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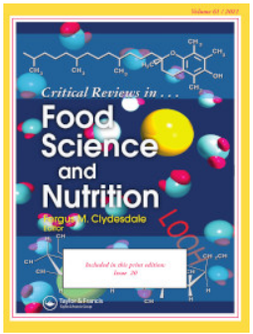

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REVIEW

Creatine supplementation and VO_2max: a systematic review and meta-analysis

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ABSTRACT

Although creatine supplementation is well-known to increase exercise performance in acute high-intensity exercises, its role in aerobic performance based on VO_2max is more controversial. Thus, we performed a systematic review and meta-analysis on the effects of creatine supplementation on VO_2max. PubMed, Cochrane, Embase, and ScienceDirect were searched for randomized controlled trials (RCTs) reporting VO_2max in creatine supplementation and placebo groups before and after supplementation. We computed a random-effects meta-analysis on VO_2max at baseline, within groups following supplementation, on changes on VO_2max between groups, and after supplementation between groups. Sensitivity analyses and meta-regression were conducted. We included 19 RCTs for a total of 424 individuals (mean age 30 years old, 82% men). VO_2max did not differ at baseline between groups (creatine and placebo). Participants in both groups were engaged in exercise interventions in most studies (80%). Using changes in VO_2max, VO_2max increased in both groups but increased less after creatine supplementation than placebo (effect size [ES] = −0.32, 95%CI = −0.51 to −0.12, p = 0.002). Comparisons after creatine supplementation confirmed a lower VO_2max in the creatine group compared to the placebo group (ES= −0.20, 95%CI = −0.39 to −0.001, p = 0.049). Meta-analysis after exclusion from meta-funnel resulted in similar outcomes in a subgroup of young and healthy participants. Meta-regressions on characteristics of supplementation, physical training, or sociodemographic were not statistically significant. Creatine supplementation has a negative effect on VO_2max, regardless of the characteristics of training, supplementation, or population characteristics.

KEYWORDS

Dietary supplement; endurance; cardiorespiratory fitness; VO_2peak; ventilatory threshold

Introduction

Creatine (Cr) is known as one of the most popular sport dietary supplements on the market, which is widely used among recreational and professional athletes, with more than $400 million in annual sales (Momaya, Fawal, and Estes 2015). The popularity of Cr supplementation (CS) can be explained by intensive marketing about its ergogenic effects, particularly in sports performance. It's primarily admitted that CS improves performance in acute and high-intensity exercises such as sprints (Dawson et al. 1995) and muscle strength (Lanhers et al. 2015; Lanhers et al. 2017) and has health and potential therapeutic benefits (Kreider and Stout 2021). Despite this, the current scientific literature diverges on its potential effects on endurance performance (Engelhardt et al. 1998; Preen et al. 2001). The gold standard to assess endurance capacities is VO_2max ("VO2 Max – an Overview | ScienceDirect Topics" 2021). VO_2max is defined as the oxygen uptake attained during maximal exercise intensity that could not be increased despite further increases in exercise workload (Hill and Lupton 1923). VO_2 is the oxygen flow taken by the lungs, transported through the blood circulation coupled with hemoglobin, and used to oxidation of energy substrates (glucose and fatty acids) in the respiratory chain of mitochondria resulting in Adenosine triphosphate (ATP) resynthesis (Bassett 2000). It was demonstrated that VO_2max is a valid index measuring the

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limits of the cardiorespiratory system (Hawkins et al. 2007) and also is one of the main outcomes that can predict performance in endurance sport. The increase in VO_2max following training results primarily from an increase in maximal cardiac output (Lanferdini et al. 2020; Kipp, Kram, and Hoogkamer 2019).

Moreover, the assessment of VO_2max through an incremental exercise test seems to be one of the most reproducible measurements, irrespective of the protocols used (Chidnok et al. 2013). Some studies have investigated the effect of CS on VO_2max (Balsom et al. 1993; Nelson et al. 2000). The ATP energy supply by the energy-rich phosphate bond of phosphocreatine (CrP) occurs during the first seconds of a high-intensity exercise in anaerobic conditions due to the limited CrP stores requiring a recovery period of several minutes before its regeneration (Gastin 2001). In prolonged efforts, Cr could interact with aerobic metabolism through several lesser-known mechanisms with a Cr phosphate shuttle facilitating the ATP transport from the mitochondria to the cytosol within the cell needing ATP for muscle contraction (Wallimann, Tokarska-Schlattner, and Schlattner 2011). It also has a role in increasing glycogen storage with an increase in GLUT4 transporters (Ju et al. 2005; Eijnde et al. 2001) and changes in cell volume with the osmotic effect of Cr (Loon et al. 2004). In addition to its role in the regulation and homeostasis of energy metabolism, CS could improve muscle activation (Da Silva Azevedo et al. 2019) and VO_2 kinetics at the same workloads (Murphy et al. 2005; Hickner et al. 2010), which may be beneficial for endurance performance. Besides its common use in sports, CS has also been evaluated in the elderly and certain pathological conditions (Canete et al. 2006; Carvalho et al. 2012). Despite these considerations, the effects of CS on aerobic performance remain uncertain. To date, no meta-analysis study has specifically focused on its effects on changes in VO_2max.

Thus, we aimed to conduct a systematic review and meta-analysis of all randomized controlled trials (RCTs) comparing the effects of CS versus placebo on VO_2max. Secondary aims were to determine the influence of population characteristics, supplementation, and physical training associated with VO_2max and assess the effects of CS on aerobic measures associated with VO_2max during incremental exercise tests (Poole and Jones 2017).

Materials and methods

Literature search

We searched for all RCTs with CS versus placebo, with a measure of VO_2max before and after supplementation. PubMed, The Cochrane Central Register of Controlled Trials (CENTRAL), Excerpta Medica Database (EMBASE), and ScienceDirect were searched until March 16th 2021, using the keywords "VO_2max" AND "creatine supplementation" (see detailed search strategy in Appendix S1). The search was not limited to specific years. No language restrictions were applied. Reference lists of all publications meeting the inclusion criteria were manually searched to identify any further studies not found through electronic searching. Two authors (DG and CL) conducted all literature searches, separately reviewed and collated the abstracts, and decided the suitability of the articles for inclusion based on the selection criteria. A third author (FD) was asked to review the articles when consensus on suitability was not met. Then, all authors reviewed the eligible articles (Figure 1). We followed the PRISMA guidelines (Appendix S7).

