

# SODIUM BICARBONATE SUPPLEMENTATION IMPROVES PERFORMANCE IN ISOMETRIC FATIGUE PROTOCOL



ORIGINAL ARTICLE  
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A SUPLEMENTAÇÃO DE BICARBONATO DE SÓDIO MELHORA O DESEMPENHO EM PROTOCOLO DE FADIGA ISOMÉTRICO

LA SUPLEMENTACIÓN DE BICARBONATO DE SODIO MEJORA EL DESEMPEÑO EN PROTOCOLO DE FATIGA ISOMÉTRICO

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## ABSTRACT

**Introduction:** Although sodium bicarbonate (NaHCO<sub>3</sub>) supplementation has been shown to decrease fatigue and improve high-intensity exercise performance, the effects on maintenance of isometric contractions are not clear. **Objective:** To investigate the effect of NaHCO<sub>3</sub> on the performance of individuals subjected to a fatigue protocol in an isometric exercise on the isokinetic dynamometer. **Methods:** Participants were 12 men in a randomized, double-blind, crossover, placebo-controlled trial. Sixteen minutes after the intake of 0.3 g/kg of body mass of NaHCO<sub>3</sub> or placebo, the participants performed an isometric fatigue protocol of right knee extension exercises during eight minutes at 70% of maximum voluntary isometric contraction. The fatigue indicator was the time point at which torque was reduced to 50% of the initial value. The length of resistance was assessed by maintaining the task over 50% of the initial torque. Lactate/blood pH concentrations and rate of perceived exertion (RPE) and pain (RPP) indexes were analyzed. The RPE of the session was evaluated 30 minutes after the test. **Results:** Blood pH was higher in pre-protocol and in the fatigue indicator after NaHCO<sub>3</sub> intake, as were the blood lactate concentrations in the fatigue indicator and at the end of the protocol ( $p < 0.001$ ). NaHCO<sub>3</sub> supplementation increased the time to fatigue and lessened the rate of decline of isometric peak torque at the end of the protocol ( $p < 0.001$ ). RPE and RPP were smaller at the end of the protocol in the NaHCO<sub>3</sub> condition, and the RPE of the session was diminished ( $p < 0.001$ ). **Conclusion:** NaHCO<sub>3</sub> supplementation enhances steady isometric contraction performance and reduces the internal load. **Level of Evidence II; Diagnostic studies - Investigation of an examination for diagnosis.**

**Keywords:** Muscle fatigue; Sodium bicarbonate; Alkalosis; Exercise.

## RESUMO

**Introdução:** A suplementação de bicarbonato de sódio (NaHCO<sub>3</sub>) tem demonstrado atenuar a fadiga e melhorar o desempenho do exercício de alta intensidade, mas os efeitos sobre a manutenção de contrações isométricas são pouco claros. **Objetivo:** Investigar o efeito do NaHCO<sub>3</sub> no desempenho de indivíduos submetidos ao protocolo de fadiga em exercício isométrico no dinamômetro isocinético. **Métodos:** Doze homens participaram do estudo randomizado, duplo-cego, cruzado e controlado por placebo. Sessenta minutos após ingestão de 0,3 g/kg de massa corporal de NaHCO<sub>3</sub> ou placebo, os participantes realizaram protocolo isométrico de fadiga dos extensores do joelho direito, com duração de oito minutos, a 70% da contração isométrica voluntária máxima. Foi considerado indicador de fadiga o momento em que o torque aplicado diminuiu para 50% do valor inicial. A duração da resistência foi avaliada com a manutenção da tarefa acima de 50% do torque inicial. As concentrações de lactato e pH do sangue, assim como os índices de percepção subjetiva de esforço (PSE) e dor (PSD) foram analisados. A PSE da sessão foi avaliada 30 minutos após o teste. **Resultados:** O pH sanguíneo foi maior pré-protocolo e no indicador de fadiga após ingestão de NaHCO<sub>3</sub>, assim como as concentrações de lactato sanguíneo no indicador da fadiga e ao final do protocolo ( $p < 0,001$ ). A suplementação de NaHCO<sub>3</sub> aumentou o tempo para atingir a fadiga e atenuou o declínio do pico de torque isométrico no final do protocolo ( $p < 0,001$ ). A PSE e PSD foram menores ao final do protocolo com NaHCO<sub>3</sub> e a PSE da sessão foi atenuada ( $p < 0,001$ ). **Conclusão:** A suplementação de NaHCO<sub>3</sub> melhora o desempenho de contrações isométricas sustentadas e atenua a carga interna. **Nível de Evidência II; Estudos diagnósticos – Investigação de um exame para diagnóstico.**

