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ORIGINAL ARTICLE

Effect of slow-release β-alanine tablets on absorption kinetics and paresthesia

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Abstract Oral β-alanine (βA) doses larger than 800 mg commonly result in unpleasant sensory symptoms (paresthesia). However, the association of form (pure vs. slow-release) with side-effects has not been fully described. The aim of this single-blinded, randomized three-arm clinical trial was to compare plasma kinetics and symptoms following βA bolus administration in solution or in slow-release tablet form. Eleven healthy adults ingested 1.6 g of a pure βA reference solution (REF), 1.6 g in slow-release βA tablets (TAB) or a placebo (PLA) after an overnight fast. During the next 6 h, urinary and plasma βA concentrations were measured and questionnaires about intensity, nature (pins and needles, itching, flushing, irritation, numbness, soreness), and spatial distribution of unusual sensations were filled in. TAB resulted in a smaller peak plasma concentration than REF (82 vs. 248 μmol L^{-1}, $p < 0.001$), delayed time to peak (1.0 vs. 0.5 h, $p < 0.01$) no difference in area under the curve, reduced loss in urine (202 vs. 663 μmol, $p < 0.0001$), and improved retention (98.9 vs. 96.3%, $p < 0.001$). Symptoms described as "pins and needles" were perceived rapidly on the skin of the arms and trunk after REF ($T_{max} = 15$ min) and their time course nearly mimicked plasma concentrations. Maximum intensity scores were weaker with TAB ("very low") than with REF ("low", $p < 0.001$), while TAB and PLA did not differ with respect to side-effects. In summary, ingesting 1.6 g βA in slow-release tablets rather than pure in solution results in slower absorption kinetics, improved whole body retention and sensory side-effects that cannot be differentiated from PLA.

Keywords β-Alanine · Supplementation · Nociception · Sensory · Side-effect

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Introduction

β-Alanine (βA) is a component of carnosine (β-alanyl-L-histidine), a cytoplasmic dipeptide which is found in skeletal muscle and other tissues. Carnosine contributes to intracellular muscle pH buffering capacity and among other functions it is thought to play a role in slowing the development of acidotic muscle fatigue (for reviews see Artioli et al. 2010; Derave et al. 2010). Most of the body carnosine is synthesized from its two constituent amino acids (β-alanine and L-histidine) (Bakardjiev and Bauer 1994), of which β-alanine is the limiting one. In humans, endogenous production of βA results from the degradation of uracil (see Tiedje et al. 2010 for review). Exogenous supply depends on the amount of animal sources in the diet. Based on β-alanyl dipeptides found in meat and fish (Abe 2000), the daily consumption of βA in the food of omnivores has been estimated to reach 0.8 g or more for a significant fraction of the population (Harris et al. 2006).

To date, βA supplementation studies have been successful in increasing muscle carnosine content by ~45−60% after 4 weeks, with the aim to improve anaerobic capacity and muscle function during high-intensity exercise (e.g. Harris et al. 2006; Derave et al. 2007;

Stellingwerff et al. 2011). Diverse exercise and supplementation modalities have been used, where daily doses of 2.4−6.4 g βA or more were consumed over several weeks (see Sale et al. 2010 for review). In a first series of such studies, Harris et al. (2006) observed that the acute administration of 3.2 g βA in solution induced transient flushing sensations on the skin (paresthesia) described as unpleasant. The subjects *"quickly complained of symptoms of flushing (described variously as an irritation of the skin and prickly sensation) which began within 20 min and lasted up to one hour. This first affected the ears, forehead and scalp, followed by the upper trunk including the arms and the back of the hands, and finally the base of the spine and buttocks"* (Harris et al. 2006). In order to circumvent these symptoms, pure βA doses were split into multiple smaller 0.8 g fractions, closer to the daily intake, which were administered throughout the day for 2−4 weeks, with only about 25% of subjects reporting very mild to mild symptoms. Similar dosing procedures (multiple small doses throughout the day) were used in follow-up supplementation studies using βA in solution or gelatine capsules (Hoffman et al. 2008; Kendrick et al. 2008, 2009; Sale et al. 2011; Stout et al. 2007; van Thienen et al. 2009; Zoeller et al. 2007), with sensory side-effects occasionally mentioned (Hill et al. 2007; Sweeney et al. (2010); and when omitted, it is not sure whether symptoms were absent or ignored.

Nonetheless, a slow-release βA tablet (TAB) form has become commercially available. As described briefly in conference proceedings, compared to pure 1.6 g βA in solution, 1.6 g βA in slow-release tablets were found to slow the rate of appearance and lower the peak value of βA in blood and did not induce paresthesia (Harris et al. 2008). Accordingly, studies have now utilized slow-release βA tablets, which allow for more practical daily supplementation and compliance by reducing the number of daily doses for a given prescription (Baguet et al. 2010; Stellingwerff et al. 2011).

Despite the increasing number of published βA supplementation and performance studies and the large amounts of βA consumed by volunteers or purchased for potential exercise, health or clinical benefits, surprisingly no detailed account of the symptomatology after ingestion of βA has been published subsequent to the initial narrative of Harris et al. (2006). Therefore, the present study was designed to compare the kinetics of plasma βA and its association with βA-induced paresthesia symptoms following the ingestion of 1.6 g βA in either slow-release tablet form or pure aqueous solution. The aims were to provide a comprehensive symptomatology of βA-induced paresthesia, to evaluate bioavailability and urinary loss, and to document the protective value of the slow-release βA form against adverse paresthesia side-effects.

Methods

Subjects

Eleven healthy, non-supplement using Caucasian volunteers (5 females and 6 males) gave their written informed consent to participate. Their characteristics for age, height, weight, lean body mass, and body fat were 26 ± 4 (SD) yrs, 1.74 ± 0.10 m, 68.1 ± 8.0 kg, 52.1 ± 9.0 kg and 19 ± 8%, respectively. The study conformed to the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Lausanne University.