Data collection

The data collection included the first author's name, publication year, study design, periods of studies, aims and outcomes of included articles, sample size, characteristics of individuals (age, gender, physical activity, and health status), supplementation protocol (type of Cr, total time of supplementation, and total dose), physical training during supplementation period, VO_2max (values in each unit, and measurement method), and performance associated with the incremental tests (ventilatory threshold, time to exhaustion, and/or maximal power output).

Outcomes

The main outcome for our meta-analysis was VO_2max. Our secondary outcomes were ventilatory threshold, time to exhaustion, and maximal power output from incremental exercise tests if measures were available.

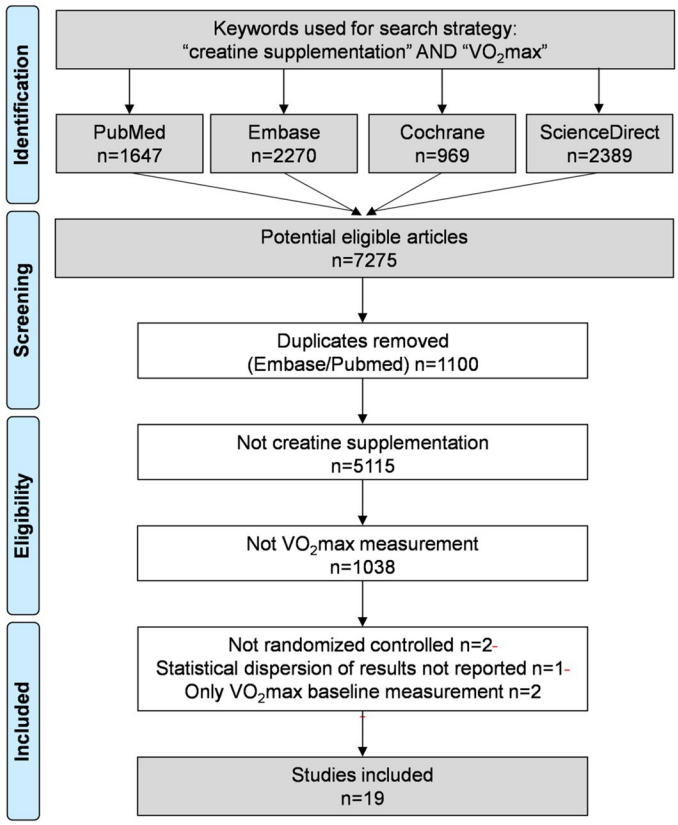

Figure 1. Search strategy.

Quality of assessment

The Scottish Intercollegiate Guidelines Network (SIGN) checklist for RCTs was used to assess the quality of reporting (Figure 2) ("Scottish Intercollegiate Guidelines Network Sign 50: A Guideline Developer's Handbook. [(Accessed on 1 August 2019)]; Available Online: http://www.Sign.Ac.Uk"). Through 11 items, the SIGN checklist evaluates internal validity and study quality. Additionally, the PEDro scale was assessed for each study (Appendix S2) ("PEDro Scale for Randomized Controlled Trials; Available Online: https://Pedro.Org.Au/"). The methodological quality of the articles was evaluated by two independent reviewers.

Statistical considerations

Data were analyzed using Stata (version 16, TX, USA). When not available, standard deviation (SD) was calculated by multiplying the standard error of the mean (SEM) by the square root of the sample size (Lee, In, and Lee 2015) or estimated from interval quartiles or minimum and maximum intervals (Wan et al. 2014). The main meta-analysis compared changes in VO_2max between groups (CS versus placebo). Changes were calculated using the method from Borenstein et al. (2009). Results were expressed as effect size (ES) (DerSimonian and Laird 1986). A decreased ES denoted a lower improvement in VO_2max in the CS compared to the placebo group. A scale for ES has been suggested with −0.2 reflecting a small effect, −0.5 a moderate effect, and −0.8 a large effect (Cohen 1988). In order to verify the strength of our results, we also computed random-effects meta-analysis on VO_2max (1) at baseline between groups (CS versus placebo) to verify that groups were comparable, (2) within groups after supplementation, (3) as well as after supplementation between groups. Each meta-analysis was stratified by type of exercise (running,

