.

Material and methods

This review was performed according to the PRISMA statement (Reporting Items for Systematic Reviews and Meta-Analyzes). A systematic bibliographic search was carried out from March to May 2021 in Medlars Online International Literature (MEDLINE) through Pubmed. Major terms were used as "caffeine", "creatine", "sodium bicarbonate" and "nitrate" using as Medical Subject Heading Terms (MesH) "dietary supplements" and "athletic performance". The search strategy used was with Boolean operators: (((((caffeine [MeSH Major Topic]) OR (creatine [MeSH Major Topic])) OR (nitrate [MeSH Major Topic])) OR (sodium bicarbonate [MeSH Major Topic])) AND (dietary supplement [MeSH Terms])) AND (athletic performance [MeSH Terms]). The search procedure was initially, review of the titles, after the abstracts and finally the entire article. The search was complemented with a review of the reference lists reflected in the articles found, to obtain additional information. The item identification process is shown in Figure 1.

255 articles were selected, of which 66 were identified with the search criteria detailed in Figure 1. The following search filters were then applied: a) Studies carried out in animals, articles from 3 years ago and in English. (excluded, n=189). b) Clinical trials and are indexed (excluded, n = 26). c) Non-relevant articles, use of other supplements in addition to caffeine, nitrate, sodium bicarbonate and creatine, subjects with pathologies, finding no benefit with supplementation (excluded, n=23). A total of 17 articles were included in this systematic review (Table 1). summarizes the main characteristics of each of the studies included in this review (references, journal, JCR, participants, design, training and supplementation, nutritional method, study results/conclusion).

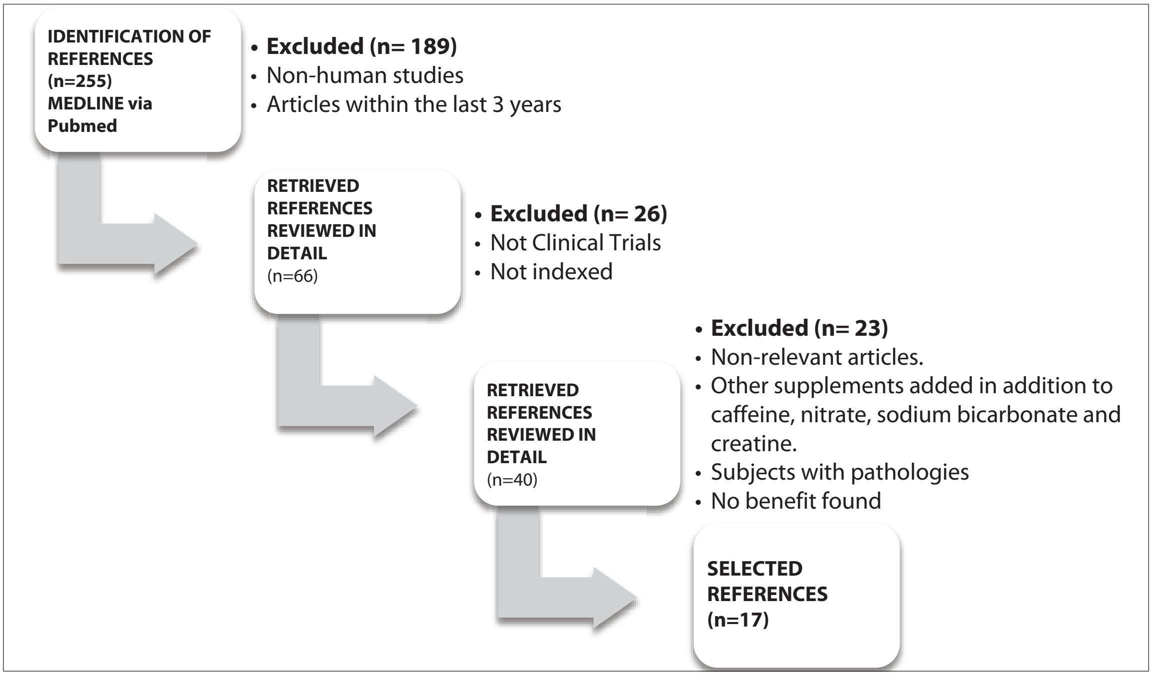

Figure 1. Flow chart of the systematic review.

Results

All the selected studies, in total 17, are clinical trials, published in English and indexed. Were published after 2018 (inclusive) with a double-blind, randomized and placebo-controlled study design and in all of them was obtained, as a result, benefit with the supplementation of each one of the studied supplements. Regarding the journal of publication (Table 2), 4 of them were published in the journal "Nutrients", 3 in the journal "Journal of the International Society of Sports Nutrition", 2 in "Journal of Strength and Conditioning Research" and "International Journal of Environmental Research and Public Health", and 1 in each of the following: "Experimental Physiology","Medicine and Science in Sport and Exercise", "Scientific reports", "Journal of Sport Medicine and Physical Fitness", "Journal of Human Kinetics" and "British Journal of Clinical Pharmacology". The following journals belonged to quartile 1 within their category in 2019: "Nutrients", "Journal of the International Society of Sports Nutrition", "Journal of Strength and Conditioning Research", "Medicine and Science in Sport and Exercise" and "Scientific reports". Within quartile 2 were "International Journal of Environmental Research and Public Health" and "British Journal of Clinical Pharmacology". To quartile 3 belonged the journals "Experimental Physiology" and "Journal of Human Kinetics", and lastly, "Journal of Sports Medicine and Physical Fitness" belonged to quartile 4.

