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Chronic lactate supplementation does not improve blood buffering capacity and repeated high-intensity exercise

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ABSTRACT:

Purpose: Since there is conflicting data on the buffering and ergogenic properties of calcium lactate (CL), we investigated the effect of chronic CL supplementation on blood pH, bicarbonate and high-intensity intermittent exercise performance. Sodium bicarbonate (SB) was used as a positive control. **Methods:** Eighteen athletes participated in this double-blind, placebo-controlled, crossover, fully counterbalanced study. All participants underwent three different treatments: placebo (PL), CL and SB. The dose was identical in all conditions: 500mg·kg^{-1}BM divided into 4 daily individual doses of 125mg·kg^{-1}BM, for five consecutive days, followed by a 2-7 day washout period. On the fifth day of supplementation, individuals undertook four 30-s Wingate bouts for upper-body with 3-min recovery between bouts. Total mechanical work (TMW) for the overall protocol and for the initial (1st+2nd) and final (3rd+4th) bouts was determined at each session. Blood pH, bicarbonate and lactate were determined at rest, immediately and 5 min after exercise. **Results:** CL supplementation did not affect performance (p>0.05 for the overall TMW as well for initial and final bouts), nor did it affect blood bicarbonate and pH prior to exercise. SB supplementation improved performance by 2.9% for overall TMW (p=0.02) and 5.9% in the 3rd+4th bouts (p=0.001). Compared to the control session, SB also promoted higher increases in blood bicarbonate than CL and PL (+0.03±0.04 *vs* +0.009±0.02 and +0.01±0.03, respectively). **Conclusions:** CL supplementation was not capable of enhancing high-intensity intermittent performance or changing extracellular buffering capacity challenging the notion that this dietary supplement is an effective buffering agent.

Keywords: buffering capacity, sodium bicarbonate, lactate, intermittent exercise, upper-body Wingate, chronic supplementation.

INTRODUCTION

During high-intensity exercise, the rate of hydrogen ion (H^+) production inside the skeletal muscle cells exceeds their neutralisation by the intracellular chemical buffers. Some of the H^+ are then exported to the blood via sodium/H^+ and monocarboxilate transporters (MCT) (Juel 1996) where they are neutralised by the blood buffering systems, in particular, blood bicarbonate (Boning et al. 2007). Nonetheless, a rapid decline in both muscle and blood pH is observed during exercise despite the presence of several pH-regulating mechanisms (Costill et al. 1983). Intramuscular H^+ accumulation and the consequential muscle acidosis have long been considered important factors contributing to fatigue (Allen et al. 2008), as they may inhibit key-enzymes of energy metabolism (Sahlin et al. 1975). The H^+ accumulation in muscle may also interfere with the calcium transient (Donaldson et al. 1978) and impair the excitation-contraction coupling process (Fabiato & Fabiato 1978). Supporting this notion, numerous human studies and meta-analyses have shown that increasing either intra- or extracellular buffering capacity via beta-alanine or sodium bicarbonate supplementation, can improve exercise capacity and performance, particularly in exercise where acidosis is limiting to performance (Carr et al. 2011; Lancha Junior et al. 2015; Peart et al. 2012) . Recently, a new nutritional strategy capable of increasing extracellular buffering capacity has gained some attention, namely acute ingestion of lactate (in the forms of polylactate, sodium lactate or calcium lactate) (Morris et al. 2011; Painelli et al. 2014).

Lactate supplementation has been postulated to increase extracellular buffering capacity. Upon ingestion, lactate is absorbed primarily in the jejunum through sodium-coupled intestinal lactate transporters (Heller & Kern 1968) and, after reaching the bloodstream, it is either converted into glucose in the liver (Hostetler et al. 1969) or oxidised in skeletal muscle (Jacobs et al. 2013). Both processes result in a net utilisation of H^+ (Brooks 1986), which could spare blood bicarbonate, thereby increasing extracellular buffering capacity. Indeed, using a 7% polylactate solution (~17.5g), Fahey et al. (1991) showed a 17% increase in blood bicarbonate (~ 2.5 to 3.0 $mmol \cdot l^{-1}$BM) while Van Montfoort et al. (2004) similarly showed a significant

25% increase in blood bicarbonate (~ 6.2 mmol·l^{-1}) with acute sodium lactate supplementation (400 mg·kg^{-1}BM). Similarly, Morris, Shafer (2011) demonstrated that acute calcium lactate supplementation (120 mg·kg^{-1}BM) induced a significant increase of 10% in blood bicarbonate (~ 2.7 mmol·l^{-1}). Thus, it appears that lactate supplementation may increase blood buffering capacity which may improve high-intensity exercise limited by acidosis.

Van Montfoort, Van Dieren (2004) showed a small improvement in exercise tolerance in a continuous run-to-exhaustion protocol lasting ~80 s while Morris, Shafer (2011) showed that acute lactate ingestion improved exercise performance by 17% in a supra-maximal exercise tolerance test following four repeated maximal exercise bouts. In contrast to these findings, an investigation from our group did not show any ergogenic effect of acute lactate ingestion on repeated supra-maximal exercise, although minor increases in blood bicarbonate were shown (Painelli, Silva 2014). Since the exercise protocol used by Painelli, Silva (2014) was highly acidotic and sensitive to detect performance improvements elicited by increased blood buffering capacity (Artioli et al. 2007; Tobias et al. 2013), these results have cast some doubt as to whether lactate is an effective buffering agent. One possible explanation for the lack of a positive effects with lactate could be related to the use of acute supplementation protocols since their alkalinising effects are transient, meaning that blood pH and bicarbonate return to baseline levels shortly after ingestion (Painelli, Silva 2014; Siegler et al. 2010). A similar pattern has also been shown following acute sodium bicarbonate ingestion (Siegler, Midgley 2010). On the other hand, chronic sodium bicarbonate supplementation has been shown to result in prolonged metabolic alkalosis with positive performance effects lasting up to 48 hours after the cessation of supplementation (McNaughton et al. 1999; Tobias, Benatti 2013). Therefore, it is plausible that the ergogenic effects of calcium lactate could become more apparent if a chronic supplementation protocol, eliciting more sustained increases in blood bicarbonate, was employed.

