ABSTRACT

Beta-alanine is a non-essential amino acid that is commonly used to improve exercise performance. It could influence the buffering of hydrogen ions produced during intense exercise and delay fatigue, providing a substrate for increased synthesis of intramuscular carnosine. This systematic review evaluates the effects of beta-alanine supplementation on maximal intensity exercise in trained, young, male individuals. Six databases were searched on August 10, 2023, to identify randomized, double-blinded, placebo-controlled trials investigating the effect of chronic beta-alanine supplementation in trained male individuals with an age range of 18-40 years. Studies evaluating exercise performance through maximal or supramaximal intensity efforts falling within the 0.5–10 min duration were included. A total of 18 individual studies were analyzed, employing 18 exercise test protocols and 15 outcome measures in 331 participants. A significant (p=0.01) result was observed with an overall effect size of 0.39 (95% CI: 0.09–0.69), in favor of beta-alanine supplementation versus placebo. Results indicate significant effects at 4 weeks of supplementation, effect size 0.34 (95% CI: 0.02–0.67, p=0.04), 4–10 minutes of maximal effort, effect size 0.55 (95% CI: 0.07–1.04, p=0.03), and a high beta-alanine dosage of 5.6–6.4 g/d, effect size 0.35 (95% CI: 0.09–0.62, p=0.009). The results provide insights into which exercise modality will benefit the most, and which dosage protocols and durations stand to provide the greatest ergogenic effects. This may be used to inform further research, and professional or recreational training design and optimization of supplementation strategies.

Keywords: beta-alanine; exercise performance; maximal intensity performance; trained males; athletic performance

1. Introduction

Physical and sports performance can benefit from various supplemental and nutritional intervention strategies. There is increasing interest in the ergogenic benefit of beta-alanine, which is the precursor to the histidine-containing dipeptide carnosine (β-alanyl-L-histidine), itself shown to have a key role in acid-base regulation during exercise, with other important health-linked roles (such as antioxidant and anti-glycation properties) also posited (Sale et al., 2013) . Carnosine is one member of the histidine-containing dipeptides found in humans, with most animals also hosting one or both of its methylated variants, anserine (also expressed in human skeletal and cardiac muscle) or ophidine (Dolan, Saunders, et al., 2019; Toviwek et al., 2022). Already established as having a pH-buffering effect, the synthesis of carnosine in muscle tissue, where it is predominantly found, is rate-limited by the availability of beta-alanine (Perim et al., 2019; Stellingwerff et al., 2012).

Beta-alanine is a non-proteinogenic, non-essential amino acid which is synthesized in relatively small amounts in the liver (Trexler et al., 2015) and can be acquired in the diet from animal, but not plant sources. Supplementation strategies have been shown to significantly increase the amounts of beta-alanine found in blood plasma, and subsequently elevate muscle carnosine content; specifically, dosages of beta-alanine ranging from 3.2 to 6.4 g/d given for 4 weeks have been shown to increase muscle carnosine levels by 42 to 66% (Harris et al., 2006).

Meta-analyses by Hobson *et al.* (R. M. Hobson et al., 2012) and Saunders *et al.* (Bryan Saunders et al., 2017) demonstrated that the greatest ergogenic effects of beta-alanine were attained in exercise lasting 0.5 to 10 minutes, where the dominant contributor to energy production is the anaerobic-glycolytic pathway (Artioli *et al.*, 2010). While the tests conducted in these meta-analyses differentiate between performance and capacity tests, they do not focus exclusively on maximal/supramaximal efforts as they also include tests which may contain significant bouts of submaximal output, particularly where concerning performance tests. It was also recently shown that beta-alanine supplementation improved output during the second level of the yo-yo intermittent performance test (Grgic, 2021), and aerobic-anaerobic transition zones during performance testing (Ojeda et al., 2020). However, it was unclear whether beta-alanine was more effective at improving exercise capacity (i.e., work done, power) or performance (i.e., time to completion/exhaustion), likely due to a lack of studies focusing on performance at the time of their publication. Furthermore, the exercise testing protocols in some of the included studies may have biased the results; for example, studies measuring maximal oxygen capacity ($\dot{V}O_{2max}$) via a cardiopulmonary exercise test (CPET) measure the total time taken for the test, which also includes the early, low-intensity stages of the test that do not stress the most relevant energy production system. Thus, it remains unclear for which types of exercise and at which exercise intensities beta-alanine supplementation is most effective.

