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

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## 29 **The antioxidant effect of beta-alanine or carnosine** 30 **supplementation on exercise- induced oxidative stress: a** 31 **systematic review and meta-analysis**

32 **Abstract:** The objective of this study was to perform a systematic review and meta-analysis of the articles  
33 that addressed the effect BA or carnosine supplementation on physical exercise (PE)-induced oxidative stress  
34 (OS). Before May 2018 we searched throughout PubMed, CAPES Periodic and SPORTDiscus human model  
35 peer review, randomized control studies with chronic BA or carnosine supplementation on PE-induced OS. A  
36 total of 128 citations were found. Only four articles met criteria for inclusion. All four studies used healthy  
37 young sedentary, recreationally active or athletic participants. After a chronic BA or carnosine  
38 supplementation, the studies evaluated PE-induced OS both immediately and several hours after exercise (0.5  
39 to 48 h). In response to PE-induced OS, when compared to placebo, BA/carnosine supplementation increased  
40 total antioxidant capacity [TAC; Effect Size (ES)= 0.35, 95% Confidence Interval (CI) 0.06 to 0.65, p=0.02]  
41 and glutathione (GSH; ES= 0.75, 95% CI 0.32 to 1.19, p=0.0007) concentrations while decreased direct OS  
42 markers (ES= -1.19, 95% CI -1.48 to -0.80, p< 0.01) and superoxide dismutase (SOD) activity (ES= -0.58,  
43 95% CI -1.10 to -0.06, p= 0.03). BA or carnosine supplementation did not prevent the increase in indirect OS  
44 markers (ES: 0.06, 95% CI -0.38 to 0.500, p=0.80). In humans, following PE-induced OS, initial treatment  
45 trials of BA or carnosine supplementation seemed to increase TAC and GSH concentrations, while decreasing  
46 SOD activity. Also, albeit mitigating the acute increase in direct OS markers (reactive nitrogen and oxygen  
47 species), treatment did not decrease measured values of indirect OS markers (peroxidation or molecule  
48 oxidation).

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50 **Keywords:** beta-alanine, carnosine, oxidative stress, antioxidant

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## 54 1. Introduction

55 It is well known that carnosine is a potent and safe antioxidant [1]. Recent animal models and humans  
56 (with type 2 diabetes) studies has been shown that carnosine supplementation can restore glutathione peroxidase  
57 (GPx) to normal levels, increase total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD)  
58 activity and reduce lipid peroxidation (LP) [1-4]. All of these changes (in CAT, GPx and SOD) are important  
59 for improvement of antioxidant system and simultaneous reduction of oxidative stress (OS) [5]. Antioxidant  
60 supplementation is commonly prescribed in disease that presents elevated ROS and RNS (reactive oxygen and  
61 nitrogen species, respectively) production, with the intention to improve the antioxidant system and decrease  
62 the OS. However, both ROS and RNS are necessary to cellular function, although its high production is  
63 detrimental, at the same time their low production is also detrimental to cellular function [6]. Therefore, the  
64 prescription of antioxidants cannot be indiscriminate.

65 Acute physical exercise (PE) is known to induce high ROS/RNS production and consequently to promote  
66 an acute OS milieu [7, 8]. Recent evidence has suggested that the acute increase in ROS/RNS production during  
67 PE is necessary to promote adaptations (e.g., improve athletic performance and  $VO_2$ max) and the improvement  
68 in the antioxidant system itself [9, 10]. It is also suggested that the use of exogenous antioxidants may be  
69 counterproductive in individuals who already have a balanced oxidant/antioxidant system [11]. However, beta-  
70 alanine (BA; a rate-limiting precursor in the synthesis of carnosine) and carnosine supplementation are popular  
71 ergogenic aids and also prescribed indiscriminately as antioxidant for athletic population. Studies with healthy  
72 humans [12-14] and animal models [15, 16] have investigated whether increased carnosine in the skeletal  
73 muscle (induced by carnosine or BA supplementation) mitigates the high ROS/RNS production (as well as  
74 acute OS milieu condition) during exercise. In animal studies, carnosine and BA supplementation were shown  
75 to effectively mitigate the OS produced by exercise [15-17]. However, in human studies, the finding were  
76 unclear. For instance, both recreationally activity men [13] and women [12] who received BA  
77 supplementation had reduced LP after an acute bout of physical exercise (wen compared to pre-  
78 supplementation, but not to placebo condition). Although, in other studies with male athletes, carnosine [18]  
79 and BA [14] supplementation did not change/mitigate the increase in LP values after an acute bout of PE,  
80 despite increasing the GSH (Glutathione) antioxidant potential when compared to pre-treatment condition. Such  
81 studies from the same laboratory showed that improvement in antioxidant system seemed to occur only in  
82 women when compared to the pre-supplementation condition [12, 13], instead other laboratories shown  
83 improvement in men [14, 18]. Therefore, it is necessary to systematize and meta-analyze studies with humans  
84 to evaluate the effectiveness of BA or carnosine supplementation as an antioxidant during PE-induced OS. If

85 BA or carnosine is an efficient antioxidant, this results can shed light to the controversial results such as  
86 impairments in endurance physical capacity [19] and  $VO_2\text{máx}$  [20, 21] found in some endurance exercise  
87 studies.