The study population was an average of 16.8 participants. Of the selected studies, 2 of them only studied women. Within the selected studies, 9 of them were based on high-level sports subjects, with intense weekly training and/or participants in competitions at the time of the study. The rest of the studies were based on subjects with experience in exercise and/or in the tests to be carried out in the study and recreationally active; several studies analysed university students of Physical Activity and Sports Sciences.

In Table 1, the information obtained from the different studies regarding training and supplementation is shown, as well as the nutritional method used. Each of them used different types of training (strength, endurance, etc.) depending on the objective of the study. Regarding the nutritional method used, only one of the studies did not contain information about it. Dietary guidelines were given so that all participants followed

Table 1. Summary of the characteristics of the selected studies.

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
San Juan et al. (2019) (6)	Nutrients	IF: 4.546 17/89 Q1	Total (n=8) Young healthy male boxers (22.0±1.778yrs, height: 1.69±0.09m, weight: 65.63± 10.79kg, (BMI): 22.69±1.31Kg/ m^2, Wingate test: 4.91±0.82kp). Spain's Olympic selection for the Tokyo 2020 Olympic Games	Double-blind, randomised, placebo-controlled design	They completed 2 equal training sessions (separated by 48h) in the laboratory at the same time interval. Beginning with initial lactate measurements, hand grip and countermovement jump, followed by Wingate test (30 seconds). Administration 75 minutes before the session of a caffeine capsule (6mg · kg -1) or sucrose (placebo).	Dietary guidelines for everyone to consume the same macronutrient content in the 48h prior to each session. List of caffeine-rich foods not to be consumed 24h prior to the end of the study.	Caffeine supplementation (6mg•kg-1) improves anaerobic performance with similar electromyographic activity and lower limb fatigue levels and increased neuromuscular efficiency in some muscles in Olympic-level boxers. In addition, caffeine consumption improves reaction speed.
Schäfer et al. (2019) (7)	Experimental Physiology	IF: 2,431 42/81 Q3	Total (n=11) Non-vegetarian males (mean age 22.6±2.8yrs; body mass, 75.8±11.5kg; peak O_2 consumption, 51.7±8.3mL min -1 kg -1; peak power output (P peak) 311±37W). Recreationally active, volunteers. Familiar with cycloergometry and exercise procedures used in the laboratory.	Randomised controlled trial	They performed an incremental cycling test with 4-5 constant-load trials to task failure (TTF) to obtain asymptote (Critical Power) and constant of curvature (W') of the power-duration relationship, followed by 3 constant-load supra-CP: 1) a TTF after placebo (PLA) supplementation; 2) a TTF after creatine supplementation and 3) a trial of equal duration to placebo after creatine supplementation. All participants ingested 4×5g day -1 of dextrose (PLA) during the first 5-day supplementation period. Prior to the second trial they ingested 4×5g day -1	Not stated	Time to task failure improved significantly with creatine supplementation just as work performed above CP increased significantly. An improvement in high-intensity cycling performance above CP following creatine supplementation is shown to not influence the magnitude of neuromuscular fatigue at task failure.

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
					of creatine monohydrate for five successive days and during the third supplementation period they ingested maintenance doses of 2g day -1 of creatine for each day between the second and third periods of the main trial. Supplements were taken at regular intervals throughout the day.		
VAN Cutsem et al. 2020 (8)	Medicine and science in sport and exercise	IF: 4,029 9/85 Q1	Total (n=14) non-blind participants (4 female, 10 male, mean age 24±3yrs. mass=74±13kg, height = 79±9cm). None with known mental or somatic disorder and categorised as low-moderate activity according to the International Physical Activity Questionnaire form.	Double-blind, randomised, controlled design	Participants performed a 90-minute mental fatigue task in two different conditions: after 7-day creatine supplementation (20g/day) and after 7-day calcium lactate supplementation (placebo) separated by a 5-week period (washout). In both conditions, a 7-min sport-specific visuomotor task, a dynamic handgrip strength endurance task and a 3-min flanker task were performed before and after the mental fatigue task. Physiological and perceptual responses were measured throughout the protocol.	Restrictions and prohibitions on food, sleep or activity (no changes in routines) during the study period. Limit alcohol consumption to no more than two drinks every 24h. Avoidance of alcohol and caffeine the day before testing. Prohibited the use of medication during and between tests.	Creatine supplementation improved physical (strength endurance) and prolonged cognitive (Stroop accuracy) performance, but did not improve mental fatigue impairments in short-term sport-specific psychomotor or cognitive (Flanker) performance.
Ansdell, P y Dekerle, J. (2020) (9)	Journal of strength and conditioning research	IF: 2,973 19/85 Q1	Total (n=10). Healthy, active male basketball players with more than 4yrs of competitive basketball experience.	Double-blind, crossover, randomised design.	All participants ingested 0.2 g•kg -1 NaHCO3 (or an equimolar placebo dose of NaCl) 90-60 min before the start of the basketball game simulation. Maximal voluntary isometric	Recommendations: avoidance of stimulant or alcohol consumption and replication of food intake during the 24h prior to the test.	Maximal voluntary isometric contraction, high and low contraction forces decreased progressively in both trials with a less pronounced decrease in maximal voluntary isometric

Table 1 (*Continued*)