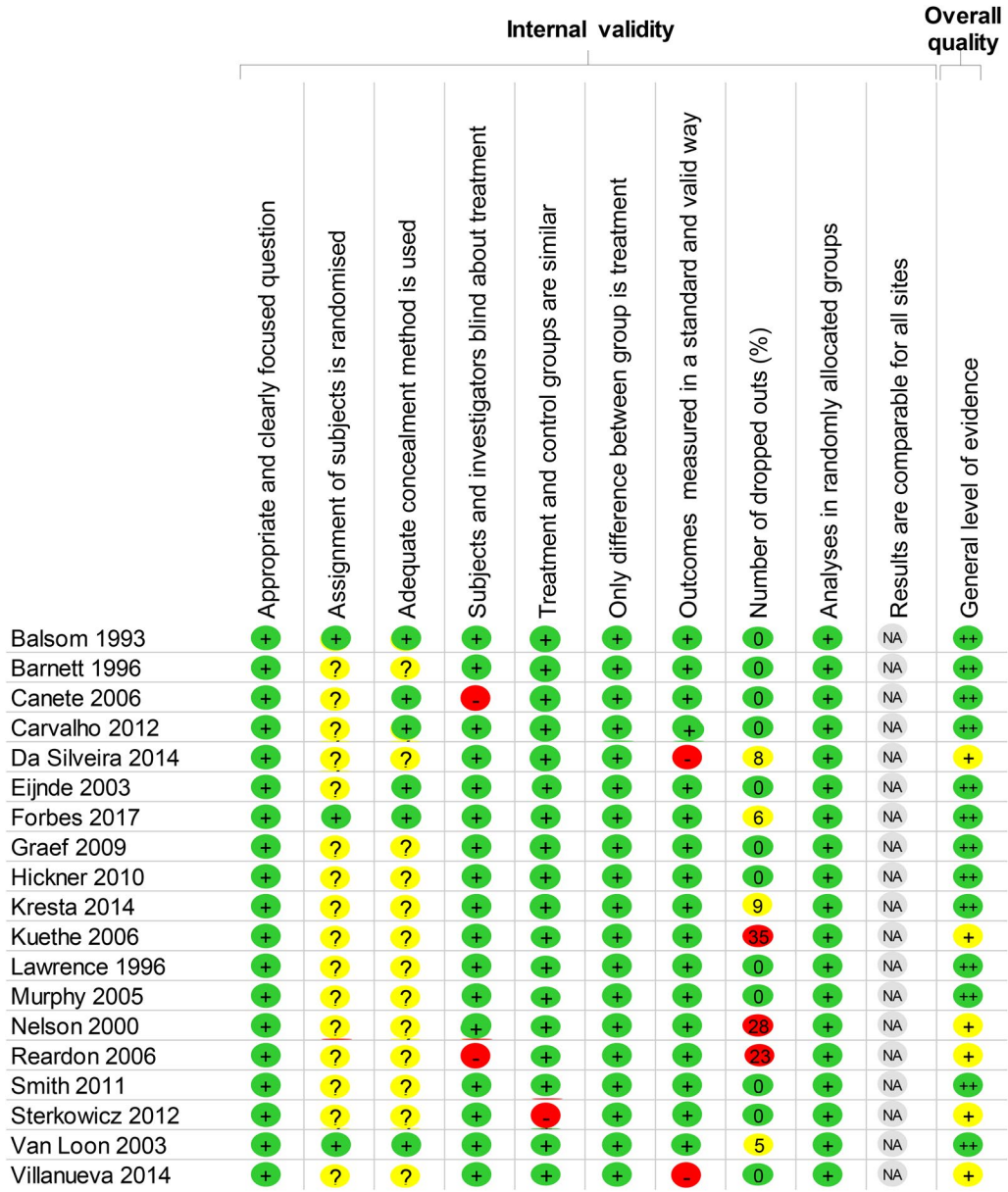

Figure 2. Quality of articles – SIGN checklist.

cycling, or rowing) and units of measurement (L/min or mL/kg/min). For studies that reported VO_2max in both units (L/min and mL/kg/min) simultaneously, we computed meta-analyses including both units (where the weight of studies would need ponderation), and sensitivity analyses, i.e., meta-analyses using only data in L/min or mL/kg/min when both units were available within the same study. Finally, we also conducted meta-analyses on the ventilatory threshold, maximal power output, and time to exhaustion on the incremental exercise tests. For all aforementioned meta-analyses, sensitivity analyses were conducted after excluding studies outside funnel plots and on young and healthy individuals. Heterogeneity between studies was also evaluated using I^2 statistic, ranging from 0 (no heterogeneity) to 100% (high heterogeneity). Meta-regressions were computed to search for putative influential factors of VO_2max, such as characteristics of the population (age, gender, training, and health), characteristics of supplementation (loading dose, total duration of supplementation, and total dose), and characteristics of training during supplementation (none, aerobic, or anaerobic). Results were expressed as regression coefficients and 95%CI. *P*-values less than 0.05 were considered statistically significant.

Results

An initial search produced a possible 4985 articles. Removal of duplicates and use of the selection criteria reduced the number of articles reporting VO_2max assessment at baseline and following CS or placebo in nineteen articles. Among them, one study reported two independent gender groups (Smith et al. 2011). All articles were written in English (Figure 1).

Quality of articles

The risk of bias in included studies was mostly low (Figure 2 and Appendix S2). However, most studies did not describe the randomization method. Some studies matched participants based on the baseline measurements. Two studies used a single-blind design (Reardon et al. 2006; Canete et al. 2006), but all participants were blinded to supplementation. Three studies declared a dropout of more than twenty percent (Nelson et al. 2000; Reardon et al. 2006; Kuethe et al. 2006). All studies mentioned ethical approval.

Study designs and aims of included articles

All studies were double-blind, randomized placebo-controlled trials. Only one study had a cross-over design (Kuethe et al. 2006). All the studies were monocentric. Five studies were conducted in Europe (Germany (Kuethe et al. 2006), Netherlands (Van Loon et al. 2003; Bert O. Eijnde et al. 2003), Poland (Sterkowicz et al. 2012) and Sweden (Balsom et al. 1993)), one in Brazil (Carvalho et al. 2012; Lázaro da Silveira et al. 2014), eight in North America (Canada (Forbes et al. 2017) and USA (Graef et al. 2009; Hickner et al. 2010; Kresta et al. 2014; Nelson et al. 2000; Smith et al. 2011; Canete et al. 2006; Villanueva, He, and Schroeder 2014)), and four in Australia (Barnett, Hinds, and Jenkins 1996; Lawrence et al. 1997; Murphy et al. 2005; Reardon et al. 2006). The duration between baseline and after supplementation ranged from 4 (Barnett, Hinds, and Jenkins 1996) to 180 days (Carvalho et al. 2012). The nineteen included studies aimed to evaluate the effect of CS on performance or quality of life in elderly and patients with heart failure (Canete et al. 2006; Carvalho et al. 2012; Kuethe et al. 2006; Villanueva, He, and Schroeder 2014), among which nine studies aimed specifically at assessing aerobic performance (Balsom et al. 1993; Nelson et al. 2000; Murphy et al. 2005; Hickner et al. 2010; Smith et al. 2011; Reardon et al. 2006; Forbes et al. 2017; Graef et al. 2009; Lawrence et al. 1997). Seventeen studies recruited healthy individuals, while two studies recruited patients with heart failure (Carvalho et al. 2012; Kuethe et al. 2006) (Table 1).

Population

The sample size ranged from 10 (Sterkowicz et al. 2012) to 46 (Eijnde et al. 2003), for a total of 424 participants (216 in the CS and 208 in the placebo group).

Gender was reported in all studies. The mean proportion of males was 81.7%, with 160 males and 56 females in the CS group versus 155 males and 53 females in the placebo group. Eleven studies included only males (Balsom et al. 1993; Barnett, Hinds, and Jenkins 1996; Lázaro da Silveira et al. 2014; Graef et al. 2009; Hickner et al. 2010; Murphy et al. 2005; Van Loon et al. 2003; Eijnde et al. 2003; Carvalho et al. 2012; Villanueva, He, and Schroeder 2014) and three only females (Canete et al. 2006; Forbes et al. 2017; Kresta et al. 2014).

Age was reported as the mean value in all studies except for three studies that reported age in the min-max interval (Balsom et al. 1993; Lázaro da Silveira et al. 2014; Reardon et al. 2006). The mean age was 29.7 years old, ranging from 20.5 (Barnett, Hinds, and Jenkins 1996) to 68 (Canete et al. 2006). Most studies (79%) had participants aged between 18 and 40 years old.

Physical activity at baseline was reported in all studies. We classified individuals as competitive or highly trained athletes (15.6%) (Sterkowicz et al. 2012; Balsom et al. 1993; Lázaro da Silveira et al. 2014; Lawrence et al. 1997), recreationally trained (54.2%) (Barnett, Hinds, and Jenkins 1996; Forbes et al. 2017; Graef et al. 2009; Hickner et al. 2010; Kresta et al. 2014; Murphy et al. 2005; Nelson et al. 2000; Reardon et al. 2006; Smith et al. 2011; Villanueva, He, and Schroeder 2014), and sedentary or have low daily physical activity (30.2%) (Van Loon et al. 2003; Bert O. Eijnde et al. 2003; Canete et al. 2006; Kuethe et al. 2006; Carvalho et al. 2012).