In the present study, we investigated the effects of chronic lactate supplementation on blood bicarbonate, pH and subsequent exercise performance using an exercise protocol designed to induce a pronounced acidosis. In order to attest that our protocol was sensitive enough to

detect the ergogenic effects of a buffering agent, chronic sodium bicarbonate supplementation was used as a positive control. We hypothesised that chronic lactate supplementation could induce a sufficiently large and sustained metabolic alkalosis capable of improving repeated high-intensity exercise performance.

METHODS

Participants

Eighteen rugby (n=11), judo (n=2) and jiu-jitsu (n=5) athletes actively training and competing at university level completed the study (age: 26 ± 5 years; body mass [BM]: 88.8 ± 6.8 kg; height: 1.78 ± 0.07 m; body fat: 18.6 ± 6.2 %). Inclusion criteria were: male athletes aged 18 to 35 years engaged in sports requiring high levels of anaerobic metabolism of the upper limbs; minimum training experience of 2 years; minimum training volume of 6 hours per week. Exclusion criteria included the use of creatine and beta-alanine in the previous 3 and 6 months, respectively, the presence of any musculoskeletal disorder and any previous use of anabolic steroids. Participants were requested to maintain similar levels of physical activity and food intake throughout the duration of the study; compliance with these requests was verbally confirmed. Participants were informed about the risks and discomforts associated with participation and thereafter provided written consent. The study was approved by the Institutional Ethics Committee (29181114.0.0000.5391).

Study Design

A double-blind, placebo-controlled, crossover, counterbalanced study was conducted. Participants visited the laboratory on six separate occasions, separated by 2-7 days, to undertake 4 bouts of the upper-body Wingate anaerobic test. The first and second visits were performed to familiarise the participants with the protocol, while the third session was undertaken following no treatment (control). The remaining three sessions were undertaken following the acute ingestion of calcium lactate, sodium bicarbonate or placebo. The order of the treatments was

chosen at random in a fully counterbalanced manner. To further confirm that the order of the tests did not influence performance, we compared overall total mechanical work (TMW) obtained in the experimental sessions between the three visits (see more details below). As expected, no significant differences were found (visit 1: 33462 ± 5122 J; visit 2: 33813 ± 5371 J; visit 3: 33436 ± 4928 J; F = 0.70, p = 0.50). All tests were performed during the same period of the day to account for circadian variation (Atkinson & Reilly 1996).

The volunteers were instructed to arrive at the laboratory in a well fed and hydrated state, without having ingested any food in the 2 h preceding the tests. In order to minimise the influence of diet on performance, athletes were requested to maintain the same diet prior to all trials and this was confirmed by the analysis of all individual's 72 h food recall prior to each test. The participants were also informed to refrain from strenuous exercise and caffeine in the 24 hours preceding the experimental sessions. Compliance with these requests was verbally confirmed before each trial. Body fat was estimated by hydrostatic weighing measuring body volume density and calculating perceptual body fat using the equation proposed by Siri (1961).

Participants performed four bouts of the Wingate upper-body anaerobic test during every session. Blood samples were collected at rest (baseline), immediately after and 5 min after the fourth bout of the Wingate test. The efficacy of the blind procedure was verified during all trials; immediately after exercise, participants were asked to report which treatment they believed they had received, and to describe all perceived side effects.

High-Intensity Intermittent Performance

High-intensity intermittent exercise performance was assessed using 4 bouts of the upper-body Wingate Anaerobic Test, a protocol that has been previously used to assess the effects of metabolic induced alkalosis on performance in athletes (Artioli, Gualano 2007; Tobias, Benatti 2013). Athletes warmed up by performing arm-cranking with no resistance for 3 minutes, followed by 1 min rest prior to the first bout. Each bout of the Wingate Test began from a static start and lasted 30 seconds; the athletes were required to perform all-out arm-cranking at maximal velocity throughout the entire 30 seconds against a fixed load equivalent to

4% BM. The volunteers were verbally encouraged during the exercise. The four bouts were interspersed by 3-minute periods of active recovery, with no load, at a self-selected cadence. The active recovery was chosen in order to avoid post-exertion vasovagal response between the Wingate bouts; previous studies using similar exercise protocols have shown no performance effects of active recovery (Franchini et al. 2003; Ouergui et al. 2014). The tests were performed on a mechanically-braked upper body ergometer; wheel velocity was measured by a set of 24 sensors and power output was calculated automatically every second by computer software (Ergometric 6.0, Cefise, Brazil). TMW was obtained and calculated for the overall test session. In order to evaluate whether our intervention was more effective during the initial or final bouts of the exercise test, TMW was also calculated for the initial (i.e., $1^{st} + 2^{nd}$) and the final (i.e., $3^{rd} + 4^{th}$) bouts. Performance decrement was measured as the percentage loss in mechanical work from the 1^{st} to the 4^{th} bout. The coefficient of variation for TMW obtained in the testing sessions was 2.62 ± 3.12%.

Supplementation protocol

Participants underwent 5 days of chronic supplementation of either 500 $mg \cdot kg^{-1} \cdot d^{-1}$BM of calcium lactate (PhD Innovation Expertise, Sao Paulo, Brazil), sodium bicarbonate (LabSynth, Sao Paulo, Brazil) or calcium carbonate (placebo; LabSynth, Sao Paulo, Brazil). The total daily dose was divided into 4 individual doses of 125 $mg \cdot kg^{-1}$ BM and all supplements were given in gelatin capsules identical in number, size and appearance. Participants were required to ingest their last dose within 4 hours before the performance assessment. In order to control adherence to the protocol, each individual dose was provided in a separate plastic bag identified with an adhesive tape. A supplementation log was given to the participants and they were asked to paste every label on the log sheet immediately after ingestion. All participants received 5% more individual doses than necessary without being informed, so adherence to supplementation could be further confirmed by the leftover capsules.

Blood sampling and lactate analysis

Venous blood samples (1 mL) were collected at baseline, immediately after and 5 minutes after the last Wingate bout for the determination of blood pH, bicarbonate, base excess and plasma lactate. Samples were taken from the antecubital vein using a heparinised syringe (BD A-Line Ca^{2+} LH ~30 I.U.) and immediately injected into an automatised blood gas analyser (Rapid Point 350®, Siemens, Germany) for pH and PCO_2 determination. Blood bicarbonate and base excess were calculated according to the Henderson–Hasselbalch equation. For plasma lactate analysis, a small aliquot (20 µL) of the sample was placed in a microtube containing the same volume of an ice-cold 2% NaF solution and homogenised. The samples were then centrifuged at 2000 g for 5 min at 4^{o} C to separate plasma from erythrocytes. Plasma was removed and stored at -80^{o}C until analysis. Plasma lactate was determined spectrophotometrically using an enzymatic-colorimetric method as supplied by a commercially available kit (Katal, Interteck, Sao Paulo, Brazil).

