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The antioxidant effect of beta-alanine or carnosine supplementation on exercise- induced oxidative stress: a systematic review and meta-analysis

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Abstract: The objective of this study was to perform a systematic review and meta-analysis of the articles that addressed the effect BA or carnosine supplementation on Physical exercise (PE)-induced oxidative stress (OS). We searched throughout PubMed, CAPES Periodic and SPORTDiscus human model peer review, randomized control studies with chronic BA or carnosine supplementation on PE-induced OS. We search papers published before May 2018. A total of 128 citations were found. Only four articles met criteria for inclusion. All four studies used healthy young (21y) sedentary, recreationally active or athletic participants. After a chorionic BA (~30 days) or carnosine (14 days) supplementation, the studies evaluated PE-induced OS both immediately and several hours after exercise (0.5 to 48 h). In response to PE-induced OS, when compared to placebo, BA/carnosine supplementation increased total antioxidant capacity [TAC; Effect Size (ES)= 0.35, 95% Confidence Interval (CI) 0.06 to 0.65, p= 0.02] and glutathione (GSH; ES= 0.75, 95% CI 0.32 to 1.19, p= 0.0007) concentrations while decreased pro-oxidant markers (ES= -1.19, 95% CI -1.48 to -0.80, p< 0.01) and superoxide dismutase (SOD) activity (ES= -0.58, 95% CI -1.10 to -0.06, p= 0.03). BA or carnosine supplementation did not prevent the increase in peroxidation markers (ES: -0.20, 95% CI -0.59 to 0.20, p= 0.33). In humans, following PE-induced OS, initial treatment trials of BA or carnosine supplementation seemed to increase TAC and GSH concentrations, while decreasing SOD activity. Also, albeit mitigating the acute increase in pro-oxidants, treatment did not decrease measured values of peroxidation markers.

Keywords: beta-alanine, carnosine, oxidative stress, antioxidant, Physical exercise

1. Introduction

The It is well known that carnosine is a potent and safe antioxidant [1]. Recent animal models and humans (with type 2 diabetes) studies has been shown that carnosine supplementation can restore glutathione peroxidase (GPx) to normal levels, increase total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD) activity and reduce lipid peroxidation (LP) [1-4]. All of these changes are important for improvement of anti-oxidant system and simultaneous reduction of oxidative stress (OS) [5].

Acute physical exercise (PE) is known to induce high reactive oxygen species (ROS) production and consequently to promote an acute OS milieu [6,7]. Studies with healthy humans [8-10] and animal models [11,12] have investigated whether increased carnosine in the body [induced by carnosine or beta-alanine (BA) supplementation] mitigates the high ROS production (as well as acute OS milieu condition) during exercise. In animal studies, carnosine and BA supplementation were shown to effectively mitigate the OS produced by exercise [11-13]. However, in human studies, the finding were unclear. For instance, both recreationally activity men [9] and women [8] who received BA supplementation had reduced OS after an acute bout of physical exercise (wen compared to baseline, but not to placebo condition). The improvement in antioxidant system seemed to occur only in women when compared to the baseline [8]. Although, in other studies with male athletes, carnosine [14] and BA [10] supplementation did not change/mitigate the LP values after an acute bout of PE, despite increasing the GSH (Glutathione) antioxidant potential when compared to pre-treatment condition.

Conditions for alterations in the antioxidant system and OS due to BA or carnosine supplementation were tested using different physical exercise interventions and enrolled participants with different physical fitness levels. In addition, different assessment times were used for PE-induced ROS and OS markers [7], also, different types of ROS, LP or antioxidant system markers assessed might have influenced the study's results [7].

In this sense, it is necessary to maintain the highest standards in relation to BA/carnosine supplementation on PE-induced ROS production and OS milieu. Thus, the purpose of this review to carry out a systematic meta-analysis of the randomized controlled studies that investigated the effects of BA or carnosine supplementation on antioxidant system, ROS and OS markers that are induced by PE in healthy individuals.

2. Methods

2.1. Search Criteria

Materials We searched throughout PubMed, CAPES Periodics and SPORTDiscus peer reviewed studies that involved human subjects and were published before May 2018. The following MeSH terms were used: beta-alanine OR carnosine AND oxidative stress OR antioxidants AND exercise (Supplemental material 1). Independently, two authors (E.F and M.R.) verified titles, abstracts, and full text for the articles identified to verify eligibility for inclusion in the present review. Discrepancies were resolved by group discussion. For the articles that were fully accessed, we searched among the references for potential studies for inclusion in the analysis. In addition, we searched Google citations for potential articles that could meet the criteria of this review. A flow diagram for publications inclusion criteria represented in Fig 1.

