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**EFFECTS OF ACUTE SODIUM BICARBONATE
SUPPLEMENTATION ON REPEATED SPRINT ABILITY IN
FEMALE FOOTBALL PLAYERS**

Dissertação com vista à obtenção do Grau de Mestre em Treino de Alto Rendimento

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O mundo está nas mãos daqueles que têm a coragem de sonhar e correr o risco de viver os seus sonhos.

Paulo Coelho

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ABSTRACT

This study sought to investigate the effects of acute sodium bicarbonate (SB) supplementation on repeated sprint ability in female football players. Eleven athletes completed a double blind randomized crossover controlled trial with 2 repeated sprint sessions (SB or placebo, PL) performed in a cycle ergometer, consisting of 3 x 6 maximal 6-s sprints performed every 30 s with 5-min recovery between sets. The first take (0.2 g/kg) of the supplement was ingested 120 minutes before the exercise protocol and the second take (0.1 g/kg) 60 minutes before. Sodium bicarbonate allowed a higher blood lactate accumulation (Max - SB: 14.0 ± 4.32 vs PL: 10.9 ± 3.55 , $p=0.010$), induced a greater elimination of CO₂ ($p=0.038$) through ventilation and showed a tendency to provide a larger muscle oxygenation. These metabolic effects did not traduce into major benefits in terms of power output and total work performed in the task other than preventing a decline in mean power output in the second set. Sodium bicarbonate also favoured neuromuscular performance, inducing a smaller decrement on jump height in a countermovement jump (SB: -0.8% vs PL: -7.4%). This research suggests that female football players may benefit from sodium bicarbonate supplementation to support the high physiological demands of the game.

Keywords: sodium bicarbonate; supplementation; repeated sprint ability; football; female athlete; performance; lactate; gas exchange; muscle oxygenation; countermovement jump

RESUMO

Este estudo procurou analisar os efeitos da suplementação aguda com bicarbonato de sódio (BS) na habilidade de sprints repetidos em jogadoras de futebol feminino. Onze atletas completaram um estudo duplo cego randomizado cruzado controlado com 2 sessões de sprints repetidos (BS ou placebo, PL) realizadas num cicloergómetro, consistindo em 3 x 6 sprints de 6 s executados a cada 30 s com 5 min de recuperação entre séries. A primeira toma (0.2 g/kg) do suplemento ocorreu 120 minutos antes do protocolo de exercício e a segunda toma (0.1 g/kg) 60 minutos antes. O BS possibilitou uma maior acumulação de lactato (Máx – BS: 14.0 ± 4.32 vs PL: 10.9 ± 3.55 , $p=0.010$), maior eliminação de CO₂ ($p=0.038$) pela ventilação e revelou uma tendência para gerar maior oxigenação muscular. Estes efeitos metabólicos não se traduziram em benefícios significativos ao nível da potência e trabalho total produzidos na tarefa para além de prevenir um declínio na potência média na segunda série. O BS também favoreceu a performance neuromuscular, induzindo um menor decréscimo na altura saltada num salto com contramovimento (BS: -0.8% vs PL: -7.4%). Os resultados sugerem que jogadoras de futebol feminino poderão beneficiar da suplementação com BS para suportar as elevadas exigências fisiológicas do jogo.

Palavras-chave: bicarbonato de sódio; suplementação; sprints repetidos; futebol; atleta feminina; desempenho; lactato; trocas gasosas; oxigenação muscular; salto com contramovimento

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ABBREVIATIONS

BMI – Body Mass Index

CMJ – Countermovement Jump

ECW – Extracellular Water

FFM – Fat Free Mass

FM – Fat Mass

HHb – Deoxygenated Hemoglobin

HR – Heart Rate

ICW – Intracellular Water

JH – Jump Height

MPO – Mean Power Output

O₂Hb – Oxygenated Hemoglobin

PL – Placebo

PPO – Peak Power Output

RER – Respiratory Exchange Ratio

RPE – Rated Perceived Exertion

RSA – Repeated Sprint Ability

RST – Repeated Sprint Task

SB – Sodium Bicarbonate

Sdec – Sprint Decrement

TBW – Total Body Water

TOI – Tissue Oxygenation Index

TW – Total Work

VE – Ventilation

CHAPTER 1: INTRODUCTION

1.1 CONTEXT AND PROBLEM

Football is characterized as an intermittent sport since it involves high intensity actions intersped with periods of lower activity (de Sousa et al., 2021; Fernandes, 2021; Lockie et al., 2020). Therefore, the ability to perform repeated sprints while requiring minimal recovery periods between efforts – repeated sprint ability (RSA) – appears to be an important aspect of team sports like football (Gonçalves et al., 2021). The large physical demands required by this type of effort, as well as the increasing number of training sessions, matches and travels per season, highlight the relevance of nutritional strategies to meet them (de Sousa et al., 2021; Fernandes, 2021). In this domain, particular interest is attributed to supplementation, attending to the global spread of the supplements market, making it a common practice among football players (Oliveira et al., 2017).

High intensity exercises induce the accumulation of H^+ , requiring the actuation of intramuscular and extracellular buffers, in order to prevent acidity from compromising the normal operation of the energetic and contractile systems (Jones et al., 2016). Increasing the alkaline reserve prior to exercise may be a strategy to enable muscle to produce more H^+ and La^- , by increasing the gradient of concentration for efflux from the muscle (Dalle et al., 2019). The increase in alkaline reserve can be accomplished with a few supplements but, considering the pros and cons of each, sodium bicarbonate seems to be the most suitable. However, the transfer of the benefits of this supplement for football match is not clear and no meta-analysis has been conducted to verify the effects on RSA (de Sousa et al., 2021). Furthermore, most studies have focused on its effects on performance but little is known about the influence it may have on pulmonary gas exchange and muscle oxygenation. Attending to the scarcity of studies that aimed to explore the efficacy of sodium bicarbonate supplementation on RSA in football, this study aims to provide new scientific evidence that enriches the existing literature in this domain.

Regarding football, there is considerably less research performed on female compared to male players and, specifically, the investigation of RSA in female footballers is very limited (de Sousa et al., 2021; Lockie et al., 2020). Furthermore, only 20% of the existing studies about sodium bicarbonate supplementation involved women as participants, of which only 7.4% provided group analyses exclusively in women (Grgic et al., 2021b; Saunders et al., 2021). Therefore, given the fact that few investigations had

women as sample of the study, since their specific morphological and physiological characteristics may induce inconsistencies in the results of the investigation that affect its validity, this study aims to contribute to the little existing evidence, launching future research perspectives for studies in sport sciences with female athletes.

1.2 AIMS

In addition to the more general objectives described above, three specific aims were defined for this research:

1. To investigate the effect of acute ingestion of sodium bicarbonate in female football players' performance on a repeated sprint task (RST). H1: Sodium bicarbonate improves mean and peak power, induces a smaller power decrement along the exercise protocol and improves work capacity, increasing general performance.
2. To investigate the effect of acute ingestion of sodium bicarbonate on pulmonary gas exchange, muscle oxygenation and blood lactate accumulation during a RST. H2: Sodium bicarbonate induces a greater elimination of CO₂ and larger muscle oxygenation and allows a higher blood lactate accumulation.
3. To investigate the effect of acute ingestion of sodium bicarbonate on the neuromuscular system before and after the RST. H3: Sodium bicarbonate induces a smaller decrement on jump height (JH) in a countermovement jump (CMJ).