Food intake assessment

To control for intervening variables, food intake was assessed during the supplementation week of each experimental condition by means of three 24-h dietary recalls undertaken on separate days (1 weekend day and 2 consecutive weekdays preceding every test day), with the aid of a visual photo album of real-sized foods and portions. The 24-h dietary recall consisted of listing the foods and beverages consumed during the 24-h before the assessment. Nutritional supplements were also recorded. Energy and macronutrient intake were analysed with Virtual Nutri software (Sao Paulo, Brazil).

Statistical Analysis

Data are presented as mean ± standard deviation. Mixed models (proc mixed, SAS 9.3) followed by single degree of freedom contrast analysis were used to examine changes in blood variables (plasma lactate, blood pH, bicarbonate and base excess), with 'treatment' and 'time' as fixed factors and 'participants' as random factors. Absolute and relative ΔTMW were calculated by subtracting control values from those obtained in each trial (i.e. calcium lactate, sodium

bicarbonate and placebo). ΔTMW was compared between treatments using repeated measures ANOVA ('treatment' as a fixed factor) followed by Tukey's post-hoc test. Repeated measures ANOVA followed by Tukey's post-hoc test were also used to compare the relative performance decrement as well as food intake data between the experimental conditions. This same procedure was employed to analyse the absolute change in TMW in the 1^{st}+2^{nd} and 3^{rd}+4^{th} bouts. Effect sizes were calculated using Cohen's d. In addition, magnitude-based inference analysis was conducted on TMW based upon the recommendations of Batterham and Hopkins (2006) to detect small effects of practical relevance. The Fischer Exact Test was used for the rate of participants who correctly guessed their allocation in the trials. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

High-Intensity Intermittent Performance

There was a main effect of 'treatment' on TMW (F = 3.40; p = 0.02) with post hoc test indicating a significant difference between sodium bicarbonate and placebo (p = 0.02; 95% CI = 61 – 2043 J). ANOVA showed that sodium bicarbonate promoted a significantly higher absolute change in TMW versus control (ΔTMW; Figure 1, Panel A) than calcium lactate (p = 0.03; 95% CI = -67 – 1827 J; ES = 0.74) and placebo (p = 0.01; 95% CI = 106 – 1999 J; ES = 0.99) whereas calcium lactate was not different from placebo (p = 0.75; 95% CI = -774 – 1119 J; ES = 0.1). There was no main effect of 'treatment' on TMW in the 1^{st} + 2^{nd} bouts (F = 0.99; p = 0.38) (Figure 1, Panel B). However, a significant main effect of 'treatment' was shown in the 3^{rd} + 4^{th} bouts (F = 7.61; p = 0.001) with sodium bicarbonate being superior to calcium lactate (p < 0.01; 95% CI = 188 – 1194 J; ES = 0.84) and placebo (p < 0.01; 95% CI = 192 – 1198 J; ES = 0.88). On the other hand, calcium lactate was not different from placebo (p > 0.01; 95% CI = -499 - 507 J; ES = 0.05) (Figure 1, Panel C). ANOVA also showed a main effect of 'treatment' on relative performance decrement (F = 4.17; p = 0.01), with sodium bicarbonate promoting a significantly greater attenuation of fatigue (-33.87 ± 9.01%) than calcium lactate (-38.66 ±

8.72%; p = 0.02; 95% CI = 0.15 - 8.61%) and placebo (-36.15 ± 8.93%; p = 0.05; 95% CI = -1.95 - 6.50%).

Individual data analysis showed that 4 out of 18 participants improved TMW above the coefficient of variation with calcium lactate during the initial bouts (1^{st}+2^{nd}), while only 3 and 2 improved with sodium bicarbonate and placebo, respectively (Figure 2, Panel A). However, 13 out of 18 improved TMW with sodium bicarbonate above the coefficient of variation during the final bouts (3^{rd}+4^{th}), while only 8 improved with calcium lactate and placebo (Figure 2, Panel B). Furthermore, compared to control, magnitude-based inference analysis showed that sodium bicarbonate had a positive and possibly beneficial effect on TMW, while both calcium lactate and placebo only had trivial and unclear effects on performance (Table 1). When taking into account only the final bouts, both calcium lactate and placebo remained with a trivial and unclear effect on performance, while sodium bicarbonate had a positive and very likely beneficial effect on TMW.

Blood Measures

Blood variables are presented in Figure 3. The Mixed Model analysis showed a significant main effect of 'time' for blood pH (F = 713.88; p < 0.0001), bicarbonate (F = 1157.73; p < 0.0001) and base excess (F = 1113.64; p < 0.0001), indicating that these variables significantly decreased from baseline to immediately post-exercise and 5 minutes post-exercise. Similarly, a significant main effect of 'time' for plasma lactate (F = 1210.45; p < 0.0001) was shown, indicating an increase from baseline to immediately post-exercise and 5 minutes post-exercise.

There was a trend towards an effect of 'treatment' on blood bicarbonate (F = 2.60; p = 0.06). No main effect of 'treatment' was found for blood pH (F = 0.46; p = 0.71), base excess (F = 1.61; p = 0.19) and plasma lactate (F = 1.72; p = 0.17). However, the ANOVA showed that, at baseline, the absolute change in blood bicarbonate and base excess were significantly greater after sodium bicarbonate supplementation compared to calcium lactate or placebo (p = 0.0015

and p = 0.0013 for blood bicarbonate, respectively; p = 0.0018 and p = 0.0039 for base excess. See Table 2). No other significant differences in blood variables were shown (Table 2).

Food Consumption Analysis

Energy intake (sodium bicarbonate: 2006 ± 556 kcal; calcium lactate: 1932 ± 451 kcal; placebo: 1951 ± 602 kcal; $p = 0.93$), carbohydrate (sodium bicarbonate: 46.6% ± 8.9%; calcium lactate: 46.6% ± 10.8%; placebo: 51.3% ± 8.8%; $p = 0.39$), lipid (sodium bicarbonate: 30.7% ± 6.2%; calcium lactate: 29.8% ± 7.0%; placebo: 27.2% ± 6.6%; $p = 0.30$), and protein (sodium bicarbonate: 22.7% ± 6.7%; calcium lactate, 21.5% ± 6.8%; placebo: 21.4% ± 5.3%; $p = 0.83$) did not significantly differ between the experimental conditions.