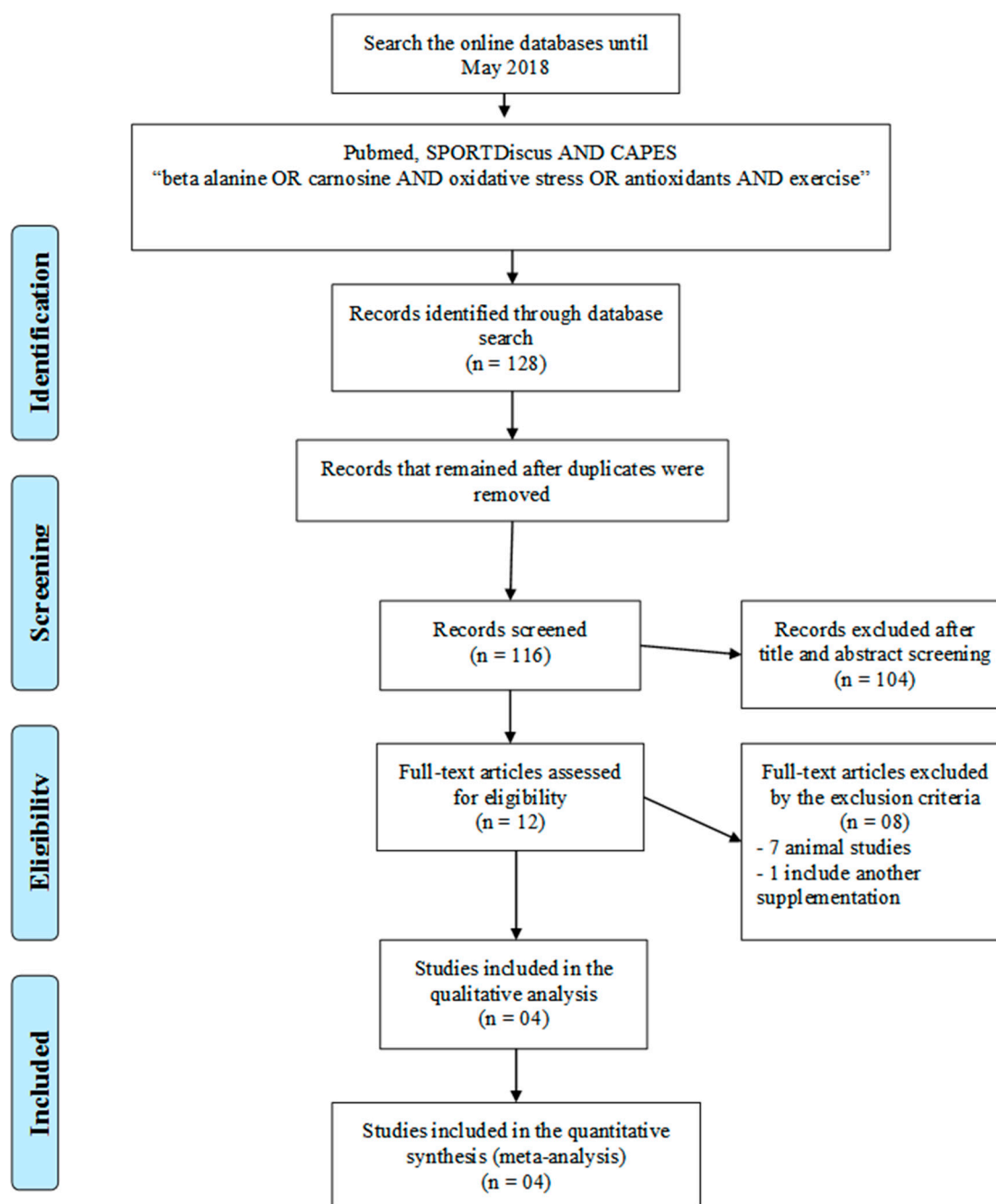
88 However, conditions for alterations in the antioxidant system and OS due to BA or carnosine  
89 supplementation were tested using different physical exercise interventions and enrolled participants with  
90 different physical fitness levels. In addition, different assessment times were used for PE-induced ROS/RNS  
91 and OS markers [8], also, different types of ROS, LP or antioxidant system markers assessed might have  
92 influenced the study's results [8]. In this sense, it is necessary to maintain the highest standards in relation to  
93 BA/carnosine supplementation on PE-induced ROS/RNS production and OS milieu. Thus, the purpose of this  
94 review to carry out a systematic meta-analysis of the randomized controlled studies that investigated the effects  
95 of BA or carnosine supplementation on antioxidant system, ROS and OS markers that are induced by PE in  
96 healthy individuals.

## 97 **2. Methods**

### 98 *2.1. Search Criteria*

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

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108

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

110

111 *2.2. Inclusion/Exclusion Criteria*

112 The inclusion criteria for the articles were (1) studies with randomized and controlled samples, (2)  
 113 language of publication either English, Portuguese or Spanish, (3) studies that performed intervention with  
 114 chronic BA ( $\geq 28$  days) or carnosine ( $> 14$  days) supplementation followed by acute PE (to induce OS). We  
 115 excluded studies that underwent other interventions in addition to BA (or carnosine) supplementation and PE  
 116 (e.g., chemotherapy, drugs or other types of antioxidant supplementation).

### 117 2.3. Identification of Eligible Studies

118 Randomized controlled studies with health human subjects that underwent chronic supplementation of BA  
119 or Carnosine ( $\geq 28$  days for BA and  $>14$  days for carnosine)[22] and have accessed OS or AO markers after  
120 acute PE. Dosage were  $\geq 1.2$  to  $\leq 6.4$  g daily for BA [23] and  $\geq 4$  g daily for carnosine supplementation [24],  
121 known as an athletic ergogenic dosage.

### 122 2.4. Data Extraction

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

### 131 2.5. Effect Size Calculation

132 For antioxidant system and OS markers (ROS/RNS, peroxidation and oxidation markers) outcome, effect  
133 size (ES) was calculated to represent the pre-exercise–post-exercise change, divided by the pre-exercise  
134 standard deviation (SD). A small sample bias adjustment was applied to each ES [25]. The following formula  
135 was used to calculate the ES with sample bias adjustment:

$$136 \quad d = \left(1 - \frac{3}{4(\text{Number of subjects} - 1) - 1}\right) \left(\frac{\text{Mean pre} - \text{Mean post}}{\text{Pre test standard deviation}}\right)$$

137 The variance around each ES was calculated using the sample size in each study and mean ES across all  
138 studies [26]. ES were classified as trivial ( $<0.2$ ), small ( $\geq 0.2$  to  $\leq 0.6$ ), moderate ( $\geq 0.6$  to  $\leq 1.2$ ), large ( $\geq 1.2$ )  
139 [27].

### 140 2.6. Statistical Analyses Results

141 Cconditions description (pre- vs. post-treatment change) are presented as mean ES followed by 95%  
142 confidence interval (CI).

143 Between conditions comparisons were performed using a random effects method. Data is displayed as  
144 mean difference with random effects, inverse of variance and 95% CI. Statistical heterogeneity of the treatment  
145 effects among studies were assessed using Cochran's Q test and the inconsistency  $I^2$  test, in which values above  
146 25% and 50% were considered indicative of moderate and high heterogeneity, respectively. Review manager  
147 5.3 was used to build the Forest plot graphs and used to carry out the statistical analysis.

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

155 Also, multiple sensitivity analyses were performed to determine if any of the results were influenced by  
156 the studies that were removed.

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### 159 3. Search results

160 The search of PubMed, SPORTDiscus and CAPES periodic provided a total of 128 citations (titles and  
161 abstracts were accessed). 116 articles were removed (both duplicates and articles that met the exclusion criteria).  
162 We examined the full text of the remaining 12 articles and only four articles [12-14, 18] were included in the  
163 review (Fig 1).

164 Seven out of eight studies excluded did not meet the criteria of human subjects (animal models were rats  
165 and mice). One study involved chronic training [4] or evaluated acute injected BA [28]. Two studies evaluated  
166 PE-induced OS, but had other antioxidants combined with BA [29] or carnosine [17] supplementation. One  
167 human study [30] was excluded because it used others AO combined with BA. Three other animal studies who  
168 were also excluded which evaluated PE-induced OS after BA/carnosine supplementation [15-17] and were  
169 therefore were used in the discussion of this review (Fig 1).

170

171 *3.1. Participant and Intervention Characteristics*

172 All studies used healthy young adults (mean age from four studies: 21y) who were sedentary,  
 173 recreationally active or trained participants. Only one study used women as subjects. Only one study used  
 174 carnosine supplementation, while the other three studies used BA supplementation. All supplementation  
 175 protocols employed chronic treatment, being 28 days for BA supplementation and 16 days for carnosine  
 176 supplementation (Table 1).

177 Exercise-induce EO involved classic Wingate test (short all-out high-intensity repeated bouts), moderate  
 178 endurance-running (70-75% of VO<sub>2</sub>max) and short high-intensity one bout (2000-m run time trial type)  
 179 exertion. All physical exercise interventions successfully and significantly induced OS (Table 1).