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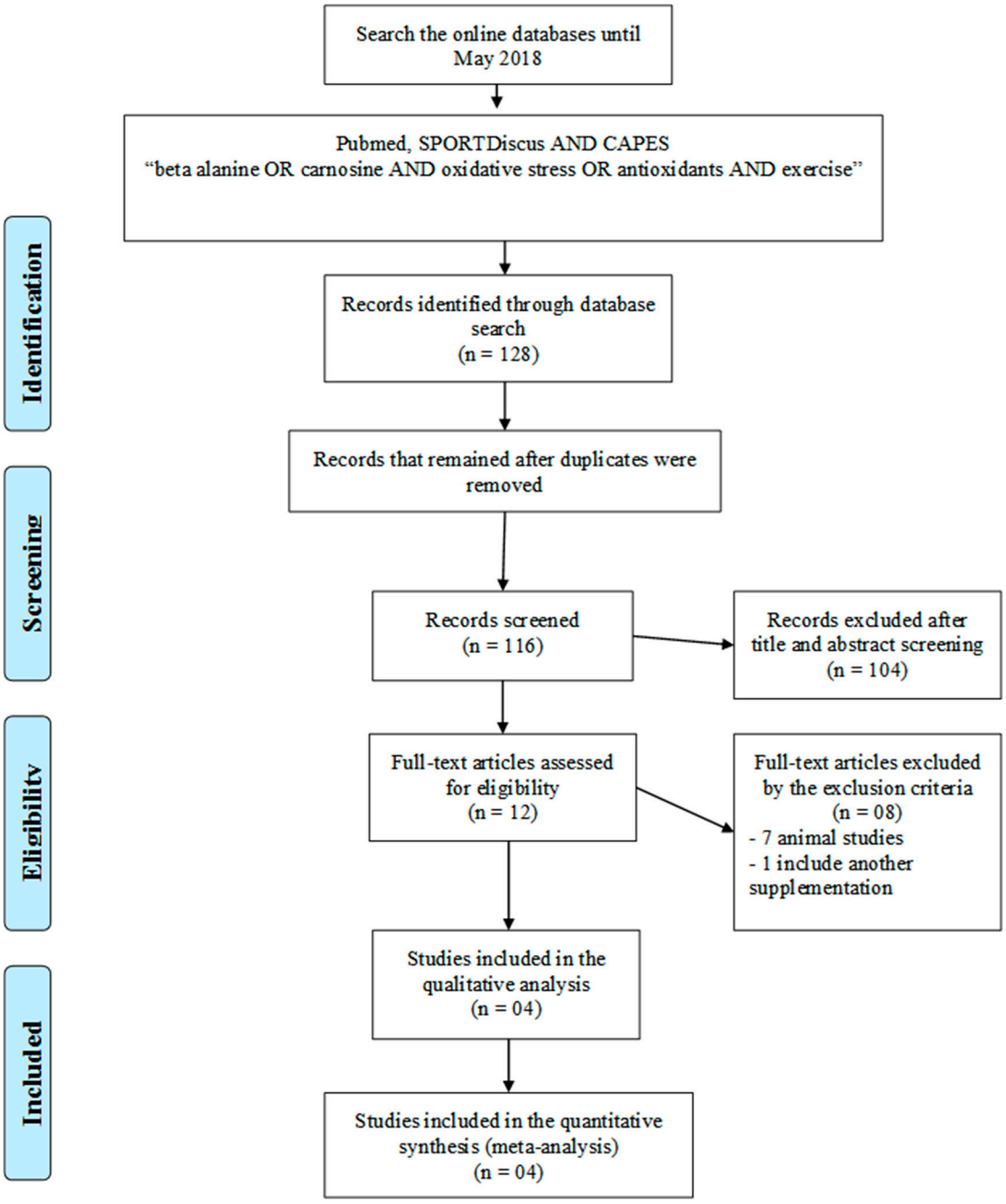

Figure 1. Flow diagram for the strategy of searching for the studies

2.2. Inclusion/Exclusion Criteria

The inclusion criteria for the articles were (1) studies with randomized and controlled samples, (2) language of publication either English, Portuguese or Spanish, (3) studies that performed intervention with chronic BA or carnosine supplementation followed by acute PE (to induce OS). We excluded studies that underwent other interventions in addition to BA (or carnosine) supplementation and PE (e.g., chemotherapy, drugs or other types of antioxidant supplementation).

2.3. Identification of Eligible Studies

Human subjects that underwent chronic supplementation of BA or Carnosine (≥ 28 days for BA and 14 days for carnosine) and acute PE for induction of OS.

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2.4. Data Extraction

Table 1 describes information on: participants descriptive information such as sex, age, training status. Participants described as Trained or Athletes were defined as those with regular training, with at least one year of experience. Participants were described as Recreational if they practiced PE at least 2-3 times per week and Sedentary if their level of PE practice was less than 1 time per week. Table 1 describes the training program (when provided); whether the study had parallel design (two groups) or the same participants (crossover); the number of participants in each group; intervention duration; daily dose supplementation and type of vehicle (i.e. capsules, tablets), dosage distribution over the course of the day and finally the moment of assessment of PE-induced pro-oxidant and OS as well as the evaluation site (intra- or extra-cellular).

2.5. Effect Size Calculation

For antioxidant system, pro-oxidant and peroxidation markers outcome, an effect size (ES) was calculated to represent the pre-exercise–post-exercise change, divided by the pre-exercise standard deviation (SD). A small sample bias adjustment was applied to each ES. The variance around each ES was calculated using the sample size in each study and mean ES across all studies [15]. ES were classified as trivial (<0.2), small (≥ 0.2 to ≤ 0.6), moderate (≥ 0.6 to ≤ 1.2), large (≥ 1.2) [16]

2.6. Statistical Analyses Results

Calculations was performed using a random effects method. Data is displayed as mean difference with random effects, inverse of variance and 95% confidence interval. Statistical heterogeneity of the treatment effects among studies were assessed using Cochran's Q test and the inconsistency I^2 test, in which values above 25% and 50% were considered indicative of moderate and high heterogeneity, respectively. Review manager 5.3 was used to build the Forest plot graphs and used to carry out the statistical analysis.