The aim of this study was to perform a systematic review and meta-analysis on the effects of beta-alanine supplementation on maximal intensity exercise output; specifically, strength and power as capacity measures, and performance time in young, male, trained individuals. Ergogenic supplements in general (muscle building, endurance enhancing) have a larger proportion of sales arising from men, as oppose to women, who generally gravitate more to weight loss supplements (Austin et al., 2017); taking this discrepancy into account, and in an effort to maintain a more homogeneous group to minimize bias, studies focusing on men were selected over those focusing on women or mixed groups, to be included in this study. Maximal intensity exercise is defined herein as efforts which reach muscular failure during strength and power tests at which no further, technically proficient, repetition can be executed, or efforts in which voluntary output of required (maximal/supramaximal) intensity must be sustained for a given duration. Thus, this study did not examine the effects of beta-alanine supplementation on sub-maximal exercise. The Secondary aims of this study were to add resolution and nuance to the data sets, by applying sub-analyses on supplementation duration and dosage, as well as the duration of the tests being performed.

2. Methods

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021), with the question determined according to PICO (Population, Intervention, Comparator, and Outcomes). The protocol of this systematic review was registered at the Open Science Framework (https://doi.org/10.17605/OSF.IO/AYZ5K).

2.1. Literature Search

Relevant articles were identified via electronic search using six databases (PubMed, Google Scholar, Cochrane Library of Science, Scopus, Web of Science, and ScienceDirect). Key search terms 'beta-alanine' and 'β-alanine' were concatenated with 'trained male individuals', 'maximal intensity exercise', and 'athletic performance', as well as ("trained males" OR "sports performance" OR "athletic performance" OR "maximal intensity") AND ("beta-alanine" OR "β-alanine"). The terms were combined with the databases' filter for controlled trials of interventions on humans. Screening was initiated with a title and abstract search against key search terms. Duplicates and non-published articles were removed after importing to Microsoft Excel. All remaining studies (titles and abstract) were screened against inclusion/exclusion criteria, with unclear studies remaining at this stage. The remaining articles were then retrieved and thoroughly assessed against criteria. All reviewers participated in this process and discussed any studies which needed further scrutiny before finalizing the studies being included.

2.2. Study Selection

The inclusion criteria of this systematic review were as follows: (i) human study, (ii) placebo-controlled, double-blinded, randomized study, (iii) male participants, (iv) participants supplemented with beta-alanine, (v) participants who were physically active or recreationally active with consistent training more than 3 times per week for at least 6 months, and professional/semi-professional/recreational athletes, (vi) participants 18 to 40 years of age, (vii) studies that investigated exercise involving maximal or supramaximal intensity efforts of 0.5–10 minute duration, exercise tests involving a single bout of sustained effort, or shorter intervals of high-intensity effort interrupted by brief recovery periods, and (viii) peer-reviewed studies published in English. The exclusion criteria were: (i) non-randomized clinical studies, (ii) untrained, inactive or unhealthy participants, (iii) participants under the age of 18 or above the age of 40, (iv) participants supplementing other dietary or ergogenic supplements with beta-alanine, (v) unspecified supplementation duration, (vi) studies that investigated outcomes not-relevant to exercise output, (vii) PhD theses, comments, editorials or reviews, and (viii) cross-over designed studies that failed to report an appropriate wash-out period; studies have shown a decrease in supplemented levels of muscle carnosine at a rate of approximately 2%/week following cessation of supplementation protocol, with durations of up to 16 weeks being shown necessary for complete return to baseline levels (Audrey Baguet et al., 2009). Thus, 16 weeks was set as an appropriate washout period. Females were excluded due to the possibility of bias arising from the inherently lower levels of carnosine present in these participants (Wim Derave et al., 2010), which can result in females experiencing a greater increase in intramuscular carnosine (Glenn et al., 2015).