1.3 DISSERTATION STRUCTURE

In order to establish a fluid and logical reasoning between topics, this dissertation is organized in 6 chapters. Chapter 1 provides a general overview of the state of the art and the definition of the problem, from which the aims of this research were established, which are also described in this chapter. Still in Chapter 1, in order to clarify the organization of this work, the structure of the dissertation is also presented. Chapter 2 contains a literature review of the topic starting with a brief introduction to RSA in

football and continuing with the importance of supplementation to improve performance, focusing the most common supplements used by football players. Then, a physiological overview of sodium bicarbonate is presented, with particular reference to its mechanisms of actuation, limitations of using it as an ergogenic supplement, performance/physiological related effects and factors influencing its effects on the body. The review ends with a specific topic about the female athlete, highlighting the relevance of involving female athletes in sport sciences research and mentioning some specific methodological considerations for studies with women. In addition to a general overview of the participants and description of the experimental design, Chapter 3 also includes the description of how anthropometry and body composition were accessed, supplementation protocol, questionnaire of the gastrointestinal effects of sodium bicarbonate, repeated sprints protocol, measurements and neuromuscular fatigue assessment, as well as an explanation of how data was analysed. Chapter 4 presents the results of the study in regard to participants characterization and gastrointestinal symptoms induced by the supplement, performance in the RST, rated perceived exertion (RPE), lactate accumulation, pulmonary gas exchange and heart rate (HR), muscle oxygenation and neuromuscular fatigue. In Chapter 5, the main findings of the research are discussed, considering the results of other studies. The last chapter of the dissertation, Chapter 6, presents the main conclusions of this investigation, the limitations to have in consideration and possible suggestions for future research in this domain.

CHAPTER 2: LITERATURE REVIEW

2.1 A BRIEF INTRODUCTION TO FOOTBALL: REPEATED SPRINT ABILITY

2.1.1 Repeated sprint ability as a predictor of success in football

Football is the most popular sport in the world and its physical demands have been increasing over the past few decades, attending to the growing number of matches per season (Dobrowolski et al., 2020; Oliveira et al., 2017). In fact, elite footballers have been exposed to greater training volumes and competitions than ever before in history (de Sousa et al., 2021). The challenges associated with the increase in the number of football players worldwide triggers an increased concern in understanding the characteristics of these players, their physiological and physical demands and their training processes (Gonçalves et al., 2021).

Football can be generally defined as an intermittent sport with high intensity periods intersped with periods of lower activity (de Sousa et al., 2021; Fernandes, 2021; Lockie et al., 2020). Low intensity activity usually involves walking or jogging and high intensity actions relate to sprinting, changes of direction, tackles or jumping, as well as accelerations and decelerations (Fernandes, 2021; Gonçalves et al., 2021; Lockie et al., 2020; Oliveira et al., 2017). Low intensity movements are predominant (70% of the match) but high intensity actions (about 150 to 250 during a match) (Oliveira et al., 2017) acquire special relevance since they are usually associated with key match situations like ball possession, offensive attacks and goal scoring chances (Gonçalves et al., 2021; Lockie et al., 2020). The ability to support maximal or near maximal efforts intersped with brief recovery intervals is thus a determinant factor in performance (Miller et al., 2016). A football player's ability to recover quickly from repeated maximal sprint efforts will dictate his/her ability to stay involved in match play and may determine overall effectiveness (Lockie et al., 2020).

The ability to perform repeated sprints while requiring minimal recovery periods between efforts (RSA) appears to be an important aspect of team sports (Gonçalves et al., 2021). RSA is indeed an essential factor for determining success in football, not belittling other qualities like technical and tactical skills (Gonçalves et al., 2021). In theoretical terms, we can define RSA as the capacity to repeatedly produce maximal or near maximal efforts, intersped with brief recovery intervals of rest or low to moderate intensity activity

(Lockie et al., 2020; Girard et al., 2011). A better understanding of the factors contributing to fatigue during RSA should be the first step in order to design interventions, such as training programs or ergogenic aids that could delay the onset of fatigue, enhance RSA and improve physical match performance in team sport athletes (Girard et al., 2011).

2.1.2 Factors contributing to fatigue during repeated sprints

In general terms, fatigue resistance during repeated sprints is directly dependent on the exercise mode since higher decrement scores during cycling protocols have been reported when compared with running protocols. Furthermore, it is also dependent on resistive load and on the distribution and duration of the work periods and the recovery pattern, as well as on the presence or absence of previous exercise. Other factors such as sex, age, playing position or training status may also influence RSA. Being female, young or aerobically trained has typically been associated with smaller decrement scores (Girard et al., 2011).

Specifically, RSA can be limited by muscular and neural factors, as well as additional factors such as stiffness regulation or specific environmental conditions (heat and altitude, for example). At the muscular level, muscle excitability, limitations in energy supply and acidosis are the most important factors to have in consideration in this domain. In regard to acidosis, the considerable increases in muscle and blood H^+ accumulation that occur during repeated sprints may affect sprinting performance via adverse effects on the contractile machinery and/or through the inhibition of ATP derived from glycolysis, possibly via negative effects on its regulatory enzymes. At the neural level, factors such as neural drive, traducing the ability to fully activate the contracting musculature, and muscle recruitment strategies, associated with an increase in the relative contribution of type I muscle fibers along the exercise protocol, may also contribute to fatigue during repeated sprint exercise (Girard et al., 2011).

Repeated sprint ability (RSA) is indeed a complex quality, influenced by anaerobic and aerobic metabolism that is correlated with motor unit activation and is essentially dependent on H^+ buffering and oxidative capacity for phosphocreatine recovery, which together provide the ability to repeat sprints (Gonçalves et al., 2021). Therefore, RSA in football can be influenced by a well-developed neuromuscular system,

lower limb strength and power, aerobic capacity and even agility (Gonçalves et al., 2021; Lockie et al., 2020). Training the various factors that influence RSA is a useful strategy to improve this quality, contributing to a better performance (Bishop et al., 2011).

Numerous tests can be used to access RSA in football but most of them usually involve 6 to 12 maximal efforts, completed every 15 to 30 seconds, with distances that vary between 20 and 40 meters (Lockie et al., 2020). According to Miller et al. (2016), a single set of 5 to 15 sprints lasting no more than 10 seconds, intersped with a recovery of 60 seconds or less can be used to define repeated sprint exercise which typically manifests fatigue. The main aspect to have in consideration in the definition of a protocol to evaluate RSA is the ability of the test to simulate intermittent exercise and then identify a player's capacity to maintain maximal effort and recovery during multiple successive sprinting efforts (Gonçalves et al., 2021). Also, the number of repetitions, sprint duration and recovery times must be specific to the requirements of the athlete's sport to have the best application for testing RSA (Lockie et al., 2020).