**Descritores:** Fadiga muscular; Bicarbonato de sódio; Alcalose; Exercício.

## RESUMEN

**Introducción:** La suplementación de bicarbonato de sodio (NaHCO<sub>3</sub>) ha demostrado atenuar la fatiga y mejorar el desempeño del ejercicio de alta intensidad, pero los efectos sobre el mantenimiento de contracciones isométricas son poco claros. **Objetivo:** Investigar el efecto de NaHCO<sub>3</sub> en el desempeño de individuos sometidos al protocolo de fadiga en ejercicio isométrico en el dinamómetro isocinético. **Métodos:** Doce hombres participaron del estudio aleatorizado, doble ciego, cruzado y controlado por placebo. Sesenta minutos después de la ingestión de 0,3 g/kg de masa corporal de NaHCO<sub>3</sub> o placebo, los participantes realizaron protocolo isométrico de fatiga de los extensores de la rodilla derecha

con duración de ocho minutos a 70% de la contracción isométrica voluntaria máxima. Se consideró indicador de fatiga el momento en que el torque aplicado disminuyó para 50% del valor inicial. La duración de la resistencia fue evaluada con el mantenimiento de la tarea por encima del 50% del torque inicial. Fueron analizadas las concentraciones de lactato y pH sanguíneos, así como los índices de percepción subjetiva de esfuerzo (PSE) y dolor (PSD). La PSE de la sesión fue evaluada 30 minutos después del test. Resultados: El pH sanguíneo fue mayor pre-protocolo y en el indicador de fatiga después de la ingestión de NaHCO<sub>3</sub>, así como las concentraciones de lactato sanguíneo en el indicador de la fatiga y al final del protocolo ( $p < 0,001$ ). La suplementación de NaHCO<sub>3</sub> aumentó el tiempo para alcanzar la fatiga y atenuó el declive del pico de torque isométrico al final del protocolo ( $p < 0,001$ ). La PSE y PSD fueron menores al final del protocolo con NaHCO<sub>3</sub> y la PSE de la sesión fue atenuada ( $p < 0,001$ ). Conclusión: La suplementación de NaHCO<sub>3</sub> mejora el rendimiento de las contracciones isométricas sostenidas y atenúa la carga interna. **Nivel de Evidencia II; Estudios diagnósticos - Investigación de un examen para diagnóstico.**

**Descriptor:** Fatiga muscular; Bicarbonato de sodio; Alcalosis; Ejercicio.

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## INTRODUCTION

Muscular fatigue (MF) is a complex, multifactorial phenomenon and, depending upon the task done, with various mechanisms that could impact on the strength reduction magnitude.<sup>1,2</sup> Specifically, in the high-intensity exercises with the predominance of the glycolytic system, the high hydrolysis of ATP results in the increasing of hydrogen ions concentrations ( $[H^+]$ ) followed by a pH decreasing. This condition is named metabolic acidosis and could lead to a series of mechanisms affecting the contraction-relaxation muscle's process and, consequently, could be considered as an important MF indicator.<sup>3,4</sup> Although MF is understood as a decrement in the ability to yield strength and muscular strength, when the task involves supporting a maximum contraction, the performance decrease is parallel to MF increase. Nevertheless, when the exercise asks for submaximal contractions, it is possible that the emergence of MF will not be associated with the effort interruption. As many activities in daily life and many exercise modalities involve submaximal contractions, the arising of MF could not be a limitation to the ability of an individual to perform the exercise, which means that submaximal contractions could be kept after the beginning of MF.<sup>1</sup>

In this sense, the use of alkaline substances has been widely employed in various sports modalities and high-intensity exercise protocols, trying to improve the performance by decreasing MF.<sup>5-9</sup> The sodium bicarbonate (NaHCO<sub>3</sub>) intake increases the blood concentration of sodium bicarbonate, which favors the H<sup>+</sup> and lactate efflux of the muscle cell and, in this way, decelerate the acidification process.<sup>10,11</sup>

A series of studies<sup>5-9</sup> have shown that the intake of NaHCO<sub>3</sub> improve the performance in high-intensity exercises, mainly the ones of intermittent nature, although other researchers have found divergent results.<sup>12-14</sup>

Amongst these factors, the use of ergogenic substances in isometric exercises has been poorly evaluated.<sup>15-17</sup> Maughan et al.,<sup>17</sup> assessing the effects of alkalosis induced by NaHCO<sub>3</sub> intake in the exercise performance in healthy people, found a significantly increase in performance on loads related to 20%, but not to 50 and 80% of the maximal voluntary contraction of knee extensors. Hunter et al.,<sup>16</sup> found a lower reduction of strength during the maximal sustained contraction of 50s after an extended cycling exercise with individuals trained in the modality when NaHCO<sub>3</sub> was used compared to the placebo.