2.3. Data Extraction

Three reviewers (K.A., G.G., and S.A.) extracted the data from the eligible articles. The information extracted was as follows: study design, participant characteristics (number, sports or physical activity), group characteristics (number of participants and age), beta-alanine group (dosage), placebo (PL)/comparison group (form and dosage), study duration, outcome measures, testing protocol, load, and results.

2.4. Statistical Analysis

For the meta-analysis, descriptive and statistical analysis was performed using Review Manager 5.4.1 (Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014). The meta-analysis compared differences between beta-alanine and PL supplementation, and all quantitative data were processed as continuous measurements. The information analysed for each study included the number of participants, standardised mean difference (SMD) and standard error of SMD of the intervention (beta-alanine) and control (PL) groups. Where available, means and other data were extracted directly from the studies. In cases where data was omitted and authors did not respond to requests for additional data sets, the standard deviation of the difference between means (SD_{change}) for each group of each study was calculated using *Formula 1*;

$$SD_{change} = \sqrt{\left|(SD_{pre})^2 + (SD_{post})^2 - (2 * r * SD_{pre} * SD_{post})\right|}$$

where SD_{pre} and SD_{post} represent the standard deviation of the pre- and post-supplementation means, and r represents the correlation coefficient of the data, set at 0.75 to facilitate a conservative estimate (Rosenthal, 1991). A second meta-analysis using identical data, but assuming r to be 0.50 was also performed for comparison (Figure 7). The DerSimonian and Laird inverse-variance model was used to estimate variance, p-values, and confidence intervals.

The overlap of confidence intervals (95% CI) of outcome measurements from the included studies was used to determine statistical heterogeneity, represented by Cochran's Q (chi-square test) and I^2. The percentage of observed total variation between studies was indicated by the I^2 statistic showing real heterogeneity as opposed to sampling error. I^2 value is separated into three categories: low heterogeneity (25-50%), moderate (50-75%), and high (>75%)(Grant & Hunter, 2006).

2.5. Quality and risk of bias assessment

Quality assessment and the risk of bias was performed by all reviewers, in accordance with the Revised Cochrane Collaboration's tool for assessing risk of bias in randomized trials (RoB2) (J. P. T. Higgins et al., 2011). The tool comprises six domains: bias arising from the randomization process; bias arising from period and carryover effects; bias due to deviations from intended interventions; bias due to missing outcome data; bias in measurement of the outcome; and bias in selection of the reported result; and. For each domain, a possible risk-of-bias judgment was low risk, some concerns and high risk, as well as not applicable. A sensitivity test of the studies with highest bias are included (Supplementary File, Figure 4).

3. Results

3.1. Study selection

After searching the six databases, 1478 articles were identified, of which 328 were not randomized controlled trials (RCTs). The remaining were filtered by title, and irrelevant (n=594) and duplicated (n=427) articles were excluded. All reviewers (GG, KA, SA, EM) took part in the screening process. The remaining 129 articles were retrieved, of which 70 were excluded because they failed to meet inclusion criteria. One report was not retrieved as it was written in Spanish. A total of 58 were then assessed for eligibility, of which 32 were excluded. Finally, a total of 26 articles were eligible for inclusion in the qualitative synthesis of this systematic review. Eight articles were not eligible for inclusion in the meta-analysis because the authors did not mention means and/or SDs of their data. The article selection process is shown in Figure 1. The characteristics of the included articles are summarized in Table 1.