180

181 *3.2. Antioxidant, direct and indirect OS assessment after BA or carnosine supplementation in exercise-*  
 182 *induced oxidative stress*

183 As direct OS markers were ROS and RNS (i.e., H<sub>2</sub>O<sub>2</sub>, Hydrogen peroxide, 3-Nitro, 3-nitrotyrosine and  
 184 nitric oxide) and PL or molecules oxidation markers as indirect OS (i.e., 8-ISO, 8-isoprostane; MDA,  
 185 malondialdehyde and; PC, protein carbonyl, GSSG, oxidized glutathione). Antioxidant markers were GSH  
 186 (glutathione), SOD (superoxide dismutase) and TAC (total antioxidant capacity. All assessment were from  
 187 blood samples. Therefore, DNA (8-ISO), protein (PC) and cell damage (3-Nitro) as well as lipid peroxidation  
 188 (MDA) were assessed as indirect markers of OS. H<sub>2</sub>O<sub>2</sub> and NO were assessed as direct OS markers. SOD was  
 189 assessed as endogenous AO; TAC, GSH and GSSG were assessed as exogenous AO. All four studies evaluated  
 190 PE-Induced OS post-supplementation immediately after exercise. Three out of four studies repeated the  
 191 assessment after 30 min [18], 2h, 4h [12, 13], 24h and 48h [18] post exercise (Table 1).

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

Study	Experimental design	Exercise training or Exercise induce OS	OS or AO markers (method of assessment)
Belviranli at al. [14]	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	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	GSSG, PC and MDA, SOD; SOD, TAC and GSSG (colorimetric assay)*



	plasma OS and AO markers were analyzed before and after Wingate test (WTs) sessions.	period of supplementation	
Smith-Ryan et al. [13]	25 healthy recreationally active males (age, $21.9 \pm 3.4$ y; height, $177.6 \pm 5.4$ cm; weight, $78.8 \pm 9.7$ kg) randomly assigned to 28 days of PL or BA (1,6g 3x day, sustained release) supplementation. Blood plasma OS and AO 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 (ELISA)*; SOD, TAC, and GSH (colorimetric assay)*
Smith-Ryan et al. [12]	26 healthy recreationally active women (age, $21.7 \pm 1.9$ y; height, $165.0 \pm 5.7$ cm; weight, $61.9 \pm 6.7$ kg) randomly assigned to 28 days of PL or BA (1,6g 3x day, sustained release) supplementation. Blood plasma OS and AO 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 (ELISA)*; SOD, TAC and GSH (colorimetric assay)*
Slowinska-Lisowska et al. [18]	14 elite kayakers and canoeists athletes (age, $21.2 \pm 1.3$ y; height, $177.4 \pm 7.9$ cm; weight, $78.9 \pm 8.9$ kg) in a crossover way assigned to 16 days of PL and Carnosine (2g 2x day) supplementation. Washout was four weeks. Blood plasma OS and AO 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).	GSH <sup>#</sup> , GSSG <sup>#</sup> , TAC <sup>#</sup> , NO <sup>#</sup> , H <sub>2</sub> O <sub>2</sub> <sup>#</sup> and SOD* (colorimetric assay); 8-ISSO* and 3-Nitro <sup>#</sup> (ELISA).

193 **Note:** 3-Nitro, 3-nitrotyrosine; 8-ISO, 8-isoprostane; BA, beta-alanine; GSH, glutathione; GSSG, oxidized  
 194 glutathione; H<sub>2</sub>O<sub>2</sub>, Hydrogen peroxide; MDA, malondialdehyde; OS, oxidative stress; PC, protein carbonyl; PL,  
 195 placebo; SOD, superoxide dismutase; TAC, total antioxidant capacity. Symbols (\*, #) represent the same fabricant  
 196 commercial assay kit.

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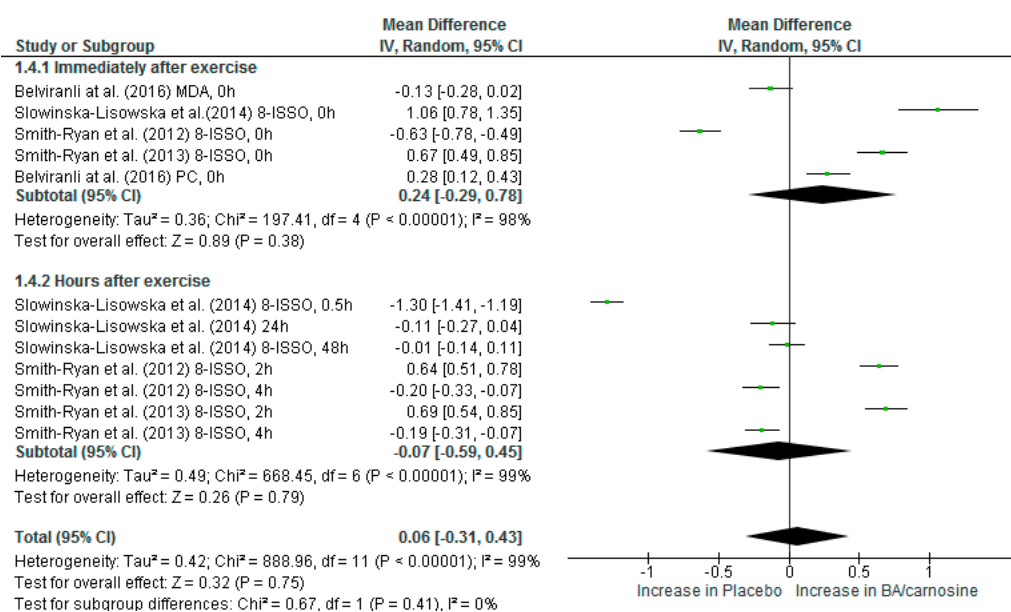
### 198 3.3. Meta-analysis

#### 199 3.3.1. Oxidative stress markers

200 Exercise induced large increase in indirect OS markers (PC, MDA, 8-ISO) in both conditions, immediately  
 201 after exercise (BA/carnosine ES= -1.32, 95% CI -3.52 to 0.88; placebo ES= -1.07, 95% CI -2.63 to 0.49) and  
 202 moderate decrease hours after exercise (BA/carnosine ES= 0.61, 95% CI -0.12 to 1.35; placebo ES= 0.54, 95%  
 203 CI -0.35 to 1.44)). Overall between conditions comparison (pooled ES) suggest no difference between  
 204 conditions (difference ES= 0.06, 95% CI -0.38 to 0.50,  $p=0.80$ ,  $I^2=99\%$ ) in response to exercise. Also, there

205 is no difference immediately after exercise (difference ES: 0.24, 95% CI -0.29 to 0.78,  $p=0.38$ ) or hours after  
 206 exercise (difference ES: -0.07, 95% CI -0.59 to 0.45,  $p=0.79$ ).

207



208

209 **Figure 2.** Forest plot of the indirect oxidative stress markers induced by physical exercise after  
 210 BA/carnosine or placebo supplementation. Acronyms: 3-Nitro, 3-nitrotyrosine; 8-ISO, 8-isoprostane;  
 211 MDA, malondialdehyde; PC, protein carbonyl. Note: Autor's name and year of study publication is  
 212 followed by the oxidative stress marker and moment (hours) of assessment after exercise.

