β-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis

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ABSTRACT

Objective To conduct a systematic review and meta-analysis of the evidence on the effects of β-alanine supplementation on exercise capacity and performance.

Design This study was designed in accordance with PRISMA guidelines. A 3-level mixed effects model was employed to model effect sizes and account for dependencies within data.

Data sources 3 databases (PubMed, Google Scholar, Web of Science) were searched using a number of terms ('β-alanine' and 'Beta-alanine' combined with 'supplementation', 'exercise', 'training', 'athlete', 'performance' and 'carnosine').

Eligibility criteria for selecting studies Inclusion/exclusion criteria limited articles to double-blinded, placebo-controlled studies investigating the effects of β-alanine supplementation on an exercise measure. All healthy participant populations were considered, while supplementation protocols were restricted to chronic ingestion. Cross-over designs were excluded due to the long washout period for skeletal muscle carnosine following supplementation. A single outcome measure was extracted for each exercise protocol and converted to effect sizes for meta-analyses.

Results 40 individual studies employing 65 different exercise protocols and totalling 70 exercise measures in 1461 participants were included in the analyses. A significant overall effect size of 0.18 (95% CI 0.08 to 0.28) was shown. Meta-regression demonstrated that exercise duration significantly (p=0.004) moderated effect sizes. Subgroup analyses also identified the type of exercise as a significant (p=0.013) moderator of effect sizes within an exercise time frame of 0.5–10 min with greater effect sizes for exercise capacity (0.4998 (95% CI 0.246 to 0.753)) versus performance (0.1078 (95% CI −0.201 to 0.416)). There was no moderating effect of training status (p=0.559), intermittent or continuous exercise (p=0.436) or total amount of β-alanine ingested (p=0.438). Co-supplementation with sodium bicarbonate resulted in the largest effect size when compared with placebo (0.43 (95% CI 0.22 to 0.64)).

Summary/conclusions β-alanine had a significant overall effect while subgroup analyses revealed a number of modifying factors. These data allow individuals to make informed decisions as to the likelihood of an ergogenic effect with β-alanine supplementation based on their chosen exercise modality.

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INTRODUCTION

The purported pleiotropic effects of carnosine (β-alanyl-L-histidine) have attracted interest in this histidine-containing dipeptide in recent years due to its potential ergogenic and therapeutic benefits.[1] Its abundance in skeletal muscle suggests that it plays an important role during exercise, with a key physiological role considered to be intracellular acid–base regulation, although further roles for carnosine have also been suggested, such as protection against oxidative damage, glycation and regulation of calcium sensitivity (for review, see ref. 2). The rate limiting factor to muscle carnosine synthesis is the availability of β-alanine from the diet,[3] with supplementation shown to increase skeletal muscle concentrations in the upper and lower limbs.[3–9] This has led to investigations into the effects of β-alanine supplementation, and thus increased muscle carnosine concentration, on capacity and performance within a variety of exercise tests and sample populations.

A previous meta-analysis[10] showed the efficacy of β-alanine supplementation on exercise with a significant effect on capacity but not performance tests, although the authors acknowledged that this might have been due to the lack of studies employing a performance measure at the time. The exercise time frame in which β-alanine was most effective was 1–4 min, with no effect on exercise of <1 min in duration. Although a positive effect was shown on exercise of more than 4 min in duration, there was a lack of protocols employing long-duration continuous exercise; the majority of the longer duration exercise studies employed incremental protocols of such low intensity during the initial stages that they were unlikely to produce any significant changes that result in fatigue. Therefore, the duration of such tests is somewhat deceptive since it is only the latter stages that may be susceptible to changes in muscle carnosine with supplementation. Furthermore, in line with the primary suggested role of muscle carnosine, a 0.5–10 min time frame may be more appropriate since acidosis following 20 s of exercise may already contribute to fatigue,[11] while exercise of 7–8 min in duration has been shown to have a high contribution from glycolytic energy sources.[12] Thus, determination of the effect of β-alanine within these different exercise durations would further contribute to the evidence base as to the main physiological role of carnosine in skeletal muscle during exercise.

It would be of interest to investigate the ability of β-alanine to improve exercise measures in different sample populations (ie, trained and non-trained) and exercise modes (ie, whole body and isolated limb exercise) since its efficacy for trained individuals[13 14] and whole body exercise has been

questioned.[15] Furthermore, since over half of professional footballers reported taking β-alanine,[16] determination of its effect on intermittent as well as continuous exercise is warranted. Additionally, since muscle carnosine increases are dose dependent,[17] it appears reasonable to suggest that the total amount ingested or supplementation duration may influence any exercise gains. Co-supplementation of β-alanine with sodium bicarbonate may lead to additive gains via increased intracellular (from increased muscle carnosine concentrations) and extracellular (from increased blood bicarbonate concentrations) buffering capacity.[18] Therefore, it would be of interest to determine the effect of co-supplementation of β-alanine with sodium bicarbonate to determine whether any improvements are additive and exceed those with β-alanine alone.

The aim of this study was to perform a systematic review and meta-analysis on the effects of β-alanine supplementation, accounting for a number of potential modifying factors including exercise duration, type and mode, sample population, total amount ingested and study length. Furthermore, the combined effects of sodium bicarbonate and β-alanine were also investigated.

METHODS

Study eligibility

The protocol for this study was designed in accordance with PRISMA guidelines,[19] and the question determined in respect of PICO (Population, Intervention, Comparator and Outcomes). The *population* included healthy human males and females of any age. Recreationally active and professional athletes were considered for inclusion. Studies conducted with diseased-state participants were excluded. The *intervention* must have employed a chronic (>1 day) β-alanine supplementation protocol. Since the net retention of β-alanine used for muscle carnosine synthesis over 4 weeks of supplementation is 6.0–6.5% over the dose range 3.2–6.4 g/day (estimated from refs. 3, 20), acute supplementation protocols were not included since a single dose of β-alanine would not result in any meaningful increases in muscle carnosine. No cross-over designs were included in the analyses if they did not demonstrate a complete washout of muscle carnosine, given the long washout period for muscle carnosine (up to 16 weeks) following doses of between 1.6 and 4.8 g/day of β-alanine.[6 17] Although Danaher *et al*[21] reported no significant differences in muscle carnosine concentration following 12 weeks of washout, a lack of statistical significance does not exclude the possibility that carnosine remained elevated in some individuals. Thus, following a discussion and agreement between all reviewers, this study was not included in any of the analyses. In relation to the *comparator*, the protocol for this study determined that only double-blinded, placebo-controlled studies were included. Studies that reported on *outcomes* based on exercise performance and capacity were considered for inclusion. For the purpose of this review, exercise capacity tests were defined as those requiring exertion to the point of volitional fatigue, while performance-based tests were defined as those requiring pacing strategies that may not necessarily elicit maximal exertion. Only peer-reviewed, original human studies published between 2002 and February 2016 were included within this review. We applied a date restriction of 2002 to coincide with the publication of the first paper to investigate the association between muscle carnosine and exercise.[22] Where data were incomplete, authors were contacted to obtain the relevant information. Data from one study were not received,[23] meaning that this study was not included in the analyses.

