Is individualization of sodium bicarbonate ingestion on time to peak necessary?

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ABSTRACT

Purpose: To describe thereliability of blood bicarbonate pharmacokinetics in response to sodium bicarbonate (SB) supplementation across multiple occasions and assess, using putative thresholds, whether individual variation indicated a need for individualised ingestion timings. **Methods:** Thirteen men (age 27±5 y; body mass (BM) 77.4±10.5 kg; height 1.75 ±0.06 m) ingested 0.3 $g \cdot kg^{-1}$BM SB in gelatine capsules on 3 occasions. One hour after a standardised meal, venous blood was obtained before and every 10 min following ingestion for 3 h, then every 20 min for a further hour. Time-to-peak (Tmax), absolute-peak (Cmax), absolute-peak-change (ΔCmax) and area under the curve (AUC) were analysed using mixed models, intraclass correlation coefficient (ICC), coefficient of variation (CV) and typical error. Individual variation in pharmacokinetic responses was assessed using Bayesian simulation with multilevel models with random intercepts. **Results:** No significant differences between sessions were shown for blood bicarbonate regarding Cmax, ΔCmax or AUC ($p>0.05$), although Tmax occurred earlier in SB2 (127±36 min) than in SB1 (169±54 min, $p=0.0088$) and SB3 (159±42 min, $p=0.05$). ICC, CV and typical error showed moderate to poor reliability. Bayesian modelling estimated that >80% of individuals from the population experience elevated blood bicarbonate levels above +5 $mmol \cdot L^{-1}$ between 75-240 min after ingestion, and between 90-225 min above +6 $mmol \cdot L^{-1}$. **Conclusion:** Assessing SB supplementation using discrete values showed only moderate reliability at the group level, and poor reliability at the individual level, while Tmax was not reproducible. However, when analysed as modelled curves, a 0.3 $g \cdot kg^{-1}$BM dose was shown to create a long-lasting window of ergogenic potential, challenging the notion that SB ingestion individualised to time-to-peak is a necessary strategy, at least when SB is ingested in capsules.

Key words: time-course; ergogenic supplement; bioavailability; blood bicarbonate; reproducibility.

INTRODUCTION

Sodium bicarbonate (SB) is an effective nutritional supplement to improve exercise performance and capacity during high intensity exercise (1-3). Acute ingestion of SB incurs an increase in blood pH and bicarbonate within approximately 30-60 mins which lasts up to several hours (4, 5).The metabolic alkalosis induced by SB ingestion leads to an increased efflux of lactate and hydrogen ions (H^+) out of the working muscles during exercise (6), which can delay the negative impact of muscle acidosis on contractile processes (7) and improve exercise performance.

Despite the known ergogenic potential of SB supplementation, recent studies are moving away from typical mean group analyses towards individualised approaches (8, 9). This is due to the identification of factors that may moderate the ergogenic effect of SB, including variability in blood responses following SB ingestion. The time course of blood bicarbonate responses to acute SB ingestion indicates large variability between individuals, with peak bicarbonate concentration occurring between 75 and 180 minutes when ingested in capsules (4) and between 10 and 140 minutes (5, 10) in solution using the commonly employed relative dose of 0.3 $g \cdot kg^{-1}$ body mass (BM) of SB. Coupled with recent evidence demonstrating consistent intraindividual response to the same dose taken on different days, it has been suggested that the optimal time to perform exercise would be at this time at which blood bicarbonate peaks (8). However, only one study to date has investigated the reproducibility of these blood response across two sessions providing SB in solution (5). In addition, the time-course responses to SB ingestion when meal ingestion is controlled remain unknown, a procedure that is likely used by most athletes in real competitive situations. Thus, more information about the consistency of the time-course responses to SB ingestion is warranted, particularly after the ingestion of a standardised meal.

The ergogenic effects of SB have been suggested to be dependent on a minimum increase of circulating bicarbonate, with an increase of +5 mmol·L^{-1} being considered a zone of potential ergogenic benefit, and increases above 6 mmol·L^{-1} being almost certainly ergogenic (4, 11, 12). It is currently unclear whether the absolute increases at time to peak differ substantially from those generally seen at standardised time points. The mean +6.5 ± 1.3 mmol·L^{-1} increase shown at time to peak by Gough, et al. (13) is similar to the increases shown following 60 min (+6.1 Dias, et al. (14); +5.1 Jones, et al. (4); +5.7 Gough, et al. (8)), 90 min (+6.5 Jones, et al. (4) +6.1 Gough, et al. (8) and 120 min (+6.5 Jones, et al. (4); +5.6 Gough, et al. (8)) with the same 0.3 g·kg^{-1}BM dose. Furthermore, blood bicarbonate concentration was not shown to be different 60-, 120- and 180-min following SB supplementation in gelatine capsules (15), which raises questions as to whether ingestion timing is an important factor for the ergogenic effects of SB in this form. It remains to be determined whether blood bicarbonate is consistently increased close to peak, or above +6 mmol·L^{-1}, for prolonged periods.

Although time to peak in blood bicarbonate has been touted as a strategy to optimise SB ingestion (13), there are several limitations that may preclude its applicability to actual training or competition settings. Firstly, it requires athletes or coaches to have access to a reliable blood gas analyser and to perform a subsequent time-course measurement of blood bicarbonate responses to SB ingestion over several hours. This procedure is laborious, costly and not easily accessible for most athletes. Secondly, time to peak assumes that the increases in circulating SB are substantially greater when blood bicarbonate peaks than at standard time points, instead of assuming that blood bicarbonate will fluctuate around the peak value for a period of time. An in-depth analysis of the blood bicarbonate responses to SB ingestion could reveal whether the "window of ergogenicity" is limited to a fixed time point or extends across a broad time period following SB ingestion. This could provide important practical information for athletes as to whether determination of time-to-peak is a necessary strategy.

To address these controversies, the aims of this investigation were to describe and determine the reliability of orally ingested SB pharmacokinetics over 4 hours using multiple testing occasions (including a placebo trial). A secondary aim of this study was to assess whether individual variation in orally ingested SB pharmacokinetics indicated a need for individualised ingestion timings. Our hypothesis was that SB ingestion would result in a sustained increase in blood bicarbonate above the purported ergogenic thresholds. We also hypothesised that this pattern would result in inconsistent responses in Tmax, potentially challenging the need for individualised ingestion timings.

METHODS

Participants

Twenty-four young, physically active, healthy men were screened for eligibility; three of them did not meet inclusion criteria, and six other candidates did not wish to partake in the study. Fifteen participants enrolled in the study, but one withdrew after the first session due to personal reasons while a second participant withdrew after the third session due to gastric distress associated with SB ingestion. Therefore, complete data were obtained for 13 participants and used in all analyses herein reported (age = 27 ± 5 years; BM = 77.4 ± 10.5 kg; height = 1.75 ± 0.06 m; body mass index = 25.2 ± 2.9 $kg \cdot m^2$). Inclusion criteria were defined *a priori* as: healthy men aged 18 to 35 years. Exclusion criteria were defined *a priori* as: smoking, use of medications that may alter stomach pH and any diagnosed condition that could affect the gastrointestinal and blood pH balance. All volunteers were informed about the discomforts and risks associated with participation and thereafter provided written consent. The study was approved by the Institutional Ethics Committee (29181114.0.0000.5391).

