 doi:10.20944/preprints201811.0189.v3

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

Elias de França[1], Fábio Santos Lira[2], Marcio Flávio Ruaro[1], Vinicius Barroso Hirota[3], Paula A. Faria Waziry[4,5], André Rinaldi Fukushima[1,6], Maria Luiza de Jesus Miranda[1], Érico Chagas Caperuto[1]*

[1] GEPAME – Metabolism of exercise Research and Study group, Department of Physical Education, São Judas University, São Paulo, Brazil
[2] Exercise and Immunometabolism Research Group, Department of Physical Education, São Paulo State University (UNESP), Presidente Prudente, Brazil
[3] Universidade Estácio de Sá, Carapicuiba, Brazil
[4] Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, Florida, United States of America
[5] Veterans Affairs Medical Center, Miami, Florida, United States of America
[6] Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil.

* Correspondence: ericocaperuto@gmail.com

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). Before May 2018 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. A total of 128 citations were found. Only four articles met criteria for inclusion. All four studies used healthy young sedentary, recreationally active or athletic participants. After a chronic 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 direct OS 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 indirect OS 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 direct OS species (reactive nitrogen and oxygen species), treatment did not decrease measured values of indirect OS markers (peroxidation or molecule oxidation).

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

1. Introduction

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

 doi:10.20944/preprints201811.0189.v3

(CAT), superoxide dismutase (SOD) activity and reduce lipid peroxidation (LP) [1-4]. All of these changes (in CAT, GPx and SOD) are important for improvement of antioxidant system and simultaneous reduction of oxidative stress (OS) [5]. Antioxidant supplementation is commonly prescribed in disease that presents elevated ROS and RNS (reactive oxygen and nitrogen species, respectively) production, with the intention to improve the antioxidant system and decrease the OS. However, both ROS and RNS are necessary to cellular function, although its high production is detrimental, at the same time their low production is also detrimental to cellular function [6]. Therefore, the prescription of antioxidants cannot be indiscriminate.

Acute physical exercise (PE) is known to induce high ROS/RNS production and consequently to promote an acute OS milieu [7,8]. Recent evidence has suggested that the acute increase in ROS/RNS production during PE is necessary to promote adaptations (e.g., improve athletic performance and VO_2max) and the improvement in the antioxidant system itself [9,10]. It is also suggested that the use of exogenous antioxidants may be counterproductive in individuals who already have a balanced oxidant/antioxidant system [11]. However, beta-alanine (BA; a rate-limiting precursor in the synthesis of carnosine) and carnosine supplementation are popular ergogenic aids and also prescribed indiscriminately as antioxidant for athletic population. Studies with healthy humans [12-14] and animal models [15,16] have investigated whether increased carnosine in the skeletal muscle (induced by carnosine or BA supplementation) mitigates the high ROS/RNS 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 [15-17]. However, in human studies, the finding were unclear. For instance, both recreationally activity men [13] and women [12] who received BA supplementation had reduced LP after an acute bout of physical exercise (wen compared to pre-supplementation, but not to placebo condition). Although, in other studies with male athletes, carnosine [18] and BA [14] supplementation did not change/mitigate the increase in LP values after an acute bout of PE, despite increasing the GSH (Glutathione) antioxidant potential when compared to pre-treatment condition. Such studies from the same laboratory showed that improvement in antioxidant system seemed to occur only in women when compared to the pre-supplementation condition [12,13], instead other laboratories shown improvement in men [14,18]. Therefore, it is necessary to systematize and meta-analyze studies with humans to evaluate the effectiveness of BA or carnosine supplementation as an antioxidant during PE-induced OS. If BA or carnosine is an efficient antioxidant, this results can shed light to the controversial results such as impairments in endurance physical capacity [19] and $VO_2máx$ [20,21] found in some endurance exercise studies.

However, 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/RNS and OS markers [8], also, different types of ROS, LP or antioxidant system markers assessed might have influenced the study's results [8]. In this sense, it is necessary to maintain the highest standards in relation to BA/carnosine supplementation on PE-induced ROS/RNS 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

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 (Appendix 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

 doi:10.20944/preprints201811.0189.v3

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.