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
					contractions of the knee extensors and high and low frequency doublet contractions were recorded before and after each quarter of the game for both tests.		contraction during sodium bicarbonate intervention. High and low contraction forces were also significantly higher when supplemented with sodium bicarbonate. Sodium bicarbonate supplementation decreased the development of fatigue by protecting the contractile elements of the muscle fibres.
Liubertas et al. (2020) (10)	Journal of the International Society of Sports Nutrition	IF: 5.068 14/89 Q1	Total (n=13) Male volunteers from the Lithuanian University of Sport. Experimental group: mean age 21.3±0.8yrs, height 180.5±8.3cm, weight 84.3±23.7kg, BMI: 25.6±5.8Kg/m^2, relative fat mass 18.9±5.6%. Placebo group: mean age 21.9± 1.9yrs, height 182.1±5.9cm, weight 88.1±12.5kg, BMI 26.5±2.8Kg/m^2, relative fat mass 13.1±3.8%.	Double-blind, randomised, placebo-controlled design	At the first visit to the laboratory, anthropometric measurements were taken and the exercise test cycling with augmentation (ICE) was performed. After 48 hours of rest they repeated the test, 1 hour after taking the supplement or placebo. For the next 4 days they took the supplement (or placebo) at breakfast and the next day they performed another test, after taking the supplement (or placebo) 1 hour before the test. The experimental group took a 60g supplement in the form of an oat bar, honey, vanilla and 4g of Amaranthus hypochondriacus concentrate. The placebo group consumed the same oat bar, but without the additive (amaranth).	They were asked not to change their nutritional habits for the duration of the study.	Long-term supplementation with amaranth dietary nitrate (NO_3) may improve aerobic capacity during the cycling exercise test with increase (ICE) in physically active young men, and may be recommended as a supplement in the last week prior to competition in endurance exercise. Peak ICE power, maximum oxygen consumption and first ventilatory threshold increased significantly after consumption of dietary amaranth for 6 days (from 4.44±0.50 to 4.55±0.43W/kg; from 37.7±2.7 to 41.2±5.4mL/kg/min and from 178.6±30.3 to 188.6±35.2W, $p<0.05$, respectively).

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
Durkalec-Michalski et al. (2018) (11)	Nutrients	IF: 4.546 17/89 Q1	Total (n=49) 18 women and 31 men were included in the study. Athletes of the Polish National Wrestling Team and/or top-level wrestlers in national competitions. All healthy, with valid and up-to-date medical certificate, with at least 4yrs of experience in the sport and with a minimum of 4 training sessions per week.	Randomised, double-blind, placebo-controlled, parallel-group design.	Wrestlers were randomised to a progressive dose of up to 100mg/kg Na bicarbonate or placebo over a 10-day period. Before and after supplementation the athletes completed the exercise protocol: test 1) Wingate test, 2) manikin throw and test 3) second Wingate test. On rest days, supplements were taken in the morning, afternoon and evening. Blood samples were taken before and after the tests. No gastrointestinal side effects were reported during the study period.	No changes in lifestyle, training, diet or supplementation (in addition to that provided) and/or medication were allowed during the study period.	The index that improved with the administration of sodium bicarbonate over placebo was the peak power time in the second Wingate test, which decreased from 3.44±1.98 to 2.35±1.17s. There were no differences in blood glucose or lactate concentrations. The loading regimen established eliminated gastrointestinal symptoms, although doses could be small to improve anaerobic power and performance in this sport. Shortening the time to reach peak power during fatigue may be important and is one of the variables conditioning success in combat sports.
Durkalec-Michalski et al. (2020) (12)	Scientific reports	IF: 3.998 17/71 Q1	Total (n=51) 33 male and 18 female athletes. All were members of the Polish National Wrestling Team and/or competed in Polish competitions at the highest level. Healthy subjects, with valid medical clearance, minimum 4yrs of experience in the sport and practicing the sport at least 4 times a week (training).	Randomised, double-blind, placebo-controlled, parallel-group design.	Athletes randomly ingested sodium bicarbonate at low concentrations (up to 100mg/kg body weight) or placebo for 10 days. Before and after supplementation they performed a sequenced exercise protocol: 1) Wingate test, 2) manikin throw test and 3) second Wingate test. On rest days, supplements were taken in the morning, afternoon and evening. Blood samples were taken before and after the tests.	No changes in lifestyle, training, diet or supplementation (other than that provided) and/or medication were allowed during the study period.	Total throws completed increased in males, improving in the midsection of the 30-second Wingate test with sodium bicarbonate supplementation. No significant differences were found for peak power, power drop and average power. The interaction with gender was significant for average power, peak power and power drop, in every second of the Wingate tests, as well as in the dummy throw test. The response to sodium bicarbonate

Table 1 (*Continued*)