Study Design

This was a crossover, placebo-controlled study in which volunteers visited the laboratory on four separate occasions, 2-7 days apart, to receive SB (on 3 occasions) or placebo (PL, on 1 occasion). To control for order effects, treatments were randomly assigned to each visit in a balanced fashion using the Latin square. Participants were requested to refrain from strenuous physical activity and alcohol intake in the 24h preceding each visit. They were also instructed to maintain a similar pattern of food intake on all days prior to the tests. Compliance with these requests was verbally confirmed with all participants. The participants arrived at the laboratory in the morning after an overnight fast, and a standardised breakfast (energy: 563 kcal; protein: 9.3 g; carbohydrate: 89.6 g; fats: 8.9 g) was served to avoid variations in blood responses due to differences in food intake prior to the tests. One hour following the breakfast, blood samples were taken before and during 4 hours after the ingestion of SB or PL.

Supplementation protocol

Sodium bicarbonate (0.3 $g \cdot kg^{-1}$ BM; Farmácia Analítica, Rio de Janeiro, Brazil) was given on three different visits while an identical number of capsules was provided in PL (each capsule containing 56 mg of corn flour; Farmácia Analítica, Rio de Janeiro, Brazil). Supplements were given in gelatine capsules identical in size and appearance. Participants had 5 minutes to ingest all capsules. After ingestion of the last capsule, a stopwatch was started to control the exact times at which blood samples were to be taken.

Blood sampling

The cephalic vein was cannulated (catheter 20 G Safelet Nipro) and kept warm with the use of a forearm thermal blanket maintained at 48°C throughout the entire 4 h sampling period. A venous blood sample was taken for the determination of baseline blood parameters (i.e., before ingestion). The participants then ingested SB or PL in gelatine capsules along with 400 ml of water and then 100 ml per hour throughout. Following ingestion, blood samples were

taken every 10 minutes for 3 hours, and then every 20 minutes in the 4th hour. Blood samples (1 ml) were collected in heparinised syringes and immediately analysed for pH and pCO2 using a blood gas analyser (RAPIDLab 348, Siemens, Germany). Quality controls were performed each experimental day prior to data collection. Blood bicarbonate was calculated using the Henderson-Hasselbalch equation. The inter-assay coefficient of variation of blood bicarbonate was 6.4% (determined over the 4-h period during the PL trial). Blood bicarbonate was defined *a priori* as the primary outcome.

Side-effects

Side-effects were recorded at the same time-points as blood collection using an adapted questionnaire (16). Participants rated the intensity of the following 13 symptoms from 0 (no effect) to 10 (very intense effect): nausea, dizziness, headache, flatulence, urge to defecate, belching, heartburn, bloating, stomach cramps, intestinal cramps, urge to vomit, vomiting, and diarrhoea.

Statistical Analysis

Data are presented as mean ± standard deviation. Area under the curve (AUC) was calculated for bicarbonate and pH using the trapezoid method. Mixed models (proc mixed, SAS University Edition) were used to compare the following variables between visits: baseline, time to peak (Tmax, defined as the first time in minutes that bicarbonate and pH variables took to reach its highest value), absolute peak (Cmax, defined as the highest value in bicarbonate and pH variables), absolute peak change (ΔCmax, defined as the absolute difference between baseline and Cmax) and AUC. Individuals were considered random factors and session (3 levels; SB1, SB2, SB3) and time (blood collection time points) were fixed factors. Mixed models were also used to compare blood bicarbonate concentration at Tmax and 60, 90 and 120 minutes after ingestion. To account for the time series nature of the data and subsequent underlying structure, four different covariance structures (Compound Symmetry,

Autoregressive, Toeplitz and Unstructured) were tested to verify the model that best fit to each data set, according to the Bayesian Information Criterion (lowest BIC value). Pairwise comparisons adjusted by Tukey-Kraemer were used when a significant F-value was observed. Intraclass correlation coefficient (ICC), typical error using data from the 3 SB trials to determine within-subject reliability. Test-retest coefficient of variations (CV) were calculated using the mean square root method (17). The frequency of side-effects reported between visits, irrespective of intensity and duration, was analysed using the chi-square test. Side-effect scores for the 13 symptoms were summed within each visit and compared between visits using the Friedman Test. Statistical significance was accepted at $p \leq 0.05$. Inter-trial reliability was assessed by calculating typical errors (sigma) and ICCs from level 0 and level 1 residuals in the mixed models. Since all blood pH data and analysis were similar that of blood bicarbonate, herein we report blood bicarbonate data only although blood pH data is included as supplemental material (Supplemental Digital Content 1 – Figure – Blood pH responses).

To describe individual variation in the pharmacokinetic responses to orally ingested SB and assess the need for individualised ingestion timings, a Bayesian perspective was adopted. A Bayesian perspective best facilitated probabilistic questions such as the probability of an individual's blood bicarbonate level exceeding a given absolute increase (i.e. +5 or +6 $mmol \cdot L^{-1}$) or percentage increase within specific time windows. Using data collected across the participants' three active testing sessions, Bayesian multilevel models with random intercepts and slopes were fitted using the brms package (18) in the programming language R. In contrast to treating observed data as independent points, it was assumed that changes in blood bicarbonate after SB ingestion followed a smooth response that could be adequately described by a polynomial function. Linear, quadratic, cubic and quartic models were fitted, with Watanabe-Akaike Information Criterion (WAIC) used to identify a cubic model as the best fit for further evaluation. The Bayesian analysis required specification of prior beliefs regarding

model parameters. To reflect a lack of prior information, default improper flat priors were selected for population-level regression parameters and the LKJ-prior selected for the multivariate normal distribution covariance matrix between group-level parameters. Posterior estimates of size n=10,000 were generated for each parameter using MCMC sampling with 4 chains and 3,500 iterations (warmup = 1,000 iterations). These posterior estimates described the typical (e.g. median) blood bicarbonate response representative of the group. To explore the likely range and distribution of responses across individuals from a similar population, posterior estimates were used to probabilistically sample regression parameters from a multivariate normal distribution. For each parameter set (n=10,000), 100 individual blood bicarbonate traces (each a cubic polynomial) were produced and the total pool of 1 million traces used to estimate probabilities that an individual's blood bicarbonate increased above +5 and +6 $mmol \cdot L^{-1}$. A threshold of 80% probability was selected to assist with interpretation of results and identify time windows where for practical purposes it could be concluded that the vast majority of individuals met the criteria.

RESULTS

Reliability

Blood bicarbonate at baseline was not different between sessions (SB1 = 25.7 ± 2.4; SB2 = 25.0 ± 2.0; SB3 = 26.0 ± 1.7; PL = 25.4 ± 2.1 $mmol \cdot L^{-1}$; F = 0.74; p = 0.5348; Figure 1). Reliability statistics were calculated for baseline (TE: 1.7 units, ICC: 0.26), Cmax (TE: 2.0 units, ICC: 0.20), DeltaCmax (TE: 2.5 units, ICC: 0.1) and Tmax (TE: 38.7 units, ICC: 0.34). ICCs, typical error and CVs calculated for blood bicarbonate between sessions are presented in Table 1.