Search strategy and quality assessment

An electronic search of the literature was undertaken by BS using three databases (PubMed, Google Scholar and Web of Science) to identify all relevant articles. The search terms 'β-alanine' and 'beta-alanine' were individually concatenated with 'supplementation', 'exercise', 'training', 'athlete', 'performance' and 'carnosine'; search terms were restricted to the title of the articles. Following the removal of duplicates, a two-phase search strategy was subsequently employed by three independent reviewers (BS, GGA and KES). Phase one assessed the eligibility of the title and abstract of every hit generated from the search terms against the inclusion/exclusion criteria. Studies that had questionable suitability were included at this stage and the final decision was reached at the next phase (phase 2). In phase 2, full articles were retrieved and assessed against the eligibility criteria. Reference lists of relevant original and review articles were screened to ensure that all relevant studies were included. Any differences of opinion relating to study eligibility were resolved through discussion. The search strategy is summarised in figure 1. The final search was completed in February 2016.

A funnel plot (see online supplementary figure S1) was used to visually determine publication bias; BS and BG performed this according to standard recommendations.[24] Some data appeared to contribute to some asymmetry in the plot, with one study having a very low sample size (β-alanine only group (BA)=5, placebo group (PL)=2).[25] High standard errors shown for the other two[26 27] are most likely due to high interindividual responses within the respective studies. It was concluded that there was no publication bias and inclusion of all study estimates was warranted. BS and KES independently reviewed the strength of the evidence provided by all studies using the criteria outlined in the Consolidated Standards of reporting Trials (CONSORT statement;[28 29] online supplementary table S1). Any differences between reviewers were resolved by discussion until an agreement was reached. Items 3b, 6b, 7b, 14a, 14b, 17b, 18, 23 and 24 were removed, as they were not applicable to any of the studies. The results of the CONSORT assessment were not used to exclude any study that did not meet the standards or requirements but provide an objective measure of each study's adherence to the minimum set of recommendations for reporting randomised trials as determined by CONSORT.

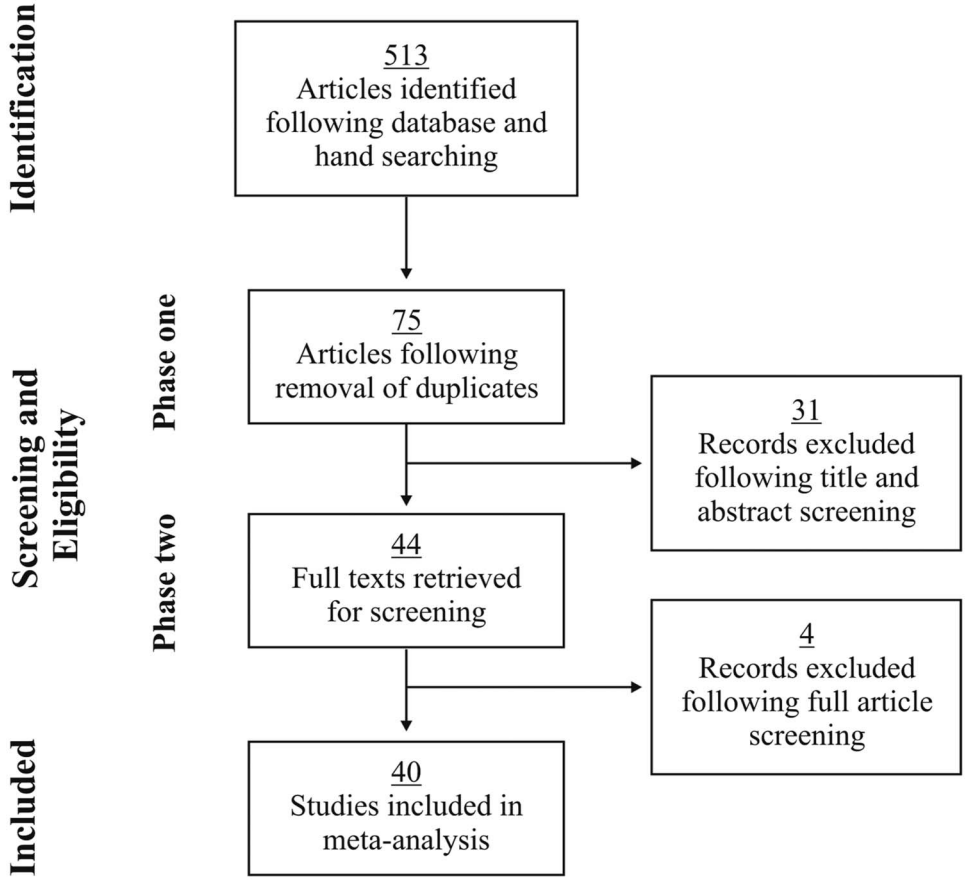
Data extraction and variable categorisation

Data extraction was conducted by BS using a standardised and prepiloted data extraction form. A number of protocols resulted in several outcome measures of the same exercise test; in order to avoid duplication bias, only a solitary outcome measure from each exercise protocol was included. Thus, where a single exercise protocol resulted in one or more outcome measures, the outcome measure included was based on a hierarchy agreed on by all reviewers to ensure consistency in data extraction. Data were extracted according to availability, prioritising exercise measures over physiological measures, according to the following hierarchical profile:

1. Total work done;
2. Mean output throughout the test (ie, mean power output; mean velocity; mean height);
3. Time to completion (performance test)/time to exhaustion (capacity test);
4. Oxygen consumption (ie, maximal (VO_{2max}) or peak (VO_{2peak}) oxygen uptake).

Where multiple time points following supplementation existed (ie, 0–4 and 0–10 weeks), postsupplementation outcome data

Figure 1 Search strategy summary.



were extracted from the final time point available. This was performed to ensure the highest possible accumulation of muscle carnosine due to β-alanine supplementation and thus, theoretically, the most likely time point to show any effect.

Following extraction, data were allocated into categories to allow for subgroup analyses. This included separating data by exercise duration according to the approach of Hobson *et al*[10] (*duration 1*), namely 0–1 min, 1–4 min and in excess of 4 min, which were chosen to consider the proposed physiological mechanisms of carnosine, in particular pH buffering. Additional subgroup analyses were performed according to exercise duration using the following categories (*duration 2*): 0–0.5 min; 0.5–10 min; 10 min+. The lower limit was chosen since Bogdanis *et al*[11] suggested that sprint performance may be affected by the acidosis that occurred during the initial 20 s of exercise. Although maximal H^+ accumulation in blood occurs after ~4 min,[30] total anaerobic energy contribution during the 4 km cycling time trial (lasting ~6 min) is ~25%,[31] while Stellingwerff *et al*[12] showed that non-oxidative glycolysis contributes ~12% towards total energy contribution during 2000 m rowing (lasting in excess of 7 min). Thus, to ensure inclusion of all exercise with a substantial contribution from anaerobic energy sources, an upper limit of 10 min was determined. All exercise in excess of 10 min, with a likely predominantly aerobic energy contribution, made up the final category.