Study overview

This was a single-blind, randomized, triple-crossover study where three different treatments [two β-alanine supplements and a placebo (PLA)] were acutely administered once. The primary outcome was pharmacokinetics (plasma βA and sensory responses) over a 6-h period. Whole body retention was estimated from βA urinary outflow, while symptoms of paresthesia were obtained after ingestion of each product by questionnaires, which were filled-out in time-course parallel with blood sampling.

Products

The reference product (REF) was 1.6 g (17.96 mmol) βA in aqueous solution (Carnosyn™, Natural Alternatives International (NAI), San Marcos CA). The test product (TAB) was a slowly releasing 1.6 g βA dose in tablet form (2 × 800 mg tablets, NAI), which contained hydroxypropyl methylcellulose, stearic acid, magnesium stearate, and silicon dioxide as excipients. The different food forms and the anticipation of different sensory side-effects prevented double-blinding of the study. In order to improve masking, the PLA was a typical 30 g energy bar with no βA. Analytical recovery of βA was 98% in REF and 95% in TAB products. Each test product was ingested with a total adjusted volume of 250 ml water.

Experimental

Subjects were instructed on how to fill in the various questionnaires (as described later). They provided questionnaire-based background information on sensations, mood, and anxiety state. On each of the three testing days, separated by weekly intervals, subjects arrived at the laboratory after an overnight fast, with water consumption allowed ad libitum. After voiding, an antecubital catheter was inserted with low saline flow (0.5 ml min^{-1}) and a baseline blood sample collected. The test product was then ingested (0 min = initiation of eating) in the only presence

of a technician assigned to this task. Blood was subsequently sampled at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, and 360 min. Intensity, nature, and sites of occurrence of unusual sensory symptoms were reported in writing, privately and in silence, within five separate time-dependent questionnaires at the time of each blood draw (questionnaires 1−5, Q1−Q5), which took less than 5 min. Subjects remained seated, with a break at 2 h to urinate (0−2 h urine pool). 150 ml of water was consumed at 2 h and at 4 h, while a standardized vegetarian snack was taken after the 4 h blood draw. At 6 h the catheter was removed and subjects urinated again (2–6 h urine pool). Finally, three more questionnaires (questionnaires 6−8, Q6−Q8) were administered retrospectively, evaluating the most intense grade of sensations of the elapsed session and to reveal any association of perceptions with personality traits. The staff in charge of administering questionnaires and drawing blood were unaware of the treatment order.

Analysis of β-alanine

β-alanine was determined in plasma, urine, and in REF and TAB products by a blinded analyst. After deproteinization and filtration (Tan and Gajra 2006; Van Kuilenburg et al. 2004), free amino acids from supernatants were separated by ion exchange chromatography, and βA was quantified using dual wavelength photometric detection following ninhydrin post column derivatization (Biochrom 30 Amino acid Analyzer, Biochrom Ltd, Cambridge, UK; PEEK Li columns, Laborservice Onken GmbH, D-Gründau). Repeatability of the assay was 3%, with a limit of quantitation of 15 μmol L^{-1}. The amount of βA retained was calculated as the amount ingested minus the cumulated amount excreted in urine over 6 h.

Sensory and psychological data

The sensory questionnaires were based on established procedures to evaluate perceptual ratings, but were not tested for reliability and validity for this study. This is why multiple assessments with distinct scale types (visual analogue Q1, category scale Q2, linear scale Q3) were used for the perception of intensity. The distribution of sensations on the body questionnaire (Q4) was created for this study and was not validated. The psychological data (Q6, Q7, and Q8) were obtained through the published validated questionnaires given in reference. All questionnaires except Q5 (Flush) were self-administered, Q1−Q5 immediately after each blood draw and Q6−Q8 only at the session end. Questionnaires were presented in the same sequence every time. After filling a questionnaire, the page was turned and the subject was not allowed to browse backward to review. Quality of records was reviewed by the nurse both during and at the end of each session, so that any incoherence or confusion was remedied without delay.

Time-dependent questionnaires (Q1−Q5)

Q1. The Visual Analogue Score (VAS) focused on the perceived intensity of symptoms. It consisted of a horizontal, continuous 9-cm line with vertical marks 2 mm from each end, labeled "no unusual sensation" to "most intense sensation imaginable". Low and high ends of the scale were arbitrarily placed on either the left or on the right side at successive times to reduce habituation. A box was placed next to the low end where subjects acknowledged with a tick their awareness of the direction of the scale. The record was a vertical line drawn at the level most fitting with perception intensity. VAS was expressed as percent of scale length from the low end.

Q2. The Intensity of Sensation Score (ISS) was also about symptom intensity. It featured a discrete scale similar to the widely used Borg category-ratio scale, which is valid for assessing non-linear changes in the perception of intensity (Noble et al. 1983). The scale consisted of 15 boxes (0−14) labeled from "absent" (bottom box) to "unbearable" (top box). The records (a tick in the best fitting box from 1 to 14) were grouped further into very low- (1−3), low- (4−5), moderate- (6−8), and intense- (9–14) intensity categories, such that no ticked box was ever misplaced into a category name of a lower severity grade than its label.

Q3. The Qualitative Light Symptoms Inventory (QLSI) qualified the nature of the sensation from within a subset of six descriptive attributes: "pins and needles and/or tingles", "tickling and/or itching", "flush and/or shiver", "tactile hypersensitivity and/or irritation", "numbness and/or insensitivity", and "pain and/or soreness". Next to each attribute was a 5-level scale from 0 = "absent" to 4 = "extremely intense". Subjects recorded the most fitting level for each descriptor. An additional category allowed free text descriptions.