213

214 Independent analysis of 8-ISSO showed a large increase in immediately after PE in both condition  
 215 (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  
 216 moderate decrease in both conditions following hours after PE (BA/carnosine ES= 0.62, 95% CI -0.12 to 1.35;  
 217 placebo ES= 0.54, 95% CI -0.35 to 1.45). Between condition comparison reveal non-significant immediately  
 218 after exercise (difference ES= 0.36, 95% CI -0.70 to 1.42,  $p=0.51$ ,  $I^2=99\%$ ) or hours after exercise (difference  
 219 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  
 220 ( $I^2=0\%$ ,  $p=0.48$ ), however when we exclude the Smith et al. [12] study (00 hour post exercise), there is a  
 221 significant effect of time of assessment ( $I^2=87.2\%$ ,  $p<0.01$ ), indicating a decrease in plasma 8-ISSO  
 222 concentration hours after exercise.

223

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

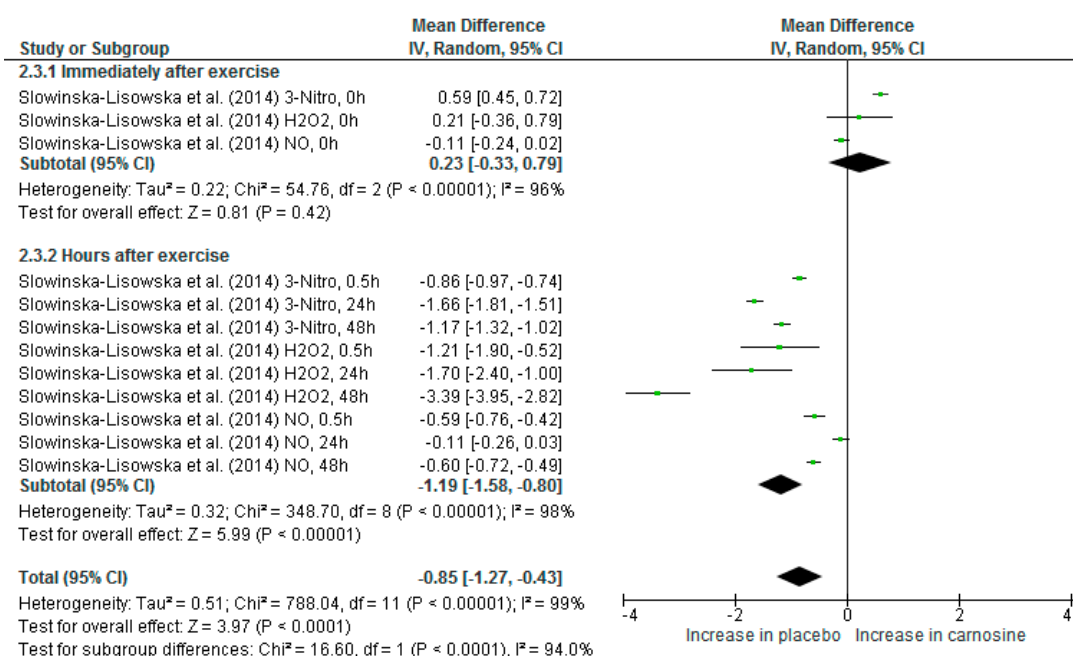
224

Only the study by Slowinska-Lisowska et al. [18] performed direct OS markers assessment immediately

225

after plasma collection. Data reanalysis of this study (Fig 3) suggests that exercise induced large increase in

226 direct OS markers (ROS/RNS) in both conditions, immediately after exercise (BA/carnosine ES= -1.22, 95%  
 227 CI -1.77 to -0.68; placebo ES= -0.89, 95% CI -1.48 to -0.29). However, hours after exercise increase in  
 228 ROS/RNS were moderate for carnosine and large for placebo condition (BA/carnosine ES= -1.02, 95% CI -  
 229 1.49 to -0.46; placebo ES= -1.74, 95% CI -2.12 to -1.36). Comparisons between conditions suggested that  
 230 carnosine did not mitigate the increase in ROS/RNS production immediately after exercise (difference ES: 0.23,  
 231 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  
 232 involving the later hours after the exercise, carnosine was shown to mitigate the increase in ROS/RNS  
 233 (difference ES= -1.19, 95% CI -1.48 to -0.80,  $p < 0.01$ ,  $I^2 = 98\%$ ). There is a significant sub-group (immediately  
 234 after exercise vs. hours after exercise) difference ( $I^2 = 94\%$ ,  $p < 0.01$ ) on ROS/RNS markers, see Fig 3.



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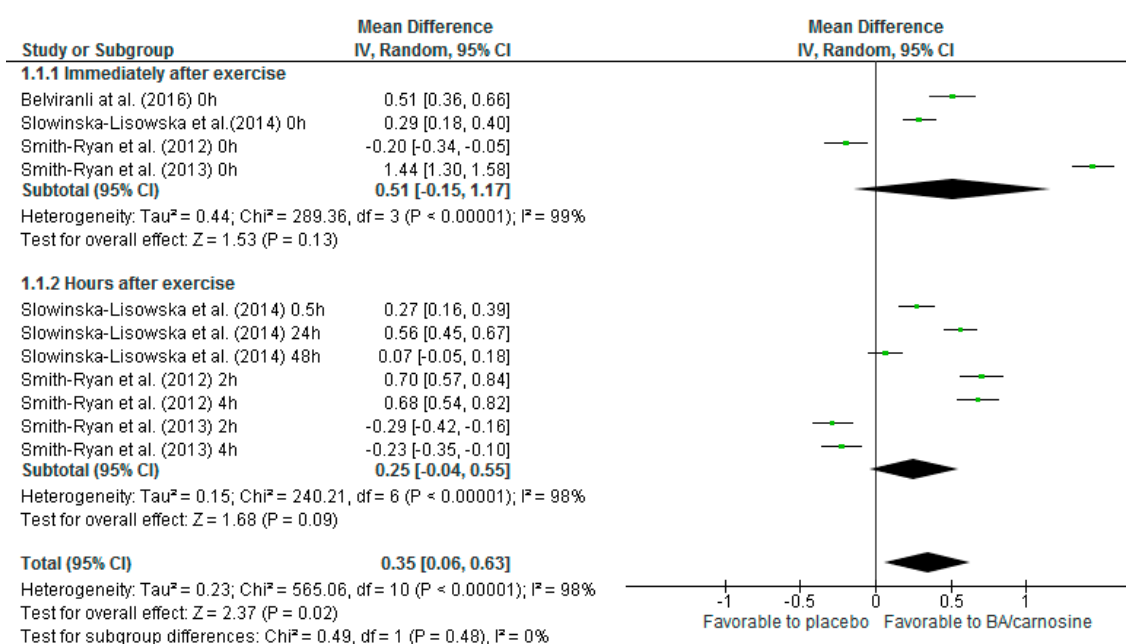
236 **Figure 3.** Forest plot of the plasma reactive oxygen and nitrogen species induced by physical exercise  
 237 after carnosine or placebo supplementation. Acronyms: 3-Nitro, 3-nitrotyrosine; H2O2, Hydrogen  
 238 peroxide; NO, nitric oxide. Note: Autor's name and year of study publication is followed by the oxidative  
 239 stress marker and moment (hours) of assessment after exercise.