Additional outcomes were seldomly reported, such as echocardiographic measures (Murphy et al. 2005; Kuethe et al. 2006) or cellular changes using muscular biopsy (Hickner et al. 2010; Reardon et al. 2006; Van Loon et al. 2003; Eijnde et al. 2003; Kresta et al. 2014) (Table 1). Twelve

Table 1. Characteristics of included studies.

	Population					Intervention					VO_2 assessment	
								g/day – n/day – n days		Total		VO_2max
Study	Country	*n*	%Men	Age	Physical activity level	Training	Type Cr	Loading dose	Maintenance dose	dose	Exercise test	or VO_2peak
Balsom et al. (1993)	Sweden	14	100	19–37	Competition (runners)	Aerobic	Cr monohydrate	0	5 – 1 – 6	30	Treadmill	VO_2peak
Barnett, Hinds, and Jenkins (1996)	Australia	17	100	20.5	Recreation	Aerobic	Cr monohydrate	0	5 – 4 – 4	20	Bicycle ergometer	VO_2peak
Canete et al. (2006)	USA	16	0	68	Sedentary	None	Cr monohydrate	19 – 3 – 7	0	130	Bicycle ergometer	VO_2peak
Carvalho et al. (2012)	Brazil	33	100	55.7	Sedentary (heart failure)	None	NA	0	5 – 1 – 180	900	Bicycle ergometer	VO_2peak
Lázaro da Silveira et al. (2014)	Brazil	22	100	18-30	Competition (military)	Aerobic (MTP)	Cr monohydrate	25 – 3 – 7	2,5 – 1 – 86	385	Cooper test	estimated
Eijnde et al. (2003)	Netherlands	46	100	55–75	Sedentary	Aerobic (FTP)	Cr monohydrate	0	5 – 5 – 182	910	Bicycle ergometer	VO_2peak
Forbes et al. (2017)	Canada	17	0	23	Recreation	Anaerobic (HIIT)	Cr monohydrate	19 – 2 – 5	6,5 – 1 – 23	245	Bicycle ergometer	VO_2peak
Graef et al. (2009)	USA	33	100	22.6	Recreation	Anaerobic (HIIT)	Cr citrate	0	10 – 2 – 30	300	Bicycle ergometer	VO_2peak
Hickner et al. (2010)	USA	12	100	27.2	Recreation (cyclists)	Aerobic	Cr monohydrate	0	3 – 1 – 28	84	Bicycle ergometer	VO_2max
Kresta et al. (2014)	USA	15	0	21.5	Recreation	Aerobic	Cr monohydrate	18 – 4 – 7	6 – 1 – 21	252	Bicycle ergometer	VO_2peak
Kuethe et al. (2006)	Germany	13	75	58.2	Sedentary (heart failure)	None	NA	20 – 4 – 42	0	840	Treadmill	estimated
Lawrence et al. (1997)	Australia	20	50	21.2	Competition (rowers)	Aerobic	Cr monohydrate	18 – 4 – 5	0	92	Rowing ergometer	VO_2peak
Murphy et al. (2005)	Australia	18	100	24	Recreation (team sports)	Aerobic	Cr monohydrate	20 – 4 – 7	10 – 2 – 21	350	Bicycle ergometer	VO_2max
Nelson et al. (2000)	USA	36	55	23.5	Recreation	Aerobic	Cr monohydrate	20 – 4 – 7	0	140	Bicycle ergometer	VO_2peak
Reardon et al. (2006)	Australia	13	70	19–22	Recreation	Aerobic	Cr monohydrate	20 – 4 – 7	5 – 1 – 21	168	Bicycle ergometer	VO_2peak
Smith et al. (2011)	USA	55	49	21.7	Recreation	Aerobic	di-Cr citrate	20 – 4 – 5	0	100	Treadmill	VO_2max
Sterkowicz et al. (2012)	Poland	10	100	21.2	Competition (judoists)	Aerobic	Cr malate	0	5 – 1 – 42	210	Treadmill	VO_2peak
Van Loon et al. (2003)	Netherlands	20	100	20.6	Sedentary	None	Cr monohydrate	20 – 4 – 5	2	174	Bicycle ergometer	VO_2peak
Villanueva, He, and Schroeder (2014)	USA	14	100	68.1	Recreation	Anaerobic (strength)	Cr monohydrate	24 – 3 – 5	5,5 – 1 – 79	552	Bicycle ergometer	VO_2peak

Note: Age: mean or interval; FTP, fitness training program; HIIT, high intensity interval training; NA, not available; MTP, military training program.

studies assessed body mass changes (Balsom et al. 1993; Canete et al. 2006; Eijnde et al. 2003; Forbes et al. 2017; Graef et al. 2009; Hickner et al. 2010; Kresta et al. 2014; Lawrence et al. 1997; Murphy et al. 2005; Reardon et al. 2006; Smith et al. 2011; Van Loon et al. 2003).