Area under the curve was not different between SB sessions (SB1 = 1447 ± 364 $mmol \cdot min \cdot L^{-1}$; SB2 = 1468 ± 421 $mmol \cdot min \cdot L^{-1}$; SB3 = 1210 ± 520 $mmol \cdot min \cdot L^{-1}$; F = 0.87;

p = 0.43; figure 1, panel B). No significant differences between sessions were shown for blood bicarbonate regarding Cmax (SB1 = 36.8 ± 2.8 mmol·L^{-1}; SB2 = 35.5 ± 1.4 mmol·L^{-1}; SB3 = 35.2 ± 2.0 mmol·L^{-1}; F = 2.65; p = 0.10; figure 1, panel C) or ΔCmax (SB1 = 11.1 ± 2.7 mmol·L^{-1}; SB2 = 10.5 ± 2.5 mmol·L^{-1}; SB3 = 9.3 ± 2.2 mmol·L^{-1}; F = 1.30; p = 0.29, figure 1, panel D), although Tmax occurred significantly earlier in SB2 (127 ± 36 min) than in SB1 (169 ± 54 min, p = 0.0088) and SB3 (159 ± 42 min, p = 0.05; Figure 2) (main effect of session: F = 5.83; p = 0.0086) (figure 1, panel E).

Individual analysis showed substantial intra-individual variation for Tmax in blood bicarbonate following SB ingestion, despite the lack of statistical differences between sessions for mean values (figure 2). Moreover, a prolonged time period above the +5 mmol·L^{-1} (light grey blocks) and +6 mmol·L^{-1} (dark grey blocks) thresholds was shown in nearly all participants in all three sessions (figure 2).

Tmax vs. standard time points

Comparison between Tmax and standard time points showed statistically significant differences in absolute bicarbonate values between all prespecified time points (Tmax: 35.9 ± 2.2 mmol·L^{-1}; 60 min: 30.8 ± 2.4 mmol·L^{-1}; 90 min: 32.1 ± 2.6 mmol·L^{-1}; 120 min 33.0 ± 3.0 mmol·L^{-1}; F = 45.87; p < 0.0001), except for 90 *vs* 120 min (p = 0.1852). Delta change for blood bicarbonate was different between Tmax vs. all pre specified time points (all p < 0.001), but no significant differences were shown between 90 and 120 min (p = 0,1852; Figure 3).

Modelling approaches

Bayesian modelling and subsequent simulations estimate that over 80% of individuals from the population experience elevated blood bicarbonate levels greater than 5 mmol·L^{-1} between 75 and 240 min after ingestion. For absolute increases greater than 6 mmol·L^{-1}, the expected window decreased to between 90 and 225 min (Table 2). Results of the Bayesian

modelling and subsequent simulations with a multilevel cubic model are illustrated in Figure 4.

Side-effects

All participants reported one or more side-effects in each of the three SB trials, with a total of 39 symptoms being reported in SB1, 46 symptoms in SB2 and 37 symptoms in SB3. No significant differences between sessions were shown for the frequency of side-effects symptoms ($x^2 = 1.45$, $p < 0.485$). The Friedman test showed that intensity of symptoms throughout the time-course was not different between visits ($p = 0.7627$; Supplemental Digital Content 2 - Figure – Side-effects).

DISCUSSION

This study is the first to investigate a 4-h time-course response of blood bicarbonate, pH and side-effects following the ingestion of 0.3 $g \cdot kg^{-1}$BM sodium bicarbonate in gelatine capsules on 3 distinct sessions. We hypothesised that, due to the dynamic nature of blood acid-base regulation and natural fluctuation in blood bicarbonate concentration, a single time point for peak blood bicarbonate would not properly represent the sustained increase in blood bicarbonate following acute SB ingestion Jones, et al. (4). We also sought to gather further information on the within-subject consistency of blood bicarbonate responses to acute SB ingestion in gelatine capsules. Repeated administration of SB in gelatine capsules did not elicit consistent responses for bicarbonate Tmax, which is in agreement with our initial hypothesis, and potentially challenges the necessity of individualised ingestion timings. Overall, our results indicate that blood bicarbonate continuously rises for ~120-160 min after SB ingestion before reaching a plateau, with elevated values being shown until the end of the 4-h period.

The Bayesian analysis revealed an interesting pattern of elevated probabilities of increased blood bicarbonate levels (above the theoretical ergogenic threshold) from ~60 min

after ingestion to the end of the measurement period. Although performance assessment was beyond the objectives of this study, our data might challenge the notion that a single time point at which blood bicarbonate peaks is necessary to optimise the ergogenic effects of SB. Instead, the Bayesian model and reliability analyses, collectively, suggest that it is not possible to accurately determine when peak blood bicarbonate has been reached since slight variations in blood bicarbonate, including the peak values, are most likely due to random error (owing to measurement error and biological variation) around the already elevated blood bicarbonate concentrations. Therefore, it appears that the ergogenic potential of SB is likely to be in place for at least 3 hours, starting ~60 min after ingestion. This finding is consistent with a previous study that measured blood bicarbonate for 3 hours in response to SB ingestion and found a similar plateau-shaped curve of increased blood bicarbonate (4). However, our data contrasts with another similar study that showed a trend towards a rapid decline in blood bicarbonate after reaching its peak (8). Perhaps the best explanation for the difference between these studies may be related to the form of SB administration. While our study and Jones, et al. (4) provided SB in gelatine capsules and found a more sustained increase in blood bicarbonate, Gough, et al. (8) provided SB in solution and found a more rapid profile of blood bicarbonate appearance and disappearance. These differences in the shape of the blood bicarbonate curves (i.e., more sustained *vs.* rapid decline) seems to also explain why the reliability of Tmax was poor in our study (random error around a long-lasting elevation in blood bicarbonate) in contrast with a good reliability in the study by Gough et al. (sharp peak and rapid decline allow a clear identification of Tmax). There is a slight difference in pharmacokinetics when SB is ingested in capsules compared to SB ingested in solution (19), meaning any conclusions in this paper are restricted to supplementation in gelatine capsules.

Another important difference between studies is the provision of a meal before SB ingestion. While we started blood collection one hour following a standardised breakfast,

297 Gough, et al. (8) requested their participants to refrain from food 4 hours before SB ingestion. It
298 is possible that the time at which an individual consumes their pre-competition or training meal
299 influences the subsequent response to SB ingestion. Although unexplored, the influence of meal
300 ingestion on the pharmacokinetic responses to SB is of great practical implication. In our study,
301 we opted to provide a standardised breakfast to better simulate a practical training or
302 competition situation, assuming that athletes typically train or compete in a well-fed post-
303 prandial state. It must be noted, however, that although our pre-ingestion meal strategy
304 represents the responses to SB ingestion under a general post-prandial state, we did not explore
305 the impact of meal composition on these responses, which remain a largely overlooked topic of
306 investigation. Another interesting point is that our ΔCmax values (~+10 $mmol \cdot L^{-1}$) were
307 considerably higher than the +7 $mmol \cdot L^{-1}$ shown by Gough, et al. (8) when supplemented with
308 the same 0.3 $g \cdot kg^{-1}$BM dose of SB. We speculate that this too could be explained, at least in
309 part, by the timing of food intake prior to supplementation. Since our volunteers had eaten only
310 one hour before supplementation, they could have been presenting a slight metabolic alkalosis
311 due to the "alkaline tide" effect that accompanies food ingestion (20). Alternatively, the
312 presence of food in their stomach could have resulted in higher luminal pH (21), which could
313 result in less bicarbonate reacting with stomach acids, allowing more bicarbonate to enter the
314 intestine to be absorbed. Differences in blood gas analysers and in blood collection methods
315 (e.g., vein *vs.* capillary blood taken with or without arterialisation) may have also played some
316 role in the different results between studies; however, it is important to note that different
317 methods may yield different absolute values but they unlikely will result in an entirely different
318 pharmacokinetic curve.