2.2 SUPPLEMENTATION AND THE ATHLETIC PERFORMANCE

2.2.1 The relevance of supplementation to improve performance in football

The large physical demands of football highlight the importance of nutritional strategies to meet them (de Sousa et al., 2021). The constant travels that many teams undergo may lead to accumulated fatigue that, combined with inadequate recovery, can determine underperformance and increased risk of injury, so athletes must follow adequate dietary practices (Oliveira et al., 2017). In fact, focus on daily diet is of great importance, supplying the nutrients needed to cover the energy demands, optimizing adaptations and improving recovery (de Sousa et al., 2021). An optimally balanced diet is therefore one of the factors that positively influences athletic performance (Dobrowolski et al., 2020). In this domain, particular interest is attributed to supplementation since it has become a common practice among football players (Oliveira et al., 2017). The greatest loss of performance of the athletes occurs in the final 10 minutes of the second half so ergogenic supplementation can be a way to maintain it until the end

of the match (Fernandes, 2021; Oliveira et al., 2017). Furthermore, as intense travel periods are common in elite football, adjusting athletes' supplementation can be a great alternative to help maintain or even improve performance during training, matches and travels (Fernandes, 2021).

There is no single definition of what a supplement is but it can be generally defined as a food, food component, nutrient or non-food compound that is ingested in addition to each athlete's regular diet with the specific aim of achieving a health and or/performance benefit (Maughan et al., 2018; Sheridan et al., 2021). Food and Drug Administration defines dietary supplement as a product designed for ingestion that contains a dietary ingredient (such as vitamins, minerals, herbs or other botanicals, amino acids, dietary substances capable of increasing total energy intake, concentrates, metabolites, constituents or extracts) intended to add a further nutritional value to supplement the diet (Garthe & Maughan, 2018). Supplements can be divided in four main categories: formulated foods and sports foods, medical supplements, ergogenic supplements and functional food/superfoods (Garthe & Maughan, 2018; Maughan et al., 2018). For the purpose of this study, the focus will be ergogenic supplements, defined as authorized substances not having an essential nutritional role but considered adequate for potential performance benefits, improving either intense movements or endurance during training and matches and aiding recovery (de Sousa et al., 2021). The reasons behind the use of supplements are quite diverse but most authors highlight the correction/prevention of nutrient deficiencies, the provision of energy and nutrients during an exercise session, to achieve a specific direct or indirect performance benefit or for financial gains related to partnerships with brands (Garthe & Maughan, 2018; Maughan et al., 2018; Sheridan et al., 2021).

Attending to the global spread of the supplements market, some concerns have been established regarding their quality and security, alerting nutritionists to carefully analyse their utilization in order to prevent possible adverse effects (Oliveira et al., 2017). Dietary supplements are not under the same regulations nor are subject to the strict control that is applied to the pharmaceutical industry, which frequently leads to some reports of its contamination with impurities, allergens, microbiological contamination or even prohibitive substances that can lead athletes to fail a doping test, preventing them from participating in competitions (Garthe & Maughan, 2018; Maughan et al., 2018; Oliveira et al., 2017). The use of a supplement must then be carefully analysed due to the poor

quality of some supplements commonly sold in the market and given the fact that Food and Drug Administration does not have the required means to evaluate all supplements (Maughan et al., 2018). Therefore, athletes should do a careful risk analysis to see if the benefits associated with a supplement compensate the risk of inadvertent doping (Maughan et al., 2018).

Therefore, performance enhancing supplements should be considered only where a strong evidence base supports their use as safe, legal and effective and after adequacy of sports nutrition dietary practices is ensured, particularly the macro and micronutrient uptake (de Sousa et al., 2021; Maughan et al., 2018). Supplements should only be used in addition to a healthy balanced diet and not as a replacement of food (Sheridan et al., 2021), and after evaluation of individual side effects. Compared with other factors such as talent, training, tactics and motivation, nutrition has a small effect on performance, and supplements are no more than a minor part of the athlete's nutrition strategy (Garthe & Maughan, 2018). Thus, athletes should consider using dietary supplements only if specific nutrient deficiencies were identified, if the consequences of chronic inadequate energy intake have been established, during periods of weight loss or those following specific diets, if some foods are excluded from the diet due to allergies, food intolerance or food choices, prior to different interventions to optimize adaptations or if convenient during travels (Garthe & Maughan, 2018).

It should be noted that the evaluation of studies with supplementation may be difficult due to different definitions of supplements, diverse populations and level of performance, different methods of data collection and different timeframes (Garthe & Maughan, 2018). In order to overcome this difficulty, research in this domain should attend to aspects such as an adequate sample size and appropriate characteristics, to allow a good statistical power and applicability to elite athletes, simulation of the conditions that exist in real life competition, standardization of variables that may influence the results, use of a supplementation protocol, verification of the contents of the product, confirmation that the supplement was taken and induced biological response, definition of a valid and reliable evaluation protocol capable of detecting minimal changes and interpretation of the results attending to the existing limitations (Maughan et al., 2018).

2.2.2 What supplements can football players use to optimize performance?

High intensity sports like football, consisting of repetitive, short all-out exercise bouts that require a high fraction of energy input via anaerobic glycolysis, result in the accumulation of H^+ and La^- in muscle cells and extracellular space (Dalle et al., 2019; Fernandes, 2021). In fact, high intensity exercise induces the accumulation of H^+ , requiring the actuation of intramuscular buffers and dynamic buffering in order to prevent acidity from compromising energetic and contractile systems, with the main controller of pH being dynamic buffering (Jones et al., 2016). Low intramuscular acidity was reported to reduce cross bridge force, velocity and power, as well as depress myofibrillar Ca^{2+} sensitivity (Dalle et al., 2019). This is particularly important since the reduction in muscle pH induced by this sport only in the first half of a match can go from 7.24 ± 0.02 to 6.96 ± 0.03 (Macutkiewicz & Sunderland, 2018). Therefore, increasing the alkaline reserve prior to exercise may be a way to enable muscle to produce more H^+ and La^- , by increasing the gradient of concentration for efflux from the muscle (Dalle et al., 2019).

Several authors have been investigating which supplements may directly improve sports performance, highlighting caffeine, creatine, nitrate, beta alanine and sodium bicarbonate (de Sousa et al., 2021; Fernandes, 2021; Garthe & Maughan, 2018; Maughan et al., 2018; Oliveira et al., 2017; Rawson et al., 2018; Sheridan et al., 2021). To keep the amount of La^- in the skeletal muscle high for a long period, supplementation with sodium bicarbonate and/or beta alanine has been used, two buffering agents that attenuate the decline in muscle pH, decreasing acidity and improving performance (Fernandes, 2021; Heibel et al., 2018). While sodium bicarbonate improves extracellular buffering capacity, by increasing blood bicarbonate, beta alanine increases intracellular carnosine content, which contributes in approximately 20% to the total buffering capacity of the type II muscle fibers (Burke, 2017; da Silva et al., 2019; de Sousa et al., 2021; Fernandes, 2021; Grgic et al., 2021b; Maughan et al., 2018; Oliveira et al., 2017; Sheridan et al., 2021).

Ingesting sodium bicarbonate with beta alanine may be useful for an event in which substantial drops in muscle pH are experienced, increasing total buffering capacity and tolerance to acidosis (Burke, 2017; Fernandes, 2021). Therefore, both sodium bicarbonate and beta alanine may have an important role in exercises highly dependent on glycolysis, and their co-supplementation seems to be more effective than single supplementation alone (da Silva et al., 2019; Grgic et al., 2021b). The combination may

be better due to the opportunity of buffering inside the muscle cell and simultaneously in the extracellular space (Burke, 2017). Furthermore, some authors refer that beta alanine does not appear to improve single or repeated sprint capacity alone but if ingested together with sodium bicarbonate, can elevate the overall buffering capacity relevant for lowering any detrimental effect derived from increases in H^+ (de Sousa et al., 2021).