When sample size was not limited, statistical heterogeneity was explored (with Review manager 5.3) by sub-group analysis: the time of assessment (immediately vs. 0.5 to 48 hours after the exercise test). Also, multiple linear regressions throughout the stepwise method (using SPSS v. 24) were performed. For this purpose, we used ES from antioxidant system and indirect OS markers outcome as the dependent variable. The independent variables were: (1) training status, (2) sex, (3) moment of assessment, (4) antioxidant and indirect OS markers type, (5) supplementation condition (placebo, BA or carnosine), (6) exercise intensity or duration. The statistical significance level was set at $P < 0.05$.

3. Search results

The search of PubMed, SPORTDiscus and CAPES periodic provided a total of 128 citations (titles and abstracts were accessed). 116 articles were removed (both duplicates and articles that met the exclusion criteria). We examined the full text of the remaining 12 articles and only four articles [8-10,14] were included in the review (Fig 1).

Seven out of eight studies excluded did not meet the criteria of human subjects (animal models were rats and mice). Two of the excluded studies involved chronic training [4]. One study evaluated acute injected BA [17]. Two studies evaluated PE-induced OS, but had other antioxidants combined with BA [18] or carnosine [13] supplementation. One human study [19] was excluded because it used others AO combined with BA. Three other animal studies who were also excluded which evaluated PE-induced OS after BA/carnosine supplementation [11-13] and were therefore were used in the discussion of this review. (Fig 1).

3.1. Participant and Intervention Characteristics

All studies used healthy young adults (21y) who were sedentary, recreationally active or trained participants. Only one study used women as subjects. Only one study used carnosine

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supplementation, while the other three studies used BA supplementation. All supplementation protocols employed chronic treatment, being 28 days for BA supplementation and 14 days for carnosine supplementation (Table 1).

Exercise-induce EO involved classic Wingate test (short all-out high-intensity repeated bouts), moderate endurance-running (70-75% of VO_2max) and short high-intensity one bout (2000-m run time trial type) exertion. All physical exercise interventions successfully and significantly induced EO (Table 1).

Table 1. Description of studies in the systematic review and meta-analysis.

Study	Experimental design	Exercise training or Exercise induce OS	OS and AO markers
Belviranli at al. [10]	44 healthy sedentary males (age 21.7 ± 1.9 y, height 175.9 ± 5.9 cm, and body weight 70.9 ± 7.9 kg) randomly assigned to one of 4 groups: PL, BA (1,6g/d 2x day; powder), Creatine (Cr; 10g/d) or BA+Cr supplementation for 22 consecutive days, then four times per day for the following 6 days. Blood plasma OS markers were analyzed before and after Wingate test (WTs) sessions.	Three bouts of 30s Wingate test (all out, against a resistance of 75 g.kg-1 body weight) with a 2 -minute rest between bouts. The WTs session was performed before and after the period of supplementation	GSSG, PC and MDA; SOD, TAC and GSSG
Smith-Ryan et al. [9]	25 heathy recreationally active males (age, 21.9 ± 3.4 y; height, 177.6 ± 5.4 cm; weight, 78.8 ± 9.7 kg) randomly assigned to 28 days of PL or BA (1,6g 3x day, sustained release) supplementation. Blood plasma OS markers were analyzed immediately after, and at 2 and 4 hours after exercise.	40 min on a treadmill at a velocity corresponding to 70%–75% of their measured peak velocity before and after the period of supplementation.	8-ISSO; SOD, TAC, and GSH
Smith-Ryan et al. [8]	26 heathy recreationally active women (age, 21.7 ± 1.9 y; height, 165.0 ± 5.7 cm; weight, 61.9 ± 6.7 kg) randomly assigned to 28 days of PL or BA (1,6g 3x day, sustained release) supplementation. Blood plasma OS markers were analyzed immediately after, and at 2 and 4 hours after exercise.	40 min on a treadmill at a velocity corresponding to 70%–75% of their measured peak velocity before and after the period of supplementation	8-ISSO and SOD, TAC and GSH
Slowinska-Lisowska et al. [14]	14 elite kayakers and canoeists athletes (age, 21.2 ± 1.3 y; height, 177.4 ± 7.9 cm; weight, 78.9 ± 8.9 kg) in a crossover way assigned to 14 days of PL and Carnosine (2g 2x day) supplementation. Washout was four weeks. Blood plasma OS markers were analyzed immediately after (IP), and at 30min and 24h and 48h after exercise.	During supplementation period athletes underwent a 5day/wk structured schedule training (60% aerobic and 40% strength training). After supplementation athlete performer 2000-m run on kayak or canoe ergometer (exercise induce OS).	GSSG, 8-ISSO, PC, NO, H_2O_2 and 3-Nitro; TAC, SOD and GSH

Note: 3-Nitro, 3-nitrotyrosine; 8-ISO, 8-isoprostane; BA, beta-alanine; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, Hydrogen peroxide; MDA, malondialdehyde; OS, oxidative stress; PC, protein carbonyl; PL, placebo; SOD, superoxide dismutase; TAC, total antioxidant capacity.