Researches on the use of NaHCO<sub>3</sub> are concentrated in intermittent, dynamic and short duration exercises. The knowledge and the study of the action of this substance in long duration continuous isometric contraction, and of the conditions related to MF in this particular type of exercise, may contribute to the use of strategies that could reduce this phenomenon and, consequently, to improve the performance of the task.<sup>18</sup> Thus, the objective of this study was to investigate the effect of NaHCO<sub>3</sub> intake in the performance of healthy individuals submitted to a fatigue protocol in an isometric exercise in the isokinetic dynamometer.

## METHODS

After approved by the local ethics committee of Sao Judas Tadeu University, (number 786.06) and have signed the Declaration of Helsinki, 12 healthy male volunteers were recruited to participate in the study. All subjects have experience in resistance training (more than six months) and no previous injuries of the lower limbs.

All subjects went to the laboratory three times to be familiar with the procedures to perform an isokinetic evaluation by using a Biodex System 3 (Biodex, Inc., Shirley, NY) and for signing all documents. Briefly, on the first day, the subjects signed consent forms and were submitted to the familiarization process of isometric test. On the second day, the anthropometric data (height, weight and skin folds) were evaluated and a second familiarization process set was performed. On the third day, the subjects were submitted to the last familiarization procedure set. All subjects performed three maximal isometric voluntary contractions (MIVC) on the right knee extensors at 90° during five seconds and had five minutes rest between contractions to determine the maximal torque before the isometric fatigue protocol. After MIVC all subjects had to perform a high-level contraction (70% maximal voluntary contractions) maintained until eight minutes or exhaustion. During test, the volunteers were verbally encouraged to keep the force level for as long as possible, until the force value decreased to 50% of MIVC (fatigue indicator). The endurance time was evaluated (time for which a subject can maintain the requested mechanical task 50% above of MIVC).

After completing the familiarization test, the subjects were assigned in a double-blind, randomized, crossover design to consume 0.3 g/kg body mass of NaHCO<sub>3</sub> or Placebo (calcium carbonate – CaCO<sub>3</sub>). The NaHCO<sub>3</sub> was evenly distributed in gelatin capsules 60 min before exercise according to the previous studies<sup>5</sup> and separated by at least one week of washout.

Height was measured by a Cardiomed (WCS model) stadiometer, with an accuracy of 1/15/220 cm. The measurement was performed with the cursor at an angle of 90° on the scale, with the patient in a standing position with feet together in contact with Stadiometer. Total body mass was measured by a calibrated Filizola electronic scale (Personal Line Model 150) with a 100g scale and a maximum capacity of 150kg. Body mass index (BMI, kg/m<sup>2</sup>) was calculated using the equation  $BMI = \text{weight}/\text{height}^2$ .

To determine blood lactate concentration ( $[La^-]$ ) and pH, blood samples were collected from the earlobe at predetermined points throughout each test. The 25- $\mu$ L samples were immediately evaluated by electrochemical analysis in an automated device (YSI 2300, Yellow Springs, OH) and blood gas analyser (i-STAT) respectively.

Subjective rate of perceived exertion (RPE) and pain (RPP) feelings were reported before, on fatigue point and immediately after fatigue protocol (end effort) using a 0–10 Borg scale and visual analog scale, respectively.<sup>19</sup>

The internal training load for each session was evaluated by multiplication of the exercise session duration in minutes by the training intensity, indicated by the RPE through the scale according to the previous studies.<sup>20</sup> The subjects were told to choose a descriptor and a number from 0 to 10. To guarantee that the RPE mean data obtained refers to the total training, the subjects were asked to answer the following question 20 to 30 minutes after the end of the session: "How was your training today?".