Nevertheless, beta alanine needs to be ingested for a certain period of time to provide an increase in carnosine content, making sodium bicarbonate the ideal supplement to use in acute investigations (Grgic et al., 2021b). It is indeed one of the recommended supplements by the International Olympic Committee and the International Society of Sports Nutrition as a strategy for performance development in high intensity exercises (Grgic et al., 2021b; Grgic et al., 2020). Therefore, athletes from team sports like football may consider supplementation with sodium bicarbonate to acutely improve their performance (de Oliveira et al., 2021; Maughan et al., 2018). However, the transfer of its benefits for football match is not that clear and no meta-analysis has been conducted to verify the effects on RSA (de Sousa et al., 2021). This highlights the importance of better understanding how sodium bicarbonate supply affects changes in muscle anaerobic power and specific performance in team sports disciplines (Durkalec-Michalski et al., 2020a).

2.3 SODIUM BICARBONATE: PHYSIOLOGICAL OVERVIEW

2.3.1 Mechanisms of actuation of sodium bicarbonate

During high intensity exercise, the high turnover rate of ATP increases H^+ production, a by-product of anaerobic metabolism that causes fatigue (da Silva et al., 2019; Heibel et al., 2018; Macutkiewicz & Sunderland, 2018). The accumulation of H^+ causes a depression of the anaerobic pathways, since it inhibits phosphocreatine resynthesis and reduces the activity of key glycolytic enzymes (glycogen phosphorylase and phosphofructokinase), and in a competition between H^+ and Ca^{2+} for the troponin binding site, affecting contractile properties and energy homeostasis (da Silva et al., 2019; Durkalec-Michalski et al., 2020a; Grgic et al., 2020; Gurton et al., 2020; Heibel et al.,

2018; Macutkiewicz & Sunderland, 2018). In fact, H^+ accumulation produces dysfunction of the sarcoplasmic reticulum, due to decreased Ca^{2+} sensitivity, reduced myosin-actin cross bridge cycling activity and increased K^+ release, which together impede muscular myofilament function and excitation-contraction coupling (Ansdell & Deckerle, 2020).

Ingesting sodium bicarbonate elevates extracellular HCO_3^- , enlarging the gradient of concentration between extracellular and intracellular H^+ , which stimulates the La^-/H^+ cotransporter and promotes a greater efflux of these metabolites from intramuscular regions into the vascular compartment, reducing pH drop inside the muscle cells (Burke, 2017; Farias de Oliveira et al., 2020; Ferreira et al., 2019; Maughan et al., 2018; Miller et al., 2016; de Oliveira et al., 2021). In detail, in the stomach lumen, HCO_3^- reacts with HCl to form CO_2 , H_2O and Cl^- (neutralization), increasing pH. The rise in pH stimulates the Cl^-/HCO_3^- antiporter in the basolateral face of the stomach parietal cells, promoting the transport of HCO_3^- (produced in the parietal cells from CO_2 and H_2O) into the extracellular fluid and then into the vascular compartment. This is coupled with the apical H^+/K^+ -ATPase pump that secretes H^+ into the stomach lumen to restore the low pH. At the muscular level, increased extracellular pH and HCO_3^- increase the activity of monocarboxylate transporters (MCT1 and MCT4, La^- and H^+ cotransporters), promoting the efflux of H^+ and La^- out of the muscle cells. This allows higher glycolytic rates, resulting in augmented ATP production and muscle and blood lactate concentrations (Grgic et al., 2021b). Figure 1 contains a schematic representation of this process.

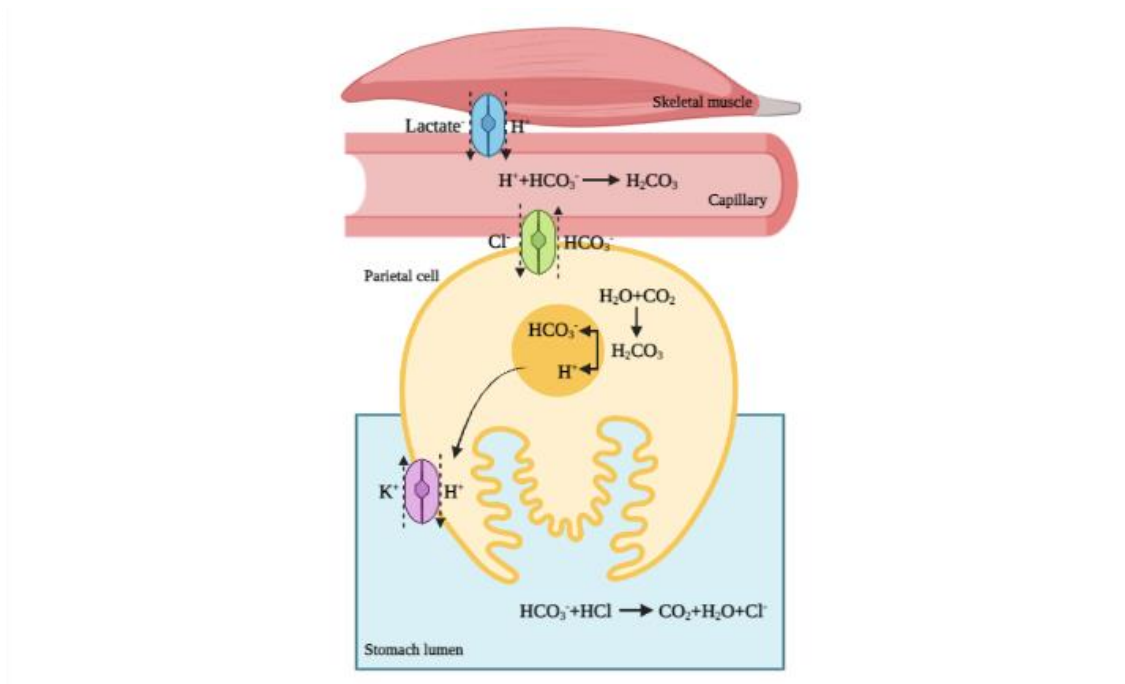


Figure 1. Mechanisms of actuation of sodium bicarbonate. Adapted from Grgic et al. (2021b).

A potential ergogenic effect of sodium bicarbonate requires a 5 mmol/L increase from baseline levels (de Oliveira et al., 2021; Farias de Oliveira et al., 2020; Grgic et al., 2020; Heibel et al., 2018). Some studies demonstrated that doses of 0.1 g/kg and 0.2 g/kg are insufficient to provide such ergogenic effect 60 minutes after ingestion (Heibel et al., 2018). On the contrary, a dose of 0.3 g/kg taken 60 to 120 minutes before exercise seems to be optimal, producing an increase in bicarbonate levels of 5 to 6 mmol/L, with positive effects on performance (de Sousa et al., 2021; Grgic et al., 2021b; Gurton et al., 2020; Heibel et al., 2018; Jones et al., 2016; Oliveira et al., 2017). Other authors suggest the ingestion of 0.3 g/kg of sodium bicarbonate in split doses 2 to 2.5 hours before exercise (Burke, 2017). Regarding ingestion timing some authors demonstrated that both increases in circulating bicarbonate and absolute concentration are similar at 60, 120 and 180 minutes post-ingestion of 0.3 g/kg of sodium bicarbonate (Farias de Oliveira et al., 2020; Grgic et al., 2021b; Heibel et al., 2018).

