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ORIGINAL CONTRIBUTION

24-Week β-alanine ingestion does not affect muscle taurine or clinical blood parameters in healthy males

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Abstract

Purpose To investigate the effects of chronic beta-alanine (BA) supplementation on muscle taurine content, blood clinical markers and sensory side-effects.

Methods Twenty-five healthy male participants (age 27 ± 4 years, height 1.75 ± 0.09 m, body mass 78.9 ± 11.7 kg) were supplemented with 6.4 g day^{-1} of sustained-release BA ($N = 16$; CarnoSyn™, NAI, USA) or placebo (PL; $N = 9$; maltodextrin) for 24 weeks. Resting muscle biopsies of the *m. vastus lateralis* were taken at 0, 12 and 24 weeks and analysed for taurine content (BA, $N = 12$; PL, $N = 6$) using high-performance liquid chromatography. Resting venous blood samples were taken every 4 weeks and analysed for markers of renal, hepatic and muscle function (BA, $N = 15$; PL, $N = 8$; aspartate transaminase; alanine aminotransferase; alkaline phosphatase; lactate dehydrogenase; albumin; globulin; creatinine; estimated glomerular filtration rate and creatine kinase).

Results There was a significant main effect of group ($p = 0.04$) on muscle taurine, with overall lower values in PL, although there was no main effect of time or interaction effect (both $p > 0.05$) and no differences between specific timepoints (week 0, BA: 33.67 ± 8.18 mmol kg^{-1} dm, PL: 27.75 ± 4.86 mmol kg^{-1} dm; week 12, BA: 35.93 ± 8.79 mmol kg^{-1} dm, PL: 27.67 ± 4.75 mmol kg^{-1} dm; week 24, BA: 35.42 ± 6.16 mmol kg^{-1} dm, PL: 31.99 ± 5.60 mmol kg^{-1} dm). There was no effect of treatment, time or any interaction effects on any blood marker (all $p > 0.05$) and no self-reported side-effects in these participants throughout the study.

Conclusions The current study showed that 24 weeks of BA supplementation at 6.4 g day^{-1} did not significantly affect muscle taurine content, clinical markers of renal, hepatic and muscle function, nor did it result in chronic sensory side-effects, in healthy individuals. Since athletes are likely to engage in chronic supplementation, these data provide important evidence to suggest that supplementation with BA at these doses for up to 24 weeks is safe for healthy individuals.

Keywords Chronic beta-alanine supplementation · Muscle taurine · Clinical markers · Safety · Side-effects

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Introduction

Beta-alanine (BA) is an effective nutritional supplement to improve exercise capacity and performance via increased muscle carnosine (β-alanyl-L-histidine) content [1]. It has been shown that supplementation with BA over a period of 4–24 weeks can increase skeletal muscle carnosine content [1–3], and the ergogenic potential of increased muscle carnosine has been demonstrated in several exercise models [4, 5]. Despite considerable solid evidence to support the use of BA to improve performance, information is lacking on the safety of long-term supplementation. Considering the increasing popularity of BA [6], it is vital to determine if

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supplementation at recommended doses could lead to any adverse outcomes.

One concern relating to chronic BA supplementation is the possible depletion of intramuscular taurine content which may negatively impact muscle function and subsequently exercise and health. Taurine is a β-sulfonic amino acid present in human tissues and performs a variety of important biological roles in skeletal muscle, such as osmoregulation, cation homeostasis, enzyme activity, receptor regulation, cell development and signalling, and cytoprotective activity [7, 8]. Trans-sarcolemmal transport of BA into muscle is predominantly mediated by *TauT* [9], a sodium- and chloride-dependent transporter [10] also responsible for the transport of taurine into muscle, meaning BA may act as an antagonist of taurine uptake, particularly if taken in concentrations exceeding habitual dietary levels (i.e., supplementation). Indeed, BA is routinely employed to induce taurine depression in rats [9, 11], albeit at far higher relative doses than those employed in human studies, leading to severe health implications and early death [12]. A moderate negative association between intramuscular taurine and carnosine with BA supplementation has been reported in humans [13]. Since we have recently shown that prolonged supplementation with BA results in a chronic downregulation of *TauT* [3], one could speculate that long-term supplementation may deplete muscle taurine content. Since this may have drastic consequences for skeletal muscle function [12], it is important to determine whether prolonged supplementation with BA at commonly employed doses results in any changes to the intramuscular taurine content.