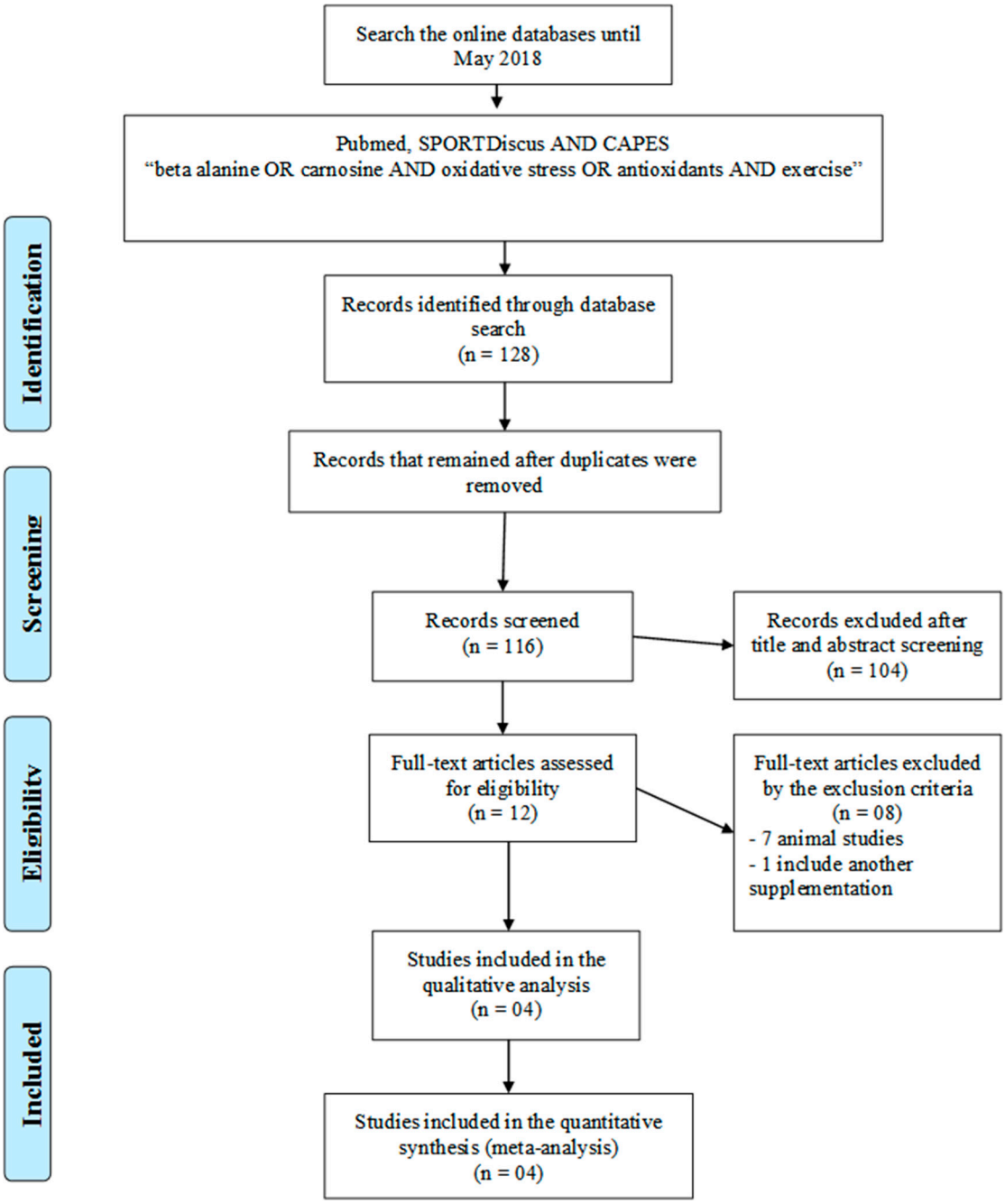


Figure 1. Flow diagram for the strategy of searching for the studies

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 (≥28 days) or carnosine (>14 days) supplementation followed by acute PE (to induce OS). We excluded studies that underwent other interventions in addition to BA (or

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 November 2018 doi:10.20944/preprints201811.0189.v3

carnosine) supplementation and PE (e.g., chemotherapy, drugs or other types of antioxidant supplementation).

2.3. Identification of Eligible Studies

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

2.4. Data Extraction

Table 1 describes participants 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. Also, 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 ROS/RNS production and OS as well as the evaluation site (intra- or extra-cellular).

2.5. Effect Size Calculation

For antioxidant system and OS markers (ROS/RNS, peroxidation and oxidation markers) outcome, 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 [25]. The following formula was used to calculate the ES with sample bias adjustment:

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

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

2.6. Statistical Analyses Results

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

Between conditions comparisons were performed using a random effects method. Data is displayed as mean difference with random effects, inverse of variance and 95% CI. 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 (BA or carnosine), (6) exercise intensity or duration. The statistical significance level was set at $P < 0.05$.

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

 doi:10.20944/preprints201811.0189.v3

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 [12-14,18] 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). One study involved chronic training [4] or evaluated acute injected BA [28]. Two studies evaluated PE-induced OS, but had other antioxidants combined with BA [29] or carnosine [17] supplementation. One human study [30] 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 [15-17] 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 (mean age from four studies: 21y) who were sedentary, recreationally active or trained participants. Only one study used women as subjects. Only one study used carnosine supplementation, while the other three studies used BA supplementation. All supplementation protocols employed chronic treatment, being 28 days for BA supplementation and 16 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 OS (Table 1).

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

As direct OS markers were ROS and RNS (i.e., H_2O_2, Hydrogen peroxide, 3-Nitro, 3-nitrotyrosine and nitric oxide) and PL or molecules oxidation markers as indirect OS (i.e., 8-ISO, 8-isoprostane; MDA, malondialdehyde and; PC, protein carbonyl, GSSG, oxidized glutathione). Antioxidant markers were GSH (glutathione), SOD (superoxide dismutase) and TAC (total antioxidant capacity. All assessment were from blood samples. Therefore, DNA (8-ISO), protein (PC) and cell damage (3-Nitro) as well as lipid peroxidation (MDA) wesre assessed as indirect markers of OS. 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 30 min [18], 2h, 4h [12,13], 24h and 48h [18] post exercise (Table 1).

189

190 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 plasma OS and AO 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; SOD, TAC and GSSG (colorimetric assay)*
Smith-Ryan et al. [13]	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 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 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 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 ± 1.3 y; height, 177.4 ± 7.9 cm; weight, 78.9 ± 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#, GSSG#, TAC#, NO#, H_2O_2# and SOD* (colorimetric assay); 8-ISSO* and 3-Nitro# (ELISA).