319 Analysis of classical timings of bicarbonate supplementation (60, 90 and 120 min post
320 ingestion) identified a progressive step pattern with significant increases over each 30 min
321 period. On average, blood bicarbonate at time to peak was 2.4 $mmol \cdot L^{-1}$ higher than that

obtained 120 minutes post ingestion. However, given typical error at baseline was estimated as 1.8 mmol·L^{-1}, differences can be explained by random errors, especially given the large number of data points measured and the probable extended plateau period. Nevertheless, mean values were very near or above the purported ergogenic thresholds in all time points. Importantly, there is currently no evidence for a linear association between the magnitude of the blood bicarbonate increase with the magnitude of the ergogenic effect of SB. Thus, one cannot assume that the higher the blood bicarbonate value, the greater the effects on performance. In fact, evidence so far points towards a minimum increase in blood bicarbonate necessary for SB to exert its ergogenic effects (4, 11, 12). In that sense, the Bayesian modelling presents a significant advance in data interpretation, as it allows for direct probabilistic questions to be addressed. For example, models can be used to estimate the probability that an individual from the population will experience an increase of at least +5 mmol·L^{-1} (or any other value) over a specified time interval. The Bayesian modelling clearly indicated a high probability for ergogenic effects (assuming the validity of the +5 and +6 mmol·L^{-1} thresholds) over a prolonged period of time although there was large inter-individual variability (Figure 2). Future research should corroborate the use of these ergogenic thresholds for exercise performance.

Another aim of our study was to confirm whether blood bicarbonate responses and, more importantly, the time to peak in blood bicarbonate, are consistent across 3 identical trials conducted on different days. Although Cmax and ΔCmax were similar between trials, we showed a significant difference in Tmax between trials, indicating poor repeatability of this measure. ICC and CV also showed moderate-to-poor reliability for these variables, especially ΔCmax and Tmax. In support of this, individual analysis also showed a considerable intra-individual variability in blood bicarbonate responses to acute SB ingestion (Figure 2). Thus, we suggest that determination of Tmax for subsequent implementation prior to exercise may not be the most suitable method when ingesting SB in gelatine capsules. This moderate-to-poor

reliability for blood bicarbonate measures shown in our study is somewhat in contrast with recent studies that showed consistent blood bicarbonate responses between trials (8, 14), but in agreement with a study that showed larger intra-individual variation in blood responses to SB ingestion (22). The large variation shown here may be a reflection of the long plateau-shaped curve we showed for blood bicarbonate, where values fluctuate around Cmax for a prolonged period, allowing the peak value to occur anytime within this period of time. This reinforces the notion that the peak value is, in our case, only slightly different than the other similarly elevated values, and that identification of a solitary peak value might represent random variation rather than a true peak value which would coincide with the best opportunity for SB to be ergogenic. Therefore, it appears likely that there is a broad window of opportunity, and not a single time point, where SB supplementation is more likely to be effective. This is supported by the Bayesian modelling used in the current study, and by previous studies showing no differences in the performance effects of SB between different time points following ingestion (23). Again, the differences between our results and those by Gough, et al. (8) might be due to different experimental settings (including pre-ingestion meal and SB being taken in capsules *vs.* dissolved in water), which might have resulted in different types of blood bicarbonate curve (*i.e.*, long-plateau *vs.* sharp increase followed by sharp decrease). Nonetheless, further work should confirm our assertions by investigating the effect of SB supplementation on exercise performance performed at various time points following supplementation.

Importantly, SB ingestion resulted in significant and frequent side-effects in all sessions, with no differences being shown between sessions. The consistent and widespread occurrence of important side-effects remains a major obstacle for SB use in practical settings, and this is yet to be solved. Future studies should look for ways to promote the ergogenic effects of SB while minimising its side-effects.

This study has some limitations. First, although we designed the experiment to have the highest possible external validity, we acknowledge that the participants remained rested for the entire experimental protocol. This means that the commencement of exercise, either a warm-up or a competition, could alter the time-responses shown herein. The exact window of ergogenic potential shown here can only be assumed to be valid if the athlete remains rested between SB ingestion and the beginning of the exercise. Future studies should examine how exercise of different intensities affect the pharmacokinetics and the time course of ergogenic properties of SB. Another limitation is the use of a single 0.3 $g \cdot kg^{-1}$ SB dose, which does not allow any extrapolation of the current findings to smaller doses (e.g., 0.2 $g \cdot kg^{-1}$) or other supplementation strategies (e.g., split-dose strategy). In fact, because previous studies showed a shorter period of blood bicarbonate elevations (above the purported ergogenic thresholds) with smaller doses (4), it is possible that time to peak remains as a relevant strategy when smaller doses are used, although this is yet to be confirmed. Indeed, the study by Gough, et al. (13) showed that individualised strategies based on time-to-peak may allow for the use of smaller doses without any measurable loss in SB ergogenicity. However, this study did not directly compare the effect of SB at time to peak with standard time points that are typically used in SB literature (e.g. 60-, 90- or 120-min following SB ingestion). Thirdly, we were unable to perform PO_2 analysis in our samples, meaning we could not ensure venous blood arterialisation, despite the use of a thermal blanket specifically designed for the arterialisation of venous blood in the forearm. Lastly, the interpretation of our data is based on the current assumption that increases in +5 and +6 $mmol \cdot L^{-1}$ in blood bicarbonate are true thresholds for SB to be ergogenic. Since we were unable to associate the pharmacokinetic data with true performance effects in our participants, some caution should be exercised when extrapolating our findings to performance.

To conclude, supplementation with SB in gelatine capsules following a standardized breakfast across three sessions showed only moderate reliability at the group level, but at the individual level, reliability appears to be poor. In particular, Tmax was not reproducible across the three sessions, suggesting it may not the most effective way by which to optimise SB supplementation. This is probably related to the long, sustained increases in blood bicarbonate following SB ingestion, so that solitary peak values are more a reflection of random error rather than true maximal increases in blood bicarbonate. Nonetheless, our data show that a 0.3 $g \cdot kg^{-1}$ BM dose results in a long-lasting (~3 hours, staring from ~60 min after SB ingestion) window of ergogenic potential considering an ergogenic threshold of +5-6 $mmol \cdot L^{-1}$ in blood bicarbonate from baseline. This challenges the notion that SB ingestion individualised to time to peak is a necessary strategy, at least when a dose of 0.3 $g \cdot kg^{-1}$ is taken in gelatine capsules.

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Figure 1. Panel A: Time course of mean blood bicarbonate (HCO_3^-) responses following supplementation, determined during each of the 3 sodium bicarbonate (SB1 black circle; SB2 dark grey square; SB3 light grey triangle) and placebo (PL; stars) trials, * different from PL; Panel B: Area under the curve (AUC) for blood bicarbonate in the 3 SB sessions; Panel C: Peak blood bicarbonate concentration (Cmax) in the 3 SB sessions; Panel D: maximum increase in blood bicarbonate from baseline (ΔCmax) in the 3 SB sessions; Panel E: Time to peak (Tmax), determined in the 3 SB sessions. Individual data are presented in circles; bars and error bars represent group mean and standard deviation, *p*-values represent adjusted within-subject effects.