The D'Agostino-Pearson test was applied to the Gaussian distribution analysis. The paired Student's t-test and One-way ANOVA followed by Bonferroni's post hoc test were performed to compare differences in fatigue protocol. Comparison analysis between inactive and active groups was performed by a repeated-measures ANOVA, followed by Bonferroni's post hoc test. Cohen's effect sizes were calculated, and evaluated based on the following criteria proposed by Rhea:<sup>21</sup> <0.50 trivial, 0.50 to 1.25 small, 1.25 to 1.9 moderate and >2 large. An alpha of 0.05 was used to determine statistical significance. All data values were expressed as mean  $\pm$  standard deviation. All analyses were performed using SPSS software (v 15.0; IBM, Armonk, NY, USA).

## RESULTS

Mean values and standard deviation of the anthropometric parameters can be seen in Table 1.

The analysis of the effects of NaHCO<sub>3</sub> supplementation did not show significant differences between placebo and NaHCO<sub>3</sub> in the variables isometric peak of torque (PT) (Placebo: 321  $\pm$  15; NaHCO<sub>3</sub>: 321  $\pm$  18 Nm) and torque development rate (Placebo: 73  $\pm$  3; NaHCO<sub>3</sub>: 76  $\pm$  4 Nm/s) as described on Figure 1A and 1B respectively.

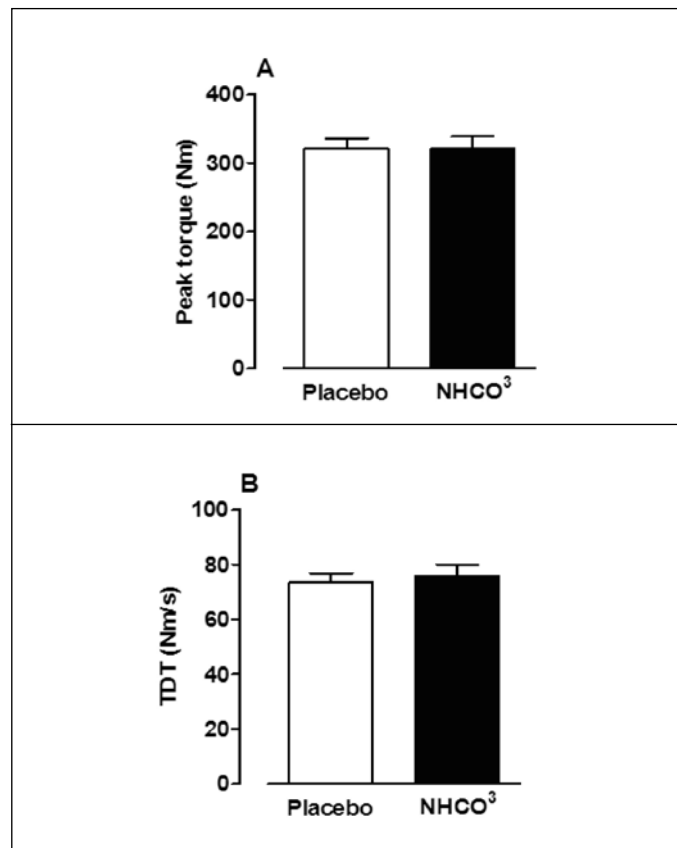
Significant differences were not found on isometric PT corresponding to 50% of the maximum peak among the interventions (Figure 2A). However, in the end effort, the NaHCO<sub>3</sub> (Fatigue: 160  $\pm$  23 Nm; End of protocol: 55  $\pm$  23 Nm) supplementation induced an improvement in the isometric PT maintenance when compared to the placebo intervention (Fatigue: 160  $\pm$  20 Nm; End effort: 16  $\pm$  8 Nm). A great effect size was found in both interventions (Placebo: 7.20; NaHCO<sub>3</sub>: 4.56), although the NaHCO<sub>3</sub> supplementation induced a smaller effect when compared to the placebo. The NaHCO<sub>3</sub> supplementation promoted greater maintenance of isometric PT (Figure 2B), delaying ( $p < 0.001$ ) the time (Placebo: 42  $\pm$  5 sec; NaHCO<sub>3</sub>: 95  $\pm$  5 sec) to reach fatigue with great effect size (10.6).