Aerobic outcomes: VO_2max and other parameters

Measurements of maximal oxygen consumption were reported in VO_2max (Balsom et al. 1993; Murphy et al. 2005; Hickner et al. 2010; Smith et al. 2011; Kuethe et al. 2006; Van Loon et al. 2003; Lázaro da Silveira et al. 2014; Villanueva, He, and Schroeder 2014) or VO_2peak (Nelson et al. 2000; Canete et al. 2006; Carvalho et al. 2012; Reardon et al. 2006; Eijnde et al. 2003; Sterkowicz et al. 2012; Forbes et al. 2017; Graef et al. 2009; Kresta et al. 2014; Barnett, Hinds, and Jenkins 1996; Lawrence et al. 1997). We used VO_2max as a generalization in this study. Nine studies measured VO_2max in L/min (Nelson et al. 2000; Murphy et al. 2005; Hickner et al. 2010; Van Loon et al. 2003; Eijnde et al. 2003; Forbes et al. 2017; Graef et al. 2009; Barnett, Hinds, and Jenkins 1996; Lawrence et al. 1997) and eleven in mL/kg/min (Balsom et al. 1993; Canete et al. 2006; Carvalho et al. 2012; Smith et al. 2011; Reardon et al. 2006; Kuethe et al. 2006; Sterkowicz et al. 2012; Lázaro da Silveira et al. 2014; Forbes et al. 2017; Kresta et al. 2014; Villanueva, He, and Schroeder 2014). Among them, one study reported both L/min and mL/kg/min (Forbes et al. 2017). Most studies measured VO_2max with gas exchange analysis during incremental exercise tests such as the bicycle ergometer test (Barnett, Hinds, and Jenkins 1996; Forbes et al. 2017; Graef et al. 2009; Hickner et al. 2010; Kresta et al. 2014; Murphy et al. 2005; Nelson et al. 2000; Reardon et al. 2006; Van Loon et al. 2003; Eijnde et al. 2003; Canete et al. 2006; Carvalho et al. 2012), treadmill test (Balsom et al. 1993; Smith et al. 2011; Sterkowicz et al. 2012; Kuethe et al. 2006; Villanueva, He, and Schroeder 2014), or rowing ergometer test (Lawrence et al. 1997). The incremental tests were as close as possible to the training habitus of participants. VO_2max was also extrapolated from sub-maximal measures of VO_2max in two studies (Ebbeling et al. 1991; Villanueva, He, and Schroeder 2014) and estimated through a 12-minute Cooper test in one study (Lázaro da Silveira et al. 2014). Ten studies also assessed ventilatory threshold, using the V-slope method (Beaver, Wasserman, and Whipp 1986) in six studies (Forbes et al. 2017; Graef et al. 2009; Nelson et al. 2000; Kuethe et al. 2006; Bert O. Eijnde et al. 2003; Carvalho et al. 2012) – that is, the point where VCO_2 production increases more than VO_2 consumption –, the "Wasserman" method (Wasserman et al. 1973) in three studies (Canete et al. 2006; Kresta et al. 2014; Smith et al. 2011) – that is, the point where VE/VO_2 increases without a concomitant increase in VE/VCO_2, and without further information in one study (Sterkowicz et al. 2012). Six studies reported time to exhaustion (Smith et al. 2011; Graef et al. 2009; Kresta et al. 2014; Murphy et al. 2005) or maximal power output (Van Loon et al. 2003; Eijnde et al. 2003; Canete et al. 2006) at the incremental tests (Table 1 and Appendix S3).

Creatine supplementation

Cr monohydrate was used in 74% of studies, Cr citrate in two studies (Graef et al. 2009; Smith et al. 2011), Cr malate in one study (Sterkowicz et al. 2012), and not reported in two studies (Carvalho et al. 2012; Kuethe et al. 2006). A loading dose was used in twelve studies, that is, a dose ≥ 20 g/day or 0.3 g/kg, which had shown the greatest effectiveness for increasing muscle total Cr content with minimal Cr losses through renal excretion (Harris, Söderlund, and Hultman 1992), split in two (Forbes et al. 2017), three (Canete et al. 2006; Lázaro da Silveira et al. 2014; Villanueva, He, and Schroeder 2014) or four (Nelson et al. 2000; Murphy et al. 2005; Smith et al. 2011; Reardon et al. 2006; Kuethe et al. 2006; Van Loon et al. 2003; Kresta et al. 2014; Lawrence et al. 1997) intakes per day, during 4–7 days for all studies except one that used a loading dose for a prolonged period (Kuethe et al. 2006). A maintenance dose ranging from 2 (Murphy et al. 2005) to 10 g/day (Murphy et al. 2005) was used in six studies, once-daily except for one study (Murphy et al. 2005). Seven studies did not use a loading dose and supplemented only with a low dose of Cr (Balsom et al. 1993; Hickner et al. 2010; Carvalho et al. 2012; Eijnde et al. 2003; Sterkowicz et al. 2012; Graef et al. 2009; Barnett, Hinds, and Jenkins 1996). In total, the CS was 285 g on average, ranging from 42 g (Barnett, Hinds, and Jenkins 1996) to 912 g (Eijnde et al. 2003), over 42 days, ranging from four days (Barnett, Hinds, and Jenkins 1996) to six months (Eijnde et al. 2003) (Table 1).

Training during supplementation

All participants performed the same physical training in all studies and differed only by supplementation (Cr or placebo). A standardized training program was proposed in six studies: high-intensity interval training (HIIT) (Graef et al. 2009; Forbes et al. 2017), resistance (Villanueva, He, and Schroeder 2014), aerobic (Reardon et al. 2006), or mixed training (Eijnde et al. 2003; Lázaro da Silveira et al. 2014). Nine studies maintained the usual physical activity of participants, i.e., running (Balsom et al. 1993), cycling (Hickner et al. 2010), rowing (Lawrence et al. 1997), team sports (Murphy et al. 2005), judo (Sterkowicz et al. 2012) or other recreational activities (Nelson et al. 2000; Smith et al. 2011; Kresta et al. 2014; Barnett, Hinds, and Jenkins 1996). Four studies included sedentary participants without exercise intervention (Van Loon et al. 2003; Canete et al. 2006; Kuethe et al. 2006; Carvalho et al. 2012). We classified exercise training as "aerobic" (68.9%), "anaerobic" (11.8%) or "none" (19.3%) (Table 1).

Meta-analysis

Considering all measures of VO_2max, there were no differences in any outcomes at baseline between-group CS and placebo groups (Appendix S4). Using changes from baseline to after the supplementation, VO_2max increased less in the CS group

than the control group (ES = −0.30, 95%CI = −0.50 to −0.11, $p = 0.003$). Stratification showed a lower increase in the CS group for VO_2max in L/min (ES= −0.39, 95%CI = −0.67 to −0.12, $p = 0.005$), and for cycling (ES= −0.33, 95%CI = −0.58 to −0.09, $p = 0.007$). Other aerobic outcomes (ventilatory threshold, time to exhaustion, maximal power output) did not differ between groups (Figure 3). Considering all measures of VO_2max and only VO_2max expressed in L/min for the single study that reported both units (Forbes et al. 2017), sensitivity analyses showed similar findings, that is, a lower increase in VO_2max in the CS compared with the control group (ES = −0.32, 95%CI = −0.52 to −0.11, $p = 0.002$) (Appendix S3). Results were nearly identical with VO_2max expressed in mL/kg/min.