Exercise protocols were categorised according to whether they measured exercise capacity or performance (*exercise type*). Capacity tests require exertion to the point of volitional exhaustion, resulting in a maximal production of H^+, whereas performance tests rely more on pacing strategies that might not elicit maximal exertion.[32] Analyses also separated investigations according to the sample populations recruited to the study (*training status*), since it has been suggested that trained individuals may be less responsive to β-alanine supplementation.[13 14] Studies were categorised as using trained individuals if the sample population was engaged in a structured training programme and whose training plan was relevant for the exercise task employed to study the effects of β-alanine; those individuals who did not fit this description (ie, recreationally active; non-trained) were categorised as non-trained. Exercise tests were additionally categorised according to whole-body or isolated limb exercise (*exercise mode*), since differences in exercise mode influence energy substrate usage[33] and oxygen uptake kinetics.[34] Differences in whole-body and isolated muscle recruitment have also been suggested to contribute to the efficacy of β-alanine due to differences in whole body versus local muscle acidosis.[15] Exercise tests were similarly categorised according to whether they employed an intermittent or continuous exercise protocol (*intermittent*). Since increases in muscle carnosine appear to be dose dependent,[17] we analysed the effect of total amount (*total amount*) and study length (*study length*) as continuous variables (in grams and days). Further subanalysis was performed on studies that investigated the effect of co-supplementation of β-alanine and sodium bicarbonate on exercise (*co-supplementation*).

Data analysis

Data were converted to observed effect sizes (d) and their variances (σ_r^2) calculated according to Morris:[35]

$$d = C\left[\frac{(M_{post,T} - M_{pre,T}) - (M_{post,P} - M_{pre,P})}{SD_{pre}}\right]$$

Table 1 All studies included in the meta-analysis

Authors and location	Participants	Supplementation protocol	Total dosage (g)	Exercise protocol
Baguet *et al*,[7] Belgium	Elite male rowers PL=9; BA=8	5.0 g/day for 49 days	245	2000 m rowing ergometry
Bellinger *et al*,[13] Australia	Trained male cyclists PL=7; BA=7	65 mg/kg BM/day for 4 weeks	~129.4	4 min maximal cycle bout
Bellinger and Minhan,[53] Australia	Trained male cyclists PL=7; BA=7	6.4 g/day for 4 weeks	179.2	1 km cycling TT 4 km cycling TT 10 km cycling TT
Carpentier *et al*,[54] Belgium	Physically active males and females PL=13; BA=14	5.6 g/day for 8 weeks	313.6	Squat jump CMJ 45 CMJ fatigue test
Chung *et al*,[55] Australia	Elite/subelite male/female swimmers PL=/; BA=/	4.8 g/day for 4 weeks followed by 3.2 g/day for 6 weeks	268.8	50–400 m swimming: Training performance Competition performance
Chung *et al*,[42] Belgium/ Australia	Trained male cyclists PL=13; BA=14	6.4 g/day for 6 weeks	268.8	1 hour cycling TT
Cochran *et al*,[56] Canada	Healthy young males PL=12; BA=12	3.2 g/day for 6 weeks	134.4	Incremental cycle test 250 kJ cycling TT Repeated sprint cycle test
del Favero *et al*,[46] Brazil	Elderly males and females PL=6; BA=12	3.2 g/day for 12 weeks	268.8	Constant load submaximal test Incremental treadmill test
Derave *et al*,[5] Belgium	Sprint-trained competitive athletes PL=7; BA=8	2.4 g/day for 4 days, 3.6 g/day for 4 days, 4.8 g/day up to 5 weeks	153.6	400 m sprint Isometric endurance Maximal isokinetic knee extensions
Ghiasvand *et al*,[57] Iran	Recreational males PL=19; BA=20	2.0 g/day for 42 days	84	Graded exercise test
Glenn *et al*,[58] USA	Female masters athletes PL=11; BA=11	3.2 g/day for 4 weeks	89.6	Lower body ISO strength Hand grip strength
Gross *et al*,[9] Switzerland	Recreational males PL=9; BA=9	3.2 g/day for 38 days	121.6	Maximal incremental cycle
Gross *et al*,[25] Switzerland	Elite male alpine skiers PL=4; BA=5	4.8 g/day for 5 weeks	168	Counter movement jump 90 s box jump test
Hill *et al*,[4] UK	Recreational males PL=8; BA=7	4.0 g/day in week 1 rising to 6.4 g/day by week 4 until week 10	414.4	Cycling capacity test at 110% of PowerMax
Hobson *et al*,[40] UK	Trained male rowers PL=10; BA=10	6.4 g/day for 28 days	179.2–192	2000 m rowing ergometry
Hoffman *et al*,[59] USA	Elite male soldiers PL=9; BA=9	6.0 g/day for 4 weeks	168	4 km run 5 CMJ 120 m sprint
Hoffman *et al*,[60] USA	Elite male soldiers PL=9; BA=9	6.0 g/day for 30 days	180	2.5 km run 1 min sprint 50 m casualty carry Repeated 30 m sprints
Howe *et al*,[61] Australia	Highly trained male cyclists PL=8; BA=8	65 mg/kg BM/day for 4 weeks	127.4	4 min maximal cycle bout
Jagim *et al*,[62] USA	Anaerobically trained athletes PL=11; BA=10	4.0 g/day for 1 week followed by 6.0 g/day for 4 weeks	196	Run to exhaustion at 115% VO_{2max} Run to exhaustion at 140% VO_{2max}
James *et al*,[41] UK	Trained male cyclists PL=9; BA=10	6.4 g/day for 4 weeks	179.2	20 km cycling TT
Jordan *et al*,[63] USA	Recreational males PL=9; BA=8	6.0 g/day for 4 weeks	168	Incremental treadmill run to exhaustion
Okudan *et al*,[64] Turkey	Untrained healthy males PL=11; BA=11	3.2 g/day for 22 days and 6.4 g/day for 6 days	108.8	3×30 s Wingates
De Salles Painelli *et al*,[47] Brazil	Male and female swimmers PL=7; BA=7	3.2 g/day for 1 week and 6.4 g/day for 3 weeks	156.8	100 m swimming 200 m swimming
De Salles Painelli *et al*,[43] Brazil	Trained male cyclists and recreational males PL=19; BA=20	6.4 g/day for 4 weeks	179.2	4×30 s cycle Wingates
Sale *et al*,[18] UK	Recreational males PL=10; BA=10	6.4 g/day for 4 weeks	179.2	Cycling capacity test at 110% of PowerMax
Sale *et al*,[65] UK	Recreational males PL=6; BA=7	6.4 g/day for 4 weeks	179.2	IKET

Continued

Table 1 Continued

Authors and location	Participants	Supplementation protocol	Total dosage (g)	Exercise protocol
Saunders *et al*,[66] UK	Elite/non-elite male games players PL=18; BA=18	6.4 g/day for 4 weeks	179.2	Loughborough Intermittent Shuttle Test
Saunders *et al*,[26] UK	Amateur male footballers PL=8; BA=9	3.2 g/day for 12 weeks	268.8	Yo-Yo Intermittent Recovery Test Level 2
Saunders *et al*,[67] UK	16 recreational males PL=8; BA=8	6.4 g/day for 4 weeks followed by 3.2 g/day for 1 week	179.2–201.6	3 sets of 5×6 s repeated sprints
Smith *et al*,[68] USA	Recreational males PL=18; BA=18	6.0 g/day for 3 weeks followed by 3.0 g/day for 3 weeks	189	Cycle capacity at 110% VO_{2max}
Smith *et al*,[69] USA	Recreational women PL=11; BA=13	4.8 g/day for 4 weeks	134.4	Graded exercise test
Smith-Ryan *et al*,[70] USA	Recreational males PL=24; BA=26	4.8 g/day for 4 weeks	134.4	Run to exhaustion at 90% PV Run to exhaustion at 100% PV Run to exhaustion at 110% PV
Smith-Ryan *et al*,[71] USA	Recreational males and females PL=15; BA=15	6.4 g/day for 4 weeks	179.2	Graded exercise test
Stout *et al*,[72] USA	Males PL=13; BA=12	6.4 g/day for 6 days, then 3.2 g/day for 22 days	108.8	Incremental cycle to exhaustion
Stout *et al*,[73] USA	Females PL=11; BA=11	3.2 g/day for 7 days, then 6.4 g/day for 21 days	156.8	Incremental cycle to exhaustion
Stout *et al*,[27] USA	Elderly males and females PL=14; BA=12	2.4 g/day for 90 days	216	Discontinuous incremental exercise protocol
Tobias *et al*,[48] Brazil	Martial arts male athletes PL=9; BA=10	6.4 g/day for 4 weeks	179.2	4×30 s upper body Wingates
Van Thienen *et al*,[74] Belgium	Well-trained male cyclists PL=8; BA=9	2 g/day for 2 weeks, 3 g/day for 2 weeks, then 4 g/day for 4 weeks	182	Incremental cycle to exhaustion 10 min cycling TT 30 s cycle sprint
Walter *et al*,[75] USA	Recreational females PL=19; BA=14	6.0 g/day for 3 weeks followed by 3.0 g/day for 3 weeks	189	Graded exercise test
Zoeller *et al*,[76] USA	Healthy males PL=13; BA=12	6.4 g/day for 6 days followed by 3.2 g/day for 22 days	108.8	Incremental cycle to exhaustion