3.2. Study characteristics

A total of 331 participants were pooled together across the studies included in the meta-analysis, with similar numbers in the beta-alanine group (n=166) and the PL group (n=165). Sporting background of the participants varied: track and field (n=25), cyclists (n=47), runners (n=18), team sports including football, soccer, basketball, hockey and rugby (n=68), recreationally active (n=58), water polo (n=22), amateur boxers (n=19), resistance trained (n=49), alpine skiers (n=9), competitive rowers (n=16).

Dosage of beta-alanine varied between studies, from 3.9 g/d to 6.4 g/d. Studies were grouped as low (3.9–4.6 g/d, n=4), medium (4.7–5.5 g/d, n=2), and high dose (5.6–6.4 g/d, n=12). These groupings (low/medium/high) are arbitrary, serving to differentiate between dose magnitudes for this review only, and are not representative of the general dose considerations found in the literature, although these groupings may be useful in practical terms, as they often approximate dose ranges available for commercial use. Supplementation protocols lasted from 4–10 weeks for the studies analyzed, grouped into duration of 4 weeks (n=9) and 5–10 weeks (n=9). For the test durations, studies analyzed were grouped as 0–1 (n=8), 1–4 (n=7), and 4–10 minutes (n=3).

3.3. Effect of beta-alanine on maximal intensity exercise

The main analysis of this review included 18 studies. Five studies measured power output (Brisola et al., 2018; Ducker et al., 2013a; Gross et al., 2014; Howe et al., 2013; Turcu et al., 2022), five measured muscle strength (Askari & Rahmaninia, 2018; de Camargo et al., 2023; Freitas et al., 2019; Kim et al., 2018; Smith et al., 2019), one measured work done (Hill et al., 2007), six measured time taken to complete an exercise task (Bellinger & Minahan, 2016b, 2016a; W Derave et al., 2007; Ducker et al., 2013c, 2013b; Jagim et al., 2013), and one measured total work sets completed (J L Maté-Muñoz et al., 2018). Since outcomes were measured using different methods and units of measurement, utilizing mean differences (MD) would have been inappropriate; therefore, global output was expressed as the composite score of all measures using standardized mean difference (SMD).

Figure 2 shows that supplementation with beta-alanine had small to moderate but significant positive effects on maximal exercise (SMD: 0.39, 95% CI:0.09-0.69; p=0.01), compared to placebo. The studies reviewed in this analysis displayed low to moderate heterogeneity (I^2=44%, p=0.02). Fourteen of the eighteen studies showed beta-alanine supplementation having a beneficial effect on maximal exercise (Bellinger & Minahan, 2016b, 2016a; Brisola et al., 2018; W Derave et al., 2007; Ducker et al., 2013c, 2013a, 2013b; Freitas et al., 2019; Hill et al., 2007; Howe et al., 2013; Kim et al., 2018; José Luis Maté-Muñoz et al., 2018; Smith et al., 2019; Turcu et al., 2022) with the study by Hill *et al.* (Hill et al., 2007) displaying a large ES (3.59). The remaining four studies showed a neutral or small detrimental effect of supplementation (Askari & Rahmaninia, 2018; de Camargo et al., 2023; Gross et al., 2014; Jagim et al., 2013).

3.4. Subgroup analysis: supplementation duration

To investigate the effect of duration of beta-alanine supplementation, a subgroup analysis was performed between studies in which supplementation lasted for four weeks (n=9) (Bellinger & Minahan, 2016a, 2016b; Brisola et al., 2018; W Derave et al., 2007; Ducker et al., 2013c, 2013a, 2013b; Freitas et al., 2019; Howe et al., 2013) or five to ten weeks (n=9) (Askari & Rahmaninia, 2018; de Camargo et al., 2023; Gross et al., 2014; Hill et al., 2007; Jagim et al., 2013; Kim et al., 2018; José Luis Maté-Muñoz et al., 2018; Smith et al., 2019; Turcu et al., 2022) (Figure 3). The lower, four week, duration of supplementation showed a smaller but significant effect on maximal intensity exercise (SMD: 0.34, 95% CI:0.02-0.67; p=0.04) when compared to placebo, and displayed no heterogeneity (I^2=0%, p=0.99), compared to the higher, 5-10 week duration of supplementation which showed a larger but statistically insignificant effect (SMD: 0.47, 95% CI:-0.14-1.07; p =0.13) compared to placebo, and moderate to large homogeneity (I^2=72%, $p < 0.0004$).