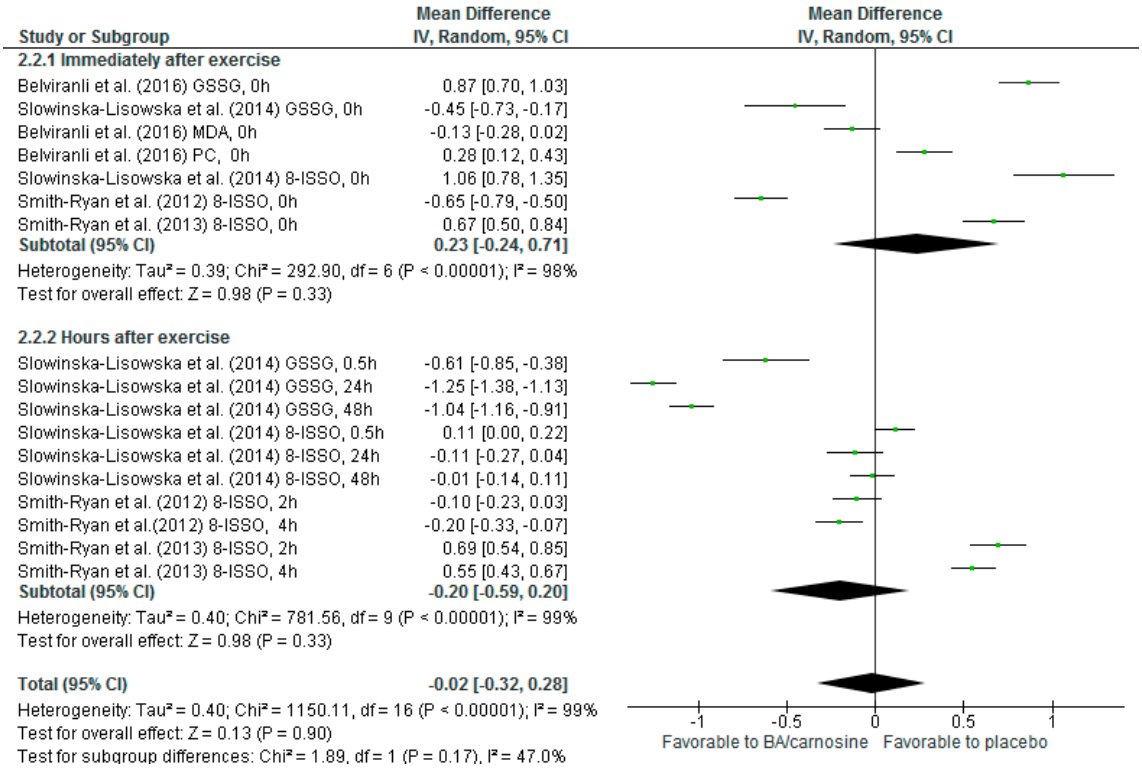
191 **Note:** 3-Nitro, 3-nitrotyrosine; 8-ISO, 8-isoprostane; BA, beta-alanine; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, Hydrogen peroxide; MDA, malondialdehyde; OS,
192 oxidative stress; PC, protein carbonyl; PL, placebo; SOD, superoxide dismutase; TAC, total antioxidant capacity. Symbols (*,#) represent the same fabricant commercial assay kit

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 November 2018 doi:10.20944/preprints201811.0189.v3

193 *3.3. Meta-analysis*

194 3.3.1. Oxidative stress markers

195 Exercise induced moderate increase in indirect OS markers (PC, MDA, 8-ISO and GSSG) in both
196 conditions (BA/carnosine ES= -0.78, 95% CI -0.19 to-1.37; placebo ES= -0.60, 95% CI -0.12 to -1.08).
197 Comparisons between conditions revealed that immediately after exercise there was a small ES and
198 non-significant increase in OS markers in the BA/carnosine group (difference ES: 0.23, 95% CI -0.24
199 to 0.71, p= 0.33). However, a small ES and non-significant decrease were observed hours after exercise
200 was favorable to the BA/carnosine condition (difference ES: -0.20, 95% CI -0.59 to 0.20, p= 0.33). Sub-
201 group analysis (immediately after exercise *vs.* hours after exercise) suggests a moderate heterogeneity
202 (I^2= 47%, p= 0.17) among indirect OS markers depending on the time of assessment (see Fig 2).
203



204

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

210

211 Independent analysis suggests large and moderate decreases in GSSG concentrations following
212 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).
213 Between group comparison showed no difference immediately after PE (difference ES= 0.21, 95% CI,
214 -1.08 to 1.51, p= 0.75, I^2= 98%), but a significant lower GSSG concentration hours after PE in
215 BA/carnosine condition (difference ES= -0.99, 95% CI, -1.28 to -0.69, p< 0.01, I^2= 76%). Sub-group
216 analysis (immediately after exercise *vs.* hours after exercise) indicates a significant effect of time of
217 assessment (I^2= 83.7%, p= 0.01).

218 Independent analysis of 8-ISSO showed a large increase in immediately after PE in both
219 condition (BA/carnosine ES= -2.15, 95% CI -6.91 to 2.60; placebo ES= -1.79, 95% CI -4.56 to 0.98,
220 respectively) and a moderate decrease in both conditions following hours after PE (BA/carnosine ES=
221 0.62, 95% CI -0.12 to 1.35; placebo ES= 0.54, 95% CI -0.35 to 1.45). Between condition comparison
222 reveal a small ES and non-significant increase in 8-ISSO immediately after exercise for BA/carnosine

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 November 2018 doi:10.20944/preprints201811.0189.v3

(difference ES= 0.36, 95% CI -0.70 to 1.42, p= 0.51, I^2= 99%) and trivial ES and non-significant 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. [12] study (00 hour post exercise), there is a significant effect of time of assessment (I^2= 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. [18] performed direct OS markers assessment immediately after plasma collection. 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 ROS/RNS 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 ROS/RNS (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 ROS/RNS markers, see Fig 3.