240

### 241 3.3.2. Antioxidants

242 ES suggests that there was a moderate increase in TAC concentration in BA/carnosine supplementations  
 243 (ES= -0.66, 95% CI -1.44 to 0.12), whereas a trivial decrease occurred in placebo supplementation (ES= 0.08,  
 244 95% CI -0.78 to 0.95) immediately after exercise, but without significant difference between them (difference

245 ES= 0.51, 95% CI -0.15 to 1.17,  $p= 0.13$ ,  $I^2=99\%$ ). Hours after exercise BA/carnosine presented a trivial  
 246 increase (ES= -0.13, 95% CI -0.78 to 0.52) and a similar small decrease occurred in the placebo condition (ES=  
 247 0.12, 95% CI -0.42 to 0.66) which showed a tend to difference between then (difference ES= -0.25, 95% CI -  
 248 0.04 to 0.55,  $p= 0.09$ ,  $I^2=98\%$ ). Overall between conditions comparison (pooled ES) suggests that BA/carnosine  
 249 supplementation increases overall TAC (difference ES= 0.35, 95% CI 0.06 to 0.65,  $p= 0.02$ ,  $I^2= 99\%$ ; Fig 4) in  
 250 response to exercise.  
 251



252

253 **Figure 4.** Forest plot of the total antioxidant capacity (TAC) change by physical exercise after  
 254 BA/carnosine or placebo supplementation. Note: Autor's name and year of study publication is followed  
 255 by the moment (hours) of assessment after exercise.

256

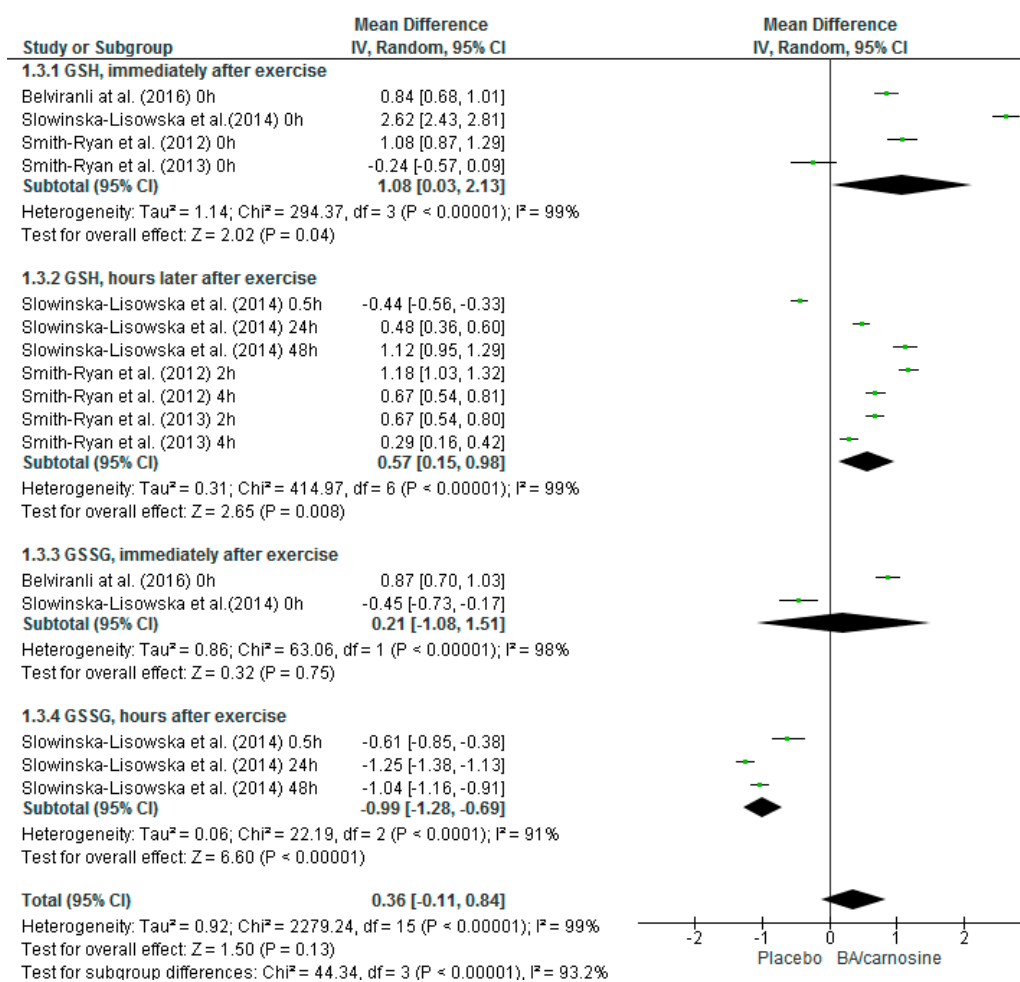
257 Immediately after exercise there were a trivial and a large GSH decreases in both conditions (BA/carnosine  
 258 ES= 0.16, 95% CI -4.68 to 4.99; placebo ES= 1.23, 95% CI -2.00 to 4.44, respectively). There were also a  
 259 moderate and a trivial increase following hours after exercise (BA/carnosine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

263

264 Also, GSSG independent analysis suggests large and moderate decreases in GSSG concentrations  
 265 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,

266 95% CI, -1.08 to 1.51,  $p=0.75$ ,  $I^2=98\%$ ), but a significant lower GSSG concentration hours after PE in  
 267 BA/carnosine condition (difference ES= -0.99, 95% CI, -1.28 to -0.69,  $p<0.01$ ,  $I^2=76\%$ ). Sub-group analysis  
 268 (immediately after exercise vs. hours after exercise) indicates a significant effect of time of assessment [ $I^2=$   
 269 83.7%,  $p=0.01$ (Fig 5)].

270 Test for subgroup difference ( $p<0.00001$ ,  $I^2=93.2\%$ ) indicates a change in GHS/GSSG ratio (Fig 5)  
 271 favorable to BA/carnosine condition.