We performed further analysis on the 5-10 weeks group, splitting them into groups that provided beta-alanine for 5-6 weeks (n=4), or 8-10 weeks (n=5). This analysis showed a small (SMD: 0.18, 95% CI:-0.43-0.79; p=0.57) and moderate (SMD: 0.79, 95% CI:-0.24-1.82; p=0.13) effect when compared to placebo, respectively, but were not significantly different. The 5-6 weeks group showed low heterogeneity (I^2=38%, p=0.18), and the 8-10 weeks group showed high heterogeneity (I^2=83%, p =0.0001).

All studies in the 4-week group reported that beta-alanine supplementation was beneficial, while five of the nine studies in the 5-10 weeks group showed beneficial effects. The remaining four studies showed neutral or small detrimental effects on maximal intensity exercise.

3.5. Subgroup analysis: exercise test duration

To investigate if beta-alanine supplementation changed maximal exercise output relative to the duration of the test, a subgroup analysis was conducted, in which the studies were divided into those which used tests lasting 0–1 minute (n=8), 1–4 minutes (n=7), and 4–10 minutes (n=3) (Figure 4).

The 0–1 minute group showed a small insignificant effect on maximal output (SMD: 0.13, 95% CI:-0.22-0.47; p=0.98) with no heterogeneity (I^2=0%, p=0.98). Despite a moderate effect size, there was no significant effect in the 1–4 minutes test duration group (SMD:0.72, 95% CI:-0.03-1.47; p = 0.06), and heterogeneity was high (I^2=75%, p=0.0005). Finally, the 4–10 minutes group showed a moderate and significant effect of beta-alanine supplementation on maximal output (SMD: 0.55, 95% CI:0.07-1.04; p=0.03) with no heterogeneity (I^2=0%, p=0.58). All results are compared to placebo.

Of the eight studies included in the 0–1 minute group, five showed beneficial effects and the remaining three showed neutral or detrimental effects. Six of the seven studies in the 1–4 minutes group showed positive effects on maximal output, and all of the three studies in the 4–10 minutes group showed beneficial effects.

3.6. Subgroup analysis: dosage

A subgroup analysis on the influence of beta-alanine dosage on maximal intensity exercise revealed significant increases with a high dose (n=12, 5.6g - 6.4 g/d), (SMD:0.35, 95%CI:0.09–0.62; *p*=0.009). Low (n=4, 3.9–4.6 g/d) and medium (n=2, 4.7– 5.5g/d) dosages had no statistically significant effects, with large (SMD:0.82, -0.78–2.43; *p*=0.32) and small (SMD:0.15, -0.48–0.78; *p*=0.65) effect sizes, respectively (Figure 5). All results are compared to placebo.

One of the studies in the low dosage group showed detrimental effects on output (Gross et al., 2014), one study (Askari & Rahmaninia, 2018) included in the medium dosage protocol displayed a neutral outcome, and two studies in the high dosage protocol (de Camargo et al., 2023; Jagim et al., 2013) reported detrimental or neutral effects. The heterogeneity between subgroups was insignificant (I^2=0%, *p*=0.70).

3.7. Risk of bias

Quality assessment and risk of bias performed according to the Revised Cochrane Collaboration's tool for assessing risk of bias in randomized trials (RoB2)(J. P. Higgins et al., 2019), demonstrated that the overall risk of bias of the included studies is low. Bias results are presented in Figure 6.