Other sensitivity analyses

Meta-analyses comparing VO_2max after CS between groups demonstrated similar results than meta-analyses on changes, that is, a significant lower VO_2max in the CS compared with the placebo group (ES= −0.20, 95%CI = −0.39 to −0.001, $p = 0.049$), as well as for VO_2max expressed in L/min (ES= −0.32, 95%CI = −0.59 to −0.04, $p = 0.022$), and a tendency for cycling (ES= −0.22, 95%CI = −0.46 to 0.02, $p = 0.074$) (Appendix S4). We also computed meta-analyses on VO_2max changes within groups showing that VO_2max increased in the placebo group (ES= 0.35, 95%CI = 0.15–0.54, $p = 0.001$), with only a tendency within the CS group (ES= 0.18, 95%CI = −0.01 to 0.37, $p = 0.060$). Despite a very limited number of studies ($n = 3$) for this parameter, maximal power output increased in the CS group (ES= 0.45, 95%CI = 0.03–0.88, $p = 0.038$). All aforementioned meta-analyses were repeated after excluding studies not evenly distributed around the base of the meta-funnel (Figure 4), and demonstrated similar findings (Appendix S5). Lastly, we also repeated all meta-analyses in the subgroup of young and healthy participants, and results were similar, that is, a lower VO_2max increase in the CS compared to the placebo group using

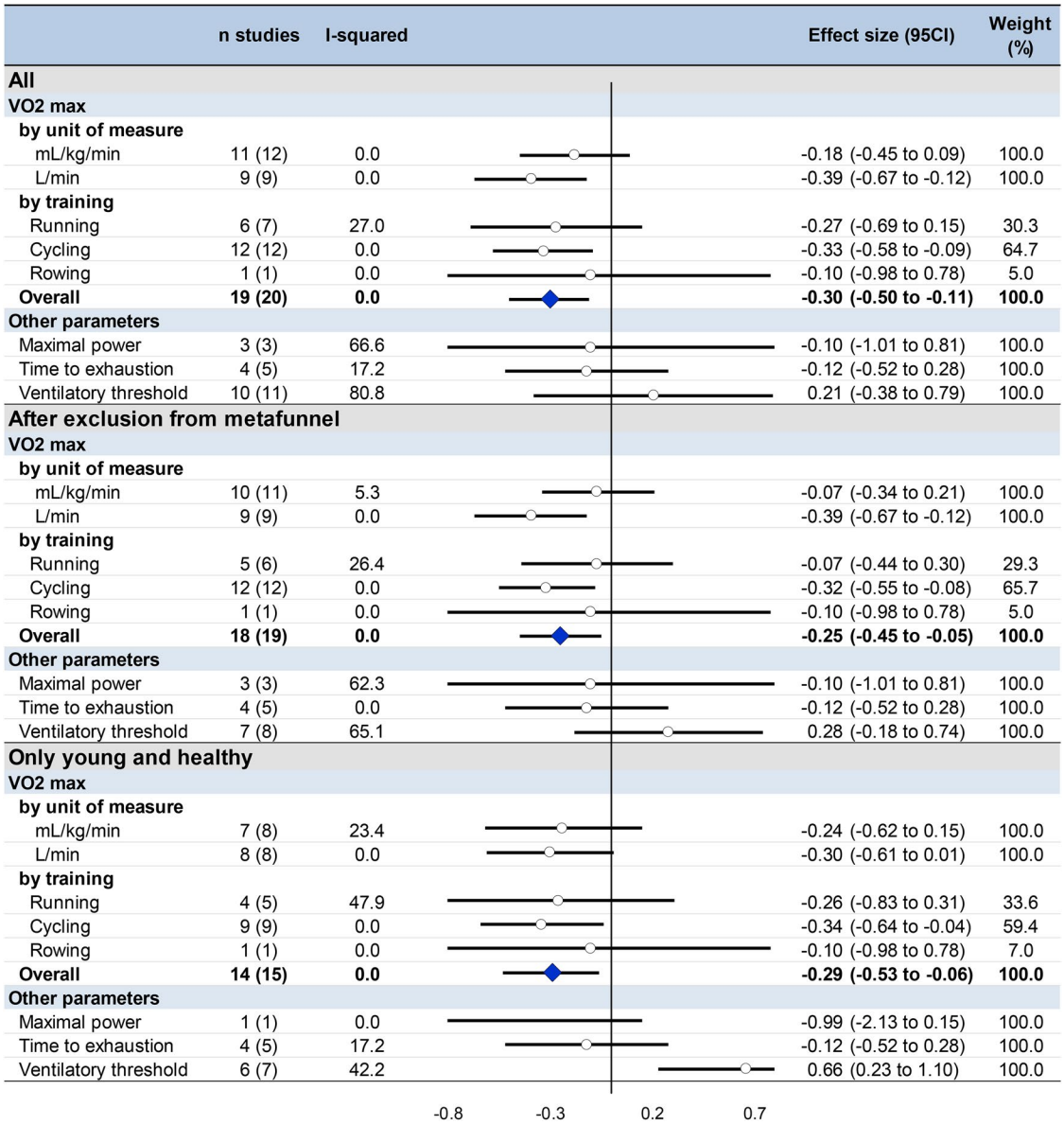

Figure 3. Meta-analysis of aerobic changes after supplementation period between groups.

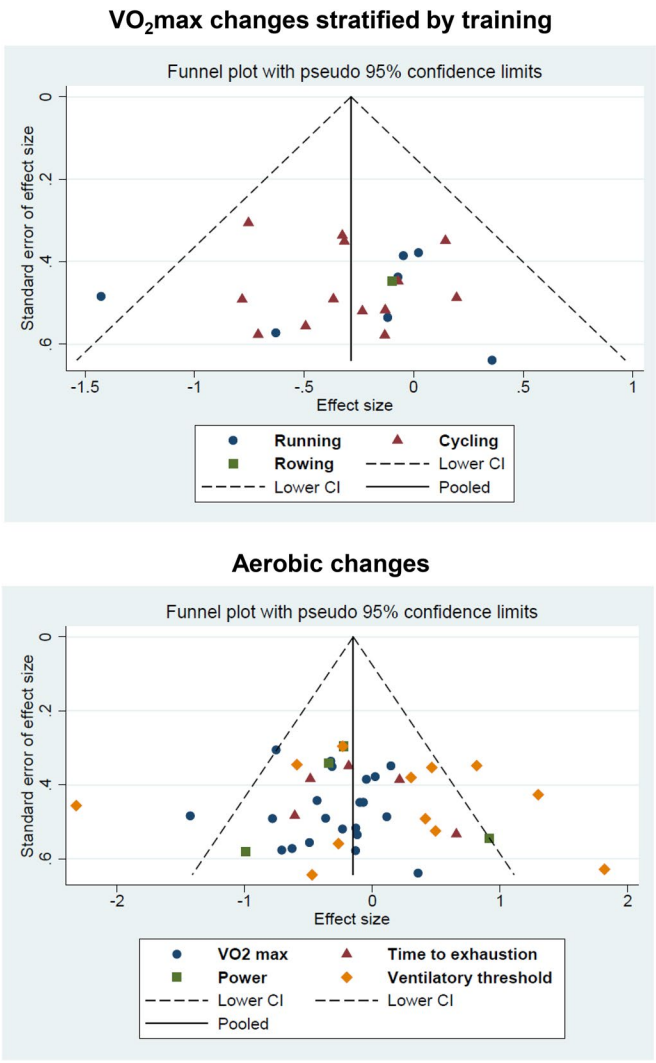

Figure 4. Funnel plots of aerobic changes after supplementation period between groups.