Q4. The body Sensitive Surface Score (SSS) highlighted spatial characteristics to identify the body areas most affected by symptoms. A schematic image of the body, featuring both front and back sides with marked rectangular areas, was used. Subjects were marked with "×" in each area where symptoms were perceived, disregarding their nature and intensity. Records (0 or 1) were counted as the sum of all marked areas for the whole body (maximum possible score = 23) and for subsets of them identifying the higher, middle, and lower parts of the body. Subsets were chosen with no consideration of an underlying neural network. Additional information derived from the McGill Pain Questionnaire (Boureau et al. 1992), using forced

single choice answers, bore on the temporality, and depth of the symptoms. Symptoms were recognized as either "brief-transient" or "periodic-intermittent" or "continuous-constant" to describe their dominant connection with time. Depth was identified as "external" or "internal".

Q5. The Flush questionnaire was based on inconspicuous visual inspection; a physician assessed changes (yes/no) in the appearance of the skin on the face, ears, and forearms (e.g. red skin, red nose, colored spots).

Session-dependent questionnaires (Q6−Q8)

Three questionnaires were filled retrospectively at the end of the test day, featuring Q6: the Profile Of Mood States (POMS), a French adaptation (Fillion and Gagnon 1999) of the abridged profile of mood states by Shacham (1983), Q7: the Questionnaire de Douleur de Saint-Antoine (QDSA), a validated French version of the McGill Pain Questionnaire (Boureau et al. 1992) that describes sensory and emotional dimensions of pain, and Q8: the Spielberger State Anxiety Inventory (SAI; Spielberger et al. 1983). Mood and anxiety were monitored to account for the potential input of affective states on sensory scores (Villemure and Bushnell 2002).

Statistics and pharmacokinetic analysis

All analyses were done in SAS 9.1 (SAS Institute, Cary NC, USA) except for the pharmacokinetic data, which were analyzed using WinNonLin 5.2.1 (Pharsight, Sunnyvale CA, USA). Normality of distribution was tested on main data using Q–Q plot and Shapiro–Wilk W test and was not rejected overall. A review of the 4- to 6-h data on a subsample of subjects indicated that the majority of the plasma βA values were below the analytical detection limit; thus blood samples were analyzed statistically up to 4 h only. For each subject and each product except PLA, analysis was conducted using the first-order compartmental model. The following parameters were estimated: peak plasma βA concentration (C_{max}, µmol L^{-1}), time to reach peak plasma βA concentration (T_{max}, h), area under the curve to infinity (AUC, µmol L^{-1} h), absorption rate constant or rate of rise to peak plasma concentration (K_a, h^{-1}), lag time until first appearance of βA in plasma (T_{lag}, h) and half-time of βA disappearance from plasma ($T_{1/2}$, h). In addition, a crossover model with visit, randomization sequence and product as fixed effects, and subjects as random effect was used to test the product effect (TAB vs. REF, TAB vs. PLA) on each parameter. Regarding each of the time-dependent Q1−Q4 questionnaires (VAS, ISS, QLSI, and SSS), maximal score (S_{max}), time to reach the maximal score and mean value over 6 h (excluding the time 0 value, S_{mean}) were determined. For each of these parameters, a similar crossover model as for the pharmacokinetic analysis was used. The scores for the end of session Q6−Q8 questionnaires (POMS, QDSA, and SAI) were analyzed by the same crossover model except that the baseline score was added as a fixed-effect. Additional correlation analysis was performed between combinations of plasma βA variables and VAS, ISS, and SSS scores. A post hoc Chi-square goodness-of-fit test was used to assess the distribution of temporality and depth of the sensations in the REF treatment. Data from Harris et al. (2008) were used to calculate the sample size based on the variance of plasma βA C_{max}. Twelve subjects had been recruited, but one was excluded from the per-protocol analysis because of unexplained, physiologically impossibly elevated plasma βA levels in the REF group (up to 600-fold higher than the average of all REF subjects). Data are presented as means ± SD with significance assumed at $p < 0.05$, unless stated otherwise.

Results

Plasma β-alanine concentrations and paresthesia

Changes in plasma βA with time and AUC are depicted in Fig. 1a and pharmacokinetic parameters are shown in Table 1. Without exception, all βA baseline concentrations were below the detection limit. Peak βA concentration after TAB was 68% lower than after REF ($p < 0.001$). TAB reached C_{max} at ~1 h, while REF peaked at the statistically ($p < 0.01$) earlier time of 30 min. Times of first appearance in plasma were similar, but the absorption rate was threefold slower with the TAB form. In contrast, AUC_{TAB} was not ($p = 0.55$) smaller than AUC_{REF}. Absence of βA after PLA was confirmed by a check analysis of the 45 min plasma samples.

The time course of sensory side-effects is also depicted in the figure. Only βA in solution (REF) produced evident sensations. The perceived intensity of unusual sensations using the continuous VAS scale (VAS_{mean}, Fig. 1b) and the intensity of the symptoms identified as pins and needles ($QLSI_{p\&n\text{-}mean}$ score, Fig. 1c) globally followed a pattern of response parallel to that of plasma βA concentration. The surge of perceived symptoms seemed to anticipate the subsequent rise in measured plasma βA, as paresthesia had reached a plateau ($QLSI_{p\&n\text{-}mean}$) or already passed peak value (VAS_{mean}) at 15 min post-ingestion, whereas plasma βA concentration was still rising sharply. By 90 min, all quantified sensations were not different from PLA.