Attending the fact that a gradient of concentration between the muscle and the vascular compartment requires some time to develop, the effect of sodium bicarbonate may be higher if time to peak bicarbonate is determined before the beginning of the activity (Miller et al., 2016). Time to peak seems to vary between 75 and 180 minutes for ingestion under the form of capsules and 10 to 140 minutes for ingestion under the form of solution (Farias de Oliveira et al., 2020; Gurton et al., 2020). Also, it should be noted

that supplementation with smaller capsules may lead to a peak more quickly (Grgic et al., 2021b). Indeed, response to sodium bicarbonate is highly individual but determining time to peak is not only time consuming but it involves multiple visits to the laboratory, also requiring sophisticated devices that raise additional costs (Dalle et al., 2019; Farias de Oliveira et al., 2020; Gurton et al., 2020). Furthermore, determination of time to peak may not be necessary since bicarbonate availability may not be fully used and moderate increases in bicarbonate concentrations are sufficient to provide performance gains (de Oliveira et al., 2021).

2.3.2 Limitations of using sodium bicarbonate as an ergogenic supplement

Sodium bicarbonate supplementation may induce some adverse side effects, like stomach cramps, bloating, nausea, vomiting, diarrhea and thirst, especially 60 to 120 minutes after ingestion (Dalle et al., 2019; de Oliveira et al., 2021; de Sousa et al., 2021; Durkalec-Michalski et al., 2020a; Grgic et al., 2021b; Grgic et al., 2020; Heibel et al., 2018; Jones et al., 2016). Switching to a smaller dose of 0.2 g/kg or ingesting the supplements together with a carbohydrate enriched meal (1.5 g of carbohydrate per kilogram of body weight) may be reliable strategies to avoid gastrointestinal distress (Grgic et al., 2021b; Gurton et al., 2020; Heibel et al., 2018; Macutkiewicz & Sunderland, 2018; Maughan et al., 2018). Also, the possibility of a dose phasing should be considered since some individuals may not tolerate the full ingestion of a single dose without the appearance of gastrointestinal perturbations (Jones et al., 2016; Maughan et al., 2018). Furthermore, researchers may also consider doing the data collection 180 minutes after the ingestion, attending to the time window where symptoms are more likely to occur (Grgic et al., 2021b; Heibel et al., 2018). It should be noted that some limitations may also surge regarding form of ingestion. While solution has several limitations, due to a bitter and salty taste of bicarbonate and compromise of the blinding, capsules have to be ingested in a large number to complete the required dose, which may be uncomfortable to some subjects (Grgic et al., 2021b).

2.3.3 Effects of sodium bicarbonate on performance and physiological variables

Previous studies demonstrated that sodium bicarbonate influences performance, speed, peak and mean muscle power, total mechanical work, strength endurance, as well as the improvement of sport specific exercise abilities in speed strength disciplines and multiple bouts exercise (Durkalec-Michalski et al., 2020a). In general, sodium bicarbonate seems to be effective in sustained high intensity events lasting 2 to 8 minutes, intermittent high intensity sports with prolonged efforts above lactate threshold (such as team sports) and sustained events just below the lactate threshold which involve high intensity efforts (Burke, 2017). Research specifically related to repeated sprint exercise showed that supplementation with sodium bicarbonate increased total work in 5% in sprint tasks in the cycle ergometer and 2% in the treadmill, although this has only been verified in some sports, which can be associated with its utilization in nonspecific gestures and questions related to athletes' technique (Macutkiewicz & Sunderland, 2018).

Exercise lasting between 30 and 90 seconds or 5 and 10 minutes may benefit from this supplement and capacity tests seem to benefit more than performance tests (de Oliveira et al., 2021). Sodium bicarbonate seems to enhance performance in about 2% in short term high intensity sprints lasting 60 seconds, with a reduced efficacy as the effort duration exceeds 10 minutes (Maughan et al., 2018). Evidence indicates that sodium bicarbonate may improve single-bout high intensity cycling and running if the exercise test is of sufficient intensity and lasts approximately between 30 seconds and 12 minutes (Grgic et al., 2021b). There is also strong evidence that sodium bicarbonate enhances performance during multiple bouts of high intensity exercise that have a high anaerobic metabolic demand and/or activities that last longer than 30 seconds but less than 10 minutes (Grgic et al., 2020). It may also enhance performance in endurance events if they include sprints during or at the end of the competition (de Oliveira et al., 2021; Grgic et al., 2021b). Based on a recent umbrella review (Grgic et al., 2021a), acute sodium bicarbonate supplementation enhances peak anaerobic power, anaerobic capacity, performance in endurance events lasting between 45 seconds and 8 minutes, muscle endurance, 2000 meters rowing performance and high intensity intermittent running, with no significant difference between sodium bicarbonate and placebo in general mean power, muscle strength or RSA.

The study of the effects of sodium bicarbonate on physiological variables, such as pH, HCO_3^- , VO_2 , VCO_2 or lactate, has not been as prevalent as the study of its effects on performance. According to the meta-analysis of Calvo et al. (2021), ingesting sodium bicarbonate improves pH, HCO_3^- and base excess in the blood during exercise. Furthermore, blood lactate is improved in anaerobic based exercise but not during aerobic tasks. Regarding respiratory parameters, sodium bicarbonate does not seem to improve VO_2 , VCO_2 or pO_2 but it improves pCO_2 during anaerobic exercise (Calvo et al., 2021). Overall, this meta-analysis suggests that sodium bicarbonate may be helpful in anaerobic activities, such as football, but makes no difference in exercises mostly dependent on the oxidative system. Nevertheless, more studies are needed to clarify the effects of this supplement on gas exchange and, as far as it is concerned, no study to date has proposed to investigate the effects of sodium bicarbonate specifically on muscle oxygenation.

2.3.4 Factors influencing the effects of sodium bicarbonate on the body

The effects on performance induced by this supplement are highly dependent on type, duration and intensity of the task since a potential ergogenic effect requires the exercise to be limited by the accumulation of H^+ (de Oliveira et al., 2021; de Sousa et al., 2021; Heibel et al., 2018; Macutkiewicz & Sunderland, 2018). The effects of sodium bicarbonate may also be affected by dose, timing of ingestion, prior exercise and training status, as well as physiological and genetic differences between athletes and different experimental designs and samples (de Oliveira et al., 2021; Heibel et al., 2018; Macutkiewicz & Sunderland, 2018; Saunders et al., 2021). Furthermore, trained athletes may have smaller gains compared with non-trained individuals and larger effects were reported when exercise protocols were performed following prior exercise (Burke, 2017; de Oliveira et al., 2021; Heibel et al., 2018). Besides, benefits may be greater in small muscle groups due to a higher blood flow, resulting in a greater H^+ exchange within the muscle (Grgic et al., 2020).

Although some studies demonstrated that chronic supplementation with sodium bicarbonate may be a strategy to enhance performance and promote aerobic adaptations associated with improved mitochondrial efficiency in slow twitch fibers, it is recommended to intermittently incorporate it only when certain training outcomes are

desired, like maximising the number of repeated efforts at top speed (Siegler et al., 2016). The protocols of acute supplementation are actually the most commonly applied in the literature and appear to be the most effective (Saunders et al., 2021). Besides, prolonged use of sodium bicarbonate may increase the risk of hypernatremia, electrolyte shifts and systemic pH changes (Durkalec-Michalski et al., 2020a). Chronic supplementation may also lead to a diminished HCO_3^- reabsorption by the kidneys in the long term and therefore abolish the physiological advantage (Dalle et al., 2019). Nevertheless, some studies did not find any benefits of supplementing with sodium bicarbonate, either acutely or chronically, which can be associated with the fact that studies in sport nutrition tend to be performed in small participant samples, leading to a low statistical power (Grgic et al., 2020). In fact, responses to supplements are highly individual and sport sciences research traditionally involves small sample sizes, so the presence of one or two outliers or contrary responders within a group can render the mean finding from the intervention using probability statistics as a non-significant outcome (Burke, 2017).