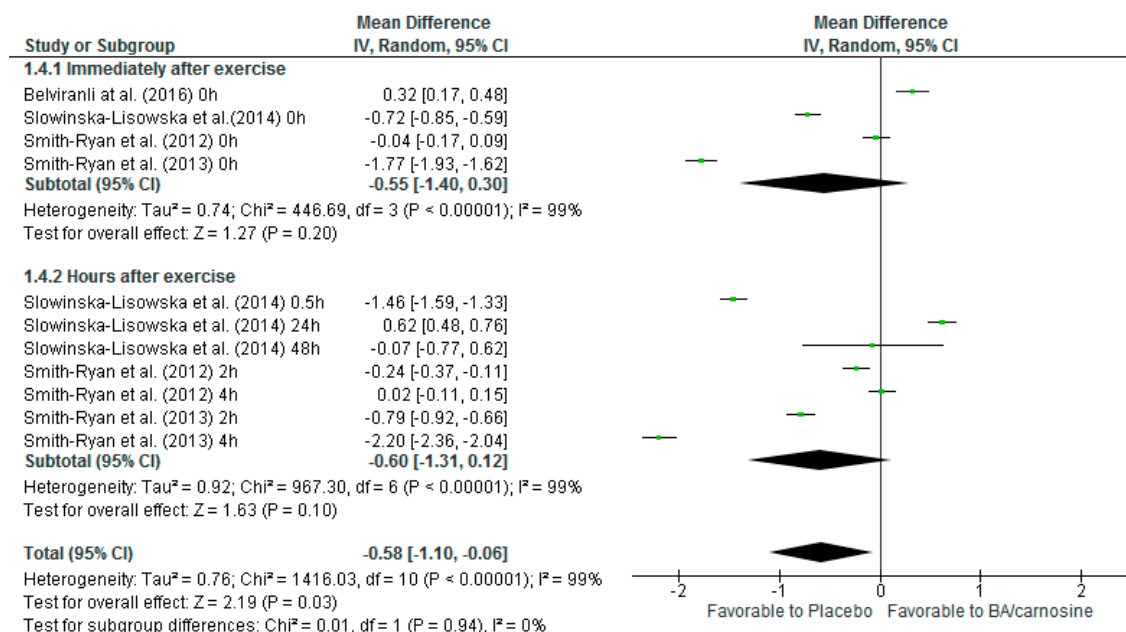
272

273 **Figure 5.** Forest plot of the glutathione (GSH) and oxidised glutathione (GSSG) ratio change by  
 274 physical exercise after BA/carnosine or placebo supplementation. Note: Autor's name and year of study  
 275 publication is followed by the moment (hours) of assessment after exercise.

276

277 Immediately after exercise, there were a trivial and a small increase in SOD activity in both conditions  
 278 (BA/carnosine ES= -0.02, 95% CI -1.15 to 1.12; placebo ES= -0.50, 95% CI -1.29 to 0.30, respectively).  
 279 Following hours after exercise, there were large increases in SOD activity for both conditions (BA/carnosine  
 280 ES= -1.39, 95% CI -4.21 to 1.41; placebo ES= -1.72, 95% CI -4.39 to 0.96). Overall between conditions

281 comparison showed that the placebo presented a moderate and significantly greater SOD activity (differences  
 282 ES= -0.58, 95% CI -1.10 to -0.06,  $p = 0.03$ ,  $I^2 = 99\%$ ; Fig 6) when compared to BA/carnosine supplementation.  
 283



284  
 285 **Figure 6.** Forest plot of the superoxide dismutase change by physical exercise after BA/carnosine  
 286 or placebo supplementation. Note: Autor's name and year of study publication is followed by the moment  
 287 (hours) of assessment after exercise.

288

### 289 3.3.3. Heterogeneity studies, multiple linear regression analysis and risk of bias.

290 Multiple linear regression shows that in indirect OS markers (8-ISO, MDA, GSSG and PC) the time of  
 291 assessment, marker type evaluated, exercise type and training status could explain 65% of ES variation ( $R^2 =$   
 292 0.650,  $p = 0.000$ ). Sex and supplementation conditions (BA or carnosine) were excluded from the model.

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

296 It was not possible to perform multiple linear regression for ROS/RNS direct markers ( $\text{H}_2\text{O}_2$ , 3-Nitro and  
 297 NO) due to insufficient data.

298 The four studies present less than three reported high or unclear risk domains (Appendix 2). Two studies  
299 (two high risk) are from the same laboratory and was unable to bling for BA condition (due to paresthesia  
300 effect).

#### 301 4. Discussion

302 The four studies included in this review observed significant increases in OS after acute physical exercise  
303 bouts. Our analyses suggest that immediately after PE-induced OS, BA or carnosine supplementation did not  
304 undermine the increase in both ROS an RNS ( $H_2O_2$ , 3-Nitro and NO) or peroxidation (8-ISSO, MDA, and PC)  
305 markers that were produced. Monitoring their levels during hours after exercise (0.5 to 48h), BA or carnosine  
306 did not appear to impose a greater decrease in 8-ISSO ( $p > 0.05$ ) when compared to placebo supplementation.  
307 Interestingly, monitoring OS levels after hours (0.5 to 48h) of PE-induced OS, carnosine treatment mitigated  
308 the increase of  $H_2O_2$ , 3-Nitro and NO production. It is important to mention that ROS/RNS ( $H_2O_2$ , 3-Nitro and  
309 NO) data were obtained from only one study (Slowinska-Lisowska et al. 2014), but such data were in  
310 accordance with previous in vitro studies [31, 32].

311 Evidence suggests that the largest post-exercise changes involving lipid, protein, glutathione and DNA  
312 oxidation occurred 1-4 days after PE (when compared with blood samples of resting condition) [8]. For instance,  
313 in an animal study that assessed PE-induced OS after 24h, it was shown that BA or carnosine supplementation  
314 decreased LP (thiobarbituric acid reactive substances and MDA markers) in skeletal muscle tissue [15, 16].  
315 The only publication that evaluated 24h post-exercise was the Slowinska- Lisowska et al. [18] study. Therefore,  
316 studies with a long follow-up period (days to weeks), thus with sufficient time to resolve an acute inflammation  
317 caused by moderate-intense exercise [33] are needed to verify whether BA or carnosine may promote clinical  
318 changes in the peroxidation markers.

319 Previous reviews [1] and recent animal studies [2-4] had already presented an antioxidant role of  
320 carnosine. When compared to placebo, our data suggested that previous BA or carnosine supplementation  
321 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  
322 0.32 to 1.19,  $p = 0.0007$ ) after PE-induced OS. These data corroborate with an animal study [16] submitted to  
323 PE-induced OS. Such study reported increased in GSH and decreased glutathione peroxidase (GPx) and  
324 glutathione reductase after exercise, suggesting that carnosine has buffering the  $H_2O_2$  production. The effect  
325 of BA and carnosine supplementation on GSSG concentrations is conflicting. Belviranli et al. [14] reported  
326 increased GSSG after PE-induced OS in sedentary individuals supplemented with BA (suggesting GSH  
327 oxidation); on the other hand, Slowinska-Lisowska et al. [18] reported decreased GSSG concentrations in



328 trained individuals supplemented with carnosine (suggesting a carnosine antioxidant effect). More research is  
329 needed to highlight the effect of BA/carnosine on GSH/GSSG ratio.

330 Both plasma TAC and GSH presented a large variation in the studies [12-14, 18], as evidenced by high  
331 heterogeneity (see Fig 4 and 5). Plasma antioxidants evaluation such as GSH and TAC after exercise practice  
332 yields conflicting results [8], however, analyzing the results from the four studies, it seems that the increase in  
333 muscle carnosine concentration influences these changes. GSH can be delivered to plasma from several tissues  
334 and this is influenced by the type of activity exerted as well as by the nutritional status of the participants [8],  
335 so, future human studies need to assess GSH from specific tissues known for its large pools of carnosine (such  
336 as the skeletal muscle) [16]. TAC assays have a limited capacity to measure the total antioxidant system  
337 capacity, excluding, for instance, the contribution of antioxidant enzymes and metal binding proteins, so  
338 changes in the TAC values probably does not reflect the carnosine antioxidant content activity in the organism.