There was a very highly significant ($p = 0.0002$) positive correlation ($R = 0.60$) between $QLSI_{p\&n\text{-}max}$ and βA C_{max}, as primarily driven by the REF data, suggesting that

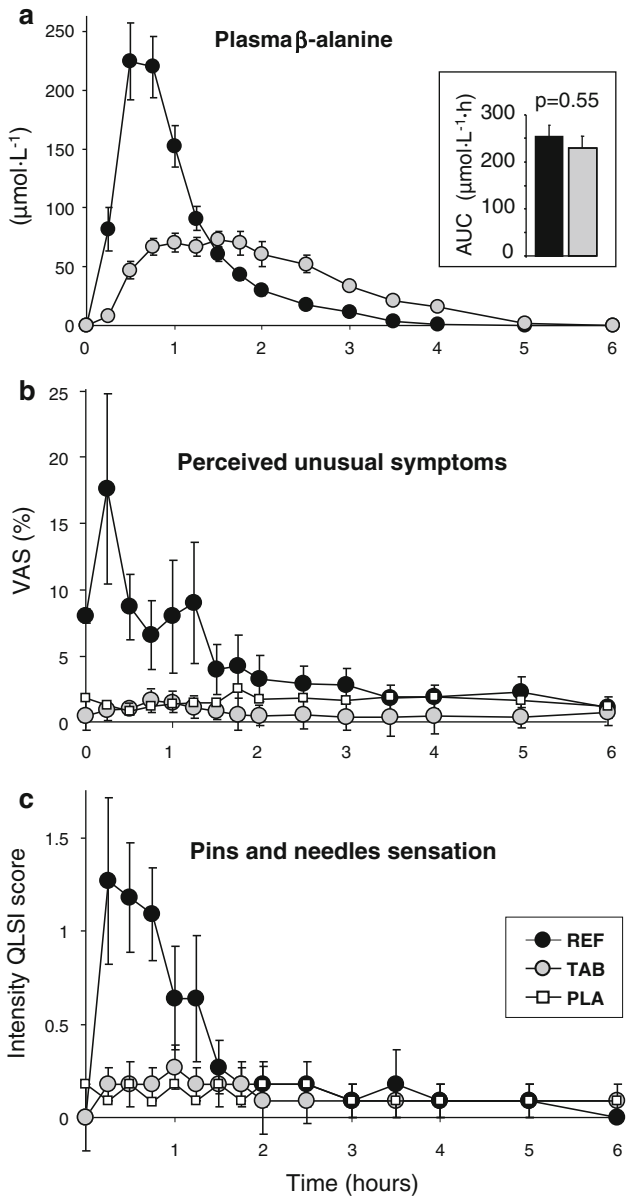

Fig. 1 **a** Plasma β-alanine concentration (μmol L^{-1}) for 6 h after the ingestion of 1.6 g βA in aqueous solution *REF* or in slow-release tablet form *TAB*. The area under the curve is depicted in the insert (μmol L h^{-1}). **b** Reported intensity of non-differentiated sensations of paresthesia using a Visual Analogue Scale (%), from 0 = "no unusual sensation" to 100 = "most intense sensation imaginable". **c** Intensity of "pins and needles" sensations using the Qualitative Light Symptom Inventory ($QLSI_{p\&n}$, scale from 0 = absent to 4 = extremely intense). Means ± SE(11)

the higher the βA peak, the higher the symptoms of pins and needles. There was a negative correlation ($R = -0.52$, $p = 0.002$) between $QLSI_{p\&n\text{-}max}$ and βA T_{max}, again mainly driven by the REF results. This suggests that the quicker the plasma peak, the more symptoms of pins and needles were observed.

Table 1 β-Alanine pharmacokinetic analysis

	TAB[a]	REF[b]	*P*
C_{max} (μmol L^{-1})	81.9 ± 27.5	248.2 ± 112.7	0.0002
T_{max} (min)	60.0 ± 16.2	29.4 ± 7.2	0.0015
AUC (μmol L^{-1} h)	229.4 ± 83.7	253.4 ± 81.1	0.5504
K_a (h^{-1})	3.55 ± 2.69	11.98 ± 7.21	0.0007
T_{lag} (h)	17.4 ± 9.6	14.4 ± 4.2	0.6606
$T_{1/2}$ (h)	80.4 ± 64.2	37.2 ± 21.6	0.0485

Means ± SD

C_{max} peak concentration, T_{max} time to peak, *AUC* area under the curve, K_a absorption rate constant, T_{lag} time until first appearance in plasma $T_{1/2}$ half-time of disappearance

[a] βA slow-release tablets

[b] βA reference aqueous solution

β-Alanine excretion and retention

There was 70% less βA excreted in urine during the entire 6-h period from TAB compared with REF (202 ± 164 vs. 663 ± 380 μmol, $p < 0.0001$, Fig. 2). Furthermore, excretion was delayed with TAB. Accordingly, only two-thirds of total βA excretion was recovered in the first 2-h urine after TAB, whereas excretion was nearly complete in the same time after REF (67 ± 22 vs. 98 ± 2%, $p < 0.0001$). When back calculating the total amount of βA retained, it was found that 98.9 ± 0.9% (1582 ± 15 mg 6 h^{-1}) of the slow-release βA was retained after TAB, which was greater ($p < 0.0001$) than the 96.3 ± 2.1% (1541 ± 34 mg 6 h^{-1}) retention of βA from the REF solution.

Severity and incidence of side-effects

Maximal reported values for symptom intensity throughout the 6-h period are listed in Table 2. The continuous (VAS_{max}) and categorical (ISS_{max}) scores of maximal intensity, the maximal intensity of the specific sensation recognized as pins and needles ($QLSI_{p\&n\text{-}max}$), all were very much smaller after TAB (classified "very, very low") than after REF ("very low", $p < 0.001$). There was no distinguishable difference between TAB and PLA. The same conclusion holds true for the S_{mean} values (VAS_{mean}, ISS_{mean}, $QLSI_{p\&n\text{-}mean}$, details not shown), but obviously at milder levels.