2.4 THE FEMALE ATHLETE

2.4.1 The relevance of using female athletes in sport sciences research

The increasing number of women in sport, visible in the percentage of 48.8% of female athletes present in the Olympic Games of Tokyo, emphasizes the need to deepen research with samples of women as object of study, accounting for the fact that little is known about the influence female gender characteristics have on performance (Elliott-Sale et al., 2021; Elliott-Sale et al., 2020). In fact, the heterogeneity in the results of studies that have involved female participants, due to the different hormonal profile each woman manifests, is responsible for the fact that most investigations in the field of sports is performed exclusively on men (Elliott-Sale et al., 2021). Regarding football, there is considerably less research performed on female compared to male players and, specifically, the investigation of RSA in female footballers is very limited (de Sousa et al., 2021; Lockie et al., 2020).

Women's football has increased in popularity and is currently one of the fastest growing sports in the world (de Sousa et al., 2021). The estimated number of female football players worldwide is 26 million and this number increased approximately 50% in recent years (Dobrowolski et al., 2020; Gonçalves et al., 2021). Over the last decade, the level of performance and professionalism within female football has progressed dramatically since many women now have full time contracts and the physical demands of the game have increased (Sheridan et al., 2021). The total distance covered by female football players during football matches is on average 10.5 km with 1.5 to 2 km completed as high intensity running (de Sousa et al., 2021; Gonçalves et al., 2021).

Many women undergoing extensive training periods may develop low energy availability, menstrual dysfunction and impaired bone health (female athlete triad), compromising performance and health by increasing the risk of stress fractures and osteopenia and osteoporosis later in life (Daily & Stumbo, 2018; de Sousa et al., 2021; Dobrowolski et al., 2020). The physiological, endocrine and anatomical differences between men and women can put female athletes at a greater risk of certain injuries, such as anterior-cruciate ligament injury, whereby the ingestion of some supplements may prevent or help in the rehabilitation process, promoting health, recovery and performance (Sheridan et al., 2021). Nevertheless, few studies have investigated the difference in response to supplements between genders, even knowing the morphological and physiological differences between men and women and the influence menstrual cycle may have on performance (Burke, 2017; de Sousa et al., 2021; Sheridan et al., 2021). Despite this gap, it is generally assumed that female athletes respond similarly to their male counterparts when circumstances are matched (Burke, 2017). Even so, because gender differences appear in substrate utilization during exercise, more research in women may clarify whether female football players would benefit from different dietary recommendations (de Sousa et al., 2021).

Regarding sodium bicarbonate supplementation, only 20% of the existing studies involved women as participants, of which only 7.4% provided group analyses exclusively in women (Grgic et al., 2021b; Saunders et al., 2021). Although this supplement is associated with improvements in performance during repeated sprints, a recurring action during football, this assumption requires more research, especially regarding the female athlete (Oliveira et al., 2017). Therefore, little is known about the influence gender may have on this domain, although it is known that women have less tolerance to high intensity

exercise, due to a smaller muscular mass and number of fast twitch fibers, as well as a diminished glycolytic capacity, due to a diminished enzyme activity, leading to less acidosis (Saunders et al., 2021).

This can implicate that women may have a smaller response to bicarbonate supplementation, due to a smaller reduction in pH (Saunders et al., 2021). Nevertheless, a meta-analysis of studies in this domain (Saunders et al., 2021) demonstrated that supplementation with sodium bicarbonate leads to considerable alterations of this substance in the blood, provoking a positive ergogenic effect on performance, of small to moderate effects, but without evidence of need for a specific supplementation to the female gender, with the general recommendations of 0.2 to 0.3 g/kg of body mass ingested 60 to 180 minutes before exercise being considered adequate. In fact, sodium bicarbonate supplementation seems to be similar across genders in regard to potential ergogenic effects (de Oliveira et al., 2021; de Sousa et al., 2021).

2.4.2 Methodological considerations for studies with female athletes

The scientific research with female athletes should attend to each woman's hormonal profile, considering the phase of the menstrual cycle in which athletes are, intra and interindividual variations and usage of hormonal contraceptive, for example (Elliott-Sale et al., 2021; Elliott-Sale et al., 2020). Research in female athletes requires then the intervention of a multidisciplinary team, especially in the presence of excessive fatigue, performance decrease and recurrent injury or illness, using questionnaire and laboratorial tests in order to foil the triad (Daily & Stumbo, 2018).

Elliott-Sale et al. (2021) suggest that one of the most important factors in research with female athletes is the consistency in the description of the participants of the study, presenting the inclusion/exclusion criteria for their selection, especially regarding hormonal parameters. According to this group of authors, for studies in which the goal is studying the influence of menstrual cycle on some variable, a biochemical confirmation of the phase of the menstrual cycle in which athletes are is required, as well as a description of its normal characteristics, but if the goal is simply to study the performance characteristics of a team, the report of the phase of the menstrual cycle seems to be sufficient. Secondary indications point to a standardization of the time of the day in which

data is collected, prior exercise, diet and consumption of caffeine, alcohol and/or tobacco, increasing precision and validity of the sample description and reducing the variability of the study (Elliott-Sale et al., 2021).

CHAPTER 3: METHODS

3.1 PARTICIPANTS

For the participation in this study some inclusion and exclusion criteria were established. To be included in the research, participants should be female football players from any of the 3 major female divisions in Portugal, aged between 18 and 30 years and able to provide informed consent. The exclusion criteria were any lower limb disabilities, severe respiratory issues and intolerance to sodium bicarbonate or any other similar supplement. The recruitment of the participants was done through contact with the technical teams of some female football clubs in the area of Lisbon and directly with female football players studying at the university. The purpose of the research, the methodological procedures and the possible risks associated with the participation in the study were explained to the athletes and they signed an informed consent. The study was approved by the Ethics Board for Research of Faculdade de Motricidade Humana (Nº.: 44/2021) and carried out in accordance with the Declaration of Helsinki.

Power and sample calculations (G-Power, Version 3.1.9.2) were based on an effect size of 0.88 (McNaughton et al., 1997) for total work performed in the sprints, a power of 0.85 and a significance of 0.05, yielding a total sample size of 11 participants. We accounted for a possible 10% dropout. After the recruitment process, 12 athletes were able to participate in the study. 1 participant withdrew due to injury leading to a total sample size of 11 athletes. The average years of practice was 7 ± 4 . A typical microcycle of the participants was composed of 3 to 5 sessions of training per week with durations that varied from 90 to 120 minutes, with a game on the weekend. At data collection date no participant was ingesting any supplements other than the ones involved in the study for at least 1 month. Of the 11 participants, 10 were naturally menstruating and 1 was using hormonal contraceptive (monophasic oral contraceptive pill).