The long-term effects of high-doses of BA on health-related markers remain poorly explored. Since the majority of BA is metabolised in the liver and kidney following supplementation [14], it is of importance to determine if chronic supplementation could overburden these organs. Previous research has shown no adverse effect on 12-lead electrocardiogram, clinical chemistry or haematological safety data after 4 weeks of BA supplementation at 3.2 g day^{-1} [2]. This was confirmed by Stellingwerff et al. [15] after 4 weeks with 3.2 g day^{-1} followed by 4 weeks of 1.6 g day^{-1}, and a total 8 weeks of 1.6 g day^{-1}. Twelve weeks of supplementation at 3.2 g day^{-1} in elderly (60–80 years old) participants also showed no adverse effects on clinical health markers [16]. However, these studies used relatively low doses ($\leq$3.2 g day^{-1}) and short supplementation periods ($\leq$12 weeks). The effects of longer term (i.e., >12 weeks) supplementation with BA at recommended doses (i.e., >3.2 g day^{-1}; [17]) on health parameters related to hepatic, renal and muscle function are unknown. Considering a chronic and high-dose approach to supplementation is likely to be taken by certain athletic populations [6], it is important to determine if higher intake may be harmful or inhibitory to health.

The only currently known side-effect of BA supplementation is paraesthesia, described as an uncomfortable sensation on the surface of the skin that occurs within 10–20 min following ingestion [2]. A number of possible mechanisms exist for paraesthesia, with the most likely being related to the Mas-related gene family of G-protein-coupled receptors, which are triggered by interactions with ligands including BA [18]. Symptoms typically arise from a high dose of BA, although they are substantially decreased when BA is taken in the form of slow-release tablets [19]. As paraesthesia does not necessarily impose any health risk per se, this symptom could be considered a side-effect instead of an adverse effect. However, given the relatively common reports of paraesthesia in short-term studies, and how little is known about the physiological consequences thereof, it is important to monitor this over a prolonged follow-up period to determine whether increased incidence or symptom aggravation occurs as a function of time.

The aim of this study was to examine the effect of 24 weeks of 6.4 g day^{-1} BA supplementation on muscle taurine content, blood parameters of renal, hepatic and muscle function, and self-reported side-effects. Our a priori hypotheses were that BA would not significantly affect blood health parameters or chronic side-effects, but that muscle taurine content would be reduced over the supplementation period, although remaining within the physiological range.

Methods

Participants

Twenty-five physically active males [age: 27 ± 4 years, height: 1.75 ± 0.09 m, body mass (BM): 78.9 ± 11.7 kg] completed the study following two drop-outs who cited personal reasons for their withdrawal. The participants were randomly allocated to either a BA or placebo (PL) group, matched for maximum cycling power output attained during an incremental cycling test to exhaustion [20]; these exercise data are not presented here [3]. Participants were fully informed of any risks and discomforts associated with the study before completing a health screen and providing informed consent. Individuals were required not to have taken any supplement in the 3 months prior to the study and had not taken BA for at least 6 months prior to the study. Participants maintained similar levels of physical activity and dietary intake for the duration of the study and compliance with this was confirmed and is presented elsewhere [3]. The study was approved by the institution's Ethical Advisory Committee and has been performed in accordance with the ethical standards in the 1964 Declaration of Helsinki and its later amendments. This was part of a larger thematic project with two distinct aims, namely, the effect of 24 weeks BA

supplementation on, (1) muscle carnosine content, carnosine-related gene expression and exercise performance, and (2) muscle taurine content, clinical markers and sensory side-effects. The remaining data are presented elsewhere [3].