In figure 3B it is shown that significant differences ( $p < 0.001$ ) in [La<sup>-</sup>] were found in the Placebo intervention (Rest: 1.08  $\pm$  0.37; Fatigue: 4.47  $\pm$  1.51; end effort: 6.92  $\pm$  1.13) as well with NaHCO<sub>3</sub> (Rest: 0.95  $\pm$  0.24; Before: 5.42  $\pm$  1.63; After: 7.71  $\pm$  0.74) and great effect size in both interventions (Placebo: 15.78; NaHCO<sub>3</sub>: 28.16). However, the NaHCO<sub>3</sub> induced hyperlactecemia both in fatigue and in the end of protocol when compared to the placebo.

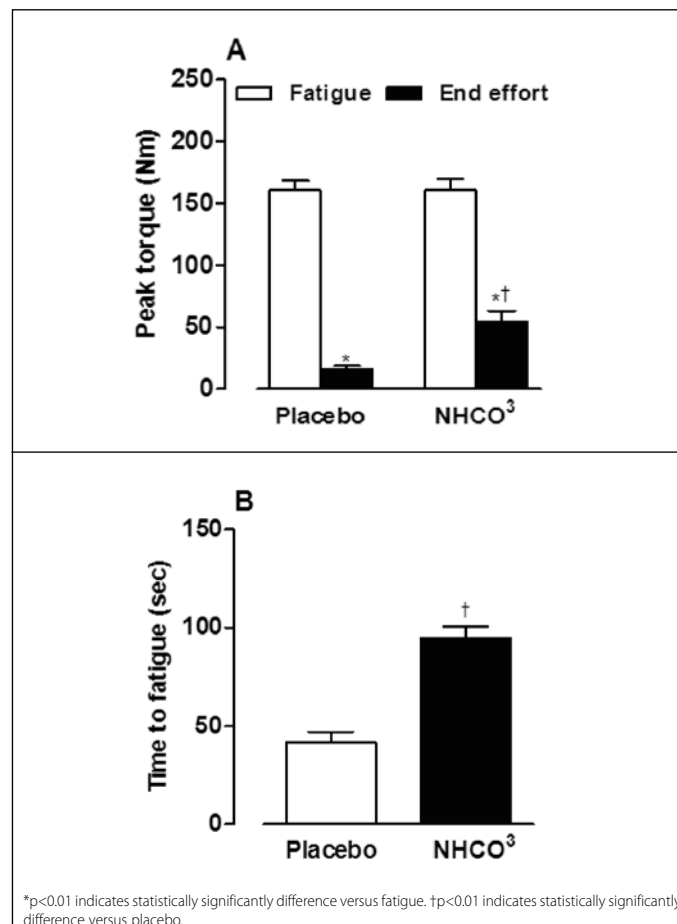
**Table 1.** Mean and standard deviation (SD) of the biometrics parameters.

	Mean $\pm$ SD	95% CI
Age (years)	32 $\pm$ 8	24.96 – 39.04
Body mass (kg)	81 $\pm$ 4	77.44 – 83.99
Height (m)	1.74 $\pm$ 0.1	1.69 – 1.79
BMI (kg/m <sup>2</sup> )	27 $\pm$ 2	25.14 – 28.29
Body fat (%)	21 $\pm$ 7	14.94 – 27.06
Fat mass (kg)	17 $\pm$ 6	11.74 – 22.83
Lean mass (kg)	63 $\pm$ 5	59.00 – 67.86

BMI: body mass index, IC: confidence interval.