Blinding Efficacy and Side Effects

There was no apparent effect of correct supplement identification or self-reported side-effects on TMW during either the initial 1^{st}+2^{nd} or final 3^{rd}+4^{th} bouts (Supplementary Figure 1). Eight out of 18 participants were able to correctly guess their supplement during the second trial, whereas 10 out of 18 correctly guessed their supplement in the first and third trials. There were no significant differences in the correct guessing rate between the trials (Fisher Exact Test: $p = 0.83$). Two, 6 and 3 individuals who correctly guessed the supplement during calcium lactate, sodium bicarbonate and placebo improved during the 1^{st}+2^{nd} bouts, while 5, 7 and 5 who correctly identified the ingested supplement did not improve TMW during these initial bouts. Six, 3 and 6 individuals who incorrectly guessed the supplement during calcium lactate, sodium bicarbonate and placebo improved during the 3^{rd}+4^{th} bouts; 6, 1 and 4 individuals who incorrectly identified the ingested supplement did not improve during the final bouts. There were only 8 reports of side effects with calcium lactate (4 of these improved TMW during the initial bouts; 5 improved TMW during the final bouts), 8 with placebo (4 of these improved TMW during the initial bouts; 5 improved TMW during the final bouts) and 13 with sodium bicarbonate (6 of these improved TMW during the initial bouts; 11 improved TMW during the

final bouts). Among the side effects, diarrhoea was the most frequent with 11 reports, followed by eructation and stomach ache both with 6 reports.

DISCUSSION

To our knowledge, this is the first study investigating the effects of chronic (five days) calcium lactate supplementation on high-intensity intermittent performance as well as blood pH and bicarbonate. Since previous studies have shown that acute lactate supplementation induced a significant increase in extracellular buffering capacity (Morris, Shafer 2011; Painelli, Silva 2014; Van Montfoort, Van Dieren 2004), we hypothesized that our chronic strategy would produce greater increases in blood variables, and hence, in exercise performance.

In contrast to our initial hypothesis, we did not show any effect of lactate supplementation either on blood pH or blood bicarbonate. These results are somewhat surprising since our chronic protocol employed high calcium lactate doses (500 $mg \cdot kg^{-1} \cdot d^{-1}$BM). However, because our positive control (i.e., sodium bicarbonate) did result in a significant increase in blood bicarbonate, we can rule out any explanation related to methodological errors. In agreement with our data, other studies have also shown little or no effect of lactate ingestion (Morris, Shafer 2011; Van Montfoort, Van Dieren 2004) or infusion (Miller et al. 2005) on blood pH. On the other hand, studies have been more consistent in showing that lactate ingestion (Morris, Shafer 2011; Painelli, Silva 2014; Van Montfoort, Van Dieren 2004) or infusion (Miller, Lindinger 2005) increases blood bicarbonate. Although differences in the type of lactate salt ingested (calcium vs. sodium) may play a role on its alkalinizing effects [Na^+ can increase strong ion difference thus having a greater impact on blood acid-base status (Miller, Lindinger 2005)], the lack of effect of lactate on blood bicarbonate in our study could not be entirely explained by the use of calcium instead of sodium lactate. In fact, calcium lactate has been shown to increase blood bicarbonate by ~3 mM (Morris, Shafer 2011), suggesting that other factors unrelated to the calcium form may explain our results. It is possible that the chronic protocol may have played some role as the alkalinizing effects of lactate might be more

transient than sodium bicarbonate. Although this might be related to the fast lactate removal from blood (Miller, Lindinger 2005), this explanation is still speculative and needs further examination.

The lack of changes in high-intensity intermittent performance in this study is likely a reflection of the absence of changes in blood bicarbonate. Increases in circulating bicarbonate resulted in a 1.7% increase in exercise capacity during a running-to-exhaustion lasting ~80s (Van Montfoort, Van Dieren 2004) while Morris, Shafer (2011) demonstrated a 17% improvement in exercise capacity during a cycling-to-exhaustion test performed immediately after 4 x 1-min bouts at 100% of maximum power output. Contrarily, Painelli et al. (2014) showed no changes in exercise performance during an upper-body repeated-bout Wingate test. Using an exercise protocol very similar to that used by Painelli, Silva (2014), the current study did not show any changes in total work or performance decrement with chronic lactate supplementation. These discrepancies on performance outcomes may also be related to the differences in exercise protocols. In this sense, time-to-exhaustion protocols usually do not have either good external validity or good reliability (Currell & Jeukendrup 2008). On the other hand, the Wingate Test is known to have good reliability (Bar-Or 1987) and exhibits heavy reliance on glycolytic metabolism (Lovell et al. 2013). Moreover, similar protocols using multiple bouts of the Wingate Test have been associated with performance and success in a variety of sports modalities (Franchini et al. 2011). The substantial fall in blood pH and bicarbonate observed after the exercise protocol in the current investigation highlights its intense and acidotic nature. As a matter of fact, previous studies have shown that our exercise protocol is sensitive to detect the ergogenic effects of buffering agents, such as beta-alanine and sodium bicarbonate (Artioli, Gualano 2007; Tobias, Benatti 2013), which was further confirmed in this study by the positive effects of sodium bicarbonate on performance. This suggests that lactate supplementation is not an effective buffering agent, at least if taken in its calcium form following a chronic protocol.

In contrast to lactate, chronic sodium bicarbonate supplementation has been widely studied and employed as an extracellular buffer (Carr, Hopkins 2011). Some of the most consistently demonstrated effects of sodium bicarbonate include increased blood pH and

bicarbonate concentration. Since H^+ removal from muscle cells is driven, among other factors, by an electrochemical gradient (Juel 2008), it has been suggested that these changes in blood acid-base status promote greater efflux of H^+ and lactate from the working muscles (Raymer et al. 2004). Consequently, there will be a reduced interference of H^+ with the contractile and energy production processes inside muscle cells (Fitts 1994; Sahlin, Harris 1975), thereby delaying fatigue onset. At baseline, chronic sodium bicarbonate supplementation successfully increased blood bicarbonate and base excess, which may have attenuated the decline in intramuscular pH during exercise. Such changes induced a significant improvement of 2.9% in TMW, which was above the calculated coefficient of variation for the test. The efficacy of sodium bicarbonate in the current study became even clearer in the final bouts, where one would expect a higher degree of muscle acidosis, and hence, a greater opportunity of action for a buffering agent. Therefore, our results agree with previous reports showing that chronic bicarbonate supplementation is an effective strategy to enhance extracellular buffering capacity (Mc Naughton & Thompson 2001; McNaughton, Backx 1999), which contrasts with the inability of a similar protocol using calcium lactate to induce the same effects.