changes between groups (ES= −0.26, 95%CI = −0.49 to −0.02, $p = 0.031$), a lower VO_2max after CS in the CS compared to the placebo group (ES = −0.26, −95%CI = 0.49 to −0.02, $p = 0.031$), an increase in VO_2max within the placebo group (ES = 0.25, 95% CI = 0.02–0.48, $p = 0.035$) and not within the CS group (ES = 0.10, 95% CI = −0.13 to 0.32, $p = 0.41$). Using changes between groups, the ventilatory threshold increased in the CS compared to the control group (ES = 0.66, 95%CI = 0.23–1.1, $p = 0.003$) (Figure 3 and Appendix S6).

Meta-regressions

The meta-regressions failed to show any influencing variables on the VO_2max, that is, the putative influence of individuals, supplementation, or training characteristics. The adverse effect of CS on VO_2max was not linked to body mass or changes in body mass, which did not significantly vary in both groups. The limited number of patients with heart failure precluded analyses on the influence of health status on VO_2max (Figure 5).

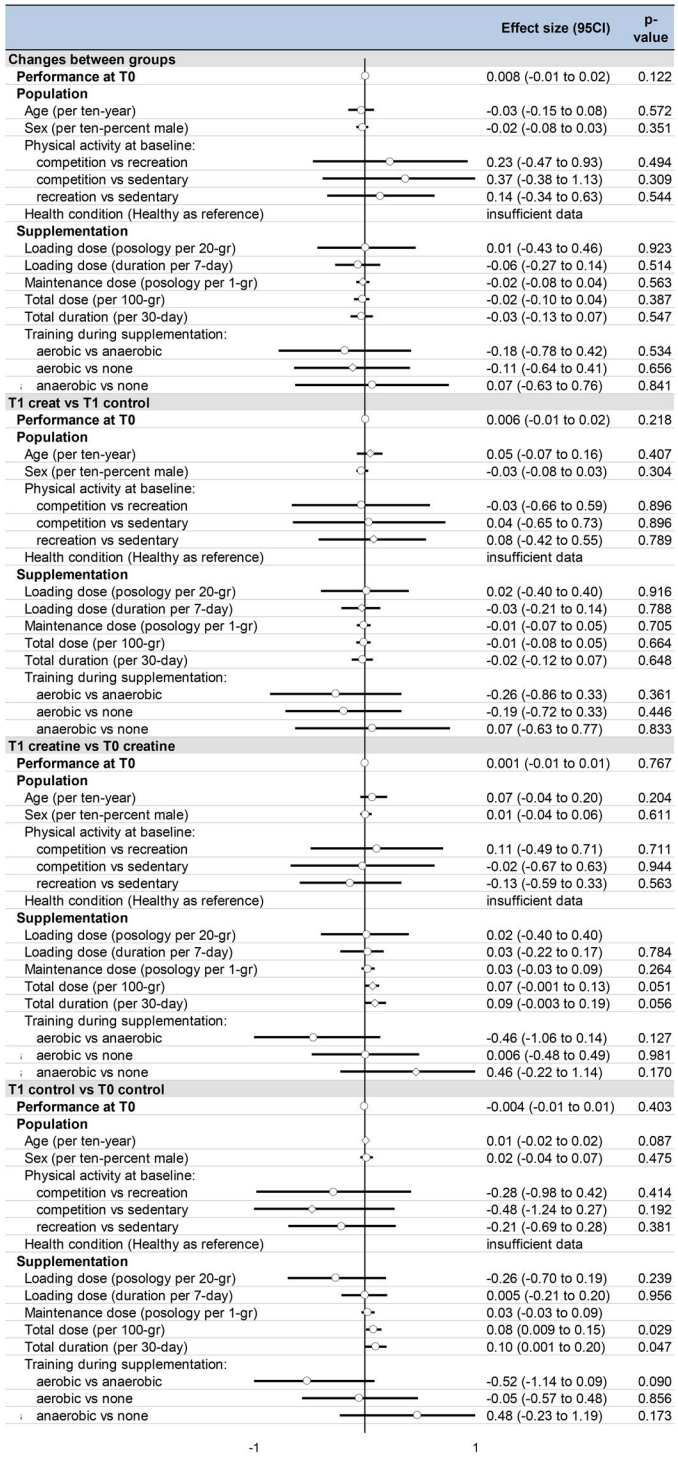

Figure 5. Meta-regressions for the main outcome (VO_2max).

Discussion

The main findings were that CS is detrimental to VO_2max, especially for cycling. If CS was also not beneficial on the maximal aerobic parameters associated with VO_2max, the ventilatory threshold seemed to be improved. Results were independent of sociodemographic or supplementation characteristics.

Cr is well-known for its ergogenic effects in acute high-intensity exercises. However, current results of our

meta-analysis led to adverse effects of CS on endurance capacity assessed with VO_2max, which increased in the placebo but not in the CS group. VO_2max limiting factors are shared between central factors for ~70% and peripheral factors for ~30% (di Prampero and Ferretti 1990). Thus, O_2 delivery and particularly maximal cardiac output are the primary limiting factors of VO_2max during exercise (Bassett 2000). Cr is known to induce water retention in tissues such as skeletal muscle (Casey et al. 1996) and, potentially, cardiomyocytes (Ipsiroglu et al. 2001). However, its cardiomyocytes' effects on contractile function and blood flow during exercise are lacking and still questionable. In addition to the cardiac function, O_2 delivery to working muscles also depends on arterial oxygen content. Even if hemoglobin levels are not modified following CS (Preen et al. 2001; Hickner et al. 2010; Robinson et al. 2000), CS can alter plasma volume during exercise (Hickner et al. 2010) effects on muscle oxygen supply and VO_2max were not fully demonstrated. Finally, the effects of CS on central limiting factors of VO_2max do not appear to be essential. This can be explained by the fact that CS rather acts at the peripheral muscle level. Since CS is well-known to improve anaerobic performance through muscle hypertrophy and the higher recruitment in fast-twitch muscle fibers (Casey et al. 1996), changes in skeletal muscle energy metabolism could have explained our negative findings on VO_2max. This hypothesis is supported by the lack of improvement in muscle oxidative capacity following CS (Van Loon et al. 2003; Thompson et al. 1996). The negative effect of CS on VO_2max should be considered, especially in endurance athletes for whom VO_2max is a determining factor of performance.