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3.2. Antioxidant and Pro-oxidants assessment after BA or carnosine supplementation in exercise-induced oxidative stress

All Pro-oxidants (3-Nitro, 3-nitrotyrosine; H_2O_2, Hydrogen peroxide and nitric oxide), peroxidation (8-ISO, 8-isoprostane; MDA, malondialdehyde and; PC, protein carbonyl) and antioxidant (GSH, glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase and; TAC, total antioxidant capacity) markers were assessed from blood samples. DNA (8-ISO), protein (PC) and cell damage (3-Nitro) as well as lipid peroxidation (MDA), indirect markers of OS were assessed. H_20_2 and NO were assessed as direct OS markers. SOD was assessed as endogenous AO; TAC, GSH and GSSG were assessed as exogenous AO. All four studies evaluated PE-Induced OS post-supplementation immediately after exercise. Three out of four studies repeated the assessment after s 30 min [14], 2h, 4h [8,9], 24h and 48h [14] post exercise (Table 1).

3.3. Meta-analysis

3.3.1. Oxidants

Exercise induced moderate increase in pro-oxidants markers (PC, MDA, 8-ISO and GSSG) in both conditions (BA/carnosine ES= -0.78, 95% CI -0.19 to-1.37; placebo ES= -0.60, 95% CI -0.12 to -1.08). Comparisons between conditions revealed that immediately after exercise there was a small increase, but not significant, in pro-oxidants markers in the BA/carnosine group (difference ES: 0.23, 95% CI -0.24 to 0.71, p= 0.33). However, a small decrease, but not significant, on peroxidation markers that were observed hours after exercise was favorable to the BA/carnosine condition (difference ES: -0.20, 95% CI -0.59 to 0.20, p= 0.33). Sub-group analysis (immediately after exercise vs. hours after exercise) suggests a moderate heterogeneity (I^2= 47%, p= 0.17) among peroxidation markers depending on the time of assessment (see Fig 2).

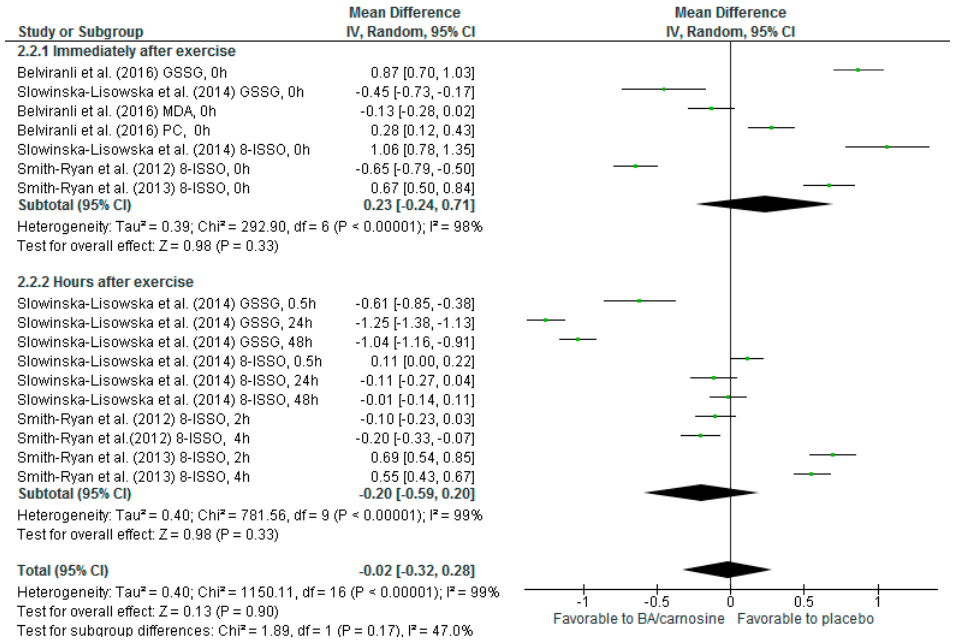

Figure 2. Forest plot of the peroxidation markers induced by physical exercise after BA/carnosine or placebo supplementation. Acronyms: 3-Nitro, 3-nitrotyrosine; 8-ISO, 8-isoprostane; GSSG, oxidised glutathione; MDA, malondialdehyde; PC, protein carbonyl.

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Independent analysis suggests large and moderate decreases in GSSG concentrations following PE (BA/carnosine ES= 1.84, 95% CI -0.63 to 4.31; placebo ES= 1.33, 95% CI -0.73 to 3.39, respectively). Between group comparison showed no difference immediately after PE (difference ES= 0.21, 95% CI, -1.08 to 1.51, p= 0.75, I^2= 98%), but a lower GSSG concentration hours after PE in BA/carnosine condition (difference ES= -0.99, 95% CI, -1.28 to -0.69, p< 0.01, I^2= 76%). Sub-group analysis (immediately after exercise vs. hours after exercise) indicates a significant effect of time of assessment (I^2= 83.7%, p= 0.01).