Figure 2. Individual data for blood bicarbonate increases after sodium bicarbonate supplementation (SB), on the three visits. Black bricks = peak bicarbonate concentration (Tmax); bricks filled with diagonal lines = +6 $mmol \cdot L^{-1}$ or above; grey bricks = +5 - +5.9 $mmol \cdot L^{-1}$.

Figure 3. Maximum increase in blood bicarbonate (HCO_3^-) from baseline at classical timing points following sodium bicarbonate supplementation (60, 90 and 120 minutes following ingestion) and at time-to-peak (Tmax; 152 ± 47 minutes) determined from the 3 SB sessions. Dotted line at 5 and 6 $mmol \cdot L^{-1}$ represents the theoretical thresholds of potential and almost certain ergogenic effects. Bars and error bars represent means and standard deviation across the three SB sessions. *p*-values represent adjusted within-subject effects.

Figure 4. Density plot of simulated (n = 1 million) cubic time-course of blood bicarbonate across a 4-h period following the acute ingestion of 0.3 $mg \cdot kg^{-1}$ body mass of sodium

bicarbonate. White triangles represent mean values from the data, and darker areas represent blood bicarbonate values with greater probabilities to occur.

Supplemental figure 1: Panel A: Time course of mean pH responses following supplementation, determined during each of the 3 sodium bicarbonate (SB1 black circle; SB2 dark grey square; SB3 light grey triangle) and placebo (PL; stars) trials, * different from PL; Panel B: Area under the curve (AUC) for pH in the 3 SB sessions; Panel C: pH peak (Cmax) in the 3 SB sessions; Panel D: maximum increase in pH from baseline (ΔCmax) in the 3 SB sessions; Panel E: Time to peak (Tmax) for pH, determined in the 3 SB sessions. Individual data are presented in circles; bars and error bars represent group mean and standard deviation.

Supplemental figure 2: Side-effects. Size of circle refers the maximal side-effects intensity related, where larger means more intense.

Table 1.[1]

Time points (min)	Intraclass correlation			Typical error (mmol·L^{-1})			Coefficient of Variation (%)		
		confidence interval			confidence interval		SB1 vs SB2	SB2 vs SB3	SB1 vs SB3
		2.5	97.5		2.5	97.5			
Baseline	0.389	0.208	0.665	1.77	1.64	1.94	5.88	6.97	7.65
10	0.330	0.080	0.681	1.44	1.10	1.96	3.31	5.72	6.38
20	0.268	0.002	0.664	1.92	1.44	2.62	9.40	8.25	8.63
30	0.218	0.002	0.611	2.09	1.60	2.79	7.63	6.16	8.66
40	0.453	0.037	0.764	1.93	1.47	2.71	6.20	4.92	8.87
50	0.335	0.010	0.698	2.11	1.63	2.85	6.28	5.40	8.75
60	0.318	0.007	0.709	2.16	1.66	2.92	7.34	6.03	6.24
70	0.367	0.016	0.726	1.70	1.29	2.33	4.90	4.76	6.38
80	0.361	0.013	0.717	1.80	1.37	2.50	4.03	6.86	5.04
90	0.388	0.042	0.740	2.26	1.73	3.10	5.91	5.05	8.55
100	0.338	0.007	0.686	2.43	1.88	3.33	6.64	7.10	7.94
110	0.263	0.003	0.645	2.81	2.16	3.79	7.46	6.76	10.24
120	0.305	0.008	0.679	2.49	1.92	3.35	7.24	4.19	8.65
130	0.266	0.003	0.645	2.68	2.08	3.62	8.23	5.89	8.65
140	0.083	<0.001	0.451	2.92	2.31	3.78	8.67	9.76	8.76
150	0.108	<0.001	0.511	2.13	1.69	2.79	6.68	6.49	5.68
160	0.123	<0.001	0.523	2.65	2.07	3.44	10.48	6.30	6.37
170	0.036	<0.001	0.307	2.40	1.91	3.14	9.67	6.99	6.26
180	0.049	<0.001	0.363	2.58	2.07	3.45	9.87	5.43	8.72
200	0.214	0.002	0.606	2.37	1.84	3.11	8.13	5.87	6.14
220	0.199	0.002	0.577	1.88	1.44	2.49	5.25	4.67	6.43
240	0.218	0.002	0.633	2.10	1.63	2.76	7.59	3.77	7.40
Cmax	0.459	0.100	0.790	1.580	1.040	2.078	5.41	4.31	6.16
ΔCmax	0.294	0.002	0.694	2.104	1.429	2.633	19.55	24.41	29.98
Tmax	0.568	0.263	0.823	31.01	20.95	41.07	32.58	20.72	21.85
AUC	0.293	0.001	0.636	347.7	244.7	423.9	26.66	25.92	33.84

[1] Reliability analyses. Intraclass coefficient correlations (ranges from 0 to 1), typical error and coefficient of variation calculated for each time point across the three sodium bicarbonate supplementation sessions and for the time-to-peak blood bicarbonate (Tmax), peak blood bicarbonate (Cmax) and maximal increase in blood bicarbonate (ΔCmax) concentration.

Table 2.[1]

Time after ingestion (min)	Probability of increases above 5 mmol·L^{-1}	Probability of increases above 6 mmol·L^{-1}
0	0%	0%
15	0%	0%
30	0%	0%
45	8.6%	0%
60	69%	14%
75	93%	60%
90	97%	86%
105	99%	93%
120	99%	95%
135	99%	96%
150	99%	96%
165	99%	95%
180	98%	94%
195	97%	92%
210	95%	88%
225	91%	80%
240	85%	70%

[1] Probability estimates (%) of elevating blood bicarbonate above 5 mmol·L^{-1} and 6 mmol·L^{-1} (from baseline) at different time points following sodium bicarbonate ingestion. Probability values were estimated using Bayesian simulation (n = 1 million).