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
							supplementation may be gender-specific and progressive at low doses. Supplementation allows for the improvement of specific wrestling performance in males, as well as helping to maintain high anaerobic power in the middle section of the 30-second Wingate test.
Hahn et al. (2018) (13)	Journal of strength and conditioning research	IF: 2,973 19/85 Q1	Total (n=14) Males, university students, practicing at least 150min of physical activity weekly, injury-free and not regular caffeine users) (Age: 21.0±0.7yrs, height: 178.5±5.1cm, weight: 77.3±9.6kg and body fat: 12.6±4.8%).	Intra-individual, double-blind, placebo-controlled, crossover design.	In the first session participants completed 3 sets of practice sprints on a non-motorised treadmill of 10-20 seconds duration. In the second session, they completed 5 sets of sprints of 15-25 seconds. In the third and fourth sessions participants ingested a portion of caffeinated beverage or placebo (in the fourth session the opposite beverage was consumed), took a 20-minute rest and completed the dynamic warm-up before starting to run. Anaerobic power was assessed by vertical jump with countermovement and a non-motorised treadmill sprint test.	Subjects completed a 2-day log prior to each experimental session, at visits 3 and 4, to confirm identical dietary patterns for each session (similar amounts of energy and macronutrients). They could not consume caffeine or alcohol 24h before the visit. Data from the dietary record were entered into the ESHA database.	In both conditions there was a significant increase in fatigue, although this was less with caffeine supplementation. This indicated that the caffeine-containing supplement improved perceived measures of fatigue, but not the power indices assessed. Therefore, caffeinated beverages may reduce perceived fatigue in acute anaerobic exercise, specifically when repeated sprints are performed.
Morales et al. (2020) (14)	Nutrients	IF: 4.546 17/89 Q1	Total (n=14) Male cyclists with at least 4yrs of cycling experience. Participation in at	Double-blind, placebo-controlled crossover design.	Four experimental trials were conducted: placebo (4 days)-placebo (acute), placebo (4 days)-caffeine (acute), caffeine (4 days)-	A validated caffeine consumption questionnaire was administered (all participants were moderate-high consumers), under the	The caffeine (4 days)-caffeine (acute) and placebo (4 days)-caffeine (acute) trials showed improvements in time and power output

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
			least 20 competitions between 2018-2019 and no history of cardiorespiratory, gastrointestinal or musculoskeletal problems in the last 3 months prior to the study. (Mean age 34.1±4.4yrs, a height of 178±9cm, weight 79.1±11.8kg, BMI 24.6±2.1kg/ m^2, VO_2max 51.5±6.3m·kg-1·min -1 and power max of 398.9±35.1 W, training volumen 202±83km/week).		caffeine (acute) and caffeine (4 days)-placebo (acute). Subjects ingested capsules containing placebo or caffeine (6mg/kg), a higher dose than usual, 1 hour before completing the 16km time trial. On the day of the test, cyclists were fasted and blood samples were taken at the laboratory, before ingestion of the supplement, 1 hour after ingestion, immediately after ingestion and after 10min of recovery. They did not exercise for 24 hours prior to the experiment. Daily training guidelines were provided.	supervision of a qualified nutritionist. At the first visit to the laboratory, a dietary assessment was performed 24h before the test. They were asked to repeat the same diet on the following days. Total energy and macronutrient intakes were determined. The TACO database and DietPro5i software were used to quantify intake and perform nutrient calculations. They were instructed how to withdraw caffeine intake (dietary sources of caffeine) during the study and were monitored by telephone, email and in person. The study included the results of the 24h recall nutritional assessment, showing energy and macronutrient intakes.	compared to the other trials. This was accompanied by increases in heart rate, minute volume, expired oxygen fraction, blood lactate concentration and reduction in expired CO_2. This indicates that caffeine, ingested by cyclists at a dose of 6mg/kg body weight for 4 days, does not induce tolerance to the ergogenic effects caused by acute ingestion on the different parameters (physiological, metabolic and performance).
Garnacho-Castaño et al. (2020) (15)	Journal of the International Society of Sports Nutrition	IF: 5.068 14/89 Q1	Total (n=12) Well-trained males (mean±SD: age=29.5±4.3yrs, body mass=79.4± 5.5kg, height =174.1± 6.6cm) with more than 2yrs of crossfit experience, regional, national and/or international level of competition, with a full back squat repetition maximum	Randomised, double-blind, crossover design.	Performed a crossfit workout after drinking 140mL of beetroot juice or placebo (prepared by a nutrition and dietetics expert). The workout consisted of repeating the same exercise routine twice (wall ball slam and full back squat with 3min rest (routine 1) or no rest (routine 2) between the two exercises. Between the two routines 3min rest. The study lasted 3 weeks	A nutrition professional established nutritional guidelines to ensure that all athletes followed a similar diet during the 48h before the start of the tests (60% carbohydrates, 25% lipids and 15% protein). Athletes were required to avoid foods high in nitrate, so they were given a list of foods to avoid 72h prior to the study. They were also prohibited from caffeine (except breakfast coffee), alcohol or other	There was a greater number of repetitions after beetroot juice intake when a 3-minute rest was set between the two exercises in routine 1. Cortisol showed a greater increase after ingestion of beetroot juice. No interaction effect on testosterone and testosterone/cortisol ratio was observed. A greater drop in oxygen saturation was observed after beetroot juice consumption. Greater

Table 1 (*Continued*)

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
			greater than 120kg, healthy, not consuming alcohol or other drugs and not using supplements at the time of the study.		in which 4 sessions were performed: 1) explanation of the procedure and details of the study, 2) 1RM test in the full back squat exercise to determine load, 3) and 4) comparison of the two experimental conditions (beetroot juice and placebo), with a period of 1 week between each session. Blood was drawn before the test (at rest) and again 3 hours later to determine lactate concentration and hormones (cortisol and testosterone).	supplements. They were prohibited from brushing their teeth, using mouthwash or chewing gum or sweets for 24h before the study.	muscle fatigue was observed with beetroot juice than with placebo. Thus beetroot juice intake improved anaerobic performance only after the recovery time between exercises.
Cuenca et al. (2018) (16)	Nutrients	IF: 4.546 17/89 Q1	Total (n=15) Males (age 22.4±1.6yrs, height 178±6cm, weight 76.9±10.3kg) with at least 18 months of resistance exercise experience, training at least 3 sessions/week and familiar with Wingate 30-second test exercises and countermovement jumps. Non-smokers and healthy.	Randomised, double-blind, placebo-controlled design.	They were asked not to do any exercise for 72 hours before each test. Session 1: preliminary assessment of body composition and knowledge of the protocol. Session 2 and 3: ingestion of 70ml of beetroot juice supplement or placebo. 3 hours later, they performed a 30-second maximal Wingate test and countermovement jumping test (pre and post Wingate). Blood samples were taken to determine lactate before and after the tests.	They were instructed on the diet to be followed the day before each test (60% carbohydrate, 30% fat and 10% protein). Dietary nitrate intake was limited by instructions on a list of nitrate-rich foods (to be avoided 48h before the trial). In the 24h before the trial, they were to avoid brushing their teeth, using mouthwash, chewing gum, taking stimulants or sweets.	Beetroot juice supplementation improved peak and average power output particularly during the first half of the 30-second peak speed test, reducing the time taken to reach peak power. Although there was an improvement in sprint performance, neuromuscular fatigue caused by exercise was similar across supplement and placebo intakes. This suggests that nitrate-rich supplements improve sprint performance without having a cumulative impact on fatigue.