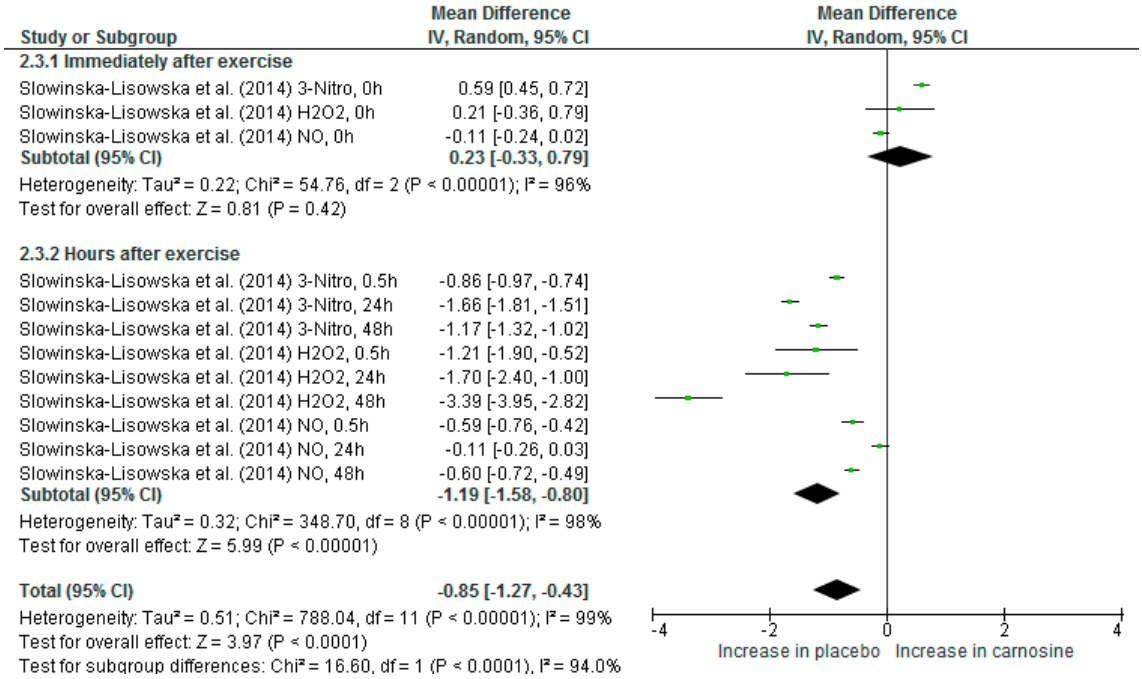


Figure 3. Forest plot of the plasma reactive oxygen and nitrogen species induced by physical exercise after carnosine or placebo supplementation. Acronyms: 3-Nitro, 3-nitrotyrosine; H2O2, Hydrogen peroxide; NO, nitric oxide. Note: Autor's name and year of study publication is followed by the oxidative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

3.3.2. Antioxidants

ES suggests that there was a moderate increase in TAC concentration in BA/carnosine supplementations (ES= -0.66, 95% CI -1.44 to 0.12), whereas a trivial decrease occurred in placebo supplementation (ES= 0.08, 95% CI -0.78 to 0.95) immediately after exercise, but without significant difference between them (difference ES= 0.51, 95% CI -0.15 to 1.17, p= 0.13, I2=99%). Hours after exercise BA/carnosine presented a trivial increase (ES= -0.13, 95% CI -0.78 to 0.52) and a similar small

 doi:10.20944/preprints201811.0189.v3

decrease occurred in the placebo condition (ES= 0.12, 95% CI -0.42 to 0.66) which showed a tend to difference between then (difference ES= -0.25, 95% CI -0.04 to 0.55, p= 0.09, I^2=98%). Overall between conditions comparison (pooled ES) suggests that BA/carnosine supplementation increases 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.

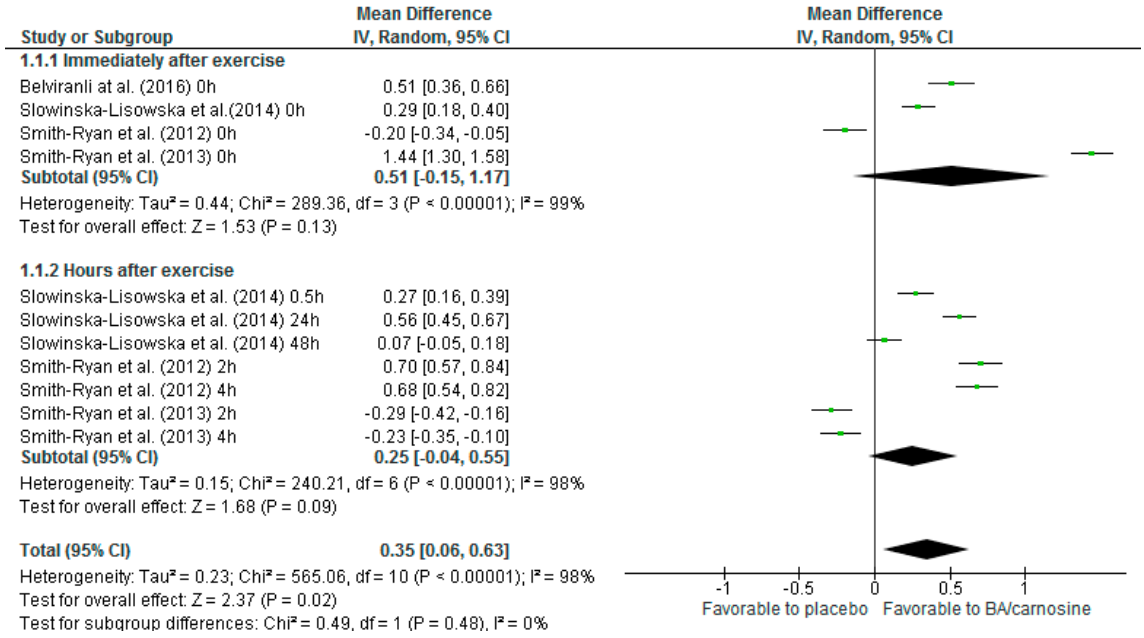


Figure 4. Forest plot of the total antioxidant capacity (TAC) change by physical exercise after BA/carnosine or placebo supplementation. Note: Autor's name and year of study publication is followed by the moment (hours) of assessment after exercise.