The percentage of subjects presenting different levels of severity of the symptoms at the different time points according to the categorical scale (ISS) is shown in Fig. 3. There were 462 opportunities to report, resulting in a 35% overall incidence rate of response when all intensity levels and treatments were grouped. Within the first 2 h post ingestion, the period when symptoms were clearly

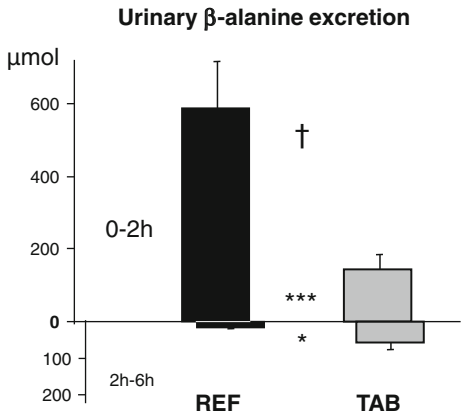

Fig. 2 β-Alanine lost in urine from 0−2 h and 2−6 h after the ingestion of 1.6 g βA in a reference solution *REF* or in a slow-release tablet *TAB*. $^{\dagger}P < 0.0001$ for the global 0−6 h treatment difference; $^{*}P < 0.05$, $^{***}P < 0.0001$ for within-period treatment differences. Means ± SE(11)

noticeable, there was no record of symptoms in the intense category; there were 14 records in the moderate-intensity category (all in REF), 17 records in the low-intensity category (REF 12, TAB 3, PLA 2), and 75 records in the very low-intensity category, which appeared to be just background noise (REF 21, TAB 26, PLA 28). The incidence rate of declared moderate- and low-intensity symptoms in the responsive treatment group (REF) was therefore 32% in the first 2 h. Severity of symptom scores and incidence were not different between TAB and PLA.

Description of the side-effects

Among the proposed selection of descriptors, "pins and needles" came out as the prevailing one for the maximal intensity score ($QLSI_{p\&n\text{-}max}$) and the incidence rate (Fig. 4). Also here, the incidence rate did not distinguish TAB from PLA: there were 2.5-fold more records of pins and needles with REF (43 records), which was the only treatment producing clear symptoms (Table 2), as compared with either TAB or PLA (17 and 18 records, respectively). When focusing on the early time period (0−2 h), after REF ingestion, 40% of all records were for "pins and needles", 24% for "tickling, itching", and the last third of all records spread over the four remaining descriptors. Questionnaire Q5 brought up no signs of flushing on the skin at any time for any treatment.

Topography of the symptoms

A summary of the body sensitive areas (SSS_{max}) is shown in Fig. 5. Accounting for the number of areas involved in each body site subscore, the main body area associated with paresthesia symptoms from pure βA supplementation was the middle body, i.e. trunk and arms. The SSS_{max} for both the whole body and the mid-body site were much smaller after TAB, with only 15−20% as many body surfaces affected as after REF (Table 2). There was no difference between TAB and PLA ($p = 0.70$). Symptoms perceived in the first 2 h after REF were unevenly perceived as periodic (61% of all records) to continuous (27%), rather than brief stimuli (12%, $p < 0.001$), and near the skin surface (73% of all records) rather than internally to the body (27%, $p < 0.01$). There was a positive correlation ($R = 0.73$, $p < 0.0001$) between the number of areas of the mid-body part that were affected and peak plasma βA (C_{max}), primarily driven by REF results, suggesting that the higher the C_{max}, the more likely that an increased number of body areas may be affected by symptoms.

Table 2 Maximal intensity scores of sensory descriptors (S_{max})

	Variable (score range)	TAB[a]	REF[b]	PLA[c]
Intensity (continuous)	VAS_{max} (0−100%)	2.9 ± 2.9	24.5 ± 22.0***	3.5 ± 5.0
Intensity (discrete)	ISS_{max} (0−14)	1.55 ± 1.92	5.18 ± 2.86***	1.27 ± 1.74
"Pins and needles"	$QLSI_{p\&n\text{-}max}$ (0−4)	0.36 ± 0.67	1.91 ± 1.22***	0.36 ± 0.67
Topography	SSS_{max} whole body (0−23)	1.0 ± 1.0	5.7 ± 4.4***	1.3 ± 1.7
	SSS_{max} mid-body (0−6)	0.4 ± 0.7	2.5 ± 1.2***	0.5 ± 1.2

Means ± SD

TAB versus REF, $^{***}P < 0.001$

TAB versus PLA, $P > 0.50$

VAS Visual analogue scale, *ISS* Intensity of sensation score, $QLSI_{p\&n}$ Qualitative light symptoms inventory, "pins and needles" descriptor, *SSS* Body Sensitive surface score

[a] *βA* slow-release tablets

[b] *βA* reference aqueous solution

[c] *PLA* food bar

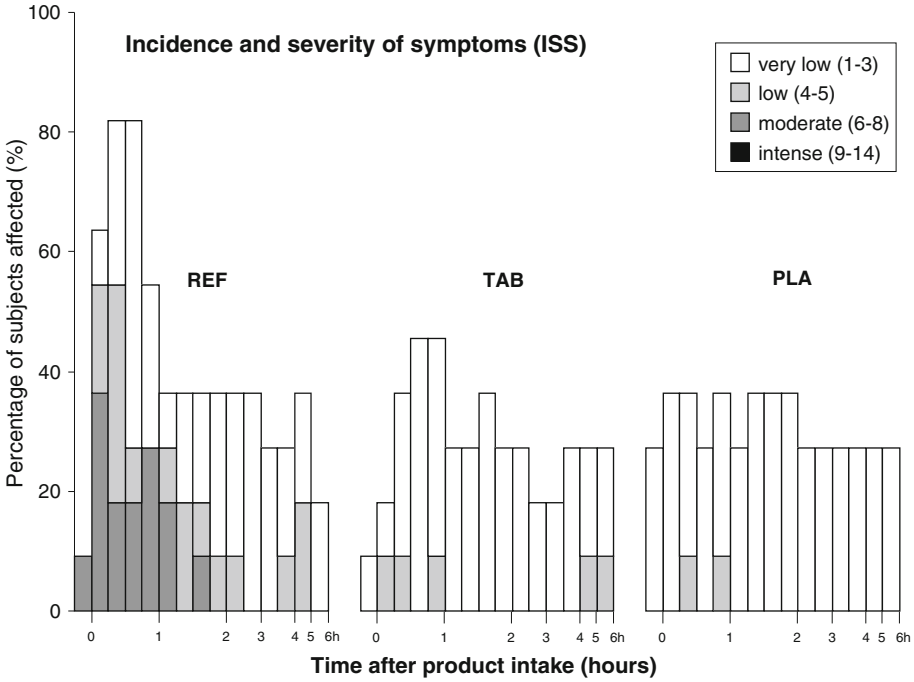

Fig. 3 Incidence (% subjects affected) and severity of symptoms after the ingestion of 1.6 g β-alanine in a reference solution *REF* or in a slow-release tablet *TAB*, or a placebo *PLA*. Severity is shown with symptom intensity scores (ISS scale from 0 = absent to 14 = unbearable) grouped into four categories, described in the legend