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
Domínguez et al. (2021) (17)	International Journal of Environmental Research and Public Health	IF:2.849 105/265 Q2	Total (n=15) Males, age: 22.60±2.16yrs; height: 1.77±0.04m; mass: 78.11±10.63kg; BMI: 24.99± 2.61kg/m^2) students of Physical Activity and Sport Sciences, with experience in resistance training, at least 3 sessions/ week of strength training in the last 18 months, 1 RM bench press greater than body weight and a full squat 1RM 1.5 times body weight. No supplementation in the 3 months prior to the study, non-smokers, healthy individuals, non-elite athletes, with Wingate test experience.	Randomised, double-blind, placebo-controlled, crossover design.	Participants ingested 6mg/kg body weight of caffeine supplement or placebo (sucrose) in the 2 experimental sessions. Within 1 hour of supplement ingestion, they completed 2 questionnaires measuring subjective vitality and mood. Performance was then assessed using a Wingate test, followed by range of perceived exertion (RPE) measurements at the general, muscular and cardiovascular levels. They were asked not to engage in physical exercise of any kind for 72 hours prior to the study.	They were instructed on the diet to be followed from the beginning of session 1 to the end of session 2. The diet was to be at the macronutrient level: 60% carbohydrate, 30% lipid and 10% protein. Intake of caffeine-rich foods was restricted and a list of foods to avoid was provided. They kept a dietary intake diary 48h before session 1 and were required to reproduce it before session 2	Caffeine supplementation increased some components of mood and subjective vitality profiles. In addition, increased peak power, average power and decreased time to reach peak power in the Wingate test were observed. Athletes reported lower muscle and overall RPE after the Wingate test. Thus, caffeine supplementation has positive psychological and physical effects in trained subjects.
Jodra et al. (2020) (18)	Journal of the International Society of Sports Nutrition	IF: 5.068 14/89 Q1	Total (n=18) 8 of them elite athletes members of the Spanish senior male boxing team, trained at the Centro de Alto Rendimiento de Madrid, Spain and participants in international competitions for more than 2yrs.	Randomised, double-blind, placebo-controlled, crossover design.	They performed 2 test sessions 48 hours before in the laboratory. In each session, half of the athletes took caffeine supplementation (6mg/kg body weight) or placebo (6mg/kg sucrose). After 1 hour's rest, they completed a profile of mood states (POMS) and subjective vitality scale (SVS)	They were instructed on their diet to eat similar macronutrient ratios (60% carbohydrate, 30% lipid and 10% protein). Intake of caffeine-rich foods was restricted by providing a list of foods to avoid for 24h prior to the study.	Caffeine supplementation showed improvements in peak W, average W and time to peak W in both elite and recreational athletes. But only elite athletes showed increases in tension, vigour and SVS scores after caffeine supplementation. Thus, caffeine supplementation improved anaerobic performance in

Table 1 (*Continued*)

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
			The other 10 subjects (recreational) were students of the Sports Science degree at the Alfonso X El Sabio University, Madrid, Spain. They had not taken supplements in the 3 months prior to the study, were non-smokers and healthy.		questionnaires. After a warm-up, they performed a Wingate test on a cycloergometer to assess anaerobic performance. Immediately afterwards, exertion was assessed using the rate of perceived exertion (RPE) scale.		both elite and recreational athletes, however, the effect of caffeine on mood and subjective vitality was greater in elite athletes.
Dumar et al. (2021) (19)	International Journal of Environmental Research and Public Health	IF: 2.849 105/265 Q2	Total (n=10) NCAA (National Collegiate Athletic Association) Division I male sprinters. Active at the time of the study.	Double-blind, counter balanced, crossover design.	Did not perform vigorous lower body exercise 24h prior to each test. Athletes performed 3 exercise tests under different conditions: Morning-placebo, Morning-beetroot juice (70mL concentrate 2h before the test) and afternoon without supplementation. After these, they completed 3x15 Wingate anaerobic tests, with 2min rest in between. Each test was separated by 72h.	They were asked to refrain from caffeine, nicotine, supplements, alcohol and mouthwash for 12h prior to testing. They were also asked to replicate their diet for each of the sessions and maintain normal sleep routines. They were also asked to avoid drinking beetroot or beetroot products during the study.	Supplementation with beetroot juice prevented losses in mean power output, anaerobic capacity and total work in the morning compared to placebo. Rate of perceived exertion was not significantly different between conditions. Heart rate was lower in the morning-beetroot juice condition compared to placebo. This suggests that anaerobic capacity suffers during the morning versus the afternoon, but beetroot juice supplementation eliminates the associated decreases in performance.
Fett et al. (2018) (20)	Journal of sport medicine and physical fitness	IF: 1.432 67/85 Q4	Total (n=8) healthy women aged 25±5yrs and BMI 20-25kg/m^2 who had done continuous resistance training	Randomised, double-blind, placebo-controlled, crossover design.	Four muscle strength tests were performed at different time points: 1RM pull down, Hack Squat, Bench Press and Knee Extension Exhaustion	They were instructed not to change their training routine during the study. They were instructed not to consume caffeine (no list ofcaffeine-rich	Caffeine supplementation improved tolerance to exhaustion and tended to improve strength in study subjects. It is likely to be useful for improving