BA, β-alanine only group; CMJ, countermovement jump; IKET, isometric knee extension test at 45% of maximum voluntary contraction; ISO, isokinetic strength; PL, placebo group; PV, peak velocity; TT, time trial; VO_{2max}, maximal oxygen uptake.

Where T refers to the BA and P refers to PL. SD_{pre} is the pooled SD and defined as:

$$SD_{pre} = \sqrt{\frac{(n_T - 1)SD^2_{pre,T} + (n_P - 1)SD^2_{pre,P}}{n_T + n_P - 2}}$$

C is a bias correction and equal to:

$$C = 1 - \frac{3}{4(n_T + n_P - 2) - 1}$$

The effect size variance σ_r^2 was calculated as

$$\sigma_r^2 = 2(1 - r_{pre-post})\left(\frac{n_T + n_P}{n_T n_P}\right)\left(\frac{n_T + n_P - 2}{n_T + n_P - 4}\right) \times \left(1 + \frac{d^2}{2(1 - r_{pre-post})(n_T + n_P)/(n_T n_P)}\right) - \left(\frac{d^2}{C^2}\right)$$

To calculate the observed effect size variance, the correlation between presupplementation and postsupplementation outcomes ($r_{pre-post}$) was required. A single correlation value was estimated for all observed effect sizes. Raw data were obtained for 9 of the 40 included studies, comprising a total of 12 outcomes. The average presupplementation to postsupplementation correlation value obtained was 0.877±0.20; a weighted average presupplementation to postsupplementation correlation value of 0.95 (0.88 to 0.97) was obtained. Therefore, a single presupplementation to postsupplementation correlation value of 0.9 was used to calculate σ_r^2. Sensitivity analyses were also carried out using a single presupplementation to postsupplementation correlation value of 0.7.

A three-level mixed effects model was used to model effect sizes and account for dependencies within the data. The basic model consisted of three regression equations, one for each level:[36]

$$d_{jk} = \beta_{jk} + r_{jk} \text{ with } r_{jk} \sim N(0, \sigma^2_{rjk}) \quad \text{(level 1 : sample)}$$

The equation at the first level states that d_{jk}, the j-th observed effect size from study k, is equal to the corresponding population value β_{jk} plus a random deviation, r_{jk}, that is normally distributed with mean zero and variance obtained as described above. The second-level equation represents the outcome level and states that the population effects for the different outcomes within a study can be decomposed into a study mean (θ_{0k}) and random residuals v_{jk}.

$$\beta_{jk} = \theta_{0k} + v_{jk} \text{ with } v_{jk} \sim N(0, \sigma^2_v) \quad \text{(level 2 : outcome)}$$

The third level is an extension of the common random-effects model and states that mean study effects θ_{0k}can vary around an overall mean γ_{00} with the random variation μ_{0k}:

$$\theta_{0k} = \gamma_{00} + \mu_{0k} \text{ with } \mu_{0k} \sim N(0, \sigma^2_\mu) \quad \text{(level 3 : study)}$$

The between study variance in the mean effect, σ^2_μ, reflects the covariance between two effect sizes from the same study. Van den Noortgate *et al*[36] previously demonstrated that the three-

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level model can provide appropriate mean effect size estimates and SE estimates across a variety of realistic situations. The model was then extended by incorporating fixed effects in an attempt to further explain the variation in observed effect sizes. The fixed effects assessed included characteristics of the outcomes ((*exercise duration*), (*exercise type*), (*training status*), (*exercise mode*)) and studies ((*total amount*), (study length)). Subgroup analyses performed on outcomes from studies providing a co-supplementation group comprising β-alanine and sodium bicarbonate were performed using a two-level meta-analysis due to limited nesting of outcomes within studies. Additionally, effect sizes were computed comparing the co-supplementation group (BASB) relative to PL and the co-supplementation group relative to BA. All data were analysed using the rma and rma.mv functions in the metafor package[37] in R (R Foundation for Statistical Computing, Vienna Austria) and significance level was previously set at $p<0.05$.

RESULTS

Included study characteristics

Forty published studies met the criteria for inclusion in the analyses (table 1). A total of 65 exercise tests were employed across the various studies resulting in 70 exercise outcome measures in 1461 participants (BA: N=746; PL: N=715). Grouping of studies and exercise protocols for the various subanalyses performed is presented in table 2.

META-ANALYSIS

Overall

Results from the meta-analysis showed a small significant mean effect size of 0.180 (95% CI 0.078 to 0.284). Substantial heterogeneity was noted with systematic variance (level 2 and level 3 variance) accounting for ~72% of the total variance (table 3). Sensitivity analysis using a presupplementation to postsupplementation correlation of 0.7 resulted in a small main effect estimate of 0.180 (95% CI 0.076 to 0.292) with a concomitant increase in sampling variance that resulted in systematic variance accounting for ~36% of the total variance.

Univariate meta-regressions

There was a significant moderating effect of (*duration 1*) (p=0.003) with results showing small significant effect sizes for durations of 1–4 min (0.210 (95% CI 0.057 to 0.362)) and 4+ min (0.233 (95% CI 0.059 to 0.407)), but not 0–1 min (0.016 (95% CI −0.223 to 0.254)). Similarly, there was a significant moderating effect of (*duration 2*) (p=0.004, figure 2) with results showing a small significant effect size for durations of 0.5–10 min (0.224 (95% CI 0.088 to 0.361)). There was a tendency towards significance for 10+ min (0.174 (95% CI −0.030 to 0.377)) but effect size was not significant for 0–0.5 min (0.040 (95% CI −0.220 to 0.300)).

There was no moderation effect distinguishing between the different levels of (*exercise type*) (p=0.256), (*training status*) (p=0.559), (*exercise mode*) (p=0.931) and (*intermittent*) (p=0.436). Similarly, no moderation effect was obtained for the continuous variable (*total amount*) (p=0.438) on effect sizes. However, there was a moderation effect of (*study length*) (p=0.005) with an apparent 0.011 increase in effect size each day beyond 20 days of supplementation (table 3). However, this result appeared to be driven by the presence of two outliers (Cook's distance 1.8 and 0.7), and when a sensitivity analysis was performed without these data points, (*study length*) no longer had a significant effect (p=0.685).