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

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 November 2018 doi:10.20944/preprints201811.0189.v3

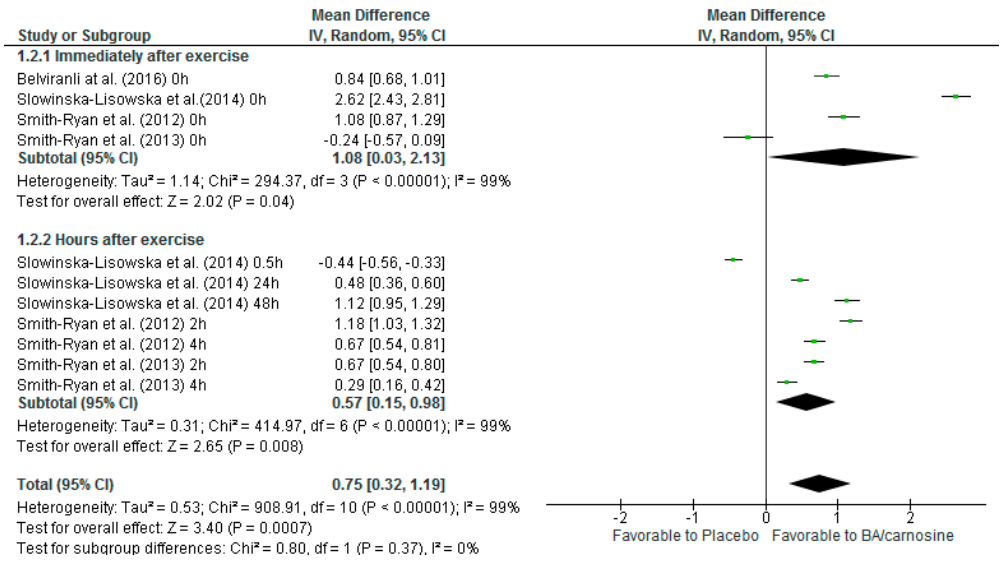


Figure 5. Forest plot of the glutathione (GSH) change by physical exercise after BA/carnosine or placebo supplementation. Note: Autor's name and year of study publication is followed by the moment (hours) of assessment after exercise.

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.

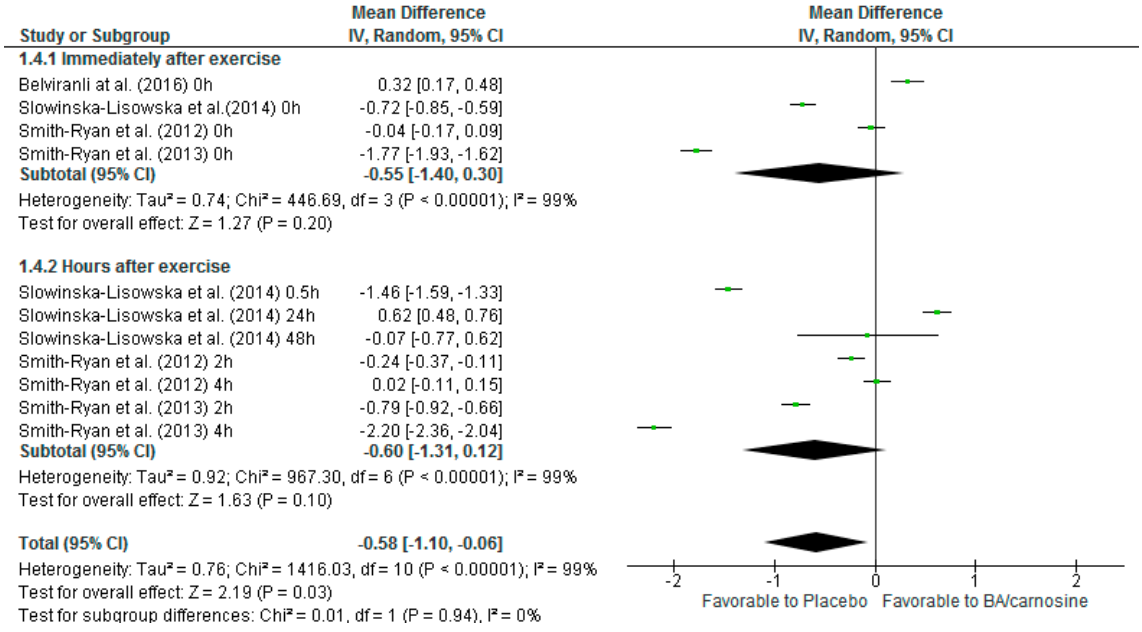


Figure 6. Forest plot of the superoxide dismutase change by physical exercise after BA/carnosine or placebo supplementation. Note: Autor's name and year of study publication is followed by the moment (hours) of assessment after exercise.

 doi:10.20944/preprints201811.0189.v3

286

287 *3.3.3. Heterogeneity studies, multiple linear regression analysis and risk of bias.*

288 Multiple linear regression shows that in indirect OS markers (8-ISO, MDA, GSSG and PC) the
289 time of assessment, marker type evaluated, exercise type and training status could explain 65% of ES
290 variation (R^2= 0.650, p= 0.000). Sex and supplementation conditions (BA or carnosine) were excluded
291 from the model.
292 Furthermore, 39% (R^2= 0.389, p= 0.000) of ES variation from antioxidant (SOD, TAC, GSH) results
293 were related to time of assessment, exercise test, training status and antioxidant marker type
294 evaluated. Sex and supplementation conditions were excluded from the model.
295 It was not possible to perform multiple linear regression for ROS/RNS direct markers (H_2O_2, 3-
296 Nitro and NO) due to insufficient data.
297 The four studies present less than three reported high or unclear risk domains (Appendix 2).
298 Two studies (two high risk) are from the same laboratory and was unable to bling for BA condition
299 (due to paresthesia effect).