Independent analysis of 8-ISSO showed a large increase in immediately after PE in both condition (BA/carnosine ES= -2.15, 95% CI -6.91 to 2.60; placebo ES= -1.79, 95% CI -4.56 to 0.98, respectively) and a moderate decrease in both conditions following hours after PE (BA/carnosine ES= 0.62, 95% CI -0.12 to 1.35; placebo ES= 0.54, 95% CI -0.35 to 1.45). Between condition compassion reveal a small and not significant increase in 8-ISSO immediately after exercise for BA/carnosine (difference ES= 0.36, 95% CI -0.70 to 1.42, p= 0.51, I^2= 99%) and trivial decrease that was measured hours after exercise (difference ES: 0.07, 95% CI -0.59 to 0.45, p= 0.79, I^2= 97%). Sub-group analysis suggests no effect of time of assessment (I^2= 0%, p= 0.48), however when we exclude the Smith et al. [8] study (00 hour post exercise), there is a significant effect of time of assessment ($I2$= 87.2%, p< 0.01).

Due to insufficient data, PC and MDA independent analysis was not performed.

Only the study by Slowinska-Lisowska et al. [14] performed direct OS markers assessment. Data reanalysis of this study (Fig 3) suggests that immediately after PE, carnosine supplementation condition (when compared to placebo) did not mitigate the increase in pro-oxidants production (difference ES: 0.23, 95% CI -0.33 to 0.79, p= 0.42, I^2= 96%; see Fig 3). On the other hand, when we compared the conditions involving the later hours after the exercise, carnosine was shown to mitigates the increase in pro-oxidants (difference ES= -1.19, 95% CI -1.48 to -0.80, p< 0.01, I^2=98%). There is a significant sub-group (immediately after exercise vs. hours after exercise) difference (I^2= 94%, p<0.01) on pro-oxidant markers, see Fig 3.

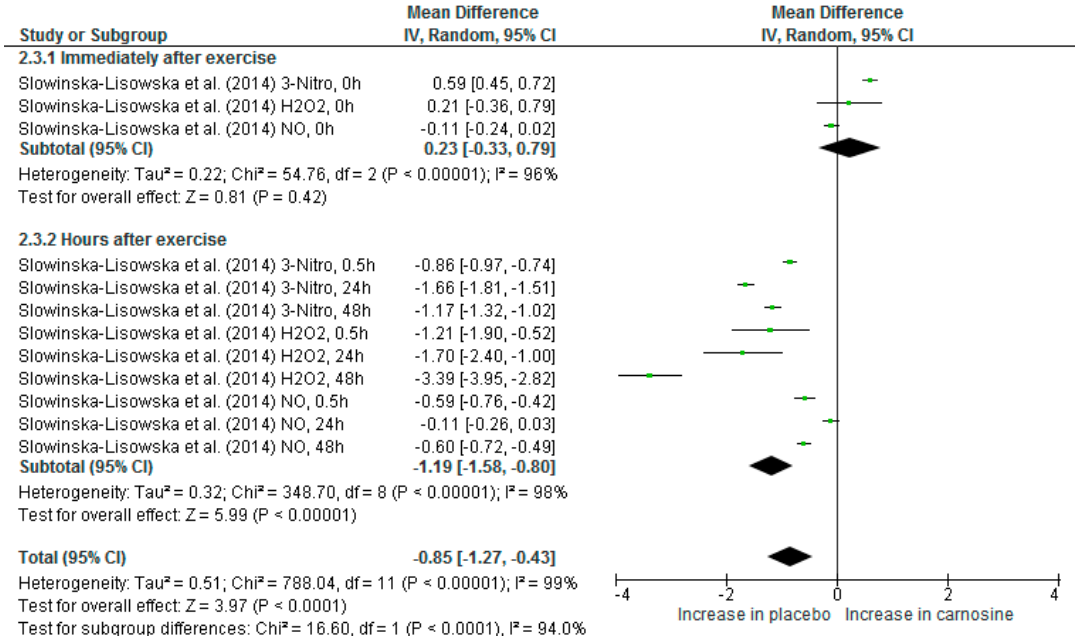

Figure 3. Forest plot of the pro-oxidants induced by physical exercise after carnosine or placebo supplementation. Acronyms: 3-Nitro, 3-nitrotyrosine; H2O2, Hydrogen peroxide; NO, nitric oxide.