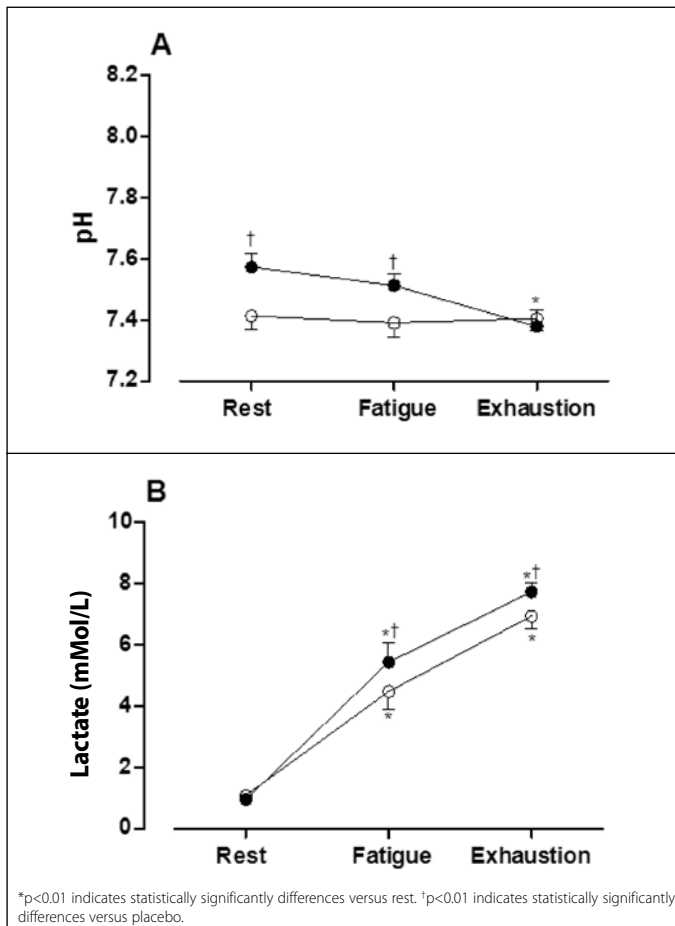


**Figure 1.** Mean and standard error of isometric peak of torque (Panel A) and torque development rate (Panel B).



\* $p < 0.01$  indicates statistically significantly difference versus fatigue. † $p < 0.01$  indicates statistically significantly difference versus placebo.

**Figure 2.** Mean and standard error of the peak of isometric torque at 50% of the isometric peak of torque (fatigue) and at the end of protocol (Panel A) and the time of sustaining strength till reach 50% of isometric peak of torque (Panel B).



**Figure 3.** Mean and mean standard deviation of pH at 50% of the isometric peak of torque and the blood lactate concentration in interventions with Placebo (○) e  $\text{NHCO}_3$  (●).

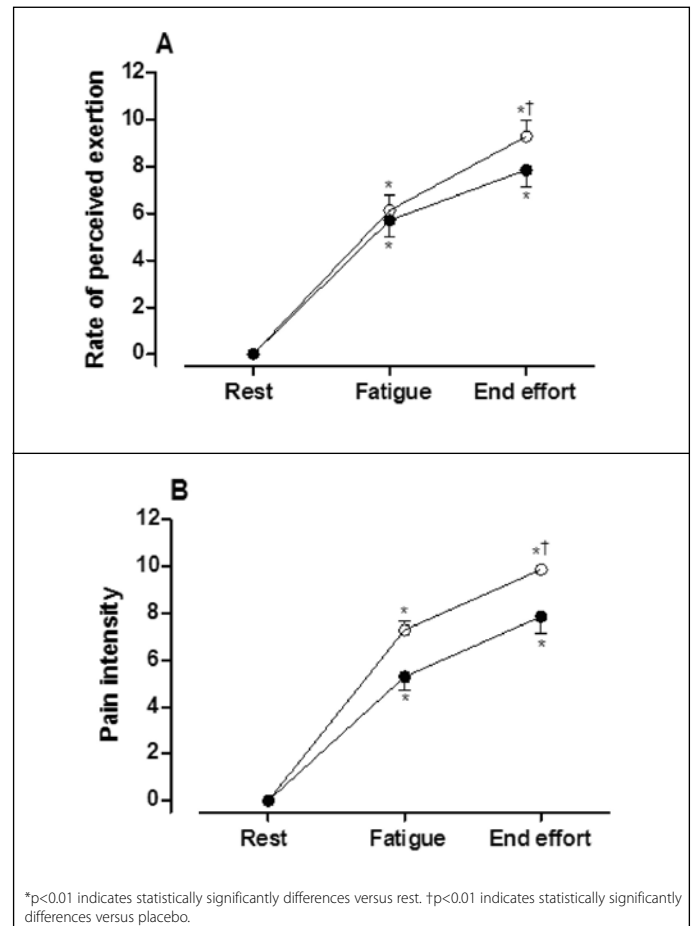
Significantly differences ( $p < 0.001$ ) found in the intervention with placebo in RPE (Fatigue:  $6.14 \pm 1.77$ ; End effort:  $9.28 \pm 1.89$ ) as well as RPP (Fatigue:  $7.28 \pm 9.85$ ; End effort:  $9.85 \pm 0.37$ ) and great effect size was found in both parameters (1.77 and 1.40) respectively. Similar results were found in RPE after the  $\text{NaHCO}_3$  intake (Fatigue:  $5.71 \pm 1.88$  < End effort:  $7.85 \pm 1.95$ ) as well as in RPP (Fatigue:  $5.28 \pm 7.85$  < End effort:  $7.85 \pm 1.95$ ) and the great effect size was found in both parameters (1.13 and 0.32). In addition, the  $\text{NaHCO}_3$  supplementation induced lower ( $p < 0.001$ ) RPE (Figure 4A) and RPP (Figure 4B) when compared to placebo.