Further analyses

Owing to the strong effect of (*duration 2*) on effect sizes, further meta-regressions were performed using (*duration 2*). Subgroup analyses were completed for the outcomes within 0.5–10 min (N=41) only. No moderation effects were shown for (*total amount*) (p=0.831), (*study length*) (p=0.309) and the different levels of (*exercise mode*) (p=0.507), (*intermittent*) (p=0.255) or (*training status*) (p=0.874). However, there was a significant moderation effect distinguishing between the two levels of (*exercise type*) (p=0.01) with higher effect sizes for exercise capacity than performance (0.4998, medium effect (95% CI 0.246 to 0.753) vs 0.1078, small effect (95% CI −0.201 to 0.416)).

Analysis of (*co-supplementation*) showed that, in comparison to PL, BASB resulted in a significant effect size of 0.433 (0.230 to 0.637; p<0.001). Furthermore, compared with BA, BASB also resulted in a significant effect size of 0.242 (0.071, 0.412; p=0.006; figure 3).

DISCUSSION

The results of this meta-analysis showed a significant positive overall effect of β-alanine supplementation on exercise, supporting the efficacy of increased muscle carnosine to improve exercise. Exercise duration was the greatest influencing factor regarding the efficacy of β-alanine supplementation. Replicating the duration time frames of Hobson *et al*[10] yielded similar results; significant effect sizes were shown for exercise 1–4 min in duration, supporting previous evidence that β-alanine is beneficial for this exercise time frame. No effect was shown for exercise protocols lasting <1 min, and considering a mechanism of action relating to increased intracellular buffering, this would make sense given that this duration of exercise is unlikely to be limited by intracellular H^+ accumulation.[38] This finding also provides some indirect evidence in opposition to the influence of increased muscle carnosine content on calcium sensitivity of the contractile apparatus or calcium release from the sarcoplasmic reticulum. This is in agreement with the recent data of Hannah *et al*[39] which showed no effect of β-alanine on maximal explosive voluntary contractions, suggesting that calcium sensitivity and release might not be the primary mechanisms by which increased carnosine improves whole muscle exercise in humans. Exercise of longer than 4 min in duration was improved with β-alanine supplementation, with greater effect sizes than exercise 1–4 min in duration (0.233 vs 0.210), though we speculate that this may be due to shorter duration protocols (6–7 min),[7 40] which may be more susceptible to increases in muscle carnosine if its primary mechanism is considered to be that of pH buffering, being grouped with those of longer duration (30–60 min).[41 42]

Although results reported within the current study agree somewhat with the categorisation of exercise durations employed by Hobson *et al*,[10] we further explored the effect of exercise duration using adapted criteria. Short duration exercise (≤0.5 min) was not benefited from supplementation (d=0.040), while effect sizes for moderate duration exercise (0.5–10 min) were significant (d=0.224). However, β-alanine was no longer shown to be effective for longer duration exercise (>10 min), although there was still a moderate effect size (d=0.174). It was supposed that these data may have been influenced by incremental protocols with a low intensity during the initial stages that were unlikely to induce fatigue. Therefore, the duration of such tests is somewhat deceptive since it is only the latter stages, conducted at higher exercise intensities, that may be susceptible to

Table 2 Categorisation of studies for subanalyses

Authors	Exercise protocol	(Duration 1) 1=0–1 min 2=1–4 min 3=4 min+	(Duration 2) 1=0–0.5 min 2=0.5–10 min 3=10 min+	(Exercise type) 1=Capacity 2=Performance	(Training status) 1=Trained 2=Non-trained	(Exercise mode) 1=Isolated limb 2=Whole body	(Intermittent) 1=Intermittent 2=Continuous	(Study length) (days)	(Total amount) (g)
Baguet *et al*[7]	2000 m rowing	3	2	2	1	2	2	49	245
Bellinger *et al*[13]	4 min maximal cycle	2	2	2	1	1	2	28	129.4
Bellinger and Minhan[53]	1 km cycling TT	2	2	2	1	1	2	28	179.2
	4 km cycling TT	3	2	2	1	1	2	28	179.2
	10 km cycling TT	3	3	2	1	1	2	28	179.2
	Supramaximal cycle	2	2	1	1	1	2	28	179.2
Carpentier *et al*[54]	Squat jump	1	1	1	2	1	2	56	313.6
	CMJ	1	1	1	2	1	2	56	313.6
	45 CMJ fatigue test	2	2	2	2	1	2	56	313.6
Chung *et al*[55]	Swimming training	2	2	2	1	2	2	70	268.8
	Swimming competition	2	2	2	1	2	2	70	268.8
Chung *et al*[42]	1 hour cycling TT	3	3	2	1	1	2	42	268.8
Cochran *et al*[56]	Incremental cycle	3	3	1	2	1	2	42	134.4
	250 kJ cycle TT	3	3	2	2	1	2	42	134.4
	Repeated cycle sprints	2	2	2	2	1	1	42	134.4
del Favero *et al*[46]	Incremental treadmill test	3	3	1	2	2	2	84	268.8
	Treadmill test	3	2	1	2	2	2	84	268.8
Derave *et al*[5]	Isometric endurance	2	2	1	1	1	2	36	153.6
	400 m running	1	2	2	1	2	2	36	153.6
	Maximal isokinetic knee extensions	1	1	1	1	1	2	36	153.6
Ghiasvand *et al*[57]	Incremental cycle test	3	3	1	2	1	2	42	84
Glenn *et al*[58]	Isokinetic strength—flexion	2	2	2	1	1	2	28	89.6
	Isokinetic strength—extension	2	2	2	1	1	2	28	89.6
	Hand grip test	1	1	1	1	1	2	28	89.6
Gross *et al*[9]	Incremental cycle test	3	3	1	2	1	2	38	121.6
Gross *et al*[25]	CMJ	1	1	1	1	1	2	35	168
	90 s box jump test	2	2	2	1	1	2	35	168
Hill *et al*[4]	$CCT_{110\%}$	2	2	1	2	1	2	70	414.4
Hobson *et al*[40]	2000 m rowing	3	2	2	1	2	2	35	192
Hoffman *et al*[59]	4 km run	3	3	2	2	2	2	28	168
	CMJ (×5)	1	1	2	2	1	2	28	168
	120 m running sprint	1	1	2	2	2	2	28	168
Hoffman *et al*[60]	2.5 km running sprint	3	3	2	2	2	2	30	180
	1 min running sprint	1	2	2	2	2	2	30	180
	Repeated 30 m sprints	1	1	2	2	2	1	30	180
	50 m casualty carry	1	1	2	2	2	2	30	180
Howe *et al*[61]	4 min maximal cycle	2	2	2	1	1	2	28	127.4
Jagim *et al*[62]	Run at 115% VO_{2max}	2	2	1	2	2	2	35	196
	Run at 140% VO_{2max}	2	2	1	2	2	2	35	196
James *et al*[41]	20 km cycling TT	3	3	2	1	1	2	28	179.2
Jordan *et al*[63]	Incremental treadmill test	3	3	1	2	2	2	28	168
Okudan *et al*[64]	3 bouts of 30 s cycling	2	2	2	2	1	1	28	108.8