3.2 EXPERIMENTAL DESIGN

The design of the study consisted in a double blinded randomized crossover trial, which means all athletes experienced the experimental condition and the control condition. The difference between conditions resided in the oral ingestion of sodium

bicarbonate (experimental) or cellulose (control) before an exercise protocol. Participants were asked to visit the laboratory in 3 non-consecutive days. Sessions were separated by a period of at least a week, choosing days that minimized impact on athletes' regular training sessions and daily routines. Data collection was done in the more convenient phase of the day for each athlete but the time of data collection was replicated within sessions. Within a period of time not exceeding 1 month, all athletes completed the 3 sessions.

The first session aimed to characterize the participants regarding weight, height, body composition and water pools, as well as allow familiarization of the athletes with the research team, the laboratory, the equipment and the exercise protocol. Then, participants performed the planned repeated sprints protocol described below in topic 3.6, after adjusting the height of the cycle ergometer seat to the height of each athlete, looking for the most comfortable position for each athlete. Before performing the exercise protocol, taking into account the fasting indications for the evaluation of body composition and water pools, athletes ingested a standardized snack, consisting of a cereal bar and a fruit gel. In the other two sessions, athletes executed the repeated sprints protocol under the influence of bicarbonate or placebo, recurring to the same cycle ergometer. Data regarding performance, RPE, lactate accumulation, pulmonary gas exchange and HR, muscle oxygenation and neuromuscular fatigue was collected during these two sessions.

3.3 ANTHROPOMETRY AND BODY COMPOSITION

In the familiarization session, after measuring subjects' weight (Seca, model 761, Hamburg, Germany) and height (Harpenden, Holtain Ltd, Crosswell, UK) to the nearest 1.0 kg and 0.1 cm, respectively, subjects were asked to lay down in a supine position for 10 minutes before undergoing a bioelectrical impedance analysis with a single frequency (50 kHz) device (BIA 101 Anniversary, Akern, Florence, Italy). The measurements were done with the lower limbs approximately 45 degrees apart from the median line of the body and the upper limbs about 30 degrees from the trunk. Before placing the 4 electrodes on the dorsal surfaces of the right hand and foot the skin was cleaned with alcohol. The source electrodes were placed on the hand, in the middle of the dorsal surface proximal

to the metacarpal-phalangeal joint, and on the foot, in the middle of the dorsal surface proximal to the metatarsal-phalangeal joint. The detector electrodes were placed on the wrist at the midline between the distal prominences of the radius and ulna and in the ankle joint at the line between the malleoli (Fukuoka et al., 2022).

The raw data resistance and reactance were registered and used to calculate fat free mass (FFM), total body water (TBW) and extracellular water (ECW) recurring to the equations presented below. Fat mass (FM) was obtained subtracting FFM from weight and intracellular water (ICW) subtracting ECW from TBW. Body mass index (BMI) was calculated as the quotient of weight and the square of height. Athletes were instructed not to eat in the previous 3 hours as well as not to ingest liquids 1 hour before the evaluation. Furthermore, they were told not to drink coffee or alcohol in the previous 4 and 24 hours, respectively, and not to involve themselves in physical exercise in the previous 12 hours.

$$FFM = - 2.261 + 0.327 * S^2/R + 0.525 * Wt + 5.462 * Sex$$

(Matias et al., 2021)

$$TBW = 0.286 + 0.195 * S^2/R + 0.385 * Wt + 5.086 * Sex$$

$$ECW = 1.579 + 0.0555 * S^2/R + 0.127 * Wt + 0.006 * S^2/Xc - 0.932 * Sex$$

(Matias et al., 2016)

Where S is stature in cm, R is resistance in ohm (Ω), Wt is weight in kg, sex is 0 if female or 1 if male and Xc is reactance in ohm (Ω).

Since only female athletes participated in this study, the equations can be reduced to the following:

$$FFM = - 2.261 + 0.327 * S^2/R + 0.525 * Wt$$

$$TBW = 0.286 + 0.195 * S^2/R + 0.385 * Wt$$

$$ECW = 1.579 + 0.0555 * S^2/R + 0.127 * Wt + 0.006 * S^2/Xc$$

3.4 SUPPLEMENTATION PROTOCOL

The ingestion of sodium bicarbonate or placebo occurred under the form of capsules in quantities of 0.3 g/kg of body mass. Supplement ingestion was divided in 2

separate doses to minimize possible gastrointestinal effects. The first take (0.2 g/kg) was done together with a carbohydrate enriched meal 120 minutes before the beginning of the exercise protocol and the second take (0.1 g/kg) occurred 60 minutes before the beginning of the exercise protocol. Participants received individualized information regarding meal content according to their eating habits and were advised to ingest liquids together with the supplements. Neither the participants nor the investigators involved in data collection were aware of the capsules content. This was restricted to the investigator responsible for randomization of the supplement intake order, which was done by block randomization.

3.5 GASTROINTESTINAL EFFECTS OF SODIUM BICARBONATE

Regarding supplementation, at each repeated sprints session, before the exercise protocol, athletes were asked to fill a questionnaire about possible gastrointestinal symptoms associated with supplement ingestion, based on the questionnaire applied by Miller et al. (2016). This questionnaire aimed to assess the level of symptoms on a scale of 0 to 10, in which 0 represented “no symptom” and 10 represented “severe symptom”, regarding nausea, flatulence, stomach cramping, belching, stomach ache, bowel urgency, diarrhea, vomiting and stomach bloating.

3.6 REPEATED SPRINTS PROTOCOL

The repeated sprints protocol was performed in a cycle ergometer (Monark Ergomedic 894 E, Monark Exercise AB, Vansbro, Sweden) and was composed of 3 sets of 6 sprints with 6 seconds duration executed at maximal velocity. Repetitions were separated by 24 seconds of active recovery and participants were conceded 5 minutes of passive recovery to rest between sets. The exercise workload was set at 4% of the body mass of each individual. The repeated sprints protocol was preceded of a 5-minute warm up in which athletes pedalled at a speed of 60 to 70 rotations per minute. After the warm up, athletes were asked to perform a single maximal sprint, registering the value of power obtained in the cycle ergometer. Then, 95% of this value was calculated and established

as the minimal target value to achieve in the first sprint of the protocol. If athletes could not achieve that value, 5 minutes of rest were conceded before repeating the process. All participants were asked to perform the sprints in an all-out performance, i.e. to give their best effort throughout the sprints and not to pace their efforts. They were given strong verbal encouragement during the entire test and were asked to keep in a sitting position during the sprints.

3.7 MEASUREMENTS ASSOCIATED WITH THE REPEATED SPRINTS TASK

3.7.1 Performance

During the experimental sessions, performance was evaluated by monitoring mean power output (MPO), peak power output (PPO) and total work (TW) in each set of the RST. MPO was obtained averaging all values of power obtained in the set while PPO corresponds to the highest value of power observed in the set. Work was assessed by the product of the mean power of each sprint and sprint duration. TW corresponds to the sum of the 6 values of work obtained in each set. The sprint decrement (Sdec) was calculated to assess power decrement during each set using the following formula and considering the peak power values of each sprint (S_i) (Girard et al., 2011):

$$Sdec (\%) = \left(1 - \frac{S_1 + S_i + \dots + S_6}{S_{best} \times \text{number of sprints}}\right) \times 100$$

3.7.2 Rated perceived exertion

Rated perceived exertion was evaluated immediately after the completion of each set of repetitions using Adapted Borg's Scale in which 0 represented "rest" and 10 represented "exhaustion" (Foster et al., 2001).