It was found significantly difference ( $p < 0.001$ ) among the internal load in the both interventions (Placebo:  $74.29 \pm 6.04$ ;  $\text{NaHCO}_3$ :  $56.0 \pm 6.53$  arbitrary units), considering the parameters more commonly used in the training prescription (Figure 5).

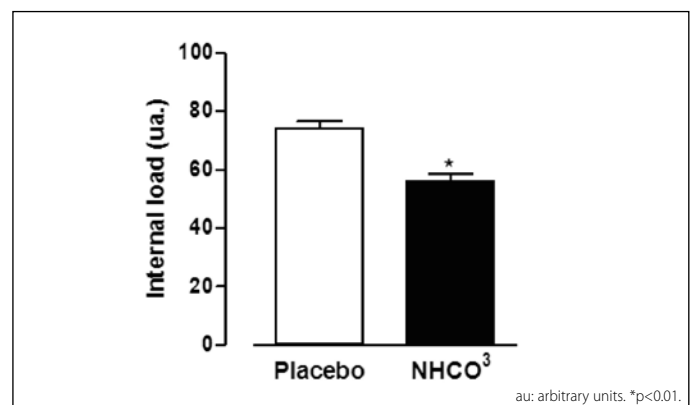
## DISCUSSION

The main findings of this study correspond to the significantly increase in the time to reach fatigue (50% of initial PT) and a smaller reduction of isometric PT at the end of protocol after the  $\text{NaHCO}_3$  supplementation. This result shows the ergogenic effect in the maintenance of the isometric strength assessed in the isokinetic dynamometer. The improvement in the exercise performance observed in this study follows other studies<sup>9,16,22-24</sup> with improvement in the performance after alkalosis induction.

It is known that the fatigue process is a multifactorial phenomenon, however in high-intensity exercises, MF is associated with the increase of  $[\text{H}^+]$  with a simultaneous decrease in the intramuscular  $\text{pH}^3$ . The best performance found after the  $\text{NaHCO}_3$  intake probably is linked to the increase of the  $\text{H}^+$  efflux of the muscle in exercise, which decreases the intramuscular acidosis and thus delays the fatigue.<sup>10</sup>



**Figure 4.** Mean  $\pm$  standard deviation of rate of perceived exertion (Panel A) and pain (Panel B) in the interventions with Placebo (○) e  $\text{NHCO}_3$  (●).



**Figure 5.** Mean  $\pm$  standard deviation of internal load in the interventions with Placebo (○) and  $\text{NHCO}_3$  (●).

The efflux of  $\text{H}^+$  from the cell into the extra cell is influenced by the  $\text{pH}$  gradient and occurs together with the lactate efflux, undertaken through the monocarboxylate transporter protein linked to the plasma membrane. Thus, the higher extracellular  $\text{pH}$  values pre-exercise and in the bout of fatigue observed after the  $\text{NaHCO}_3$  intake suggest that the  $\text{H}^+$  speed of transportation was increased as a consequence of the higher  $\text{pH}$  gradient resulting from the alkaline substance intake.<sup>25</sup>

Although increase in the blood  $[\text{La}^-]$  has been observed during the implementation of the protocol both after  $\text{NaHCO}_3$  and placebo when compared to the pre-exercise a higher efflux of lactate was observed in the bout of fatigue and at the end of protocol, when the participants were supplemented with  $\text{NaHCO}_3$ . In turn, the increased lactate efflux contributes to the intramuscular  $\text{pH}$  maintenance and, thus, to the



intensity of the exercise<sup>25</sup>. As suggested by Coombes and McNaughton,<sup>22</sup> the higher values of blood [La<sup>-</sup>] could reflect on a higher anaerobic contribution during the exercise and a higher dependence of anaerobic glycolysis to complete the protocol in the NaHCO<sub>3</sub> essay.