4. Discussion

4.1. Effect of beta-alanine on maximal intensity exercise

This review aimed to evaluate the effects of beta-alanine supplementation on maximal intensity exercise. There was a small (0.39), significant effect in favor of beta-alanine supplementation on maximal intensity exercise. The duration of beta alanine supplementation showing the greatest ergogenic benefits was 4 weeks, with the administered dosage ranging from 5.6-6.4 g/d. beta-Alanine supplementation was most effective for exercise lasting 4-10 minutes.

Our review's results are in agreement with previous systematic reviews (Berti Zanella et al., 2017; Quesnele et al., 2014) that also found improvements in the same parameters. Beyond that, our results indicate that beta-alanine supplementation can enhance both exercise performance and capacity, agreeing with previous research (Bryan Saunders et al., 2017). An older systematic review and meta-analysis by Hobson *et al.* (R. M. Hobson et al., 2012) found that beta alanine supplementation had a significant effect on capacity but did not improve performance-based measures. The difference in results could be due to the lack of studies incorporating performance measures at the time, as the authors have acknowledged (Bryan Saunders et al., 2017).

Several studies in this review showed that supplementation with beta-alanine improved short – term, high intensity exercise. Specifically, studies reported significant improvements in total work done and power, which are capacity measures (Askari & Rahmaninia, 2018; De Salles Painelli et al., 2014; W Derave et al., 2007; Ducker et al., 2013c; Gross et al., 2014; Hill et al., 2007; Kim et al., 2018; José Luis Maté-Muñoz et al., 2018; Turcu et al., 2022) and improvements in performance measures, such as time to completion/exhaustion (Bellinger & Minahan, 2016a, 2016b; Ducker et al., 2013c, 2013a; Milioni et al., 2019; B Saunders et al., 2017). However, some studies reported no significant improvements in performance or capacity after beta-alanine supplementation. Ducker, Dawson and Wallman (Ducker et al., 2013b) and Saunders *et al.* (Bryan Saunders et al., 2012) reported no changes in performance time in a repeated sprint test. No effect of beta-alanine supplementation was observed by Freitas *et al.* (Freitas et al., 2019) on maximal strength, and other authors also reported no effect (Ruth M Hobson et al., 2013; Howe et al., 2013; Tobias et al., 2013). Potential explanations are discussed within their respective sections below.

From a mechanistic perspective, beta-alanine supplementation has been demonstrated to increase muscle carnosine levels (Culbertson et al., 2010; Varanoske et al., 2017). Carnosine is an important buffer of hydrogen ions (H+), a fatiguing metabolite which accumulates during high intensity exercise, altering muscle pH from ~7.05 to as low as ~6.5 (Allen et al., 2008; Sweeney et al., 2010) negatively affecting exercise output (Culbertson et al., 2010; Ducker et al., 2013a). In addition, carnosine can increase calcium (Ca^{2+}) release from the sarcoplasmic reticulum, increasing muscular contractility and delaying fatigue, thus enhancing output (Dutka et al., 2012; Ojeda et al., 2020).

4.2. Effect of beta-alanine supplementation duration on maximal intensity exercise

Our analysis suggests that the duration of beta-alanine supplementation providing the greatest relative benefit to improving exercise output is 4 weeks. Indeed, a longer duration of supplementation did not appear to augment effects. Furthermore, when subjects were tested on a supramaximal cycling test, data suggested that the greatest enhancement in output was achieved at the end of 4 weeks of supplementation (Hill et al., 2007). Shorter supplementation periods (<4 weeks) did not significantly improve maximal intensity exercise, however, a trend towards improvements in exercise performance with smaller dosage periods has been suggested (Hoffman et al., 2008).

It is not yet clear what the optimal duration period is for increasing intramuscular carnosine concentrations (Trexler et al., 2015), but we can speculate why the 4 week duration may have led to the greatest benefits; beta alanine shares the same transporter to the cell with taurine, therefore prolonged supplementation with beta alanine may affect homeostasis (Guilherme Giannini Artioli et al., 2010; Dolan, Swinton, et al., 2019). This in turn can reverse beta alanine's influence, as reported in a study where participants were ingesting 6.4 g/d of beta alanine for 24 weeks (Dolan, Swinton, et al., 2019).