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
			for at least 12 months.		(drop-set, 100/80/60kg, repetitions). They underwent 4 tests in 4 blocks: week 1) basal, without any caffeine-based substance for at least 48 hours before; week 2) caffeine 1 (6mg/kg) 30min before; week 3) placebo (starch) 30 min before; week 4) caffeine 2 30min before. Paired t-test and repeated Tukey-Kramer ANOVA were performed.	foods/drinks to avoid is indicated).	performance in women playing sports with these physical characteristics.
Giráldez-Costas et al. (2020) (21)	Journal of Human Kinetics	IF: 1.664 55/85 Q3	Total (n=12) Young, healthy participants (9 males and 3 females) volunteers aged 18-45yrs, with low caffeine intake, experience in resistance exercise training, non-smokers, not taking supplements or medications during the month prior to the study, no family history of cardiopulmonary disease, no oral contraceptive use and no caffeine allergy.	Double-blind, placebo-controlled, randomised, counter balanced design.	After ingesting 3mg/kg bw caffeine or placebo (cellulose) one hour before, participants underwent two trials, separated by at least 5 days for full recovery. The tests consisted of a standardised minute warm-up followed by 4 sets of 8 repetitions of bench press with a load of 70% of their 1RM. Barbell speed and load in kg were checked. Average and maximal velocity and average and maximal power output were calculated, as well as time.	Participants were instructed to avoid nutritional supplements and stimulants (caffeine, alcohol or others) during the study. They were instructed to follow a similar diet and beverage intake for 24h prior to the trial.	Compared to the placebo trial, the main effect of caffeine was an increase in average and maximum bar speed. There was an increase in average strength and average power output, peak power output and work done in the caffeine trial. There was also a reduction in time to peak velocity, time to peak power, but no effect on time to peak force. So it could indicate that ingestion of 3mg/kg caffeine one hour before exercise increased average and peak bar speed, average strength and average power and peak power in training. Thus the mechanical work performed was increased.

Table 1 (*Continued*)

References	Journal	JCR Impact Factor (2019)	Participants	Design	Training and supplementation	Nutritional approach, recording and monitoring	Results/conclusion
Lara et al. (2020) (22)	British Journal of Clinical Pharmacology	IF: 3.740 71/271 Q2	Total (n=13) healthy female triathletes (age=31±6yrs; weight=58,6±7,8kg; height=1,66±0,06m, body fat=14,5±6,5%; maximum oxygen consumption=48.1± 7.3mL kg -1 min -1) participants in triathlon competitions and with an endurance training of approximately 2h/day, at least 5 days a week during the previous 2 months, caffeine consumers of less than 50mg/day in the previous 3 months, with regular menstrual cycle and no menstrual disorder, not taking oral contraceptives or medication in the previous month.	Double-blind, placebo-controlled, crossover design	They conducted 2 trials in each of the phases of the menstrual cycle (early follicular, pre-ovulatory and mid-luteal), controlled 4 months earlier using a mobile application. At each phase of the menstrual cycle they ingested a capsule containing 3mg/kg body weight of caffeine or placebo (cellulose) (after ingestion of 150mL water). 10min later they performed an adapted version of the Wingate cycloergometer test (15 seconds).	A food frequency questionnaire was administered to determine the participants' daily caffeine intake. Fluid intake and dietary guidelines were given to ensure carbohydrate bioavailability and adequate hydration in all trials. A pre-competition fluid diet/routine that met pre-trial guidelines was chosen by each athlete and replicated for each athlete.	Compared to placebo, ingestion of the caffeine supplement increased peak power during the trial in the early follicular, pre-ovulatory and mid-luteal phase. The ergogenic effect of caffeine on peak power in Wingate was similar in each of the phases of the menstrual cycle, suggesting that caffeine enhances performance in this physical test in female athletes. Therefore, it could be used as an ergogenic aid in anaerobic exercise in eumenorrhoeic women during the menstrual cycle.

Table 2. Summary of the journals where the selected studies were published.

Journal	Selected studies	Quartile to which they belonged in 2019
Nutrients	4	Q1
Journal of the International Society of Sports Nutrition	3	Q1
Journal of strength and conditioning research	2	Q1
International Journal of Environmental Research and Public Health	2	Q2
Experimental Physiology	1	Q3
Medicine and science in sport and exercise	1	Q1
Scientific reports	1	Q1
Journal of sport medicine and physical fitness	1	Q4
Journal of Human Kinetics	1	Q3
British Journal of Clinical Pharmacology	1	Q2

a homogeneous intake pattern and/or lists of foods, supplements and/or medications to avoid or prohibited hours before the study (24, 48 or 72 hours) and during its execution. A pre-study food/supplement consumption frequency questionnaire and/or dietary evaluation was conducted to learn about participants' dietary patterns and/or intake diaries during the study in 5 of the selected studies.