Consistently, has been demonstrated that the intake of 0.3 g/kg/body mass of NaHCO<sub>3</sub> increases both the pre-exercise extracellular pH and the blood [La<sup>-</sup>] during the effort.<sup>5,7-9,13,14</sup>

In connection with the initial PT and the torque development rate conducted in the MIVC test (pre-protocol, eight minutes), statistical differences were not observed between the two conditions, pointing out that the maximum strength was not affected by the NaHCO<sub>3</sub>. One possible explanation for this is that, due to the short duration of the effort (three contractions of five seconds), a significant production of H<sup>+</sup> did not occur, since in this type of effort the primary transfer channel is ATP-CP and not the glycolytic one. In this sense, the NaHCO<sub>3</sub> action is unable to affect the performance.<sup>22</sup>

The alkalosis effect in the effort perception has been investigated in research of various modalities of exercise,<sup>5,6,26</sup> but the results are conflicting. Krstrup et al.<sup>5</sup> found smaller values for only after 440 m in an intermittent test of high intensity, but not after 160 and 280m, and in the moment of exhaustion after NaHCO<sub>3</sub> supplementation when compared to the control. Nevertheless, when the participants ingested the alkaline substance, there was an improvement of 14% in the performance. In turn, Duncan et al.<sup>26</sup> did not find differences in the RPE between NaHCO<sub>3</sub> and control of trained men in resistance exercise, although there was a higher number of repetitions in the first exercise after the use of NaHCO<sub>3</sub>.

In our study, we did not find alterations in the effort perception in the bout of fatigue. However, a lesser degree of RPE was established after the eight minutes of protocol. In theory, we can suggest that centrally mediated mechanisms could be affected. Among these mechanisms, has been proposed that the metabolic alterations in the muscle in exercise result in inhibitory neural feedback through afferent fibers from

groups III and IV, which are responsive to a range of chemical stimuli.<sup>5,27</sup> Thus, the smaller RPE and the higher performance with NaHCO<sub>3</sub>, could reflect a smaller metabolic alteration (including [H<sup>+</sup>]) due to the action of this substance and, consequently, a less negative muscular feedback and a smaller effect on the descending central command for the motor neurons.<sup>27</sup> However, it is interesting to note that at the end of protocol (when the RPE was smaller in NaHCO<sub>3</sub>) pH values were not different between NaHCO<sub>3</sub> and placebo. The investigation of other metabolic variables could help to understand this phenomenon.

Concerning the RPE's session performed after 30 minutes after the end of the protocol, smaller values were observed when the participants were supplemented with NaHCO<sub>3</sub>. The session's RPE has been proposed as a valid method to monitor the exercise's intensity and allows the individual to give a global assessment about how difficult the session was.<sup>20</sup> As per our knowledge, till now there is not a research investigating the influence of NaHCO<sub>3</sub> in the answers of RPE's session. Again, it is possible that smaller metabolic alterations have occurred during the exercise in function of the higher extracellular buffered after the NaHCO<sub>3</sub> intake, which could be contributed for a full recovery and as maller perception of effort after 30 minutes of recovery.<sup>27</sup>

## CONCLUSION

The results suggest that the NaHCO<sub>3</sub> intake could improve the performance of sustained isometric contractions of knee extensors with decrease in the RPE e RPP and a smaller load. However, it is needed to conduct more research to investigate the NaHCO<sub>3</sub> effect in the internal load parameters, as, in the RPE's session, which can give information related to the recovery and help in the exercise of dynamic characteristics prescription.

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All authors declare no potential conflict of interest related to this article

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**AUTHORS' CONTRIBUTIONS:** Each author made significant individual contributions to this manuscript. CASC (0000-0002-3606-0828)\* conceived the study, acquired and interpreted data, and drafted the manuscript. RAB (0000-0001-9874-3790)\* interpreted data, and drafted and reviewed the manuscript. MAL: analyzed data and reviewed the manuscript. MMK (0000-0002-2406-4450)\* analyzed data and reviewed the manuscript. FP (0000-0002-7901-3351)\* interpreted data, and drafted and reviewed the manuscript. ALE (0000-0002-4941-6475)\*, MRRP (0000-0003-1857-6924) interpreted data, and drafted and reviewed the manuscript. AJS (0000-0002-5407-8183)\* analyzed data and reviewed the manuscript. RLC (0000-0002-6145-1337)\*, AFJ (0000-0001-8069-2366)\*, CVLST (0000-0002-8523-5794) interpreted data, and drafted and reviewed the manuscript. DSB (0000-0003-3993-8277)\* conceived the study, acquired and interpreted data, and reviewed the manuscript. \*ORCID (Open Researcher and Contributor ID).

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