339 Our data suggests that BA or carnosine supplementation can mitigate the increase of SOD activity (ES = -  
340 0.58,  $p=0.03$ ), a well-known superoxide scavenger. It is plausible that this attenuated increase of SOD activity  
341 occurs due to carnosine antioxidant effect (e.g.,  $O_2^{\cdot-}$  clearance). In vitro studies have shown that carnosine  
342 plays an effective role in decreasing ROS and RNS (e.g.  $H_2O_2$ , superoxide and NO) [31, 32]. Studies with  
343 animal training also has demonstrated that carnosine or BA supplementation mitigated SOD [19] and GPx [16]  
344 activity, when compared to control conditions. Such data are contrary to untrained animal studies [2, 3], which  
345 showed increased activity of these enzymes and decrease in PL. Such discrepancy suggest that carnosine/BA  
346 supplementation enhance antioxidant system at rest condition (*i.e.*, sedentary life style), but not during/after  
347 acute exercise. Therefore, it appears that BA or Carnosine supplementation might mitigate the increase in SOD  
348 and GPx activity induced by exercise, but has opposite effect in rest condition. Further studies are needed to  
349 explore these conflicting results. Also, further studies are needed to verify if chronic BA supplementation might  
350 down-regulate the endogenous antioxidant system during physical training.

351 The results observed in this review suggest that increase SOD activity (induced by PE) is mitigated, this  
352 occur probably due to the ability of carnosine to directly decrease ROS concentrations. Interestingly, carnosine  
353 supplementation associated with endurance training (in rats) decreased exercise tolerance (at 2 wks of training)  
354 and both SOD and lactate dehydrogenase activity in the skeletal muscle (at 4 wks of training) [19]. Therefore,  
355 future studies are needed to verify (both in an acute and chronic settings) if the changes promoted, such as  
356 increased gene expression of enzymes from the endogenous antioxidant system induced by physical exercise  
357 [9] are mitigated in the presence of BA or carnosine supplementation, as it is observed in studies with chronic  
358 [7] or acute antioxidant supplementation [28]. Moreover, BA supplementation is a well-known ergogenic agent



359 in anaerobic exercises, but not in endurance exercises [20, 34]. For instance, early evidence in human studies  
360 suggest that BA supplementation delayed lactate production, but reduce aerobic capacity [21]. Therefore, it is  
361 important to investigate if BA or Carnosine supplementation might influences negatively endurance adaptations  
362 because of their antioxidant effects [11].

363 Our ES evaluations (with antioxidant and oxidative stress markers) showed high heterogeneity. This meta-  
364 analysis pooled together studies with participants from different fitness level, enrolled in different PE-induced  
365 OS, also, different time points of different oxidative stress markers or antioxidant markers were pooled in the  
366 same ES analysis. It is well-known that time-point assessment of PE-induced OS as well as the rising in blood  
367 plasma of both oxidative stress markers or antioxidant markers are also time-dependent and this might influence  
368 our results [8]. Our sub-group analysis (immediately after exercise vs. hours after exercise- 0.5 to 48 hours)  
369 showed that the moment of assessment for both indirect (Fig 3) and direct (Fig 4) OS markers is an important  
370 confounding variable. Also, multivariable regression shows that time of assessment, the OS marker type  
371 evaluated, the exercise type and training status can explain 65% of ES variation ( $R^2= 0.650$ ,  $p= 0.000$ ). Sub-  
372 groups analysis for antioxidant (TAC, SOD, and GSH) markers did not show significant influence of time  
373 assessment. But, multivariable regression shows that only 39% ( $R^2= 0.389$ ,  $p< 0.000$ ) of ES variation from  
374 antioxidant results were from time of assessment, exercise test, training status and anti-oxidant type evaluated.  
375 This suggest that other variables (e.g. nutritional status or antioxidant system status) may be influencing this  
376 heterogeneity in antioxidant results [11]. For example, no study included in this meta-analysis mentioned that  
377 their samples were homogenized for OS or antioxidant status (deficient in oxidant status or not), so future  
378 studies with antioxidants supplementation need to homogenize their samples as deficient or not for the  
379 antioxidant system [11].

380 Practical applications can be drawn from this review. Data from this review suggests that of BA/carnosine  
381 supplementation is effective in improving the GSH/GSSG ratio, increasing TAC and decreasing ROS/RNS after  
382 PE. Therefore, by identifying these deficiencies in the antioxidant system, supplementation with these  
383 substances can help this system to suppress the exacerbate OS induced by PE. Also, future research with  
384 BA/carnosine supplementation needs to first check whether its volunteers have antioxidant system deficiencies,  
385 as this may affect the rugeness of the study [11].

#### 386 4.1. Limitations

387 This meta-analysis has several limitations. First there are only four studies, two of which are from the  
388 same laboratory, decreasing the validity and reliability of the results. Second, we included in the same analysis

389 BA and carnosine studies, the results of the carnosine study significantly influence our TAC results, but do not  
390 significantly alter the results of SOD, GSH or OS markers, in addition, meta-regression excluded the type of  
391 supplement (i.e., BA or carnosine) used as a source of heterogeneity, so BA or carnosine is not a source of  
392 heterogeneity. Third, the high heterogeneity found in this study because the studies analyzed different levels of  
393 fitness, sex and different exercise intensity/volume also decrease the reproducibility of these data, but give  
394 further evidences that these variables differ in responses to PE-induced OS. And finally, all four studies that  
395 performed the assessment of both antioxidant and OS markers in the plasma did it with the assumption that  
396 plasma measurements would reflect systemic changes [8]. Animal studies have found consistent changes in the  
397 antioxidant/oxidant ratio (in the exercise-induced ROS production) in the skeletal muscle after BA or carnosine  
398 supplementation [15-17], as the main stores of carnosine in humans are in the skeletal muscle (99%) [1], future  
399 studies need to verify such change at skeletal muscle level.

## 400 **5. Conclusions**

401 In conclusion, BA or carnosine supplementation seems to increase TAC and improve GSH/GSSG ratio,  
402 but decrease SOD activity following PE-induced OS. Also, albeit it mitigates the acute increase in ROS/RNS,  
403 it does not decrease peroxidation markers.