Continued

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Table 2 Continued

Authors	Exercise protocol	(Duration 1) 1=0–1 min 2=1–4 min 3=4 min+	(Duration 2) 1=0–0.5 min 2=0.5–10 min 3=10 min+	(Exercise type) 1=Capacity 2=Performance	(Training status) 1=Trained 2=Non-trained	(Exercise mode) 1=Isolated limb 2=Whole body	(Intermittent) 1=Intermittent 2=Continuous	(Study length) (days)	(Total amount) (g)
De Salles Painelli *et al*[47]	100 m swimming	2	2	2	1	2	2	28	156.8
	200 m swimming	2	2	2	1	2	2	28	156.8
De Salles Painelli *et al*[43]	4 bouts of 30 s cycling	2	2	2	1	1	1	28	179.2
		2	2	2	2	1	1	28	179.2
Sale *et al*[18]	$CCT_{110\%}$	2	2	1	2	1	2	28	179.2
Sale *et al*[65]	Isometric endurance	2	2	1	2	1	2	28	179.2
Saunders *et al*[66]	LIST	2	2	2	1	2	1	28	179.2
		2	2	2	2	2	1	28	179.2
Saunders *et al*[26]	Yo-Yo IR2	3	2	1	1	2	1	84	268.8
Saunders *et al*[67]	5×6 s sprints	1	1	2	2	1	1	35	201.6
Smith *et al*[68]	Cycle at 110% VO_{2max}	2	2	1	2	1	2	42	189
Smith *et al*[69]	Incremental treadmill test	3	2	1	2	2	2	28	134.4
Smith-Ryan *et al*[70]	Run to exhaustion at 110% peak velocity	2	2	1	2	2	2	28	134.4
	Run to exhaustion at 100% peak velocity	2	2	1	2	2	2	28	134.4
	Run to exhaustion at 90% peak velocity	2	2	1	2	2	2	28	134.4
Smith-Ryan *et al*[71]	Incremental cycle test	3	3	1	2	1	2	28	179.2
Stout *et al*[72]	Incremental cycle test	3	3	1	2	1	2	28	108.8
Stout *et al*[73]	Incremental cycle test	3	3	1	2	1	2	28	156.8
Stout *et al*[27]	Incremental cycle test	3	3	1	2	1	2	90	216
Tobias *et al*[48]	Upper-body Wingates	2	2	2	1	1	1	28	179.2
Van Thienen *et al*[74]	Incremental cycle test	3	3	1	1	1	2	56	182
	10 min cycling TT	3	2	2	1	1	2	56	182
	30 s cycle sprint	1	1	2	1	1	2	56	182
Walter *et al*[75]	Incremental cycle test	3	3	1	2	1	2	42	189
Zoeller *et al*[76]	Incremental cycle test	3	3	1	2	1	2	28	108.8

$CCT_{110\%}$, cycling capacity test at 110% Powermax; CMJ, countermovement jump; LIST, Loughborough Intermittent Shuttle Test; TT, time trial; VO_{2max}, maximal oxygen uptake; Yo-Yo IR2, Yo-Yo Intermittent Recovery Test Level 2.

Table 3 Results of univariate meta-regressions

Moderator	Parameter estimate	SE	QM_{df}	Between outcome variance σ^2_v (percentage of total variance)	Between study variance σ^2_v (percentage of total variance)	QE_{df}
(Duration 1)						
0–1 min	0.016	0.122	14.1_3	0.144 (71.9%)	0.001 (0.5%)	240.8_{67}
1–4 min	0.210*	0.078				
4 min+	0.233*	0.089				
(Duration 2)						
0–0.5 min	0.040	0.132	13.3_3	0.146 (72.1%)	0.001 (0.5%)	242.2_{67}
0.5–10 min	0.224*	0.070				
10 min+	0.174	0.104				
(Exercise type)						
Capacity	0.244*	0.076	13.0_2	0.142 (71.2%)	0.003 (1.5%)	243.4_{68}
Performance	0.126	0.084				
(Training status)						
Trained	0.144	0.083	12.1_2	0.145 (72.4%)	0 (0%)	244.4_{68}
Non-trained	0.207*	0.068				
(Exercise mode)						
Isolated	0.185*	0.068	11.7_2	0.146 (72.1%)	0 (0%)	244.8_{68}
Whole body	0.176*	0.084				
(Intermittent)						
Intermittent	0.284*	0.142	12.4_2	0.145 (72.4%)	0 (0%)	243.7_{68}
Continuous	0.165*	0.057				
(Total amount)						
Intercept	0.060	0.171	0.60_1	0.141 (69.4%)	0.006 (3.4%)	245.1_{68}
Gradient	0.001	0.001				
(Study length)						
Intercept	−0.217	0.160	8.06_1	0.108 (52.9%)	0.041 (20.1%)	240.8_{68}
Gradient	0.011*	0.004				

Parameter estimates and model outputs.
QE_{df}: residual heterogeneity test statistic; QM_{df}: omnibus moderator test statistic.
*$p \leq 0.05$.

changes in muscle carnosine content. Although individual studies appear to confirm this hypothesis, there were no differences in effect sizes between continuous and incremental protocols (data not shown). Nonetheless, we provide novel evidence here to suggest that exercise of 0.5–10 min in duration to be the exercise time frame in which β-alanine supplementation is most influential.

Exercise capacity and performance were both improved by supplementation, although effect sizes suggested β-alanine to be almost twice as effective at improving exercise capacity. Further meta-regressions were performed on outcomes within 0.5–10 min due to the strong effect of duration on effect sizes, and showed higher effect sizes for exercise capacity than performance indicating that exercise capacity tests within this time frame will most likely elicit the greatest improvements with β-alanine supplementation. It has previously been suggested that performance tests, such as time trials, may be influenced by intrinsic pacing,[32] potentially making them less likely to be influenced by increased muscle carnosine concentration. The majority of capacity tests included in the analyses were performed until exhaustion, most likely resulting in a maximal production of H^+, and thus are sensitive to changes in buffering capacity. As such, current data suggest that exercise capacity tests provide greater scope to identify significant effects of β-alanine supplementation, particularly within a 0.5–10 min time frame.

There has been doubt raised as to the efficacy of β-alanine in well-trained participants,[13 14] perhaps due to adaptions to training such as increased buffering capacity, which may minimise the contribution of increased muscle carnosine content in these individuals. The findings of this study showed significant effect sizes for non-trained individuals only, suggesting that they may be more susceptible to exercise improvements than their well-trained counterparts. This appears in contrast to de Salles Painelli *et al*,[43] who directly compared the efficacy of β-alanine supplementation on training status using a trained and non-trained population and showed it to be equally effective in both sets of participants. Nonetheless, there were no differences in effect sizes between trained and non-trained populations in this study, while effect sizes for the trained group were close to the overall effect of β-alanine (0.144 vs 0.180). Although it appears that trained athletes might experience smaller gains, such changes may translate into worthwhile improvements in an applied setting (ie, competition). Thus, this meta-analysis provides some evidence to support the efficacy of β-alanine in non-trained and trained populations, though improvements are likely to be greater in non-trained individuals.