300 **4. Discussion**

301 The four studies included in this review observed significant increases in OS after acute physical
302 exercise bouts. Our analyses suggest that immediately after PE-induced OS, BA or carnosine
303 supplementation did not undermine the increase in both ROS an RNS (H_2O_2, 3-Nitro and NO) or
304 peroxidation (8-ISSO, MDA, and PC) markers that were produced. Monitoring their levels during
305 hours after exercise (0.5 to 48h), BA or carnosine did not appear to impose a greater decrease in 8-
306 ISSO (p> 0.05) when compared to placebo supplementation. Interestingly, monitoring OS levels after
307 hours (0.5 to 48h) of PE-induced OS, carnosine treatment mitigated the increase of H_2O_2, 3-Nitro and
308 NO production. It is important to mention that ROS/RNS (H_2O_2, 3-Nitro and NO) data were obtained
309 from only one study (Slowinska-Lisowska et al. 2014), but such data were in accordance with
310 previous in vitro studies [31,32] .
311 Evidence suggests that the largest post-exercise changes involving lipid, protein, glutathione
312 and DNA oxidation occurred 1-4 days after PE (when compared with blood samples of resting
313 condition) [8]. For instance, in an animal study that assessed PE-induced OS after 24h, it was shown
314 that BA or carnosine supplementation decreased LP (thiobarbituric acid reactive substances and
315 MDA markers) in skeletal muscle tissue [15,16]. The only publication that evaluated 24h post-
316 exercise was the Slowinska- Lisowska et al. [18] study. Therefore, studies with a long follow-up
317 period (days to weeks), thus with sufficient time to resolve an acute inflammation caused by
318 moderate-intense exercise [33] are needed to verify whether BA or carnosine may promote clinical
319 changes in the peroxidation markers.
320 Previous reviews [1] and recent animal studies [2-4] had already presented an antioxidant role
321 of carnosine. When compared to placebo, our data suggested that previous BA or carnosine
322 supplementation increased TAC (ES= 0.35, 95% CI 0.06 to 0.65, p= 0.02; Fig 4) and increase GSH (GSH,
323 ES= 0.75, 95% CI 0.32 to 1.19, p= 0.0007) after PE-induced OS. These data corroborate with an animal
324 study [16] submitted to PE-induced OS. Such study reported increased in GSH and decreased
325 glutathione peroxidase (GPx) and glutathione reductase after exercise, suggesting that carnosine has
326 buffering the H_2O_2 production. The effect of BA and carnosine supplementation on GSSG
327 concentrations is conflicting. Belviranli et al. [14] reported increased GSSG after PE-induced OS in
328 sedentary individuals supplemented with BA (suggesting GSH oxidation); on the other hand,
329 Slowinska-Lisowska et al. [18] reported decreased GSSG concentrations in trained individuals
330 supplemented with carnosine (suggesting a carnosine antioxidant effect). More research is needed to
331 highlight the effect of BA/carnosine on GSH/GSSG ratio.
332 Both plasma TAC and GSH presented a large variation in the studies [12-14,18], as evidenced by
333 high heterogeneity (see Fig 4 and 5). Plasma antioxidants evaluation such as GSH and TAC after

exercise practice yields conflicting results [8], however, analyzing the results from the four studies, it seems that the increase in muscle carnosine concentration influences these changes. GSH can be delivered to plasma from several tissues and this is influenced by the type of activity exerted as well as by the nutritional status of the participants [8], so, future human studies need to asses GSH from specific tissues known for its large pools of carnosine (such as the skeletal muscle) [16]. TAC assays have a limited capacity to measure the total antioxidant system capacity, excluding, for instance, the contribution of antioxidant enzymes and metal binding proteins, so changes in the TAC values probably does not reflect the carnosine antioxidant content activity in the organism.

Our data suggests that BA or carnosine supplementation can mitigate the increase of SOD activity (ES= -0.58, p= 0.03), a well-known superoxide scavenger. It is plausible that this attenuated increase of SOD activity occurs due to carnosine antioxidant effect (e.g., $O2\cdot-$ clearance). In vitro studies have shown that carnosine plays an effective role in decreasing ROS and RNS (e.g. H_2O_2, superoxide and NO) [31,32]. Studies with animal training also has demonstrated that carnosine or BA supplementation mitigated SOD [19] and GPx [16] activity, when compared to control conditions. Such data are contrary to untrained animal studies [2,3], which showed increased activity of these enzymes and decrease in PL. Such discrepancy suggest that carnosine/BA supplementation enhance antioxidant system at rest condition (*i.e.*, sedentary life style), but not during/after acute exercise. Therefore, it appears that BA or Carnosine supplementation might mitigate the increase in SOD and GPx activity induced by exercise, but has opposite effect in rest condition. Further studies are needed to explore these conflicting results. Also, 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 increase SOD activity (induced by PE) is mitigated, this occur 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) [19]. 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 [9] are mitigated in the presence of BA or carnosine supplementation, as it is observed in studies with chronic [7] or acute antioxidant supplementation [28]. Moreover, BA supplementation is a well-known ergogenic agent in anaerobic exercises, but not in endurance exercises [20,34]. For instance, early evidence in human studies suggest that BA supplementation delayed lactate production, but reduce aerobic capacity [21]. Therefore, it is important to investigate if BA or Carnosine supplementation might influences negatively endurance adaptations because of their antioxidant effects [11].

Our ES evaluations (with antioxidant and oxidative stress markers) showed high heterogeneity. This meta-analysis pooled together studies with participants from different fitness level, enrolled in different PE-induced OS, also, different time points of different oxidative stress markers or antioxidant markers were pooled in the same ES analysis. It is well-known that time-point assessment of PE-induced OS as well as the rising in blood plasma of both oxidative stress markers or antioxidant markers are also time-dependent and this might influence our results [8]. Our sub-group analysis (immediately after exercise *vs.* hours after exercise- 0.5 to 48 hours) showed that the moment of assessment for both indirect (Fig 3) and direct (Fig 4) OS markers is an important confounding variable. Also, multivariable regression shows that time of assessment, the OS 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 assessment. 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 [11]. For example, no study included in this meta-analysis mentioned that their samples were homogenized for OS or antioxidant status

 doi:10.20944/preprints201811.0189.v3

(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 [11].