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407

408 **References**

409

- 410 [1] Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine.  
411 *Physiological reviews*. 2013;93:1803-45.
- 412 [2] Kim MY, Kim EJ, Kim Y-N, Choi C, Lee B-H. Effects of  $\alpha$ -lipoic acid and L-carnosine  
413 supplementation on antioxidant activities and lipid profiles in rats. *Nutrition research and*  
414 *practice*. 2011;5:421-8.
- 415 [3] Ma X, Jiang Z, Lin Y, Zheng C, Zhou G. Dietary supplementation with carnosine  
416 improves antioxidant capacity and meat quality of finishing pigs. *Journal of animal*  
417 *physiology and animal nutrition*. 2010;94.
- 418 [4] Schaalan MF, Ramadan BK, Abd Elwahab AH. Synergistic effect of carnosine on  
419 browning of adipose tissue in exercised obese rats; a focus on circulating irisin levels. *Journal*  
420 *of cellular physiology*. 2018;233:5044-57.
- 421 [5] Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *Biochimica*  
422 *et Biophysica Acta (BBA)-Biomembranes*. 2004;1660:171-99.
- 423 [6] Tan DQ, Suda T. Reactive Oxygen Species and Mitochondrial Homeostasis as Regulators  
424 of Stem Cell Fate and Function. *Antioxidants & Redox Signaling*. 2018;29:149-68.
- 425 [7] He F, Li J, Liu Z, Chuang C-C, Yang W, Zuo L. Redox mechanism of reactive oxygen  
426 species in exercise. *Frontiers in Physiology*. 2016;7.
- 427 [8] Nikolaidis MG, Jamurtas AZ, Paschalis V, Fatouros IG, Koutedakis Y, Kouretas D. The  
428 effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress. *Sports*  
429 *Medicine*. 2008;38:579-606.
- 430 [9] Henríquez-Olguín C, Díaz-Vegas A, Utreras-Mendoza Y, Campos C, Arias-Calderón M,  
431 Llanos P, et al. NOX2 inhibition impairs early muscle gene expression induced by a single  
432 exercise bout. *Frontiers in physiology*. 2016;7:282.
- 433 [10] Margaritelis NV, Theodorou AA, Paschalis V, Veskoukis AS, Dipla K, Zafeiridis A, et  
434 al. Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting  
435 redox interindividual variability. *Acta Physiologica*. 2018;222:e12898.
- 436 [11] Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Antioxidants  
437 in Personalized Nutrition and Exercise. *Advances in Nutrition*. 2018.
- 438 [12] Smith A, Stout J, Kendall K, Fukuda D, Cramer J. Exercise-induced oxidative stress:  
439 the effects of  $\beta$ -alanine supplementation in women. *Amino Acids*. 2012;43:77-90.
- 440 [13] Smith-Ryan AE, Fukuda DH, Stout JR, Kendall KL. The influence of  $\beta$ -alanine  
441 supplementation on markers of exercise-induced oxidative stress. *Applied Physiology,*  
442 *Nutrition, and Metabolism*. 2013;39:38-46.
- 443 [14] Belviranlı M, Okudan N, Revan S, Balci S, Gokbel H. Repeated supramaximal exercise-  
444 induced oxidative stress: effect of  $\beta$ -alanine plus creatine supplementation. *Asian journal of*  
445 *sports medicine*. 2016;7.
- 446 [15] Dawson Jr R, Biasetti M, Messina S, Dominy J. The cytoprotective role of taurine in  
447 exercise-induced muscle injury. *Amino acids*. 2002;22:309-24.

- 448 [16] Bortolatto GP. Efeito da carnosina e da  $\beta$ -alanina no dano produzido pelo exercício  
449 intenso no músculo sóleo de ratos: Universidade Estadual Paulista Júlio de Mesquita Filho;  
450 2015.
- 451 [17] Seifulla N, Kovalev I, Rozhkova E, Paniushkin V. [Effect of carnosine and its  
452 combination with essential amino acids on lipid peroxidation and work capacity of experimental  
453 animals]. *Eksperimental'naiia i klinicheskaia farmakologiya*. 2004;68:44-6.
- 454 [18] Slowinska-Lisowska M, Zembron-Lacny A, Rynkiewicz M, Rynkiewicz T, Kopec W.  
455 Influence of l-carnosine on pro-antioxidant status in elite kayakers and canoeists. *Acta*  
456 *Physiologica Hungarica*. 2014;101:461-70.
- 457 [19] Lee K-N, Hue J-J, Miga M, Kang BS, Kim J-S, Nam SY, et al. Effects of Carnosine on  
458 Exercise Capacity in ICR Mice. *Laboratory Animal Research*. 2008;24:617-22.
- 459 [20] Brisola GMP, Redkva PE, Pessôa Filho DM, Papoti M, Zagatto AM. Effects of 4 weeks  
460 of  $\beta$ -alanine supplementation on aerobic fitness in water polo players. *PloS one*.  
461 2018;13:e0205129.
- 462 [21] Jordan T, Lukaszuk J, Misic M, Umoren J. Effect of beta-alanine supplementation on  
463 the onset of blood lactate accumulation (OBLA) during treadmill running: Pre/post 2  
464 treatment experimental design. *Journal of the International Society of Sports Nutrition*.  
465 2010;7:20-.
- 466 [22] Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, et al. The absorption  
467 of orally supplied  $\beta$ -alanine and its effect on muscle carnosine synthesis in human vastus  
468 lateralis. *Amino Acids*. 2006;30:279-89.
- 469 [23] Caruso J, Charles J, Unruh K, Giebel R, Learmonth L, Potter W. Ergogenic Effects of  
470  $\beta$ -Alanine and Carnosine: Proposed Future Research to Quantify Their Efficacy. *Nutrients*.  
471 2012;4:585.
- 472 [24] Maemura H, Goto K, Yoshioka T, Sato M, Takahata Y, Morimatsu F, et al. Effects of  
473 carnosine and anserine supplementation on relatively high intensity endurance performance.  
474 *International Journal of Sport and Health Science*. 2006;4:86-94.
- 475 [25] Morris SB. Estimating Effect Sizes From Pretest-Posttest-Control Group Designs.  
476 *Organizational Research Methods*. 2008;11:364-86.
- 477 [26] Borenstein M, Hedges LV, Higgins JP, Rothstein HR. *Introduction to meta-analysis*:  
478 John Wiley & Sons; 2011.
- 479 [27] Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports  
480 medicine and exercise science. *Medicine+ Science in Sports+ Exercise*. 2009;41:3.
- 481 [28] Vereshchaka IV, Bulgakova NV, Maznychenko AV, Gonchar OO, Prylutskyy YI, Ritter  
482 U, et al. C60 Fullerenes Diminish Muscle Fatigue in Rats Comparable to N-acetylcysteine or  
483  $\beta$ -Alanine. *Frontiers in Physiology*. 2018;9.
- 484 [29] Pence BD, Gibbons TE, Bhattacharya TK, Mach H, Ossyra JM, Petr G, et al. Effects of  
485 exercise and dietary epigallocatechin gallate and  $\beta$ -alanine on skeletal muscle in aged mice.  
486 *Applied Physiology, Nutrition, and Metabolism*. 2015;41:181-90.
- 487 [30] Rozhkova E, Ordzhonikidze Z, Druzhinin A, Seifulla N, Paniushkin V, Kuznetsov I.  
488 Using carnosine and natural antioxidants for the prophylaxis of acute post-loading oxidative  
489 stress. *Eksperimental'naiia i klinicheskaia farmakologiya*. 2007;70:44-6.

- 490 [31] Nicoletti VG, Santoro AM, Grasso G, Vagliasindi LI, Giuffrida ML, Cuppari C, et al.  
491 Carnosine interaction with nitric oxide and astroglial cell protection. *Journal of neuroscience*  
492 *research*. 2007;85:2239-45.
- 493 [32] Miceli V, Pampalone M, Frazziano G, Grasso G, Rizzarelli E, Ricordi C, et al. Carnosine  
494 protects pancreatic beta cells and islets against oxidative stress damage. *Molecular and*  
495 *Cellular Endocrinology*. 2018;474:105-18.
- 496 [33] Ho ATV, Palla AR, Blake MR, Yucel ND, Wang YX, Magnusson KEG, et al.  
497 Prostaglandin E2 is essential for efficacious skeletal muscle stem-cell function, augmenting  
498 regeneration and strength. *Proceedings of the National Academy of Sciences*.  
499 2017;114:6675-84.
- 500 [34] Trexler ET, Smith-Ryan AE, Stout JR, Hoffman JR, Wilborn CD, Sale C, et al.  
501 International society of sports nutrition position stand: Beta-Alanine. *Journal of the*  
502 *International Society of Sports Nutrition*. 2015;12:1-14.