Figure 1

Click here to access/download;Figure;Figure 1.jpg

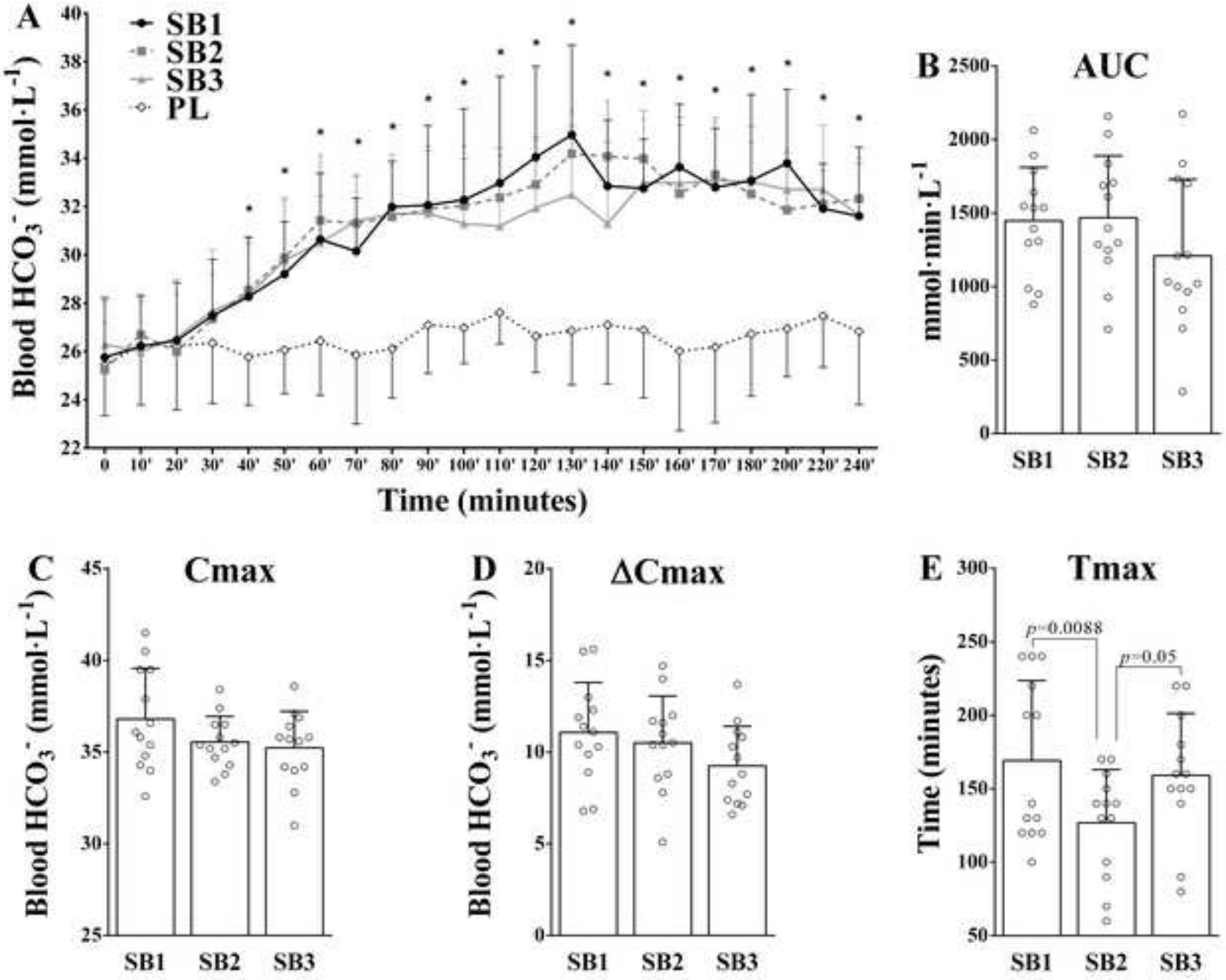

Figure 2

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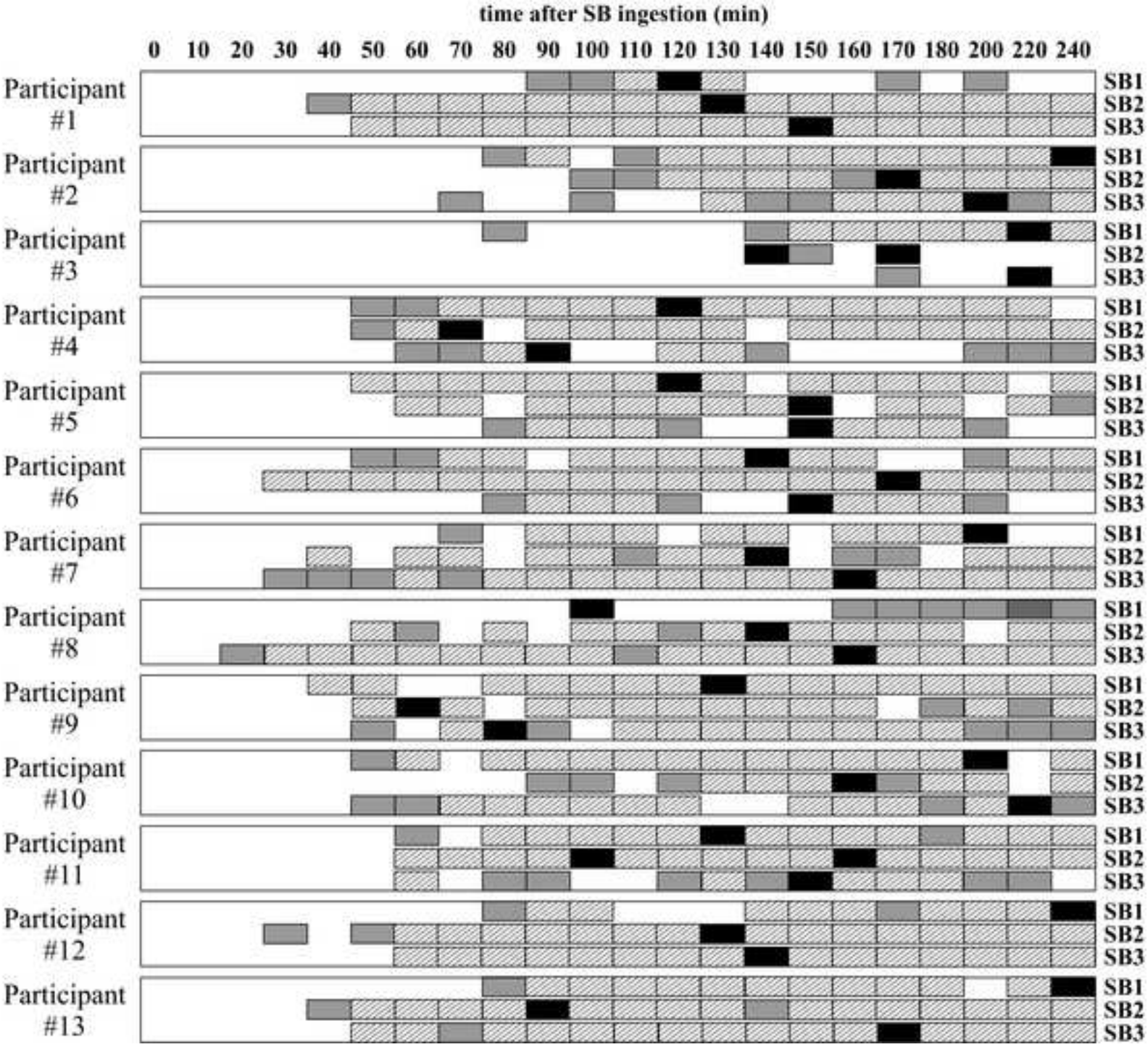