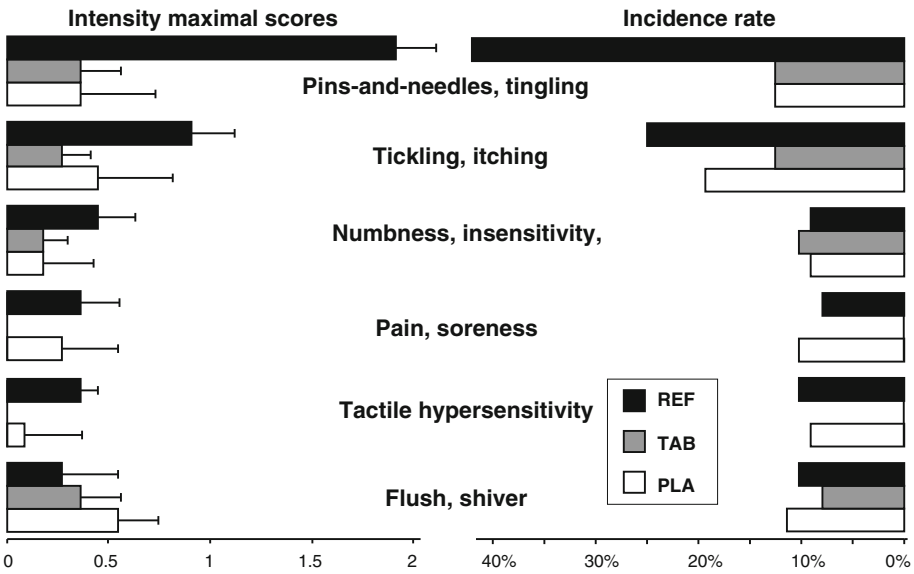

Fig. 4 Description of reported symptoms after the ingestion of 1.6 g β-alanine in a reference solution *REF* or in a slow-release tablet *TAB*, or a placebo *PLA*, using the Qualitative light symptom inventory QLSI. *Left* Maximal intensity scores $QLSI_{max}$, 0–4 scale (0–6 h). Means ± SE(11). *Right* Incidence rate in % of reporting opportunities (0–2 h). Pins-and-needles was the most frequent sensation and it occurred after REF only

Retrospective questionnaires

The QDSA questionnaire was used to retrospectively evaluate any painful features of the sensations perceived in the study. There was no statistical difference in QDSA scores, either between TAB and REF (sensory dimension $p = 0.09$, affective dimension $p = 0.29$) or between TAB and PLA (sensory $p = 0.52$, affective $p = 0.81$; data not shown). There was no statistical difference in either mood (POMS score: TAB vs. REF $p = 0.14$, TAB vs. PLA $p = 0.30$) or anxiety (SAI score) for TAB vs. REF ($p = 0.11$), whereas the SAI score for TAB vs. PLA reached significance (28.6 ± 7.5 vs. 26.3 ± 6.5, $p = 0.024$).

Discussion

The key finding of this study, which is in line with the pilot report of Harris et al. (2008), is the absence of any difference in any sensory outcome between the oral consumption of a 1.6-g dose of slow-release βA and the PLA. Paresthesia symptoms were comprehensively probed through multiple dimensions (severity by independent measures, qualitative identifiers, incidence), which all were decreased to the level of the PLA by the slow-release formulation.

For most individuals, 400 mg βA ingested acutely is symptom free and this dose was frequently used in

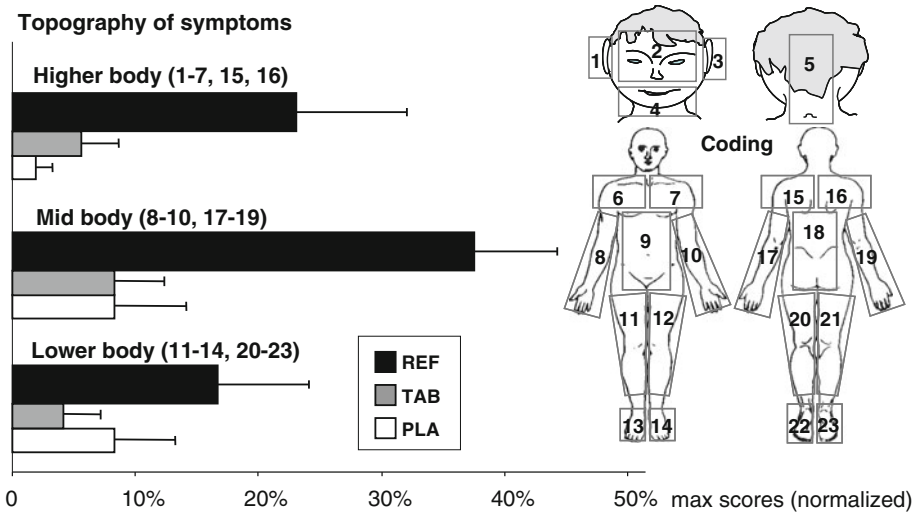

Fig. 5 Topography of β-alanine-induced sensations. Data shown are the maximal reported values of the body surface sensitive score (SSS_{max}), expressed as % of the number of areas per site, for the higher body (areas #1−7, 15, 16), mid body (areas #8−10, 17−19) and lower body (areas #11−14, 20−23; note: rectangular areas were not numbered on the test form). Means ± SE(11)