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213 3.3.2. Antioxidants

214 ES suggests that there was a moderate increase in TAC concentration in BA/carnosine
215 supplementations (ES= -0.66, 95% CI -1.44 to 0.12), whereas a trivial decrease occurred in placebo
216 supplementation was observed (ES= 0.08, 95% CI -0.78 to 0.95) immediately after exercise, but without
217 significant difference between them (difference ES= 0.51, 95% CI -0.15 to 1.17, p=0.13, I2=99%). Hours
218 after exercise BA/carnosine presented a trivial increase (ES= -0.13, 95% CI -0.78 to 0.52) and a similar
219 small decrease occurred in the placebo condition (ES= 0.12, 95% CI -0.42 to 0.66) which showed a tend
220 to difference between then (difference ES= -0.25, 95% CI -0.04 to 0.55, p= 0.09, I^2=98%). Overall
221 between conditions comparison (pooled ES) suggests that BA/carnosine supplementation increases
222 overall TAC (difference ES= 0.35, 95% CI 0.06 to 0.65, p= 0.02, I^2= 99%; Fig 4) in response to exercise.
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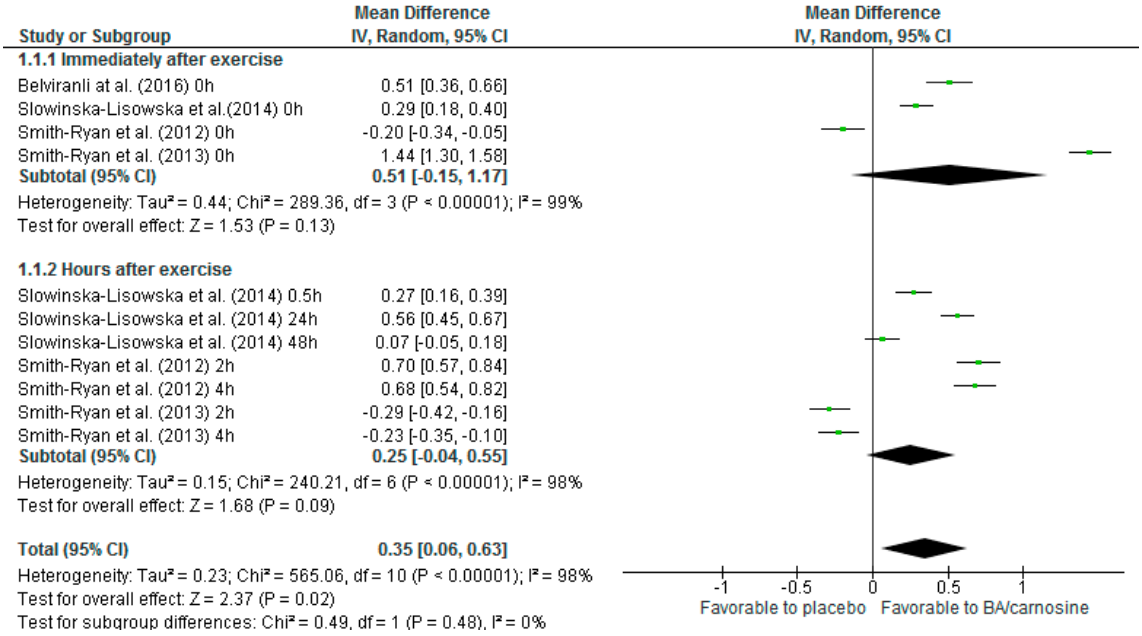

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225 **Figure 4.** Forest plot of the total antioxidant capacity change by physical exercise after BA/carnosine
226 or placebo supplementation.

227
228
229 Immediately after exercise there were a trivial and a large GSH decreases in both conditions
230 (BA/carnosine ES= 0.16, 95% CI -4.68 to 4.99; placebo ES= 1.23, 95% CI -2.00 to 4.44, respectively).
231 There were also a moderate and a trivial increase following hours after exercise (BA/carnosine ES= -
232 0.69, 95% CI -1.61 to 0.22; placebo ES= -0.12, 95% CI -0.99 to 0.77, respectively). Between conditions
233 comparison presented a significant difference in GSH concentration (favorable to BA condition) both
234 immediately after and several hours following exercise [Overall ES difference= 0.75, 95% CI 0.32 to
235 1.19, p= 0.0007, I^2= 99% (Fig 5a)].

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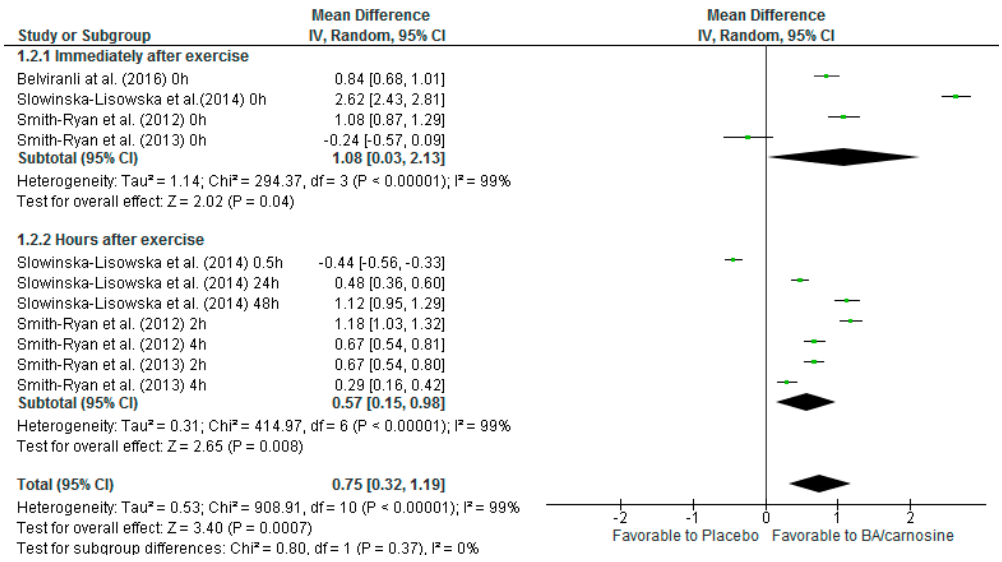

Figure 5. Forest plot of the glutathione change by physical exercise after BA/carnosine or placebo supplementation.