A recent study has suggested that a minimum increase of 5-6 $mmol \cdot L^{-1}$ in blood bicarbonate is necessary for a potential ergogenic effect, while increases in excess of 6 $mmol \cdot L^{-1}$ will almost certainly result in an ergogenic effect (Carr, Hopkins 2011). In the present study we employed a chronic supplementation protocol for both sodium bicarbonate and calcium lactate with the participants being required to ingest the last dose 4 h prior to attending to the laboratory. This period was intentionally chosen so any effect would be due to chronic supplementation rather than acute ingestion of the supplements. The mean absolute increase in blood bicarbonate promoted by sodium bicarbonate and calcium lactate were 2.5 ± 2.0 and -0.1 ± 2.3 $mmol \cdot L^{-1}$, respectively. To our surprise, no effects of sodium bicarbonate on blood pH were observed, which contrasts with previous findings (McNaughton, Backx 1999). Despite the smaller increase in blood bicarbonate compared to the literature and the lack of effect on pH, only one participant did not respond to sodium bicarbonate supplementation (who also did not report a 100% adherence to the supplementation protocol), suggesting that increased blood pH

is not a prerequisite for an ergogenic effect with sodium bicarbonate provided that there is an increase in blood bicarbonate. In fact, other investigations have already shown performance improvements following increased blood bicarbonate despite no changes in blood pH (Morris, Shafer 2011).

Supplementation with the substances used in this study may cause side effects, especially gastrointestinal discomfort (Carr, Hopkins 2011; Peart, Siegler 2012). This may potentially interfere with the double-blind design and affect the ergogenic effects of the supplement. In the current study, the higher incidence of side effects occurred with sodium bicarbonate supplementation, although most of the participants did not complain about the severity of symptoms. However, this could have made it easier for participants to guess the supplement they were taking. The Fisher's exact test, however, showed that our double-blind design was effective. Moreover, individual analysis (Supplementary Figure 1) shows that neither supplement identification nor the occurrence of side effects had any interference with the performance effects.

In conclusion, chronic calcium lactate supplementation was neither able change blood pH and bicarbonate nor presented ergogenic effects on high-intensity intermittent performance.

PERSPECTIVES

The present study shows that chronic calcium lactate is not an effective supplement to improve blood buffering capacity and does not enhance high-intensity exercise performance; our results further confirm that chronic sodium bicarbonate supplementation is effective at both improving blood buffering capacity and exercise performance. In view of the conflicting data in the literature when an acute lactate ingestion protocol is used, the applicability of calcium lactate acutely ingested in a sporting context needs to be further examined. The use of a positive control such as sodium bicarbonate seems to be relevant to more precisely attest or refute its applicability.

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REFERENCES

Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiological reviews*. 2008: **88**: 287-332.

Artioli GG, Gualano B, Coelho DF, Benatti FB, Gailey AW, Lancha AH, Jr. Does sodium-bicarbonate ingestion improve simulated judo performance? *International journal of sport nutrition and exercise metabolism*. 2007: **17**: 206-217.

Atkinson G, Reilly T. Circadian variation in sports performance. *Sports medicine*. 1996: **21**: 292-312.

Bar-Or O. The Wingate anaerobic test. An update on methodology, reliability and validity. *Sports medicine*. 1987: **4**: 381-394.

Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. *International journal of sports physiology and performance*. 2006: **1**: 50-57.

Boning D, Klarholz C, Himmelsbach B, Hutler M, Maassen N. Extracellular bicarbonate and non-bicarbonate buffering against lactic acid during and after exercise. *European journal of applied physiology*. 2007: **100**: 457-467.

Brooks GA. The lactate shuttle during exercise and recovery. *Medicine and science in sports and exercise*. 1986: **18**: 360-368.

Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports medicine*. 2011: **41**: 801-814.

Costill DL, Barnett A, Sharp R, Fink WJ, Katz A. Leg muscle pH following sprint running. *Medicine and science in sports and exercise*. 1983: **15**: 325-329.

Currell K, Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. *Sports medicine*. 2008: **38**: 297-316.

Donaldson SK, Hermansen L, Bolles L. Differential, direct effects of H+ on Ca2+ -activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits. *Pflugers Archiv : European journal of physiology*. 1978: **376**: 55-65.

Fabiato A, Fabiato F. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *The Journal of physiology*. 1978: **276**: 233-255.

Fahey TD, Larsen JD, Brooks GA, Colvin W, Henderson S, Lary D. The effects of ingesting polylactate or glucose polymer drinks during prolonged exercise. *International journal of sport nutrition*. 1991: **1**: 249-256.

Fitts RH. Cellular mechanisms of muscle fatigue. *Physiological reviews*. 1994: **74**: 49-94.

Franchini E, Del Vecchio FB, Matsushigue KA, Artioli GG. Physiological profiles of elite judo athletes. *Sports medicine*. 2011: **41**: 147-166.

Franchini E, Yuri Takito M, Yuzo Nakamura F, Ayumi Matsushigue K, Peduti Dal'Molin Kiss MA. Effects of recovery type after a judo combat on blood lactate removal and on performance in an intermittent anaerobic task. *The Journal of sports medicine and physical fitness*. 2003: **43**: 424-431.

Heller MD, Kern F, Jr. Absorption of lactic acid from an isolated intestinal segment in the intact rat. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine*. 1968: **127**: 1103-1106.

Hostetler KY, Williams HR, Shreeve WW, Landau BR. Conversion of specifically 14 C-labeled lactate and pyruvate to glucose in man. *The Journal of biological chemistry*. 1969: **244**: 2075-2077.

Jacobs RA, Meinild AK, Nordsborg NB, Lundby C. Lactate oxidation in human skeletal muscle mitochondria. *Am J Physiol Endocrinol Metab*. 2013: **304**: E686-694.