Experimental design

Participants attended the laboratory on nine separate occasions. The first two visits were for the determination of each participant's cycling maximal power output and familiarisation of the main exercise protocol (data not presented). The remaining visits were for the completion of the main trials. One main trial was completed before supplementation, followed by one main trial every 4 weeks for 24 weeks of a double-blind supplementation programme, during which participants were randomly allocated in a 2:1 ratio to ingest BA or PL. The unbalanced design was adopted a priori to minimize the number of individuals being biopsied. The dosing regimen consisted of the consumption of two 800 mg tablets of BA (CarnoSyn™, NAI, USA) or placebo (PL; maltodextrin, NAI, USA) four times per day at 3–4 h intervals to avoid paraesthesia [2], totalling 6.4 g day^{-1}. Participants completed a log to verify compliance (BA: 95 ± 6%; PL: 93 ± 6%); one individual who supplemented with BA did not adhere to the supplementation protocol and was removed from any analyses. Blinding occurred via an outside researcher who allocated individuals into either BA or PL. Identical white bottles containing only the participants' names were provided to the researchers. All participants were informed about the associated side-effects with BA supplementation (i.e., paraesthesia) and were asked to report any perceived symptoms at every laboratory visit throughout supplementation. Participants were requested to identify which supplement they believed they had ingested following their final visit (week 24).

Experimental procedures

Participants abstained from alcohol and caffeine and strenuous exercise in the 24 h period prior to the initial trial and arrived at the laboratory a minimum of 4 h following their last consumption of food. A cannula was first inserted into the antecubital vein to allow for venous blood collection, performed with the individual in a supine position. The participants then underwent a muscle biopsy of the *m. vastus lateralis*.

Muscle biopsies

Muscle biopsies were taken at rest using a 5 mm biopsy Allandale needle (Northern Hospital Supplies, Edinburgh, UK) adapted from Bergstrom [21], described in detail elsewhere [22]. Briefly, following local anaesthesia (lidocaine 1%) of the skin, the individual's dominant leg was prepared for skeletal muscle biopsy sampling via an incision along the *m. vastus lateralis*. Approximately 50 mg of muscle was taken and snap-frozen in liquid nitrogen and stored at − 80 °C until subsequent analysis. Biopsies followed the same standardised pattern across individuals. The location of the initial biopsy was at a point 25 cm proximal from the tuberositas tibiae and 5 cm lateral from the midline of the femoral course; the second incision was performed adjacent (~ 1 cm) to the first. The following incisions were made superior to the previous ones; since this was part of a larger project, all participants underwent a total of seven muscle biopsies every 4 weeks which resulted in three pairs of parallel incisions and one single incision at the most superior point. Samples taken at weeks 0, 12 and 24 were analysed for muscle taurine content.

Chromatographic determination of taurine in whole muscle

Muscle samples were lyophilised and powdered before a ~ 6 mg amount was combined with 300 µl of G22 (solution of 0.5 M perchloric acid [$HClO_4$] and 1 mM EDTA) and homogenized using vortex for 15 min. Samples were then centrifuged for 3 min at 3000 rev·min^{-1} at 4 °C. The resultant supernatant was weighed and used to calculate the extract volume accordingly (volume of supernatant divided by 1.025), which was subsequently multiplied by 2.5 to obtain the volume of G23 (2.1 M solution of potassium bicarbonate; $KHCO_3$) to be added. The G23 was added to the solution while vortexing to allow a salt precipitate to form according to the following reaction: $KHCO_3 + HClO_4 \rightarrow KClO_4 + CO_2 + H_2O$. The resultant CO_2 was dissipated by vortexing before the sample was centrifuged for 3 min at 3000 rev·min^{-1} at 4 °C. The resultant supernatant was extracted using a centrifugal filter (0.2 µm), checked to ensure a pH close to 7 and then stored at − 80 °C until HPLC analysis.