Figure 3

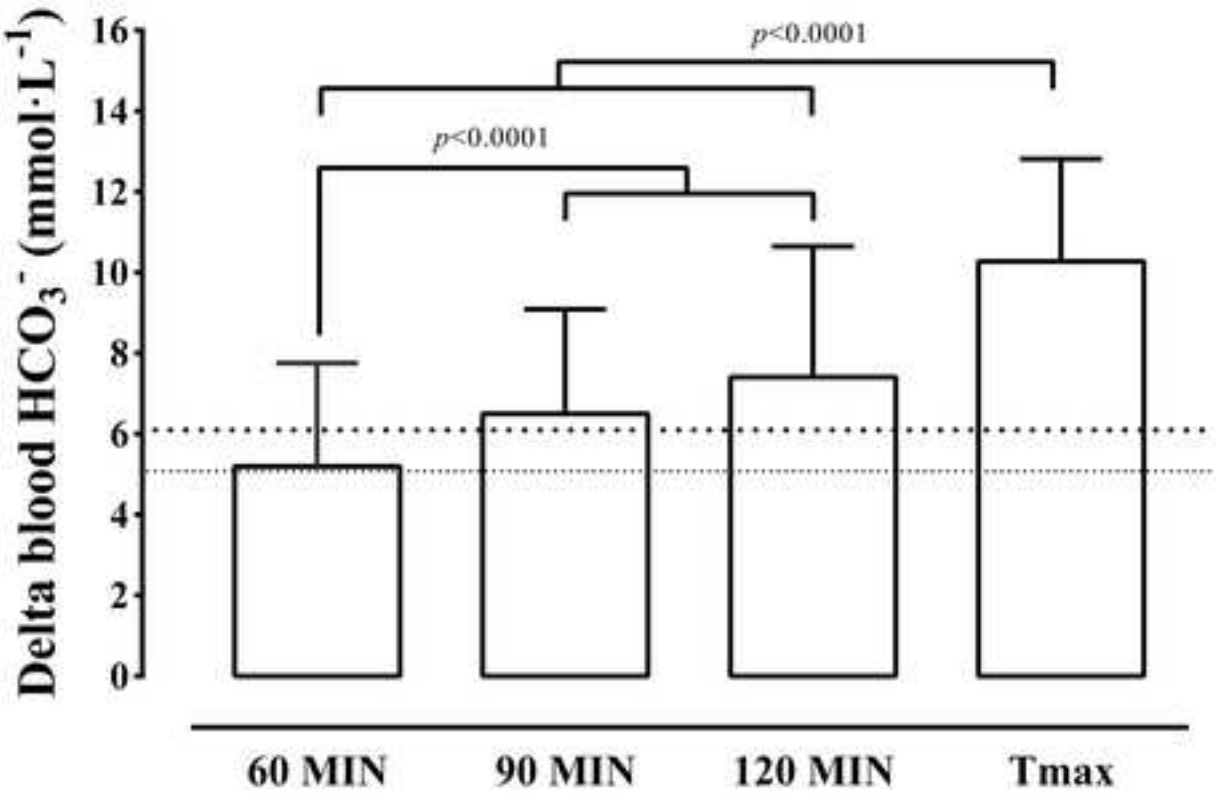

Figure 4

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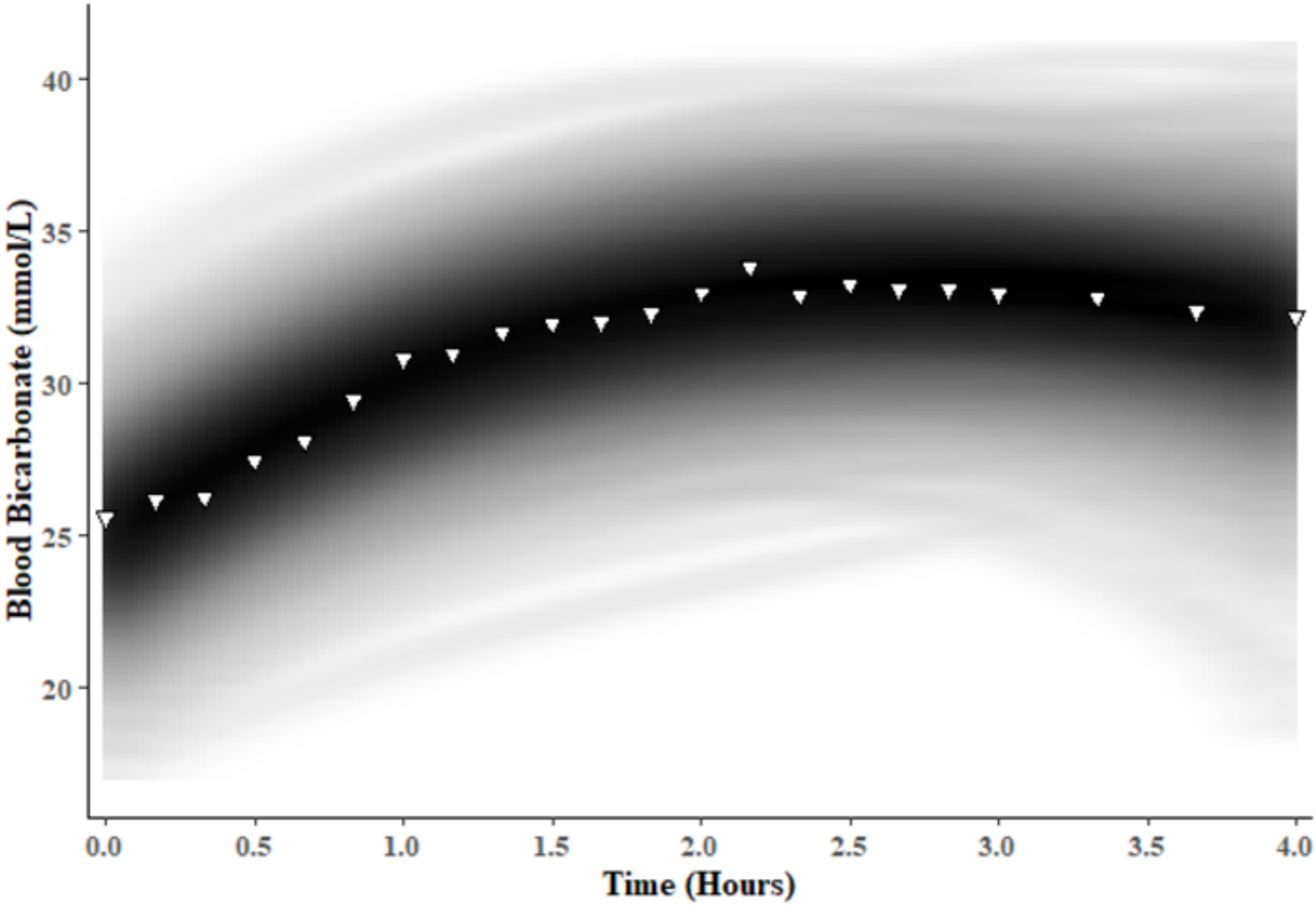
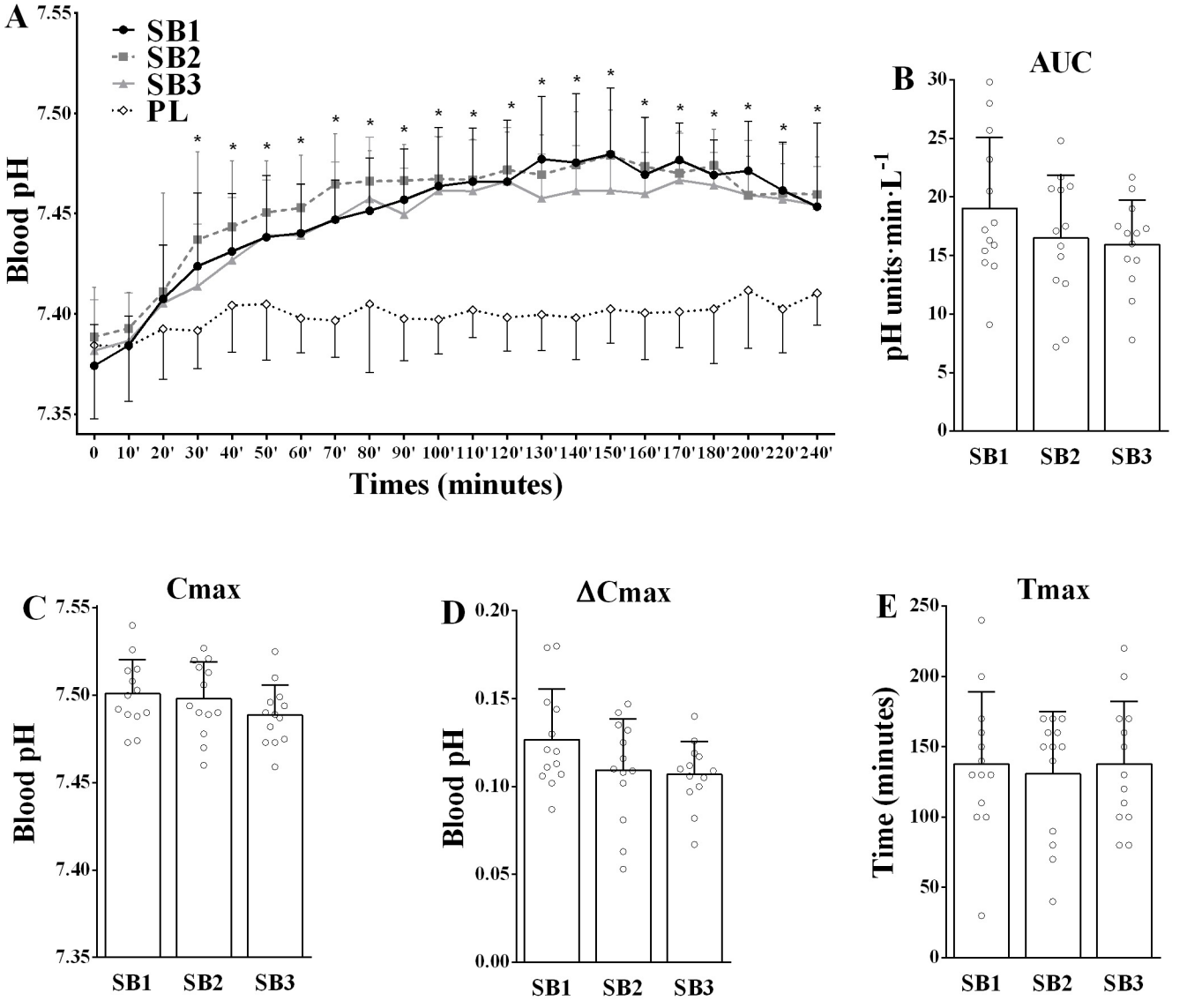
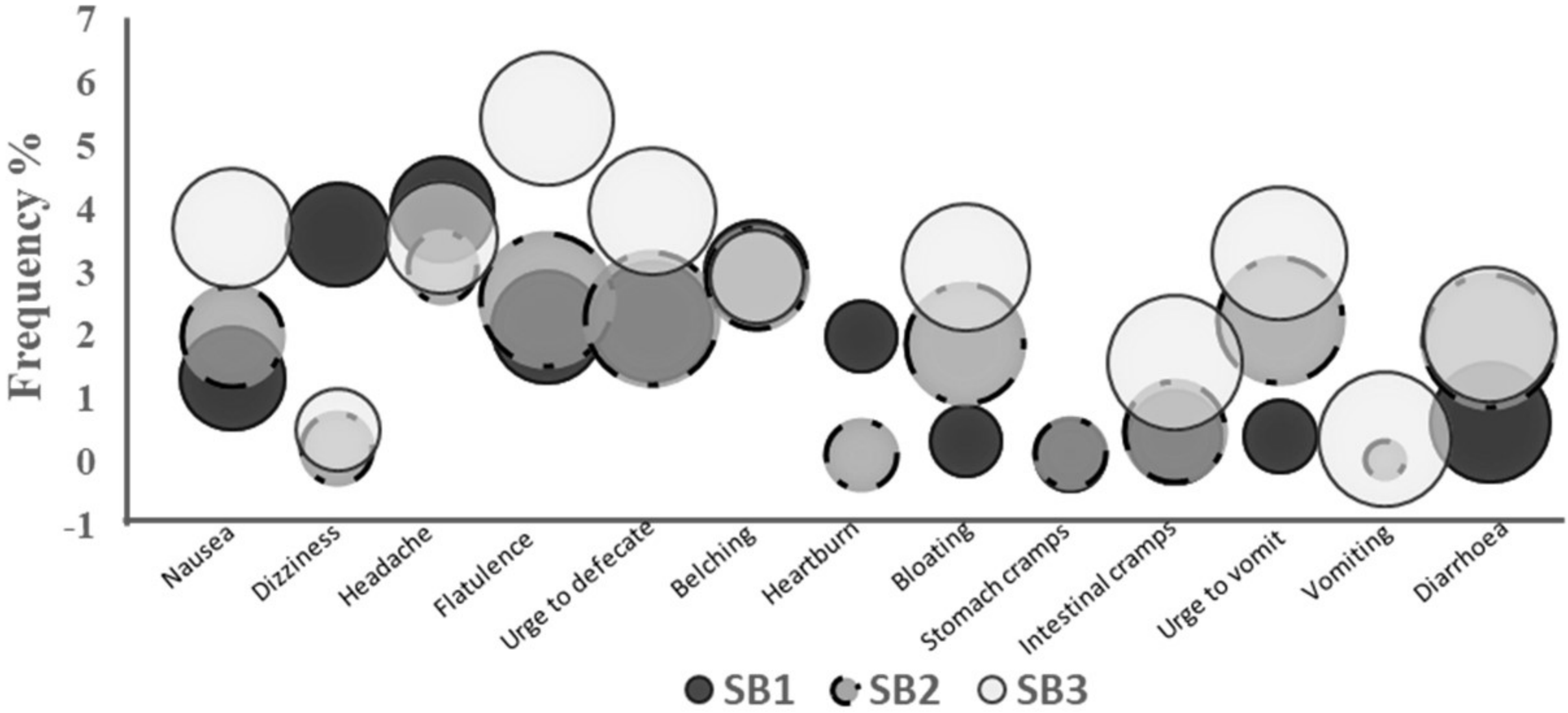



A
SB1
SB2
SB3
PL
Blood pH
7.55
7.50
7.45
7.40
7.35
0 10' 20' 30' 40' 50' 60' 70' 80' 90' 100' 110' 120' 130' 140' 150' 160' 170' 180' 200' 220' 240'
Times (minutes)
B
AUC
pH units·min·L-1
30
25
20
15
10
5
0
SB1 SB2 SB3
C
Cmax
Blood pH
7.55
7.50
7.45
7.40
7.35
SB1 SB2 SB3
D
ΔCmax
Blood pH
0.20
0.15
0.10
0.05
0.00
SB1 SB2 SB3
E
Tmax
Time (minutes)
250
200
150
100
50
0
SB1 SB2 SB3


Frequency %
7
6
5
4
3
2
1
0
-1
Nausea
Dizziness
Headache
Flatulence
Urge to defecate
Belching
Heartburn
Bloating
Stomach cramps
Intestinal cramps
Urge to vomit
Vomiting
Diarrhoea
SB1
SB2
SB3