Juel C. Lactate/proton co-transport in skeletal muscle: regulation and importance for pH homeostasis. *Acta physiologica Scandinavica*. 1996: **156**: 369-374.

Juel C. Regulation of pH in human skeletal muscle: adaptations to physical activity. *Acta physiologica*. 2008: **193**: 17-24.

Lancha Junior AH, Painelli VP, Saunders B, Artioli GG. Nutritional Strategies to Modulate Intracellular and Extracellular Buffering Capacity During High-Intensity Exercise. *Sports medicine*. 2015: **45 Suppl 1**: 71-81.

Lovell D, Kerr A, Wiegand A, Solomon C, Harvey L, McLellan C. The contribution of energy systems during the upper body Wingate anaerobic test. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. 2013: **38**: 216-219.

Mc Naughton L, Thompson D. Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output. *The Journal of sports medicine and physical fitness*. 2001: **41**: 456-462.

McNaughton L, Backx K, Palmer G, Strange N. Effects of chronic bicarbonate ingestion on the performance of high-intensity work. *European journal of applied physiology and occupational physiology*. 1999: **80**: 333-336.

Miller BF, Lindinger MI, Fattor JA, Jacobs KA, Leblanc PJ, Duong M, Heigenhauser GJ, Brooks GA. Hematological and acid-base changes in men during prolonged exercise with and without sodium-lactate infusion. *Journal of applied physiology*. 2005: **98**: 856-865.

Morris DM, Shafer RS, Fairbrother KR, Woodall MW. Effects of lactate consumption on blood bicarbonate levels and performance during high-intensity exercise. *International journal of sport nutrition and exercise metabolism*. 2011: **21**: 311-317.

Ouergui I, Hammouda O, Chtourou H, Gmada N, Franchini E. Effects of recovery type after a kickboxing match on blood lactate and performance in anaerobic tests. *Asian J Sports Med*. 2014: **5**: 99-107.

Painelli VS, Saunders B, Sale C, Harris RC, Solis MY, Roschel H, Gualano B, Artioli GG, Lancha AH, Jr. Influence of training status on high-intensity intermittent performance in response to beta-alanine supplementation. *Amino acids*. 2014: **46**: 1207-1215.

Painelli VS, Silva RP, de Oliveira OM, Jr., de Oliveira LF, Benatti FB, Rabelo T, Guilherme JP, Lancha AH, Jr., Artioli GG. The effects of two different doses of calcium lactate on blood pH, bicarbonate, and repeated high-intensity exercise performance. *International journal of sport nutrition and exercise metabolism*. 2014: **24**: 286-295.

Peart DJ, Siegler JC, Vince RV. Practical recommendations for coaches and athletes: a meta-analysis of sodium bicarbonate use for athletic performance. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2012: **26**: 1975-1983.

Raymer GH, Marsh GD, Kowalchuk JM, Thompson RT. Metabolic effects of induced alkalosis during progressive forearm exercise to fatigue. *Journal of applied physiology*. 2004: **96**: 2050-2056.

Sahlin K, Harris RC, Hultman E. Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. *The Biochemical journal*. 1975: **152**: 173-180.

Siegler JC, Midgley AW, Polman RC, Lever R. Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. *Journal of strength and conditioning research / National Strength & Conditioning Association*. 2010: **24**: 2551-2557.

Siri WE. Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A, eds. *Techiques for measuring body composition*. Washington: National Academy os Science, 1961:223-244.

Tobias G, Benatti FB, Painelli VS, Roschel H, Gualano B, Sale C, Harris RC, Lancha AH, Jr., Artioli GG. Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. *Amino acids*. 2013: **45**: 309-317.

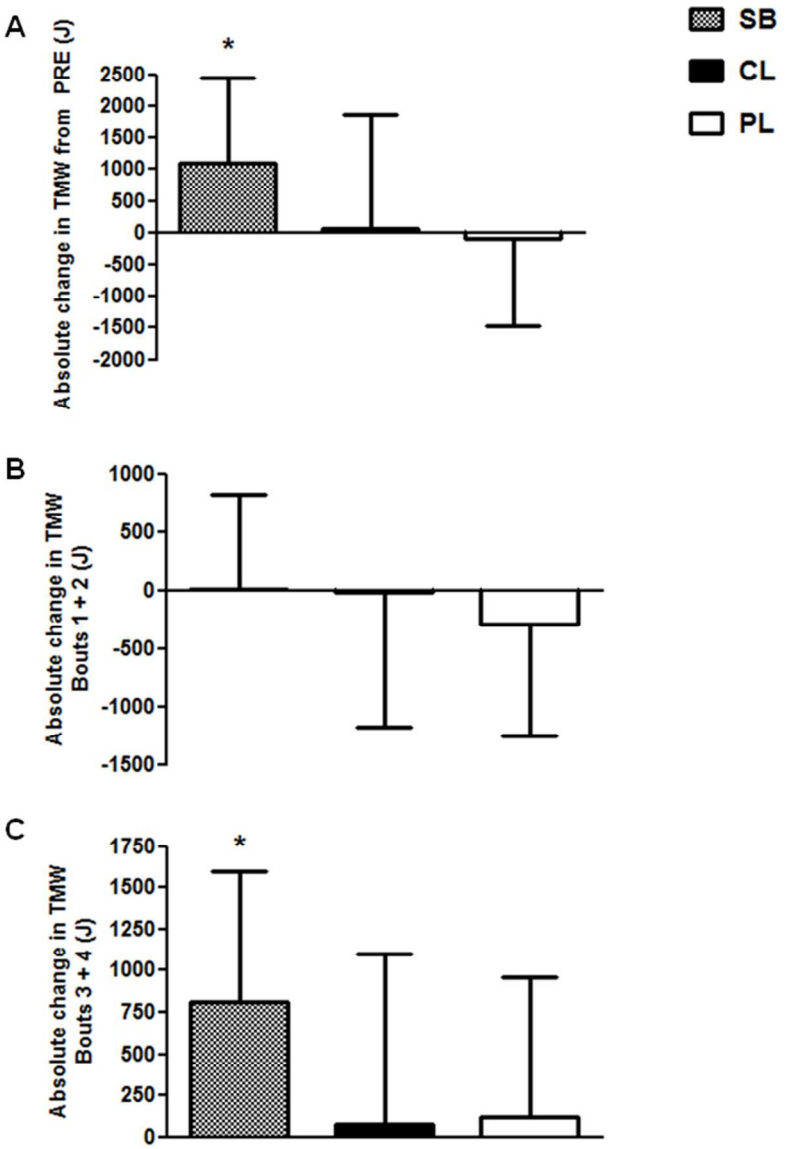
Van Montfoort MC, Van Dieren L, Hopkins WG, Shearman JP. Effects of ingestion of bicarbonate, citrate, lactate, and chloride on sprint running. *Medicine and science in sports and exercise*. 2004: **36**: 1239-1243.