Taurine content in whole muscle was measured using HPLC coupled to fluorescence detection, according to an adapted method [23, 24]. The column used for chromatographic separation was an ODS HYPERSIL column (4.6 × 150 mm, 5 µm) from Thermo Scientific (Waltham, Massachusetts, USA) attached to a universal uniguard holder with drop-in guards (4 × 10 mm, 5 µm). Prior to HPLC injection, a derivatisation agent was added to each sample. The derivatisation agent, composed of 0.5 M borate buffer (pH 10.4), ø-phthalaldehyde (40 mg/1 ml 100% ethanol) and 3-propionic acid (250:20:1 v/v/v), was prepared 24 h prior to use and stored in the dark at ~ 2 °C. Norleucine (0.25 mM) was used as an internal control for any variation in expression following derivatization. Immediately prior to injection, 12 µl of derivatization

reagent was combined with 6 µl of norleucine and 6 µl of sample extract. Due to insufficient sample, taurine analysis was only performed for 12 individuals in BA and 6 in PL.

The column was equilibrated for 5 min under initial conditions prior to each injection. The method used three mobile phases: mobile phase A: 20 mM phosphate buffer and tetrahydrofuran (995:5, v/v). Mobile phase B: 20 mM phosphate buffer, methanol and acetonitrile (500:350:150, v/v). Mobile phase C: 100% methanol. The phosphate buffer used in mobile phases A and B were made up of 5.68 g sodium phosphate dibasic (Na_2HPO_4) and 4.79 g monosodium phosphate (NaH_2PO_4) in ultrapure water made up to a volume of 2 l and adjusted to pH 6.8. Gradient elution composition was as follows: 0 min 100% solvent A; 1.5 min 100% solvent A; 17.5 min 50% A 50% B; 19.5 min 100% B; 20 min 100%B; 22 min 50% B 50% C; 24 min 100% C; 26 min 50% B 50% C; 30 min 100% B. The flow-rate was 1.6 ml·min^{-1} for 17.5 min and 0.8 ml·min^{-1} for 12.5 min; total analysis time was 30 min per sample. Taurine concentration was determined using fluorescence at excitation and emission wavelengths of 340 nm and 450 nm. All chromatography was carried out at room temperature. Quantification of concentrations was performed using peak areas, calculated by computer software coupled to the Chromatographer. Peak area values for the standard curve were plotted and a regression equation obtained from which interpolations were used to calculate content. The intra assay coefficient of variation (CV) of taurine between duplicate injections of all analyses ($N = 54$) was $5.0 \pm 4.2\%$.

Blood collection and analyses

Two resting venous blood samples of 4 and 5 ml were collected into EDTA and SST vacutainer tubes (BD, USA) for analyses of aspartate transaminase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, albumin, globulin, creatinine, creatine kinase and uric acid. Estimated glomerular filtration rate was determined using blood creatinine values using the Cockcroft–Gault formula:

$$\text{GFR} = [(140 - \text{Age}) \times \text{BM}]/72 \times \text{Creatinine}.$$

All blood samples were taken with the individuals in a supine position. All samples were stored at room temperature and submitted to a central laboratory (Clinical Hospital of São Paulo) for analysis within 4 h of collection. The hospital specialises in the clinical analyses performed with annual CVs for the blood analytes measured between 1.75 and 3.59%. The reference values provided by the hospital's central laboratory were used to classify whether each individual analyte was within the normal range of the laboratory.

Statistical analyses

All data were analysed using the SAS statistical package, (SAS 9.2, SAS Institute Inc., USA) and are presented as mean ± 1SD and 95% confidence interval (CI), unless otherwise stated. Data were analysed for normality using the Shapiro–Wilk tests and for homogeneity and variance/sphericity using the Mauchly test (all $p > 0.05$). Muscle taurine was analysed using mixed model analysis with individuals assumed as a random factor and group (2 levels; BA and PL) and time (3 levels; week 0, 12 and 24) assumed as fixed factors. Tukey–Kramer adjustments were performed whenever a significant F value was obtained, and the significance level was set a priori at $p \leq 0.05$. Blood parameters were analysed using mixed model analysis with individuals assumed as a random factor and group (2 levels; BA and PL) and time (7 levels; week 0 to week 24) assumed as fixed factors. Generalized estimating equations (GEE) were performed to compare groups regarding the proportion of participants outside of the normal range at each week. Finally, the Fischer exact test was used to compare the number of participants who correctly guessed their allocation in each group.