Immediately after exercise, there were a trivial and a small increase in SOD activity in both conditions (BA/carnosine ES= -0.02, 95% CI -1.15 to 1.12; placebo ES= -0.50, 95% CI -1.29 to 0.30, respectively). Following hours after exercise, there were large increases in SOD activity for both conditions (BA/carnosine ES= -1.39, 95% CI -4.21 to 1.41; placebo ES= -1.72, 95% CI -4.39 to 0.96). Overall between conditions comparison showed that the placebo presented a moderate and significantly greater SOD activity (differences ES= -0.58, 95% CI -1.10 to -0.06, p= 0.03, I^2= 99%; Fig 6) when compared to BA/carnosine supplementation.

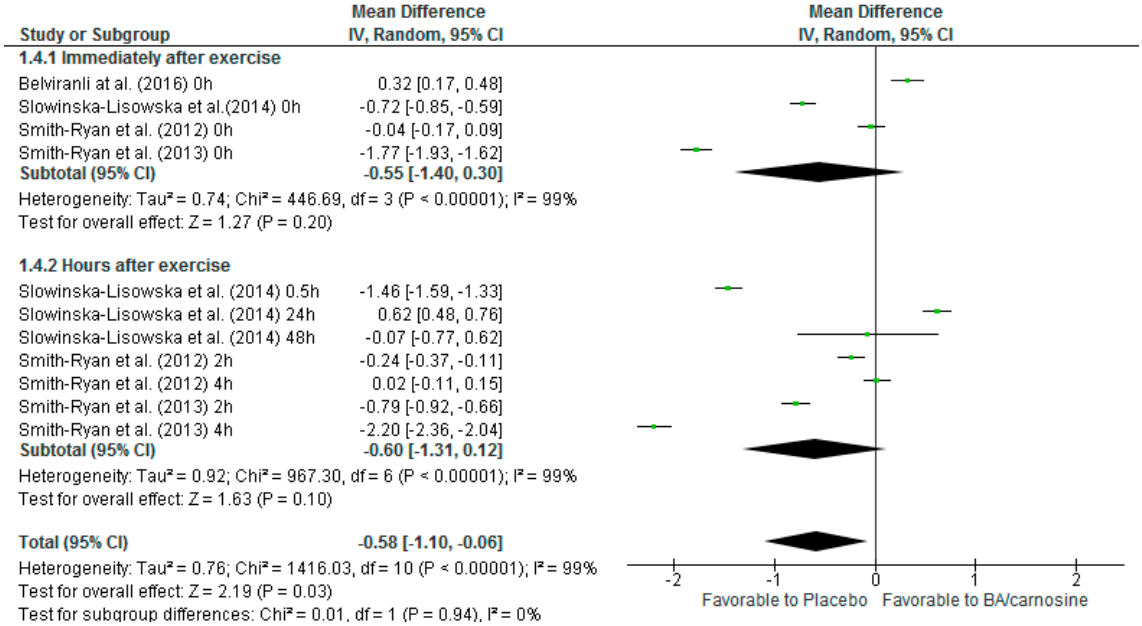

Figure 6. Forest plot of the Superoxide dismutase change by physical exercise after BA/carnosine or placebo supplementation.

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3.3.3. Heterogeneity studies and multiple linear regression analysis

Multiple linear regression shows that in peroxidation markers (8-ISO, MDA. GSSG and PC) the time of assessment, pro-oxidant marker type evaluated, exercise type and training status could explain 65% of ES variation (R^2= 0.650, p= 0.000). Sex and supplementation conditions (BA or carnosine) were excluded from the model.

Furthermore, 39% (R^2= 0.389, p= 0.000) of ES variation from antioxidant (SOD, TAC, GSH) results were related to time of assessment, exercise test, training status and anti-oxidant marker type evaluated. Sex and supplementation conditions were excluded from the model.

It was not possible to perform multiple linear regression for pro-oxidant direct markers (H_2O_2, 3-Nitro and NO) due to insufficient data.

4. Discussion

The four studies included in this review observed significant increases in OS after acute physical exercise bouts. Our analyses suggest that immediately after PE-induce OS, BA or carnosine supplementation did not undermine the increase in pro-oxidants (H_2O_2, 3-Nitro and NO) or peroxidation (8-ISSO, MDA, and PC) markers that were produced. Monitoring their levels during hours after exercise (0.5 to 48h), BA or carnosine did not appear to impose a greater decrease in 8-ISSO (p> 0.05) when compared to placebo supplementation. Interestingly, monitoring OS levels after hours (0.5 to 48h) of PE-induced, carnosine treatment mitigated the increase of H_2O_2, 3-Nitro and NO production. It is important to mention that pro-oxidants (H_2O_2, 3-Nitro and NO) data were obtained from only one study (Slowinska-Lisowska et al. 2014), but such data were in accordance with previous in vitro studies [20,21] .

Evidence suggests that the largest post-exercise changes involving lipid, protein, glutathione and DNA oxidation occurred 1-4 days after PE (when compared with blood samples of resting condition) [7]. For instance, in an animal study that assessed exercise-induce OS after 24h, it was shown that BA or carnosine supplementation decreased LP (thiobarbituric acid reactive substances and MDA markers) in skeletal muscle tissue [11,12]. The only publication that evaluated 24h post-exercise was the Slowinska- Lisowska et al. [14] study. Therefore, studies with a long follow-up period [days to weeks, therefore with sufficient time to resolve an acute inflammation caused by moderate-intense exercise [22]] are needed to verify whether BA or carnosine may promote clinical changes in the peroxidation markers.