LEGENDS FIGURES

Figure 1. Total work. Panel A: Absolute change in total work (ΔTMW) after sodium bicarbonate (SB), calcium lactate (CL) or placebo (PL) supplementation compared to control; Panel B: Absolute change in TMW after SB, CL or PL in the initial bouts (1^{st}+2^{nd}); Panel C: Absolute change in TMW after SB, CL or PL in the final bouts (3^{rd}+4^{th}). Legend: the symbol * refers to a significant difference (at $p < 0.05$) compared to the other experimental conditions.

Figure 2. Individual analysis. Panel A: Individual analysis of the relative change in total work (ΔTMW) during the initial bouts (1^{st}+2^{nd}) compared to the control session; Panel B: Individual analysis of the relative change in TMW during the final bouts (3^{rd}+4^{th}) compared to the control session. The dashed line represents the calculated variation of the exercise test.

Figure 3. Blood analysis. Chronic effects of sodium bicarbonate (SB), calcium lactate (calcium lactate) or placebo (PL) supplementation on blood levels of pH, bicarbonate, base excess and plasma lactate at baseline (BASE), immediately after the Wingate test (POST) and 5 minutes after the Wingate test (5-MIN). Legend: the symbol $^{\#}$ refers to a significant difference (at $p < 0.05$) compared BASE; the symbol $^{\&}$ refers to a significant difference (at $p < 0.05$) compared to POST; the symbol * refers to a significant difference (at $p < 0.05$) compared to the other conditions within the same moment.



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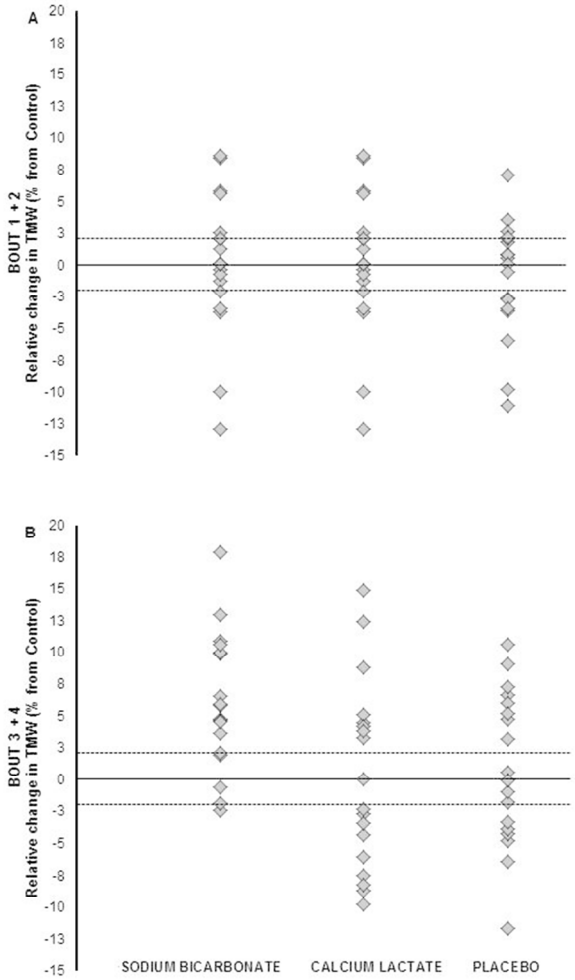

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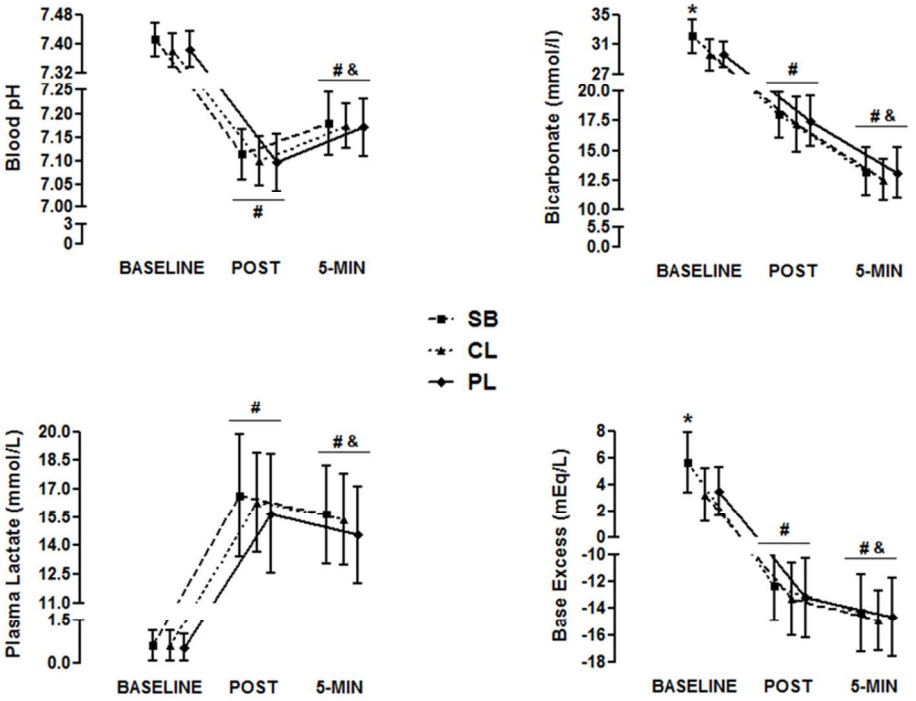

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TABLE 1.[1]