Results

Muscle taurine content

There were no significant differences in the pre-supplementation taurine content between BA (week 0: 33.67 ± 8.18 mmol kg^{-1} dm, 95% CI 29.04, 38.30) and PL (week 0: 27.75 ± 4.86 mmol kg^{-1} dm, 95% CI 25.36, 30.13; $p = 0.21$). There was a significant main effect of group ($p = 0.04$), with overall lower values in PL, although there was no main effect of time or interaction effect (both $p > 0.05$; Fig. 1) meaning there were no differences between groups at week 12 (BA: 35.93 ± 8.79 mmol kg^{-1} dm; PL: 27.67 ± 4.75 mmol kg^{-1} dm) or week 24 (BA: 35.42 ± 6.16 mmol kg^{-1} dm; PL: 31.99 ± 5.60 mmol kg^{-1} dm).

Blood parameters

One individual from PL showed abnormally high values (> 2 SDs from the mean) for several health markers at week 0 (aspartate transaminase, alanine aminotransferase, alkaline phosphatase) as indicated by the clinical analysis. Data were analysed with and without this individual and showed no differences in the main outcomes. Therefore, since this study focused on a healthy population, data for this individual were removed from any subsequent analysis in their entirety.

At week 0, there was no difference between BA and PL for any marker of health (all $p > 0.05$). There were no group, time or interaction effects for any health parameter

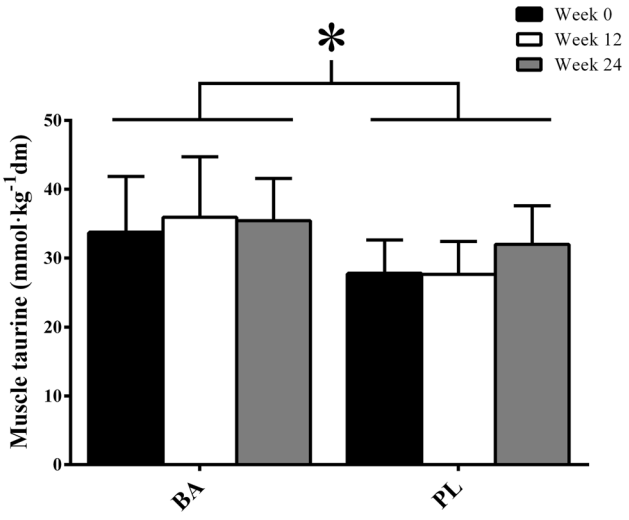

Fig. 1 Muscle taurine in the beta-alanine (BA; $N=12$) and placebo (PL; $N=6$) groups. *$p=0.04$ Main effect of group. Data are mean ± 1 SD

(all $p>0.05$; Table 1). Blood markers at every time point were within the normal range for both BA and PL (Table 1). Further analysis revealed a few individuals that showed values outside the normal profile range of the measurements at various time points (Table 2), although GEE analysis did not show any significant differences between groups regarding the proportion of individuals with values outside the normal profile range of the measurements for any of the blood markers measured (all $p>0.05$).

Self-reported side-effects and supplement identification

No self-reported side-effects occurred throughout the supplementation period in either BA or PL. Eight individuals in BA correctly identified the supplement they had ingested, while the remaining seven incorrectly believed they had ingested placebo. In PL, four individuals believed they had been allocated to the BA group, and the other four correctly identified themselves to be in PL. There were no significant differences between groups regarding supplement identification (Fisher's exact test: $p=0.51$).

Discussion

These novel data showed that 24 weeks of BA supplementation at 6.4 g day^{-1} did not (1) result in any changes in muscle taurine content throughout the supplementation period, (2) affect clinical markers of renal, hepatic and muscle function, and, (3) result in any self-reported side-effects. Since a high proportion of athletes have been shown to ingest BA [6], many of which are likely to adopt long-term and high-dose supplementation strategies, these data provide important information to suggest that this type of approach to supplementation will not negatively impact upon the health parameters measured here.