The ventilatory threshold refers to the point during exercise at which ventilation starts to increase at a faster rate than VO_2 related to the onset of lactate accumulation (Wasserman et al. 1973). The ventilatory threshold occurs at a higher percentage of VO_2max in well-trained endurance athletes (Ivy et al. 1980) and can predict endurance performance, regardless of the level of training (Bassett 2000; Reybrouck et al. 1986). The increase in the ventilatory threshold found after CS in the young and healthy follows greater exercise efficiency at submaximal workloads, resulting in decreased O_2 consumption (Nelson et al. 2000; Murphy et al. 2005; Hickner et al. 2010). Hypotheses are that CrP hydrolysis consumes hydrogen ions and might contribute to the buffering of intracellular acidosis when ATP resynthesis occurs from CrP stores (Mesa et al. 2002). Moreover, increased muscle CrP levels due to CS could delay the decrease in the ATP/ADP ratio needed to stimulate mitochondrial respiration and VO_2 (Nelson et al. 2000). An increase in mechanical efficiency could also participate (Nelson et al. 2000; Da Silva Azevedo et al. 2019). Mitochondrial changes following CS at a cellular level are yet to be explored. The effects of age in ventilatory threshold improvement remain unclear (Thomas et al. 1985). Despite being poorly studied, maximal aerobic power output did not change following CS in our meta-analysis (Canete et al. 2006; Van Loon et al. 2003; Eijnde et al. 2003), in coherence with the fact that CS rather acts on fast-twitch muscle fibers (Hill and Rowell 1996). There was no change in time to exhaustion, but an incremental exercise test targets to reach VO_2max in a limited time, and therefore this parameter is mainly dependent on the incremental protocol.

We showed that CS limited more VO_2max in cycling than in running or rowing, but it may be biased considering the greatest number of participants performing cycling. While HIIT partly involves anaerobic capacities and shows interesting results on VO_2max in the literature (Tabata et al. 1996), the lack of data for HIIT in our meta-analyses did not permit to retrieval of an influence of HIIT on VO_2max when associated with CS (Forbes et al. 2017; Graef et al. 2009). We also failed to demonstrate any relationships between volume and intensity of training on the negative effects of CS on VO_2max, despite abundant dose-response literature in anaerobic performance (Lanhers et al. 2015 and 2017). Regarding characteristics of supplementation, Cr monohydrate was mainly used, precluding further comparisons between Cr forms. Improvement in VO_2max is altered with CS regardless of the doses. We failed to demonstrate a dose-response relationship, even though CS varied from 4 days to 6 months. Even if optimal doses to increase muscle total Cr content with minimal renal Cr losses (Harris, Söderlund, and Hultman 1992), favoring anaerobic performances, mitochondrial changes following CS at a cellular level are yet to be explored. CS has been most extensively studied in young and trained males.

Interestingly, some studies also explored the putative benefits of CS in specific populations, such as in the elderly and patients with heart failure. In those populations, CS did not improve VO_2max, regardless of the type of physical activity (Canete et al. 2006; Eijnde et al. 2003; Villanueva, He, and Schroeder 2014), which seem coherent because the central limitation appears even more predominant than in young athletes. However, it should be noted that CS is known to benefit the elderly, such as improving muscular strength and functional performance (Kuethe et al. 2006) (Devries and Phillips 2014). Lastly, even if strength gains were reported to be greater in males (McNaughton, et al. 1998; Tyka et al. 2015), we did not find sex-based differences on VO_2max changes with CS, following one study that included gender comparisons (Smith et al. 2011).

This is the first meta-analysis that has focused on the effect of CS on VO_2max. Many studies did not report and included all their results in the meta-analysis. For instance, studies reported baseline measurement without measurement after supplementation for VO_2max or without its change expression (Nelson et al. 2000; McNaughton, Dalton, and Tarr 1998) or did not express dispersion of its measurement (Tyka et al. 2015). Our study has some limitations inherited from all meta-analyses (LeLorier et al. 1997), but the use of rigorous inclusion criteria, that is, double-blind RCTs limited the publication bias. Reporting bias due to the scarcity of publications with negative findings is common to all systematic reviews and meta-analyses. However, it could have reinforced our main findings. A publication bias was also assessed according to funnel plots, and sensitivity analyses showed similar results on the main outcome. Measurements of VO_2max varied among studies, which

could have led to a measurement bias. The rigorous determination of VO_2max depends on different criteria (plateau in oxygen consumption, respiratory exchange ratio > 1,1, heart rate greater than age-predicted maximal heart rate, blood lactates > 6–8 mmol or physical exhaustion (Lacour and Flandrois 1977)). In our meta-analysis, VO_2max was reported through rigorous determination by three studies (Hickner et al. 2010; Murphy et al. 2005; Smith et al. 2011). Four studies (Nelson et al. 2000; Canete et al. 2006; Forbes et al. 2017; Graef et al. 2009) assessed VO_2peak as the highest average VO_2 obtained for any period of time between 15-sec (Graef et al. 2009) and 1-min (Canete et al. 2006) without specifying if a VO_2plateau was obtained. Ten studies did not provide additional information for the determination of the maximum oxygen uptake and mentioned VO_2max (Balsom et al. 1993; Van Loon et al. 2003) or VO_2peak (Carvalho et al. 2012; Reardon et al. 2006; Kuethe et al. 2006; Eijnde et al. 2003; Sterkowicz et al. 2012; Kresta et al. 2014; Barnett, Hinds, and Jenkins 1996; Lawrence et al. 1997) without precision, and two studies estimated VO_2max (Lázaro da Silveira et al. 2014; Villanueva, He, and Schroeder 2014). To assess the impact of training associated with CS, we used a classification that may have generated a bias. Most of the performance variables included in this meta-analysis were measured in a laboratory setting, and field-based endurance performance also must be assessed.

Conclusion and practical implications

Our analysis showed the negative effects of CS on VO_2max, regardless of the supplementation protocols, physical training, and population characteristics. Considering that creatine is the most widely used dietary supplement in sport, particular attention toward its use should be paid to endurance athletes.

Author contributions

Conceived and designed the study: FD and JBBM. Conducted the systematic literature search: DG, CL. Final approval of eligible articles: all authors. Analyzed the data: DG, FD and BP. Wrote the first draft of the article: DG, FD, UU. Critical revision of the article: all authors. Integrity of the data analysis: DG, FD.

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Data availability statement

All relevant data are within the paper.

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