previous studies (Baguet et al. 2009; Derave et al. 2007; Harris et al. 2006). With 500 mg doses, no symptoms were reported and treatment identification was not different between verum and PLA (van Thienen et al. 2009). With 800 mg doses (10 mg kg^{-1}), Harris et al. (2006) acknowledged "mild symptoms of flushing" in two out of four subjects and for Hill et al. (2007), symptoms were "infrequent and mild when they occurred". With a 1.6-g dose, symptoms were recorded as "significant" in three of four subjects (Harris et al. 2006) and none were reported in other studies (Stout et al. 2007; Zoeller et al. 2007) although, as communicated later, about 20% of the subjects reported "tingling"sensations with βA in the former study (J. Stout, *personal communication*). When increasing to 2 g βA doses, Sweeney et al. (2010) reported "no side effects other than a mild prickling sensation" in the neck and the limbs. With single βA doses of 3.2 g (40 mg kg^{-1}) and greater, an apparent plasma threshold is crossed, and side-effects are perceived as "unpleasant" (Harris et al. 2006). The symptom-free slow-release galenic βA formulation utilized in this study should simplify practice and compliance, because it delivers an asymptomatic dose that was recently shown to raise muscle carnosine when administered just once a day for 2 weeks (Stellingwerff et al. 2011). Also, by eliminating perceived side-effects, it ensures proper blinding of subjects in future studies. Although not assessed in the current study, the pilot work by Harris et al. (2008) suggests that, if necessary, a dose of 3.2 g of slow-release βA could be used, and only result in "mild symptoms".

The absence of side effects with TAB was associated with a blunted and extended time course of βA concentration in plasma in comparison with REF, although the total AUC between TAB and REF were not different. This again supports the preliminary results of Harris et al. (2008) who, with very similar time courses of plasma βA concentrations, found βA peak value reduced to 54% (this study to 33%) and plasma concentration at 3 h maintained fivefold higher (this study three fold higher) with the slow-release product. However, the correlations found for symptom intensity with plasma βA C_{max} (positive) and βA T_{max} (negative) suggest that the attenuation of symptoms is explained by a combination of both a lowered maximum plasma concentration and a sustained release of βA. The threshold of βA plasma concentration for the appearance of paresthesia was situated at 75−80 µmol L^{-1} in REF, as paresthesia was highest at 15 min, when mean βA concentration was 82 µmol L^{-1}. Conversely in TAB, symptoms were absent despite the fact that βA concentrations reached and remained around 60−75 µmol L^{-1} for nearly 1 h. Interestingly, sensory saturation took place quickly at ~15 min (Fig. 1b, c), and despite a further 2.7-fold rise of plasma βA from 15 to 30 min after the fast REF solution (Fig. 1a), there was no further increase in paresthesia reporting, in fact more like an attenuation of the perceived symptoms. This paradox could be hypothetically due to psychological anticipatory factors, to inhibitory feedback at the receptor level or independent mechanism(s) through some pathway involving βA. However, it must be remembered as a limitation of this study that most sensory data were collected using non-validated tests.

A novel finding of the current study was the threefold lower urinary βA loss after supplementation of TAB vs. REF (Fig. 2). Similar effects have previously been found when creatine, another dietary supplement, was ingested in a food matrix with viscous properties that delay gastrointestinal absorption (Deldicque et al. 2008). Since the primary benefit of increased muscle carnosine loading due to βA supplementation is not an immediate effect, but via the sustained net accumulation of carnosine in muscles over several weeks, the improved whole body βA retention suggested by urine data adds value beyond the similar

bioequivalence pointed out by the AUC. The recent finding of increased muscle carnosine after only 2 weeks of supplementation with TAB (Stellingwerff et al. 2011) lends further support to the efficacy of slow-release βA for muscle carnosine storage.

There are at least five recognized receptor sites for βA and the mechanism responsible for the sensitization of nociceptive neurons has not been unequivocally clarified (Crozier et al. 2007; Tiedje et al. 2010). As summarized by Sale et al. (2010), candidates include (a) βA-activated strychnine-sensitive glycine receptor sites, in association with glutamate sensitive *N*-methyl-D-aspartate receptors in the brain and the central nervous system, and (b) the mas-related gene family of G protein-coupled receptors, in dorsal root ganglia neurons ending in the skin, which are triggered by interactions with specific ligands such as βA. The symptoms observed in this study after the pure 1.6 g βA solution were limited to "pins and needles, tingling", and, to a small extent, to a semantically close and possibly confusing "tickling, itching" sensation. They never exceeded the "very low" severity grade. With higher doses, a broader array of symptoms have been described, including steady burning and electric shock sensations (Harris et al. 2008). Furthermore, with these higher doses prickly sensations are also more intense, affecting different individuals variably on the skin and around the body in moving and vibrating patterns. The QDSA (modified pain questionnaire) did not highlight any sign of ordinary pain. Interestingly, the often used qualification of "flushing" for βA-induced paresthesia (e.g. Harris et al. 2006) was neither perceived as a sensation by subjects (Fig. 4) nor spotted as a redness of the skin by the external observer in the current study. For most individuals "flushing" would not be the proper description of the primary side-effect at the moderate βA dose used in this study. The absence of any visible cutaneous vasodilation is also coherent with a mechanism of action of βA that differs from that causing flushing after consumption of niacin, which activates prostaglandin release in capillaries (Kamanna et al. 2009). Instead, the primary and sole reported sensation in the current study was the tingling sensation of "pins and needles". Finally, as reviewed by Artioli et al. (2010) and Derave et al. (2010), every study to date has shown that any symptoms of paresthesia due to βA appear to be normal and transient manifestations that dissipate in 1−2 h. To date, chronic βA supplementation has produced no other side-effects and neither biochemical nor hematological alteration after repeated daily administrations (Harris et al. 2006; Stellingwerff et al. 2011). In conclusion, the ingestion of slow-release βA tablets (2 × 0.8 g) is a practical, symptom-free, probably effective and apparently safe mode of βA supplementation.