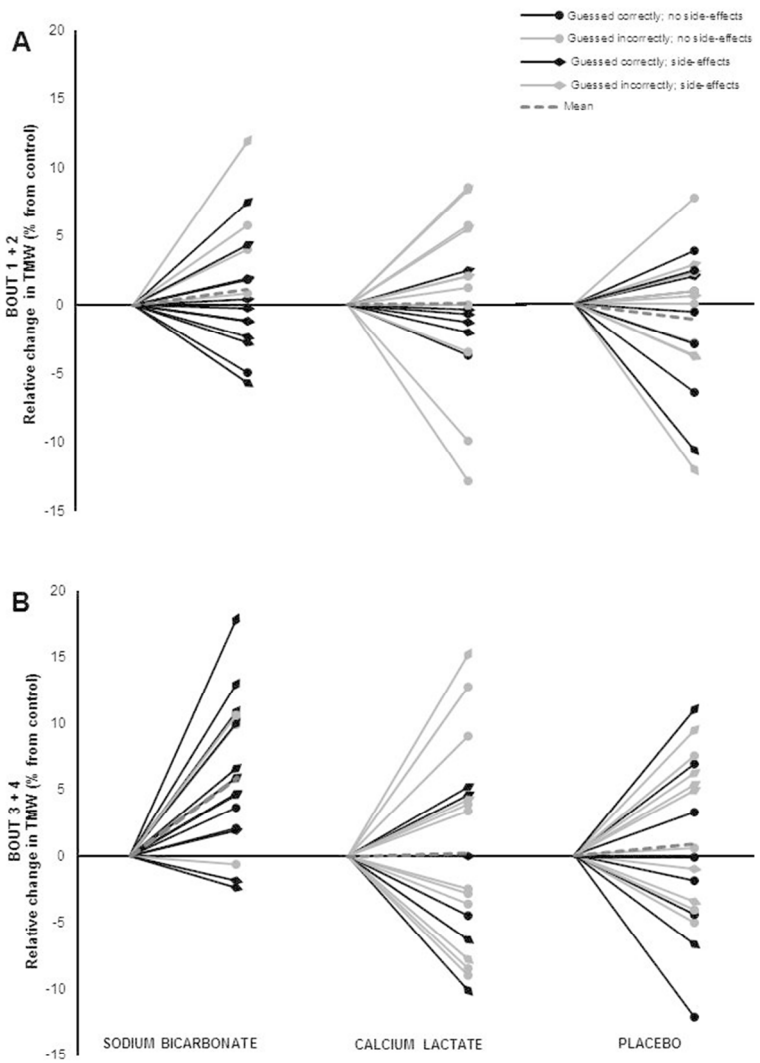
	Difference (%)	Chances of treatment being positive (%)	Chances of treatment being trivial (%)	Chances of treatment being negative (%)
Total Mechanical Work				
SB vs. CON	+ 2.86	64	36	0
CL vs. CON	+ 0.13	0	100	0
PL vs. CON	- 0.02	0	99	1
SB vs. CL	+ 2.16	43	57	0
SB vs. PL	+ 2.64	57	43	0
CL vs. PL	- 0.004	0	100	0
Total Mechanical Work (Bouts 1+2)				
SB vs. CON	+ 0.13	0	99	0
CL vs. CON	+ 0.12	2	95	3
PL vs. CON	- 1.01	0	85	15
SB vs. CL	+ 0.19	1	98	1
SB vs. PL	+ 1.31	17	83	0
CL vs. PL	+ 0.96	15	85	0
Total Mechanical Work (Bouts 3+4)				
SB vs. CON	+ 5.93	96	4	0
CL vs. CON	+ 0.21	1	99	0
PL vs. CON	+ 0.87	6	93	1
SB vs. CL	+ 5.40	83	17	0
SB vs. PL	+ 5.22	86	14	0
CL vs. PL	- 0.20	49	2	49

[1] Table 1. Magnitude-based inferences for total mechanical work across conditions.
Legend: SB = sodium bicarbonate; CL = calcium lactate; PL = placebo; CON = control session.

TABLE 2.[1]

	Baseline	Immediately post-exercise	5 minutes post-exercise
pH			
Sodium Bicarbonate	+ 0.035 ± 0.048	+ 0.033 ± 0.046	+ 0.026 ± 0.065
Calcium Lactate	+ 0.009 ± 0.029	+ 0.006 ± 0.046	+ 0.004 ± 0.053
Placebo	+ 0.019 ± 0.038	+ 0.016 ± 0.044	+ 0.013 ± 0.039
Bicarbonate (mmol·L^{-1})			
Sodium Bicarbonate	+ 2.5 ± 2.0*	+ 1.7 ± 2.1	+ 1.1 ± 1.7
Calcium Lactate	- 0.1 ± 2.3	+ 0.6 ± 2.3	- 0.66 ± 2.4
Placebo	- 0.3 ± 1.7	+ 1.2 ± 1.3	+ 0.25 ± 2.7
Base excess (mEq·L^{-1})			
Sodium Bicarbonate	+ 2.7 ± 2.1*	+ 2.2 ± 2.6	+ 1.5 ± 2.7
Calcium Lactate	+ 0.1 ± 1.6	+ 0.6 ± 2.4	- 0.4 ± 2.9
Placebo	+ 0.1 ± 1.6	+ 1.3 ± 1.9	+ 0.5 ± 2.9
Lactate (mmol·L^{-1})			
Sodium Bicarbonate	+ 0.3 ± 0.5	+ 1.7 ± 3.6	+ 1.9 ± 3.4
Calcium Lactate	+ 0.2 ± 0.5	+ 1.4 ± 3.9	+ 1.2 ± 2.8
Placebo	+ 0.1 ± 0.4	+ 0.8 ± 4.1	+ 0.8 ± 3.6

[1] Table 2. Absolute changes in blood pH, bicarbonate, base excess and lactate from control session across the moments.
Legend: * means a significant ($p < 0.05$) difference from the other conditions at the same moment.



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Supplementary Figure 1

Figure 1. Individual analysis side effects and blinding. Panel A: Side effects, blinding and individual relative change in total work (ΔTMW) compared to control session during the initial bouts (1^{st}+2^{nd}); Panel B: Side effects, blinding and individual relative change in TMW compared to control session during the final bouts (3^{rd}+4^{th}). Legend: Individuals in black lines correctly guessed the ingested supplement; individuals in gray lines incorrectly guessed the ingested supplement; the dashed line refers to the mean relative change in TMW with the treatment; individuals in diamond had side effects; individuals in circle did not report any side effects. Overall, these data suggest no apparent effect of either correctly guessing the allocation or self-reported side effects upon exercise performance.