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

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 OS 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. And finally, all four studies that performed the assessment of both antioxidant and OS markers in the plasma did it with the assumption that plasma measurements would reflect systemic changes [8]. Animal studies have found consistent changes in the antioxidant/oxidant ratio (in the exercise-induced ROS production) in the skeletal muscle after BA or carnosine supplementation [15-17], as the main stores of carnosine in humans are in the skeletal muscle (99%) [1], future studies need to verify such change at skeletal muscle level.

5. Conclusions

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

Supplementary Materials: The following are available online.

Author Contributions: Paper conceptualization, E.F., M.L.J.M. and E.C.C.; data extraction and methodology design, M.F.R., E.F., M.L.J.M and E.C.C.; Statistical analysis, E.F. and E.C.C.; writing—original draft preparation, E.F., F.S.F., M.L.J.M. and E.C.C.; writing—review and editing, A.R.F., P.A.F.W; supervision, E.C.; project administration, E.F. and E.C..

Acknowledgments: The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarship support.

Conflicts of Interest: The authors report no conflicts of interest associated with this manuscript.

References

1. Boldyrev, A.A.; Aldini, G.; Derave, W. Physiology and pathophysiology of carnosine. *Physiological reviews* **2013**, *93*, 1803-1845.
2. Kim, M.Y.; Kim, E.J.; Kim, Y.-N.; Choi, C.; Lee, B.-H. Effects of α-lipoic acid and L-carnosine supplementation on antioxidant activities and lipid profiles in rats. *Nutrition research and practice* **2011**, *5*, 421-428.
3. Ma, X.; Jiang, Z.; Lin, Y.; Zheng, C.; Zhou, G. Dietary supplementation with carnosine improves antioxidant capacity and meat quality of finishing pigs. *Journal of animal physiology and animal nutrition* **2010**, *94*.
4. Schaalan, M.F.; Ramadan, B.K.; Abd Elwahab, A.H. Synergistic effect of carnosine on browning of adipose tissue in exercised obese rats; a focus on circulating irisin levels. *Journal of cellular physiology* **2018**, *233*, 5044-5057.
5. Turunen, M.; Olsson, J.; Dallner, G. Metabolism and function of coenzyme Q. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2004**, *1660*, 171-199.
6. Tan, D.Q.; Suda, T. Reactive Oxygen Species and Mitochondrial Homeostasis as Regulators of Stem Cell Fate and Function. *Antioxidants & Redox Signaling* **2018**, *29*, 149-168, doi:10.1089/ars.2017.7273.
7. He, F.; Li, J.; Liu, Z.; Chuang, C.-C.; Yang, W.; Zuo, L. Redox mechanism of reactive oxygen species in exercise. *Frontiers in Physiology* **2016**, *7*.
8. Nikolaidis, M.G.; Jamurtas, A.Z.; Paschalis, V.; Fatouros, I.G.; Koutedakis, Y.; Kouretas, D. The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress. *Sports Medicine* **2008**, *38*, 579-606.
9. Henríquez-Olguín, C.; Díaz-Vegas, A.; Utreras-Mendoza, Y.; Campos, C.; Arias-Calderón, M.; Llanos, P.; Contreras-Ferrat, A.; Espinosa, A.; Altamirano, F.; Jaimovich, E. NOX2 inhibition impairs early muscle gene expression induced by a single exercise bout. *Frontiers in physiology* **2016**, *7*, 282.
10. Margaritelis, N.V.; Theodorou, A.A.; Paschalis, V.; Veskoukis, A.S.; Dipla, K.; Zafeiridis, A.; Panayiotou, G.; Vrabas, I.S.; Kyparos, A.; Nikolaidis, M.G. Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting redox interindividual variability. *Acta Physiologica* **2018**, *222*, e12898, doi:doi:10.1111/apha.12898.
11. Margaritelis, N.V.; Paschalis, V.; Theodorou, A.A.; Kyparos, A.; Nikolaidis, M.G. Antioxidants in Personalized Nutrition and Exercise. *Advances in Nutrition* **2018**.
12. Smith, A.; Stout, J.; Kendall, K.; Fukuda, D.; Cramer, J. Exercise-induced oxidative stress: the effects of β-alanine supplementation in women. *Amino Acids* **2012**, *43*, 77-90.
13. Smith-Ryan, A.E.; Fukuda, D.H.; Stout, J.R.; Kendall, K.L. The influence of β-alanine supplementation on markers of exercise-induced oxidative stress. *Applied Physiology, Nutrition, and Metabolism* **2013**, *39*, 38-46.