4.3. Mediation of beta-alanine's effect by test duration

Meta-analysis results demonstrated that beta alanine supplementation improved exercise output in tests lasting 4–10 minutes, but not for tests lasting 0-1 minute or 1-4 minutes, although the SMD was moderate for the latter. These results are in contrast to previous research indicating that tests lasting from 1-4 minutes benefited most from beta alanine supplementation (R. M. Hobson et al., 2012; Bryan Saunders et al., 2017). During exercise of this duration at high intensity, the predominant energetic pathway is the anaerobic glycolytic pathway, where H+ is generated, leading to a decrease in pH, that can precipitate fatigue (Ojeda et al., 2020).

A possible explanation for our findings could be the nature of the tests employed in studies. Performance tests were intermittent, relying upon pacing strategies. They included short periods of maximal intensity exercise followed by light intensity recovery periods, possibly masking beta alanine's ergogenic effect at the early stages of the tests. Maximal blood H+ accumulation occurs after approximately 4 minutes of intense exercise (Bryan Saunders et al., 2017). When maximal exercise is intermittent, it takes longer for H+ to accumulate, extending the time needed for beta alanine supplementation to manifest its effects.

Beta alanine supplementation did not enhance performance in shorter tests lasting 0–1 minute, possibly because exercise of this duration is not limited by acidosis (Guilherme Giannini Artioli et al., 2010). According to Saunders *et al.* (Bryan Saunders et al., 2017), a 0.5-10 minute time frame could be more applicable for carnosine's acid buffering role since physical activity lasting 7-8 minutes still relies heavily on energy from glycolysis. Total anaerobic energy contribution during a 4km cycling test lasting around 6 minutes is about 25%, while total energy contribution from glycolysis during 2000m rowing, lasting more than 7 minutes, is about 12% (Bryan Saunders et al., 2017).

Additionally, it is worth mentioning that duration of the tests itself may have an effect on the reliability of precision of the assessments. Hopkins et al., (2001) showed that measures of reliability can vary based on the nature of the tests being conducted (i.e., field test of sprint running vs. mean power on isokinetic ergometers) as a function of measurement error, expressed as coefficient of variation (CV). It stands to reason that similar effects may be observed as the duration of a test varies, and more research on this topic is required.

4.4 Effect of beta-alanine dosage

Dosages were categorized into three groups, low (3.9-4.6 g/d), medium (4.7-5.5 g/d) and high (5.6-6.4 g/d). The most significant changes in exercise output were observed with high dosages of beta-alanine supplementation, at 5.6-6.4 g/d. This dosage range has been shown to increase muscle carnosine concentrations by up to 64 and 80% at 4 weeks and 10 weeks, respectively (Trexler et al., 2015).

This is in line with research that demonstrated carnosine synthesis in human skeletal muscle is dependent on beta-alanine availability (Sale et al., 2010). Additionally, in the absence of a known threshold of intramuscular carnosine storage, it is reasonable to assume that higher beta-alanine dosages result in higher muscle carnosine concentrations, and this equates to greater muscle buffering capacity during high-intensity exercise (R. M. Hobson et al., 2012). Estimations suggest that carnosine contributes up to 10% of the total buffering capacity in muscle (Guilherme Giannini Artioli et al., 2010). After beta alanine supplementation, total buffering capacity can reach up to 15%, and if only type II muscle fibers are taken into consideration, carnosine contribution can be greater than 25% (Guilherme Giannini Artioli et al., 2010).

However, Huerta Ojeda *et al.* (Ojeda et al., 2020) found in their systematic review and meta-analysis that even dosages as low as 1.5 g/d could result in changes in physical output. This variability in results regarding beta-alanine dosage could be due to the exercise test or outcomes measured and requires further research. Ducker, Dawson and Wallman (Ducker et al., 2013a) state that a range of 3 to 6 g/d of beta-alanine supplementation for a duration of 4 weeks can increase intramuscular carnosine concentrations from 30–80%, with the range of dosage supplementation likely being proportional to carnosine concentration increase.