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References

Abe H (2000) Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. Biochem (Mosc) 65:757–765

Artioli GG, Gualano B, Smith A, Stout J, Lancha AH Jr (2010) Role of beta-alanine supplementation on muscle carnosine and exercise performance. Med Sci Sports Exerc. 42:1162–1173

Baguet A, Reyngoudt H, Pottier A, Everaert I, Callens S, Achten E, Derave W (2009) Carnosine loading and washout in human skeletal muscles. J Appl Physiol 106:837–842

Baguet A, Bourgois J, Vanhee L, Achten E, Derave W (2010) Important role of muscle carnosine in rowing performance. J Appl Physiol 109:1096–1101

Bakardjiev A, Bauer K (1994) Transport of beta-alanine and biosynthesis of carnosine by skeletal muscle cells in primary culture. Eur J Biochem 225:617–623

Boureau F, Luu M, Doubrere JF (1992) Comparative study of the validity of four French McGill Pain Questionnaire (MPQ) versions. Pain 50:59–65

Crozier RA, Ajit SK, Kaftan EJ, Pausch MH (2007) MrgD activation inhibits KCNQ/M-currents and contributes to enhanced neuronal excitability. J Neurosci 27:4492–4496

Deldicque L, Decombaz J, Zbinden FH, Vuichoud J, Poortmans JR, Francaux M (2008) Kinetics of creatine ingested as a food ingredient. Eur J Appl Physiol 102:133–143

Derave W, Ozdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, Achten E (2007) Beta-alanine supplementation augment muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. J Appl Physiol 103:1736–1743

Derave W, Everaert I, Beeckman S, Baguet A (2010) Muscle carnosine metabolism and beta-alanine supplementation in relation to exercise and training. Sports Med 40:247–263

Fillion L, Gagnon P (1999) French adaptation of the shortened version of the Profile of Mood States. Psychol Rep 84:188–190

Harris RC, Jones G, Wise JA. The plasma concentration-time profile of b-alanine using a controlled-release formulation (Carnosyn™). FASEB J. 2008; 22:709.1[abstr.]

Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, Wise JA (2006) The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. Amino Acids 30:279–289

Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK, Wise JA (2007) Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. Amino Acids 32:225–233

Hoffman J, Ratamess NA, Ross R, Kang J, Magrelli J, Neese K, Faigenbaum AD, Wise JA (2008) Beta-alanine and the hormonal response to exercise. Int J Sports Med 29:952–958

Kamanna VS, Ganji SH, Kashyap ML (2009) The mechanism and mitigation of niacin-induced flushing. Int J Clin Pract 63:1369–1377

Kendrick IP, Harris RC, Kim HJ, Kim CK, Dang VH, Lam TQ, Bui TT, Smith M, Wise JA (2008) The effects of 10 weeks of

resistance training combined with beta-alanine supplementation on whole body strength, force production, muscular endurance and body composition. Amino Acids 34:547–554

Kendrick IP, Kim HJ, Harris RC, Kim CK, Dang VH, Lam TQ, Bui TT, Wise JA (2009) The effect of 4 weeks beta-alanine supplementation and isokinetic training on carnosine concentrations in type I and II human skeletal muscle fibres. Eur J Appl Physiol 106:131–138

Noble BJ, Borg GA, Jacobs I, Ceci R, Kaiser P (1983) A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. Med Sci Sports Exerc 15:523–528

Sale C, Saunders B, Harris RC (2010) Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. Amino Acids 39:321–333

Sale C, Saunders B, Hudson S, Wise JA, Harris RC, Sunderland CD (2011) Effect of beta-alanine plus sodium bicarbonate on high-intensity cycling capacity. Med Sci Sports Exerc 43:1972–1978

Shacham S (1983) A shortened version of the Profile of Mood States. J Pers Assess 47:305–306

Spielberger CD, Gorsuch RL, Lushene PR, Vagg PR, Jacobs AG. (1983) Manual for the State-Trait Anxiety Inventory STAI (Form Y): self-evaluation questionnaire. Consulting Psychologists Press, Inc, Palo Alto

Stellingwerff T, Anwander H, Egger A, Buehler T, Kreis R, Decombaz J, Boesch C (2011) Effect of two beta-alanine dosing protocols on muscle carnosine synthesis and washout. Amino Acids. doi:10.1007/s00726-011-1054-4

Stout JR, Cramer JT, Zoeller RF, Torok D, Costa P, Hoffman JR, Harris RC, O'Kroy J (2007) Effects of beta-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. Amino Acids 32:381–386

Sweeney KM, Wright GA, Glenn BA, Doberstein ST (2010) The effect of beta-alanine supplementation on power performance during repeated sprint activity. J Strength Cond Res 24:79–87

Tan IK, Gajra B (2006) Plasma and urine amino acid profiles in a healthy adult population of Singapore. Ann Acad Med Singapore 35:468–475

Tiedje KE, Stevens K, Barnes S, Weaver DF (2010) Beta-alanine as a small molecule neurotransmitter. Neurochem Int 57:177–188

Van Kuilenburg AB, Stroomer AE, van LH, Abeling NG, Van Gennip AH (2004) New insights in dihydropyrimidine dehydrogenase deficiency: a pivotal role for beta-aminoisobutyric acid? Biochem J 379:119–124

Van Thienen R, Van Proeyen K, Vanden Eynde B, Puype J, Lefere T, Hespel P (2009) Beta-alanine improves sprint performance in endurance cycling. Med Sci Sports Exerc 41:898–903

Villemure C, Bushnell MC (2002) Cognitive modulation of pain: how do attention and emotion influence pain processing? Pain 95:195–199

Zoeller RF, Stout JR, O'kroy JA, Torok DJ, Mielke M (2007) Effects of 28 days of beta-alanine and creatine monohydrate supplementation on aerobic power, ventilatory and lactate thresholds, and time to exhaustion. Amino Acids 33:505–510