The efficacy of β-alanine may be influenced by the mode of exercise performed, specifically whole-body versus isolated limb activity. Smaller muscle groups, such as upper-body muscles, may be more subjected to local acidosis than whole body exercise.[44] In addition, increased muscle tension imposed by the type of muscle contraction can mechanically reduce peripheral blood flow and oxygen extraction,[45] which may lead to an increased muscle acidosis. Therefore, it has been suggested that differences in muscle recruitment between activities such as

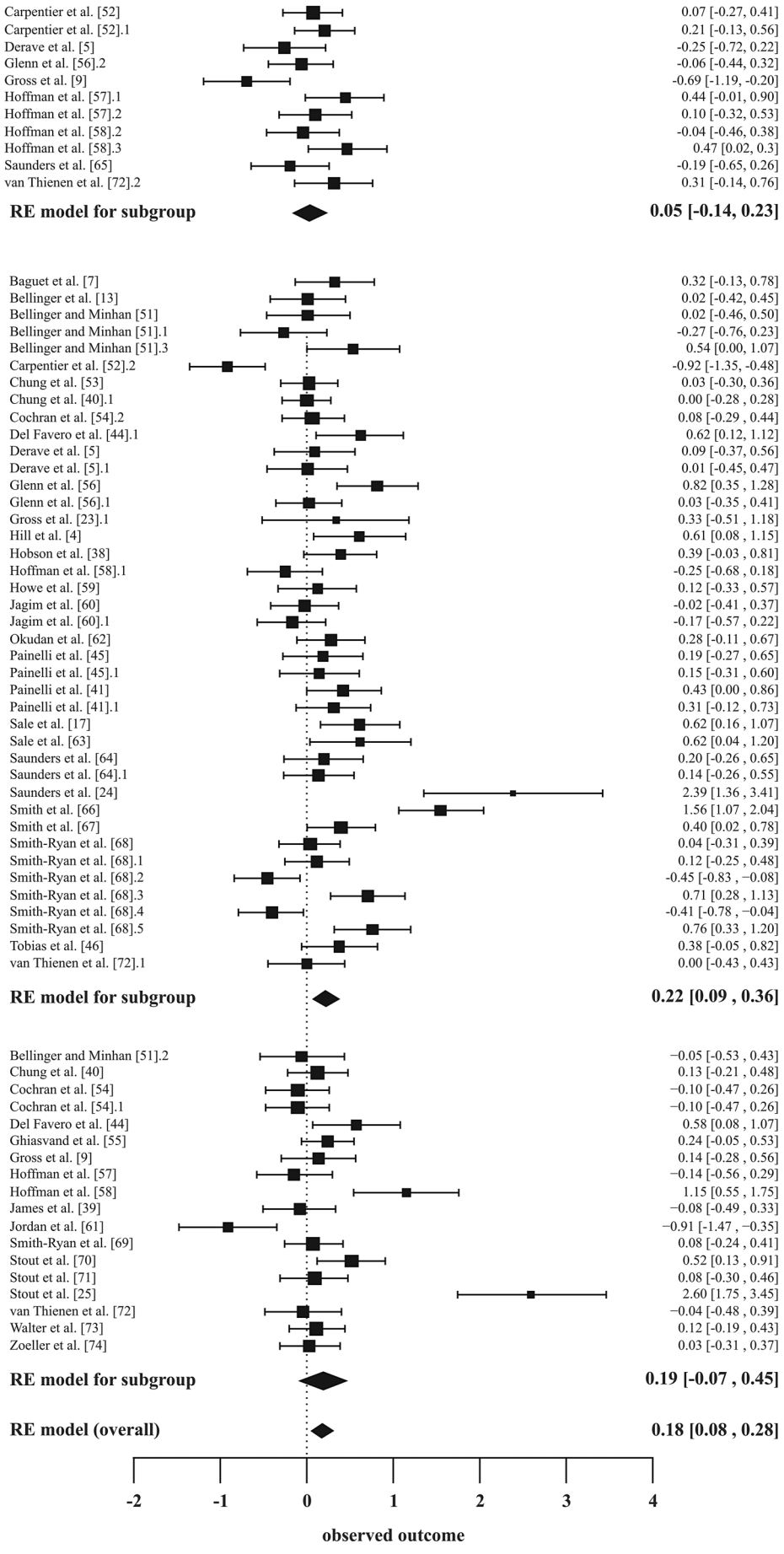

Figure 2 Forest plot of the results of the random-effects (RE) meta-analysis shown as mean effect sizes with 95% CIs. Data are separated according to (*duration 2*). The diamonds below each section represent the overall mean effect within the various subgroups; the diamond below the graph represents the overall effect of β-alanine only group (BA) versus placebo group (PL).

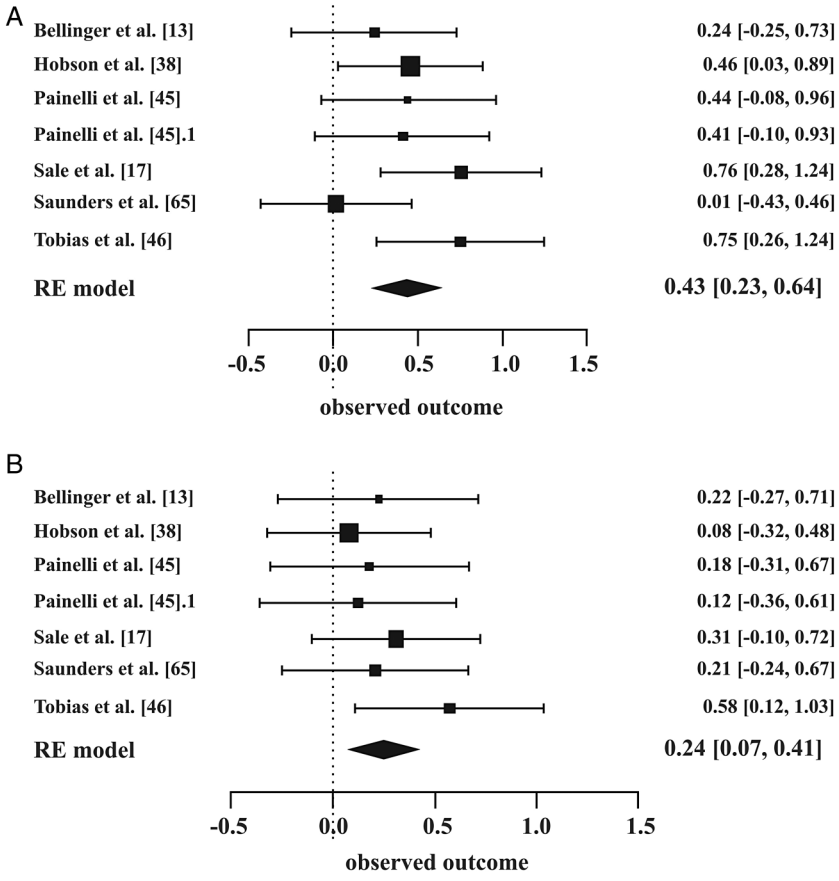

Figure 3 Forest plot of the results of the random-effects (RE) meta-analysis for (A) BASB versus placebo group (PL) and (B) BASB versus β-alanine only group (BA). Data points are shown as mean effect sizes with 95% CIs. The diamonds represent the overall mean effect within the subgroups.

cycling and running may lead to discrepancies in the apparent efficacy of the supplement.[15] The current data suggest, however, that the efficacy of β-alanine is not affected by the mode of exercise and that both isolated limb and whole-body exercise are equally likely to benefit. Indeed, individual studies do provide evidence that whole-body type tasks can be improved by β-alanine supplementation.[40 46 47] Nonetheless, well-controlled experiments specifically designed to address whether there is a greater effect of local versus whole body acidosis are warranted. Additionally, intermittent and continuous protocols were both equally improved with β-alanine. However, most intermittent tests were not sport-specific protocols, and thus further research investigating the effect of β-alanine within applied sport-specific settings is warranted.