Our data showed that chronic BA supplementation did not significantly affect the muscle taurine content, extending previous work following shorter supplementation periods at lower doses [1, 2]. Since BA has been shown to decrease muscle taurine in rats [11, 25], leading to the impairment of skeletal muscle function [12, 26, 27] and even early death [12], it was important to determine whether prolonged supplementation could similarly lead to a decreased muscle taurine content in humans, and if so, the subsequent effects thereof. We showed no changes in taurine content, despite chronic supplementation resulting in a downregulation of taurine transporter expression in these individuals [3]. Muscle taurine was similarly unaffected following downregulation of the *TauT* transporter with 7 days of taurine supplementation [28], suggesting that the skeletal muscle taurine pool is tightly regulated and that homeostasis can be maintained even when the expression of the *TauT* gene is affected. Indeed, muscle taurine content appears unaffected by exercise [28] and aging [29], while the current data suggest that it is unchanged with chronic BA supplementation in humans at high doses used by athletes [1, 2, 30]. The mechanisms through which muscle taurine content is kept constant in humans should be further investigated.

There was substantial interindividual variation in the initial taurine content (22–54 mmol kg^{-1}), as has been shown with muscle carnosine [3], although whole muscle taurine was not correlated to muscle carnosine in the present study (data not shown). Contrary to changes in muscle carnosine with BA supplementation [1–3], muscle taurine was unaffected, although some isolated individuals, irrespective of supplementation, showed some changes in muscle taurine content. We could speculate that slight differences in the location of the pre-to-post biopsy sites could partially explain this individual variability, since type I fibres have been shown to have up to four times the taurine content shown in type II fibres [31]. Despite a considerable range in the absolute content, muscle taurine appears unaffected by BA at the current and most commonly employed dose of 6.4 g day^{-1}.

Twenty-four weeks of BA supplementation did not significantly alter clinical markers of health, as measured by several markers of renal, hepatic and muscle function. This builds upon previous studies showing that lower doses of BA for between 4 and 12 weeks, up to a total of 268.8 g of BA, do not affect blood clinical chemistry [2, 15, 16]; in the current study, participants ingested a total of 1075.2 g of BA over 24 weeks. Although a few isolated individual blood marker values were changed throughout the follow-up

Table 1 Clinical blood parameters (mean ± 1 SD) in the β-alanine (BA; $N=15$) and placebo (PL; $N=8$) groups every 4 weeks over 24 weeks of supplementation