14. Belviranli, M.; Okudan, N.; Revan, S.; Balci, S.; Gokbel, H. Repeated supramaximal exercise-induced oxidative stress: effect of β-alanine plus creatine supplementation. *Asian journal of sports medicine* **2016**, *7*.
15. Dawson Jr, R.; Biasetti, M.; Messina, S.; Dominy, J. The cytoprotective role of taurine in exercise-induced muscle injury. *Amino acids* **2002**, *22*, 309-324.
16. Bortolatto, G.P. Efeito da carnosina e da β-alanina no dano produzido pelo exercício intenso no músculo sóleo de ratos. Universidade Estadual Paulista Júlio de Mesquita Filho, 2015.
17. Seĭfulla, N.; Kovalev, I.; Rozhkova, E.; Paniushkin, V. [Effect of carnosine and its combination with essentiale on lipid peroxidation and work capacity of experimental animals]. *Eksperimental'naia i klinicheskaia farmakologiia* **2004**, *68*, 44-46.
18. Slowinska-Lisowska, M.; Zembron-Lacny, A.; Rynkiewicz, M.; Rynkiewicz, T.; Kopec, W. Influence of l-carnosine on pro-antioxidant status in elite kayakers and canoeists. *Acta Physiologica Hungarica* **2014**, *101*, 461-470.
19. Lee, K.-N.; Hue, J.-J.; Miga, M.; Kang, B.S.; Kim, J.-S.; Nam, S.Y.; Yun, Y.W.; Jeong, J.-H.; Lee, B.J. Effects of Carnosine on Exercise Capacity in ICR Mice. *Laboratory Animal Research* **2008**, *24*, 617-622.
20. Brisola, G.M.P.; Redkva, P.E.; Pessôa Filho, D.M.; Papoti, M.; Zagatto, A.M. Effects of 4 weeks of β-alanine supplementation on aerobic fitness in water polo players. *PloS one* **2018**, *13*, e0205129.
21. Jordan, T.; Lukaszuk, J.; Misic, M.; Umoren, J. Effect of beta-alanine supplementation on the onset of blood lactate accumulation (OBLA) during treadmill running: Pre/post 2 treatment experimental design. *Journal of the International Society of Sports Nutrition* **2010**, *7*, 20-20, doi:10.1186/1550-2783-7-20.
22. Harris, R.C.; Tallon, M.J.; Dunnett, M.; Boobis, L.; Coakley, J.; Kim, H.J.; Fallowfield, J.L.; Hill, C.A.; Sale, C.; Wise, J.A. The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids* **2006**, *30*, 279-289, doi:10.1007/s00726-006-0299-9.
23. Caruso, J.; Charles, J.; Unruh, K.; Giebel, R.; Learmonth, L.; Potter, W. Ergogenic Effects of β-Alanine and Carnosine: Proposed Future Research to Quantify Their Efficacy. *Nutrients* **2012**, *4*, 585.
24. Maemura, H.; Goto, K.; Yoshioka, T.; Sato, M.; Takahata, Y.; Morimatsu, F.; Takamatsu, K. Effects of carnosine and anserine supplementation on relatively high intensity endurance performance. *International Journal of Sport and Health Science* **2006**, *4*, 86-94.
25. Morris, S.B. Estimating Effect Sizes From Pretest-Posttest-Control Group Designs. *Organizational Research Methods* **2008**, *11*, 364-386, doi:10.1177/1094428106291059.
26. Borenstein, M.; Hedges, L.V.; Higgins, J.P.; Rothstein, H.R. *Introduction to meta-analysis*; John Wiley & Sons: 2011.
27. Hopkins, W.; Marshall, S.; Batterham, A.; Hanin, J. Progressive statistics for studies in sports medicine and exercise science. *Medicine+ Science in Sports+ Exercise* **2009**, *41*, 3.

28. Vereshchaka, I.V.; Bulgakova, N.V.; Maznychenko, A.V.; Gonchar, O.O.; Prylutskyy, Y.I.; Ritter, U.; Moska, W.; Tomiak, T.; Nozdrenko, D.M.; Mishchenko, I.V., et al. C60 Fullerenes Diminish Muscle Fatigue in Rats Comparable to N-acetylcysteine or β-Alanine. *Frontiers in Physiology* **2018**, *9*, doi:10.3389/fphys.2018.00517.
29. Pence, B.D.; Gibbons, T.E.; Bhattacharya, T.K.; Mach, H.; Ossyra, J.M.; Petr, G.; Martin, S.A.; Wang, L.; Rubakhin, S.S.; Sweedler, J.V. Effects of exercise and dietary epigallocatechin gallate and β-alanine on skeletal muscle in aged mice. *Applied Physiology, Nutrition, and Metabolism* **2015**, *41*, 181-190.
30. Rozhkova, E.; Ordzhonikidze, Z.; Druzhinin, A.; Seĭfulla, N.; Paniushkin, V.; Kuznetsov, I. Using carnosine and natural antioxidants for the prophylaxis of acute post-loading oxidative stress. *Eksperimental'naia i klinicheskaia farmakologiia* **2007**, *70*, 44-46.
31. Nicoletti, V.G.; Santoro, A.M.; Grasso, G.; Vagliasindi, L.I.; Giuffrida, M.L.; Cuppari, C.; Purrello, V.S.; Stella, A.M.G.; Rizzarelli, E. Carnosine interaction with nitric oxide and astroglial cell protection. *Journal of neuroscience research* **2007**, *85*, 2239-2245.
32. Miceli, V.; Pampalone, M.; Frazziano, G.; Grasso, G.; Rizzarelli, E.; Ricordi, C.; Casu, A.; Iannolo, G.; Conaldi, P.G. Carnosine protects pancreatic beta cells and islets against oxidative stress damage. *Molecular and Cellular Endocrinology* **2018**, *474*, 105-118, doi:https://doi.org/10.1016/j.mce.2018.02.016.
33. Ho, A.T.V.; Palla, A.R.; Blake, M.R.; Yucel, N.D.; Wang, Y.X.; Magnusson, K.E.G.; Holbrook, C.A.; Kraft, P.E.; Delp, S.L.; Blau, H.M. Prostaglandin E2 is essential for efficacious skeletal muscle stem-cell function, augmenting regeneration and strength. *Proceedings of the National Academy of Sciences* **2017**, *114*, 6675-6684, doi:10.1073/pnas.1705420114.
34. Trexler, E.T.; Smith-Ryan, A.E.; Stout, J.R.; Hoffman, J.R.; Wilborn, C.D.; Sale, C.; Kreider, R.B.; Jäger, R.; Earnest, C.P.; Bannock, L. International society of sports nutrition position stand: Beta-Alanine. *Journal of the International Society of Sports Nutrition* **2015**, *12*, 1-14.