Neither the total amount ingested nor the supplementation duration mediated the effect of β-alanine supplementation on exercise outcomes. These results may be due to the wide range of dosing strategies employed, meaning that individuals performed exercise at various stages of increased muscle carnosine content. Although Stellingwerff *et al*[17] showed that increases in muscle carnosine concentration were dependent on the total amount of β-alanine consumed, their study employed two low doses (1.6 and 3.2 g/day), whereas most studies included in this meta-analysis employed higher doses. The time course of carnosine accumulation with doses in excess of 3.2 g/day is yet to be determined and, given that most studies did not directly measure muscle carnosine content, it is not possible to determine whether individuals performed exercise following similar increases in muscle carnosine, although this would seem unlikely. This meta-analysis showed that the total amount of β-alanine ingested does not directly influence its efficacy, making it difficult to make specific recommendations as to the optimal dosing strategy for individuals. Nonetheless, supplementation of 1.6 g/day for as little as 2 weeks has been shown to increase muscle carnosine,[17] while improvements in exercise have been shown at doses ranging from 3.2–6.4 g/day for 4–12 weeks.[4 26] Further research should determine the required dosing strategy to attain maximal carnosine accumulation in muscle and whether maximal exercise improvements occur at an identical time point.

It has been suggested that co-supplementation of β-alanine with sodium bicarbonate may result in further exercise gains over β-alanine alone, due to an increase in intracellular and extracellular buffering capacity. Six of the nine studies encountered in our search strategy investigating co-supplementation were included in the analysis. The majority of studies that employed acute doses of sodium bicarbonate have shown co-supplementation to result in non-significant effects on performance.[13 18 47] The only study that employed a chronic (7 days) sodium bicarbonate supplementation phase showed a significant effect of the additional sodium bicarbonate supplementation with β-alanine over β-alanine alone.[48] This may be due to the multiple-bout nature of the test, which most likely resulted in a higher acidosis than the continuous exercise protocols employed in other studies;[30 49] or due to the chronic supplementation protocol employed, which may have resulted in a more pronounced blood alkalosis,[50] although the authors did not measure this directly. Nonetheless, the current meta-analytic data suggest that co-supplementation of β-alanine with sodium bicarbonate can lead to additional gains over those with β-alanine alone. The reason for the apparent disparity between our findings and the individual study results may be due to the statistical power of the studies undertaken. Studies investigating the effects of combined supplementation have used relatively small sample sizes, which may have hindered standard

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analytical approaches, with many of these studies also including a magnitude-based inferences approach,[51] showing small but meaningful additional improvements with co-supplementation.[18 40] Taken together, these data suggest that the co-supplementation of β-alanine with sodium bicarbonate results in further benefits than supplementation with β-alanine alone. Nonetheless, further investigation is required since the current data are based on the results of six individual studies only.

This study is not without its limitations. All outcome measures were converted to effect sizes to standardise the data and subsequently perform our meta-analyses. It has previously been suggested that converting data to mean power may be more appropriate so that meta-analysed performance effects could be applied directly to athletic performance and practical recommendations for coaches and athletes.[52] However, the sheer variety of exercise protocols included in this analysis meant that it was not possible to convert all available data into power output. Furthermore, we feel that our analytical approach provides a comprehensive and clear analysis of the data, clarifying its significance to different athletic populations. Although our choice of subanalyses and categorisation of exercise tests revealed insightful information on the ergogenic properties of β-alanine supplementation, we acknowledge that alternative subanalyses could have been performed and that our definitions to categorise studies into these subanalyses may differ from other authors. However, we believe our choices to be relevant from a practical standpoint (ie, in a sport context) and we have attempted to support this with an evidence base.

In conclusion, β-alanine supplementation had a significant overall ergogenic effect on exercise. Exercise duration was the greatest influencing factor on the efficacy of β-alanine supplementation, with a time frame of 0.5–10 min likely to result in the greatest gains, while very short duration exercise clearly results in no benefits. Exercise capacity resulted in greater effect sizes than exercise performance; this was particularly apparent for exercise lasting 0.5–10 min. The efficacy of β-alanine for non-trained individuals was clearly demonstrated, while lower effect sizes were shown for trained populations, though these may still translate into competitive gains. Isolated limb versus whole body exercise benefited equally from β-alanine supplementation. The co-supplementation of β-alanine and sodium bicarbonate resulted in the largest effect sizes and also resulted in greater gains than β-alanine alone.

From a practical standpoint, these data allow individuals to make informed decisions as to the likelihood of an ergogenic effect with β-alanine supplementation based on their chosen exercise modality (eg, 400, 800 and 1500 m running; 4 km cycling; 100, 200 and 400 m swimming) and coaches can support claims as to the efficacy of this supplement to their athletes. Athletic individuals can also save time and resources if they are unlikely to obtain any exercise gains due to their preferred sport (eg, 100 m running; 25 m swimming), although improvements with supplementation during training should not be dismissed since this will most likely incorporate repeated exercise bouts and activity of longer duration than competition. Individuals are advised to supplement daily with β-alanine for a minimum of 2–4 weeks at a dose of 3.2–6.4 g/day ingested at several time points throughout the day (0.8–1.6 g every 3–4 hour) to avoid acute side effects. Although the current data support the efficacy of β-alanine supplementation during certain types of exercise, the exact mechanisms by which increased carnosine contributes to improved exercise outcomes remains to be experimentally demonstrated. Further studies should aim to determine the exact contribution of carnosine to buffering capacity and the changes therein following dietary supplementation of β-alanine. Additionally, it would be of interest to determine the greatest exercise gains due to increased muscle carnosine concentration using more prolonged supplementation protocols, and whether gains directly mirror changes in intracellular carnosine. The current meta-analysis provides support to design the most appropriate protocols to determine changes in muscle carnosine concentration with β-alanine (ie, exercise capacity test of 0.5–10 min).

Key points

- Carnosine (β-alanyl-L-histidine) is abundant in skeletal muscle, suggesting that it plays an important role during exercise, with a key physiological role considered to be intracellular pH regulation.
- β-alanine supplementation can increase muscle carnosine concentration and improve exercise capacity and performance.
- There may be a number of confounding factors, including exercise type, duration and mode and population, which may influence the effects of β-alanine supplementation.

These are the novel findings from the study

- There was an overall ergogenic effect of β-alanine supplementation on exercise.
- Exercise duration was the greatest factor influencing effect sizes: a duration of 0.5–10 min results in the greatest gains while very short duration exercise (<0.5 min) clearly results in no benefits.
- Effect sizes for exercise capacity were larger than for performance, particularly during exercise lasting from 0.5 to 10 min in duration.
- The effect of β-alanine on trained individuals showed smaller effect sizes than on non-trained individuals, while isolated limb and whole body exercise were shown to equally benefit from β-alanine.
- Co-supplementation of β-alanine and sodium bicarbonate, to increase both intracellular and extracellular buffering capacity, was shown to result in additional improvements above β-alanine alone.

Contributors BS, BG and CS contributed to the conception and design of the study. BS, KES and GGA contributed to the development of the search strategy analysis and to the acquisition of data. BS, PAS, ED and HR performed the data analysis and interpretation of data. BS was the principal writer of the article while all authors contributed to the drafting of the article and revising it critically. All authors approved the final version to be submitted.

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