Previous reviews [1] and recent animal studies [2-4] had already presented an antioxidant role of carnosine. When compared to placebo, our data suggested that previous BA or carnosine supplementation increased TAC (ES= 0.35, 95% CI 0.06 to 0.65, p= 0.02; Fig 4) and increase GSH (GSH, ES= 0.75, 95% CI 0.32 to 1.19, p= 0.0007) after PE-induced OS. These data corroborate with an animal study [12] submitted to PE-induced OS. Such study reported increased in GSH and decreased glutathione peroxidase (GPx) and glutathione reductase after exercise, suggesting that carnosine has buffering the H_2O_2 production. The effect of BA and carnosine supplementation on GSSG concentrations is conflicting. Belviranli et al. [10] reported increased GSSG after PE induces OS in sedentary individuals supplemented with BA (suggesting GSH oxidation); on the other hand, Slowinska-Lisowska et al. [14] reported decreased GSSG concentrations in trained individuals supplemented with carnosine (suggesting a carnosine antioxidant effect). More researcher is needed to highlight the effect of BA/carnosine on GSH/GSSG ratio.

Our data suggests that BA or carnosina supplementation can mitigate the increase of SOD activity (ES= -0.58, p= 0.03), a well-know superoxide scavenger. It is plausible that this attenuated increase of SOD activity occurs due to carnosine antioxidant effect (e.g., O2·- clearance). In vitro studies has been showed that carnosine plays an effective role in decreasing ROS and reactive nitrogen species (e.g. H_2O_2, superoxide and NO) [20,21]. Studies with animal training also has demonstrated that carnosine or BA supplementation decreased SOD [23] and GPx [12] activity, when compared to control conditions. These data are contrary to untrained animal studies [2,3]. Therefore, it appears that BA or Carnosine supplementation might mitigate the increase in SOD and

GPx activity induced by exercise. Further studies are needed to verify if chronic BA supplementation might down-regulate the endogenous antioxidant system during physical training.

The results observed in this review suggest that an acute PE increase of SOD activity is mitigated, probably due to the ability of carnosine to directly decrease ROS concentrations. Interestingly, carnosine supplementation associated with endurance training (in rats) decreased exercise tolerance (at 2 wks of training) and both SOD and lactate dehydrogenase activity in the skeletal muscle (at 4 wks of training) [23]. Therefore, future studies are needed to verify (both in an acute and chronic settings) if the changes promoted, such as increased gene expression of enzymes from the endogenous antioxidant system induced by physical exercise [24], are mitigated in the presence of BA or carnosine supplementation, as it is observed in studies with chronic [6] or acute antioxidant supplementation [17]. Moreover, BA supplementation is a well-known ergogenic agent in anaerobic exercises, but not in endurance exercises [25,26]. For instance, early evidence in human studies suggest that BA supplementation delayed lactate production, but reduce aerobic capacity [27]. Therefore, it is important to investigate if BA or Carnosine supplementation might influences negatively endurance adaptations because of their antioxidant effects [28].

Our ES evaluations (both antioxidant and prooxidant) showed a high heterogeneity. This meta-analysis pooled together studies with participants from different fitness level, enrolled in different PE-induced OS, also, different time point of pro- or antioxidant markers were pooled in the same ES analysis. It is well-know that time-point assessment of PE-induced OS as well as the resining in blood plasma of pro- or antioxidant markers type are also time-dependent and this might influence our results [7]. Our sub-group analysis (immediately after exercise vs. hours after exercise- 0.5 to 48 hours) showed that the moment of assessment for peroxidation (Fig 3) and pro-oxidant (Fig 4) markers is an important confounding variable. Also, multivariable regression shows that time of assessment, the pro-oxidant marker type evaluated, the exercise type and training status can explain 65% of ES variation (R^2= 0.650, p= 0.000). Sub-groups analysis for antioxidant (TAC, SOD, and GSH) markers did not show significant influence of time of assessed. But, multivariable regression shows that only 39% (R^2= 0.389, p< 0.000) of ES variation from antioxidant results were from time of assessment, exercise test, training status and anti-oxidant type evaluated. This suggest that other variables (e.g. nutritional status or antioxidant system status) may be influencing this heterogeneity in antioxidant results [28]. For example, no study included in this meta-analysis mentioned that their samples were homogenized for pro- or antioxidant status (deficient in oxidant status or not), so future studies with antioxidants supplementation need to homogenize their samples as deficient or not for the antioxidant system [28].

4.1. Limitations

This meta-analysis has several limitations. First there are only four studies, two of which are from the same laboratory, decreasing the validity and reliability of the results. Second, we included in the same analysis BA and carnosine studies, the results of the carnosine study significantly influence our TAC results, but do not significantly alter the results of SOD, GSH or pro-oxidant markers, in addition, meta-regression excluded the type of supplement (i.e., BA or carnosine) used as a source of heterogeneity, so BA or carnosine is not a source of heterogeneity. Third, the high heterogeneity found in this study because the studies analyzed different levels of fitness, sex and different exercise intensity/volume also decrease the reproducibility of these data, but give further evidences that these variables differ in responses to PE-induced OS.

5. Conclusions

In conclusion, following PE-induced OS previews BA or carnosine supplementation seems to increase TAC and improve GHS/GSSG ratio, but decrease SOD activity. Also, albeit to mitigate the acute increase in pro-oxidant, it does not decrease peroxidation markers.

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