5. Strengths and limitations

To date, this is the most comprehensive meta-analysis on the effects of beta-alanine supplementation, covering a generalized analysis as well as various subgroupings including supplementation duration, exercise test duration, and dosage. A major strength of this research is the high quality of the studies included. Overall, the included studies had a low risk of bias, with only one study exhibiting a high risk of bias, in just two domains. Almost all of the studies mention that they controlled for nutritional supplementation and ergogenic aid use during the intervention period, and excluded participants that consumed any supplement that could affect the results of the intervention 3 months prior to the study.

Females may have been excluded because they inherently have lower levels of carnosine (A Baguet et al., 2010) and therefore mixed gender groups could bias results. Furthermore, all studies excluded vegetarians, as they may have lower basal carnosine stores (A Baguet et al., 2010). The findings of this review are therefore only applicable to omnivorous males. A limitation is the low number of studies available for review that provided beta-alanine, without the addition of other ingredients. Additionally, any conclusions from this investigation only pertain to maximal intensity outcomes, such as strength, power, and performance time, as this review did not focus on sub-maximal outcomes, or exercise lasting 0.5 minutes or >10 minutes in duration.

6. Practical applications

Based on our findings, beta-alanine supplementation could be used by coaches, strength and conditioning trainers, and athletes across a broad spectrum of sports and activities as an ergogenic aid to enhance physical exercise output. Supplementation is likely to be most beneficial for athletes in high-intensity sports lasting from 4-10 minutes, such as middle-distance runners, rowers, swimmers, and combat sports' athletes. For Maximal benefits, the dosage should range from 5.6-6.4 g/d, for a supplementation period of 4 weeks. In order to avoid paresthesia that may occur after beta-alanine supplementation, it is recommended to ingest beta-alanine in smaller doses of 1.6 g throughout the day (Trexler et al., 2015) and to consume 2 g/kg of carbohydrates an hour before beta-alanine supplementation (Ojeda et al., 2020). This study can also serve as a foundation for further research into those areas of beta-alanine supplementation which still need clarification, such as acute dosing protocols, and whether or not upper limits to cellular carnosine saturation exist, and their subsequent effects on exercise/sports performance and physiology.

7. Conclusions and future perspectives

This systematic review and meta-analysis demonstrated that beta-alanine supplementation can significantly enhance maximal intensity exercise, as measured by changes in power, strength, work done, performance time, and total sets performed. Our analysis suggests that such effects are observed with a dosage of 5.6 g - 6.4 g/d for 4 weeks, when exercise performance lasts 4-10 minutes. This highlights the need for more high quality, newer systematic reviews since the 4-10 minute timeframe is not in agreement with previous systematic reviews (R. M. Hobson et al., 2012; Bryan Saunders et al., 2017).

Nevertheless, heterogeneous results from individual studies necessitate the need for further research. Also, some studies suggest that beta-alanine supplementation can enhance maximal intensity exercise with lower, acute doses (Ducker et al., 2013a; Ojeda et al., 2020) even when ingested one hour prior to testing (Ojeda et al., 2020). Therefore, dosage and duration of beta-alanine supplementation needs to be further investigated, as well as the minimal effective acute dose. More research should be conducted with female participants, both athletes and sports enthusiasts, since studies concerning beta-alanine supplementation in females are currently scarce compared to those in males.

Declarations

Availability of data and material: Data available within the article or its supplementary materials

Author Contributions: Conceptualization: RZ and AAR. Methodology, investigation, data curation and formal analysis, writing - original draft: GDG, KA, SA, and EAM. Writing - Review & Editing: RZ, AAR, KP, and TC. Project administration: AAR, GDG. Supervision: TC and EC. All authors read and approved the final version.

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