Discussion

Regarding the study population, the mean number of study participants was 16.8 subjects. This corresponds to a sample size that is generally quite small, which makes it neither significant nor representative and therefore the conclusions can hardly be extrapolated (23–25). Regarding sex, most of the studies (n = 11) were conducted on men and only 2 of them on women, so drawing conclusions for women on supplementation and sports performance would not be statistically significant, taking into account that there are numerous differences in terms of physiological characteristics between men and women (26), such as in body composition, metabolism of various micronutrients (calcium and iron), size of organs and devices, age of maturation, menstruation, pregnancy, etc. These differences affect the response to exercise, psychological aspects and, therefore, sports performance (27). Only 9 of the 17 studies were based on athletes considered high performance, ensuring a demanding level of training and participation in competitions. The rest were based on people with experience in exercise and/or the test to be evaluated for said study, recreational sports practitioners or university students of Physical Activity and Sports Sciences who are not considered high-performance athletes. Taking into account that a high-performance athlete has different physiological, psychological, biochemical and anthropometric characteristics than athletes who are not, in addition to lifestyle, circadian rhythms (28) and particular diet, and considering that all these characteristics change depending on the period of the season in which the athlete is (preparatory, specific, pre-competitive, competitive or recovery), reaching conclusions about the improvement of sports performance based on athletes not considered high performance could simply be a mistake due to a methodological issue in the actual choice of the sample studied, different from the population under study (29).

Regarding training and supplementation, a wide variety of information has been found in this review. Each study evaluated different determinants of sports performance (anaerobic performance, fatigue levels, aerobic performance, neuromuscular efficiency, time to task failure, work performed above critical power, strength endurance, cognitive performance, mental fatigue, etc), so the training, supplementation and tests carried out depended on the objectives of the study in each case. Regarding the nutritional method used, the registration and monitoring of the participants' diet, some of them provided uncontrolled, neither measured nor quantified dietary guidelines, which the participants had to follow hours before the study (24-48 hours) to ensure that everyone consumed the same or similar macronutrient and energy content, as well as a list of foods to avoid (foods rich in caffeine, nitrates, alcohol, etc.) (6,17,30). In this unsuccessful attempt, methodologically speaking, to control food intake, no tolerance control was reported prior to supplementation in the study subjects of the substances to be supplemented and that was the reason for the list of foods to avoid. The nutritional habit of a subject generates different levels of tolerance that could influence the post-supplementation result as a confounding variable (31,32). Likewise, in no case were the actual intakes of each supplemented substance, added to the estimate of the possible amount of these substances ingested through food, compared with the levels of maximum tolerable intake (UL) for that age group and gender, of those substances and other nutrients affected by the dietary intervention, the results obtained by a supervening toxic situation or nutritional imbalances may be influenced. These could be affecting the correct metabolic functioning and therefore, the supposed performance measured to conclude the effectiveness of the supplement (33), without neglecting the health risk to which the subject under study is subjected, not reported by the authors in any of the reviewed papers, even though the authors have declared acceptance of an ethics committee. Other studies only provided the list of prohibited foods, without a statement of methodological guarantee or control that the subject would carry out this limitation or prohibition during the study (9,20).

A few, in addition to the list of foods to avoid, established restrictions and prohibitions on eating so that there were no changes in the routine during the period of the study (8,16,19,21). These restrictions and prohibitions were made in a theoretical way and based on the existing bibliography without taking into account the previous intake of the subject, not being the same the subject who must stop consuming food that he/she came eating normally, then that subject who the restriction or prohibition does not imply any change in your daily routine because you are not a habitual consumer of what is restricted or prohibited (33). In other studies, the participants were only asked not to change eating habits, lifestyle, sleep, exercise, medication or supplementation during the time that the study lasted (10-12), without knowing, or quantifying, prior to the intervention, in no case these habits that the authors asked not to change, generating a vague population description of those habits and variables that, due to the authors' interest in recommending it, seem to influence the results and therefore could determine a great limitation of the study and a quantitative lack of control of possible confounding variables.

In only one study did the subjects complete a 2-day registration before each session to confirm similar or identical dietary patterns between participants (amounts of energy and macronutrients), in addition to the list of foods to avoid (13), without including in the study the nutritional assessment of these records that would be decisive to know to correctly interpret the results of the supplementation. In another study, participants were given a validated caffeine consumption questionnaire supervised by a qualified nutritionist, in addition to a dietary evaluation 24 hours before the test on the first visit. A single 24-hour memory is methodologically speaking inconsistent, but even so, the nutritional assessment of that 24-hour memory (energy and macronutrient intake) was included in the study, which is crucial to know in order to correctly interpret the results of supplementation. Foods to avoid with caffeine were also instructed during the study and were monitored by phone, email, and in-person (14).

Only one of the studies indicates that it was a nutrition professional who established nutritional guidelines to ensure that all participants followed a similar diet in terms of energy and macronutrients. A record of their intake was made 48 hours before the tests by means of dietary intake diaries, in addition to providing a list of foods, medications, and supplements to avoid (15). Even so, the nutritional assessment that would be decisive to know in order to correctly interpret the supplementation results was not included in the study. In the detailed reading of the work, we interpret that the nutritional guidelines were established from a qualitative aspect, difficult to quantify, without being able to make a recommendation adapted to the subject even having common criteria in the diet and generating a large number of confounding variables that could increase the limitations of the study. In one of the studies conducted on women, a food consumption frequency questionnaire was carried out to know the participants' daily caffeine consumption, and the semi-quantification of a qualitative retrospective method should be validated with another quantitative method that would support the intake. quantified daily of this substance through food (34,35). In addition to giving them guidelines on fluid intake and diet to ensure the bioavailability of carbohydrates and adequate hydration in the trials. Diet and fluid intake were replicated in each of the trials. It does not report any data on nutrient intake (36–38). Only one of the studies did not contain any information regarding the nutritional method, registration and monitoring of the participants' diet (7).