Parameter	Units	Normal range	Group	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Aspartate transaminase	(U l^{-1})	< 39	BA	20 ± 6	23 ± 7	25 ± 11	22 ± 7	28 ± 15	26 ± 14	26 ± 8
			PLA	19 ± 4	23 ± 4	25 ± 9	22 ± 5	23 ± 4	23 ± 4	20 ± 2
Alanine aminotransferase	(U l^{-1})	< 41	BA	23 ± 11	28 ± 17	28 ± 13	27 ± 11	27 ± 12	31 ± 12	27 ± 12
			PLA	25 ± 12	25 ± 10	26 ± 14	24 ± 10	24 ± 6	22 ± 6	24 ± 7
Alkaline phosphatase	(U l^{-1})	40–129	BA	62 ± 15	67 ± 17	66 ± 15	61 ± 15	63 ± 16	65 ± 18	67 ± 18
			PLA	79 ± 21	71 ± 23	67 ± 26	65 ± 25	65 ± 23	62 ± 21	63 ± 23
Lactate dehydrogenase	(U l^{-1})	135–325	BA	237 ± 82	256 ± 82	246 ± 87	261 ± 101	272 ± 131	265 ± 134	271 ± 130
			PLA	228 ± 88	231 ± 78	228 ± 83	233 ± 91	247 ± 98	260 ± 108	215 ± 74
Total protein	(g dl^{-1})	5.1–8.5	BA	7.1 ± 0.4	7.1 ± 0.3	7.2 ± 0.4	7.3 ± 0.7	7.3 ± 0.3	7.2 ± 0.6	7.1 ± 0.5
			PLA	7.2 ± 0.3	7.1 ± 0.2	7.2 ± 0.3	7.2 ± 0.5	7.3 ± 0.4	7.1 ± 0.4	7.1 ± 0.5
Albumin	(g dl^{-1})	3.4–5.0	BA	4.8 ± 0.3	4.7 ± 0.2	4.8 ± 0.3	4.7 ± 0.4	4.7 ± 0.2	4.7 ± 0.4	4.8 ± 0.3
			PLA	4.8 ± 0.2	4.7 ± 0.3	4.8 ± 0.2	4.7 ± 0.3	4.6 ± 0.2	4.6 ± 0.3	4.7 ± 0.2
Globulin	(g dl^{-1})	1.7–3.5	BA	2.4 ± 0.4	2.4 ± 0.2	2.4 ± 0.3	2.6 ± 0.5	2.5 ± 0.3	2.5 ± 0.4	2.4 ± 0.3
			PLA	2.4 ± 0.4	2.4 ± 0.2	2.4 ± 0.3	2.5 ± 0.5	2.7 ± 0.4	2.5 ± 0.3	2.4 ± 0.4
Uric acid	(mg dl^{-1})	3.4–7.0	BA	5.4 ± 0.8	5.3 ± 1.0	5.7 ± 0.9	5.7 ± 1.0	5.6 ± 1.1	5.6 ± 0.9	5.7 ± 1.1
			PL	5.1 ± 1.1	5.4 ± 1.4	5.4 ± 0.9	5.0 ± 1.2	5.2 ± 0.9	5.5 ± 1.2	5.6 ± 0.9
Creatinine	(mg dl^{-1})	0.70–1.20	BA	1.06 ± 0.13	1.04 ± 0.15	1.02 ± 0.13	1.03 ± 0.15	1.07 ± 0.13	1.06 ± 0.18	1.06 ± 0.19
			PLA	1.01 ± 0.15	0.91 ± 0.11	1.08 ± 0.22	0.98 ± 0.12	0.99 ± 0.13	1.04 ± 0.15	0.99 ± 0.13
GFR (estimated)	(ml min^{-1})	> 70	BA	119 ± 27	121 ± 25	123 ± 23	124 ± 31	118 ± 27	120 ± 26	121 ± 31
			PLA	121 ± 24	134 ± 27	115 ± 25	124 ± 17	124 ± 26	117 ± 19	123 ± 20
Creatine kinase	(U l^{-1})	39–308	BA	205 ± 120	191 ± 95	200 ± 116	233 ± 120	215 ± 132	242 ± 162	259 ± 168
			PL	184 ± 87	243 ± 103	180 ± 97	228 ± 150	205 ± 120	253 ± 154	185 ± 83
Platelets	(ml mm^{-3})	150–450	BA	199 ± 58	217 ± 58	214 ± 72	210 ± 66	213 ± 51	205 ± 64	207 ± 56
			PLA	191 ± 51	208 ± 35	235 ± 58	208 ± 52	210 ± 44	203 ± 35	215 ± 38

Table 2 Number of individuals in each group at every time point that fell above or below the normal range

Marker	Group	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Aspartate transaminase	BA	0/15	0/15	1/15	0/15	1/15	2/15	0/15
	PL	0/8	0/8	1/8	0/8	0/8	0/8	0/8
Alanine aminotransferase	BA	2/15	3/15	2/15	1/15	2/15	3/15	2/15
	PL	1/8	1/8	1/8	1/8	0/8	0/8	0/8
Alkaline phosphatase	BA	1/15	1/15	1/15	0/15	1/15	1/15	1/15
	PL	0/8	0/8	0/8	0/8	0/8	1/8	1/8
Lactate dehydrogenase	BA	2/15	2/15	1/15	3/15	2/15	4/15	2/15
	PL	1/8	1/8	1/8	1/8	2/8	2/8	1/8
Total protein	BA	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	PL	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Albumin	BA	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	PL	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Globulin	BA	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	PL	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Uric acid	BA	0/15	1/15	1/15	2/15	1/15	1/15	1/15
	PL	1/8	2/8	1/8	1/8	0/8	1/8	0/8
Creatinine	BA	3/15	2/15	1/15	2/15	1/15	3/15	1/15
	PL	1/8	0/8	1/8	0/8	0/8	2/8	0/8
Estimated GFR	BA	0/15	0/15	0/15	0/15	0/15	0/15	0/15
	PL	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Creatine kinase	BA	3/15	2/15	1/15	4/15	3/15	4/15	5/15
	PL	1/8	2/8	2/8	3/8	2/8	3/8	1/8
Platelets	BA	1/15	1/15	1/15	0/15	1/15	1/15	1/15
	PL	0/8	0/8	0/8	0/8	0/8	0/8	0/8