It has been observed that it is difficult to find studies where an assessment of the nutritional status, dietary record or follow-up of the participants' diet is carried out before and during the intervention. This methodological absence of nutritional variables generates a great limitation in those studies that attempt to observe through a nutritional intervention with supplements the possible benefit obtained from them in sports performance (33).

It is necessary to know in advance what is the nutritional status and eating habits of those who are going to participate in these trials in order to reach more accurate conclusions. Therefore, it would be advisable to previously carry out dietary surveys (24-hour recall, food consumption frequency questionnaire, hydration questionnaire and dietary intake diary), anthropometric, biochemical and hematological determinations, in addition to designing feeding and dietary guidelines to follow with a methodological guarantee of its compliance, together with the supplementation guidelines chosen (36–39). It should be noted in most of the selected studies that the hydration status of the participants is not taken into account, nor the hydration patterns before, during and after the trials. Hydration, along with a balanced and balanced diet, are the basis for meeting the nutritional requirements of most athletes, these two factors being the ones that most contribute to the development of fatigue during exercise. Therefore, to optimize sports performance, before considering supplementation, it is necessary for the athlete to be well fed, properly nourished and continuously hydrated (1,40).

After the results found in the different studies discussed in this review, new questions arise: a) If the study population is small, how can extrapolatable conclusions be reached? b) If the nutritional status, eating habits, hydration and lifestyle of athletes are not known, how can one know if supplementation is necessary or not and what is the real effect of it? c) If most of the studies are based on the effects of supplementation in men, how can one know the effect of this in women, taking into account their biological differences with men?. The main strength of this review is that it provides current and complete information on the methodology used in studies on sports supplementation. However, it is not without limitations. Some studies may have a lack of information in this regard. Another limitation is the heterogeneity of the studies as they studied different types of supplements and effects on performance by measuring different indicators of sports performance, so the methodology was very different.

Following the results found in the studies discussed in this review and the new questions that have arisen from them, we propose to carry out a nutritional review of the methodology applied in sports supplementation studies. Supplements are marketed for athletes as well as for the general population. They are used without knowing with certainty the benefits they offer, since, as we have seen in this review, it is difficult to find studies where a nutritional assessment of the athlete, dietary record or monitoring of the diet of the study subjects before and during the intervention is carried out. This means that there is a methodological absence of nutritional variables, generating limitations in the studies that aim to observe the possible benefit in sports performance through nutritional intervention with supplements. It would be necessary to carry out nutritional studies in healthy athletes (men and women) affiliated to High Performance Centres (adequate sample size). Validated questionnaires should be conducted to determine the nutritional status and eating habits of the participants prior to the supplementation intervention. The questionnaires should be: 24-hour recall, food consumption frequency questionnaire, hydration questionnaire, dietary intake diary, socio-demographic characteristics questionnaire (e.g. age, sex, marital status, profession, educational level, sport modality, level and phase of season) and lifestyle habits questionnaire (e.g. drinking, smoking, supplement consumption). In addition, it is considered important to carry out anthropometric, biochemical and haematological determinations; and to design food and dietary guidelines (controlled, measured and quantified) to be followed, with a methodological guarantee of compliance with these guidelines by the participants in addition to the supplementation guidelines that are determined.

The tolerance of the substances to be supplemented in the study subjects should be controlled and the intake of these substances (through food or supplements) compared with the maximum tolerable intake (UL) levels for each age group and gender, in order to avoid that the results may be influenced by a toxic situation or nutritional imbalances that may affect the correct functioning of the metabolism and therefore the performance to be measured to reach accurate conclusions, taking into account the risk for the health to which the subject under study can be subjected. The questionnaires could be administered to the participants through a document to be completed under the supervision of a qualified dietitian-nutritionist and signing the informed consent to participate in the study, which must be carried out in accordance with the Helsinki declaration (October 2020) and approved by an ethics committee.

The data obtained from the food consumption frequency questionnaires should be processed using food composition tables and diet computer programs capable of calculating nutrient intake in g or mL from daily food intakes.

It would be of great importance to include in the study the results of the nutritional assessment carried out on the participants, in order to be able to correctly interpret the results of the supplementation.

Conclusions

The sample size of the available and selected studies was mostly small (mean 16.8 subjects), which makes it neither significant nor representative and therefore the conclusions of difficult studies can be extrapolated. The studies were conducted on men (only two of them on women), so drawing conclusions about supplementation and performance for women is complicated, taking into account the differences in terms of physiological characteristics between men and women. Several of the selected studies were not based on high-performance athletes, so they will reach conclusions about the improvement of sports performance based on athletes not considered high-performance could be an error, taking into account the different physiological, psychological, biochemical characteristics and anthropometric among them. It is difficult to find studies where an assessment of the nutritional status, dietary record, or monitoring of the diet of the study subjects is carried out before and during the intervention. This methodological absence of nutritional variables being a great limitation in the studies that have as objective observes through a nutritional intervention with supplements the benefit obtained from them in sports performance. To optimize sports performance, it is necessary for the athlete to be well-fed, properly nourished and continuously hydrated, so it would be necessary to previously know what the nutritional and hydration status is, as well as the eating, supplementation and lifestyle habits of the participants in these trials, in order to reach more accurate conclusions.

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