period, this is unlikely due to BA supplementation, since these were similar in both the BA and PL groups and GEE analysis revealed no significant difference in the number of points outside the normal range between groups for any marker. Furthermore, most of the individuals who showed markers outside of the normal range at timepoints throughout the supplementation period were those who had slightly elevated levels at week 0 (i.e., prior to the supplementation period). It appears that BA supplementation at 6.4 g day^{-1} could result in minor, non-clinically meaningful changes in some of these markers depending on individual differences (e.g., baseline health markers, dietary intake). These data show that prolonged supplementation with BA at the dose given for 24 weeks did not have negative health implications for healthy volunteers, as measured by these indices of renal, hepatic and muscle function.

There were no self-reported side-effects of paraesthesia in the present study, suggesting that the sustained-release tablets were successful in avoiding a quick and large release of BA into the circulation, which causes this uncomfortable sensation. Symptoms of paraesthesia have previously been shown to be dose-dependent; ingestion of a 40 mg kg^{-1} BM dose of BA in powder form resulted in substantially uncomfortable symptoms of paraesthesia [2], while a lower 20 mg kg^{-1} BM, which would equate to an equivalent single dose used in the current study in an 80 kg individual, and 10 mg·kg^{-1} BM similarly resulted in symptoms but less intense. Decombaz et al. [19] have previously shown that administration of 1.6 g of BA in slow-release tablets resulted in significantly lower sensory side-effects than the same BA dose administered as a solution, and that the symptoms associated with the slow-release tablets could not be differentiated from those associated with placebo ingestion. Several individuals in their study reported minor incidence of symptoms, which is contrary to the participants in the current study. This may be due to the specific aims of the two studies; Decombaz et al. [19] explicitly investigated the occurrence of side-effects and participants were required to respond to a questionnaire at a number of time points following acute ingestion of BA. Had the participants in our study been required to respond to a questionnaire, it is possible we may have shown small incidences of paraesthesia. Nonetheless, the results of our study suggest that ingesting 1.6 g of sustained-release BA interspersed by a minimum of 3 h throughout the day is unlikely to result in any perceivable side-effects.

We did not determine muscle L-histidine in the current study, which may have provided insight into the chronic effects of BA on this intramuscular amino acid, which is essential for carnosine synthesis. It has been suggested that a reduction in muscle histidine could affect muscle protein synthesis [30] or histamine kinetics [15], which may impact muscle function and impair exercise. Twenty-three days of BA supplementation at 6 g day^{-1} has been shown to reduce

the intramuscular L-histidine content [30], and this reduced muscle histidine availability has been suggested to impair the efficiency of carnosine loading with BA as supplementation is extended over time. However, these data have not been replicated, with no change in histidine following BA supplementation at 12 g day^{-1} for 2 weeks [32] and 6 g day^{-1} for 4 weeks [32–34].

The current study showed that 24 weeks of BA supplementation at 6.4 g day^{-1} did not negatively impact muscle taurine content, clinical markers of renal, hepatic or muscle function, nor did it result in any chronic sensory side-effects. Since athletes are likely to engage in prolonged periods of supplementation, these data provide important evidence to suggest that, if they are healthy individuals, they can safely supplement with BA at these high doses for up to 24 weeks. Further work should elucidate the tolerable upper intake level that poses no risk for adverse effects to the general population.

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