3.7.3 Lactate accumulation

A sample of capillary blood was collected from the ear lobe for lactate concentration assessment using a lactate measurer (Lactate Pro 2, KDK Corporation, Kyoto, Japan) before the beginning of the repeated sprints protocol, 1 minute after the end of each set and at minute 1 and every 2 minutes after the RST was completed until the value started to decrease. Regarding the values obtained after the RST, the maximal value achieved was considered for analysis.

3.7.4 Pulmonary gas exchange and heart rate

During the entire task, VO_2 , VCO_2 , respiratory exchange ratio (RER) and ventilation (VE) were measured breath-by-breath using a gas analyser (MetaMax 3B, Cortex Biophysik, Leipzig, Germany). HR was evaluated continuously during the exercise protocol using a HR monitor (H7, Polar Electro Oy, Kempele, Finland). The gas analyser was previously calibrated according to the manufacturer's instructions and the measurement of variables started 3 minutes before the beginning of the exercise protocol in order to stabilize its values. Data was interpolated second by second. The maximum 6-second moving average of relative VO_2 and the integral of VO_2 and VCO_2 were calculated for each set of the exercise protocol. For HR, RER and VE, was considered the maximum value obtained after calculating the 6-second moving average for each variable.

3.7.5 Muscle oxygenation

Oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb) and tissue oxygenation index (TOI) were evaluated continuously throughout the whole exercise protocol recurring to the near-infrared spectroscopy technique (Niro-200NX, Hamamatsu Phototonics, Hamamatsu, Japan). The probe was placed in the bulkier portion of the vastus lateralis of the quadriceps muscle after hair removal and cleaning of the area with alcohol. A dark band was placed around the probe to avoid the influence of external light. The device settings were adjusted to the fat fold of that muscle area, which was measured

using a calliper (Slim Guide, Creative Health Products, Plymouth MICH, USA). The zero set of the measurement occurred 2 minutes before the beginning of the exercise protocol to stabilize the values of each variable. Data was collected twice per second. Since the resting state was used as reference, the values of O₂Hb are negative. The integral of O₂Hb and HHb and the 6-second moving average of TOI were calculated for each set of the exercise protocol. The maximum value obtained in the 6-second moving average was considered for TOI.

3.8 NEUROMUSCULAR FATIGUE ASSESSMENT

With the aim of understanding the impact of sodium bicarbonate intake in association with the performance in a RST at the neuromuscular level, athletes were asked to execute a CMJ in a contact mat (Chronojump Boscosystems, Software Version 2.2.0) before and after the repeated sprints protocol, in both supplement sessions, accessing values of JH. The CMJ protocol was composed of 3 jumps. Subjects were asked to place their hands on the hips and maintain them there throughout the test. When ready, athletes squatted down until the knees were bent at 90 degrees, then immediately jumped vertically as high as possible, landing back on the mat with both feet at the same time. It was conceded participants 10 seconds to rest between trials and the best jump was considered for analysis.

3.9 DATA ANALYSIS

Statistical analysis was performed recurring to IBM SPSS Statistics (Version 27.0, NY, IBM) and R software (version 4.2.0, open-source code, General Public License). Mean and standard deviation were used to summarize the data. Normal distribution of variables was tested using Shapiro-Wilk test. If normal distribution was verified, the data was analysed using a 2-way analysis of variance (ANOVA) considering 2 within factors: the supplement condition and the timing of measurement. When there were more than 2 moments of measurement, sphericity was tested using Mauchly's test. If the sphericity

assumption was not verified, the Greenhouse-Geisser correction was considered when analysing the data from ANOVA. In the cases where normal distribution was not verified, the statistical analysis was performed with the nparLD module of the R software to perform a non-parametric 2-way ANOVA-type test. Both the parametric and the non-parametric ANOVA tests analyse the effects of each factor separately and the interaction between factors.

Whenever a statistical significance was observed in the parametric ANOVA test, post-hoc paired comparisons were performed with the Bonferroni correction to localize the differences, having in consideration the existence or not of interaction between the 2 factors. Statistical significance was set at $p < 0.05$. Whenever a statistical significance was observed in the non-parametric 2-way ANOVA-type test, a Friedman test and the post-hoc paired comparisons with the Bonferroni correction were performed to localize the differences between moments of evaluation in each supplementation condition and a Wilcoxon test was used to compare the 2 conditions at each moment of evaluation. Statistical significance was adjusted according with the number of tests performed: $p < 0.05/2$ for the time factor, as 2 Friedman tests were performed and the Bonferroni correction was already introduced in the post-hoc paired comparisons, and $p < 0.05/3$ for the condition factor, as 3 Wilcoxon tests were performed.

Although athletes were equally randomized for each group, some had to repeat 1 of the sessions due to an equipment failure, so the planned supplement intake order suffered modifications. Therefore, in order to access the carryover effect, for each variable, the sum of the values of all moments of measurement of each participant was calculated and the results for the participants that performed the supplement intake as placebo-sodium bicarbonate were compared with the ones for the participants that performed the supplement intake as sodium bicarbonate-placebo using a Mann-Whitney test. After the Mann-Whitney test was performed, no carryover effect was observed in any of the variables.

CHAPTER 4: RESULTS

4.1 PARTICIPANTS CHARACTERIZATION AND GASTROINTESTINAL SYMPTOMS INDUCED BY THE SUPPLEMENTS

Data regarding age, weight, height, BMI, body composition, specifically FFM and absolute and percentage FM, and water pools, specifically TBW, ECW and ICW, are presented in Table 1. Values represent mean \pm standard deviation.

Table 1. Participants characterization.

<i>Parameter</i>	<i>Mean \pm standard deviation</i>
Age (years)	20 \pm 2
Weight (kg)	56 \pm 6
Height (m)	1.633 \pm 0.057
BMI (kg/m ²)	21.0 \pm 2.10
FFM (kg)	43.8 \pm 4.24
FM (kg)	12.2 \pm 2.03
% FM	21.7 \pm 2.42
TBW (kg)	31.8 \pm 2.91
ECW (kg)	13.9 \pm 1.10
ICW (kg)	17.9 \pm 1.84

In what concerns to the menstrual cycle, in the placebo session, 2 athletes reported being in the follicular phase of the cycle and 8 in the luteal one. In the experimental session, 4 athletes reported being in the follicular phase, 5 in the luteal phase and 1 in ovulation. 1 athlete reported not having a regular menstrual cycle so she could not identify the phase of the cycle in any of the sessions.

In regard to the gastrointestinal symptoms induced by the supplements, 3 athletes reported 1 symptom each, associated with sodium bicarbonate ingestion, respectively belching (rate 4 in a scale of 0 to 10), stomach ache (rate 4) and stomach cramping (rate 2), but none of them mentioned the symptoms were and impediment to performing the protocol.

4.2 PERFORMANCE IN THE REPEATED SPRINT TASK

Table 2 presents the results of performance regarding MPO and PPO, Sdec and TW at each set in the RST in both supplementation conditions. Also, schematic representations of these results are presented in Figures 2, 3, 4 and 5.

Table 2. Results of performance in the RST: MPO and PPO, Sdec and TW.

Variable	PL			SB		
	S1	S2	S3	S1	S2	S3
MPO (W)	296±35.0*	285±31.7	288±28.0	288±28.5	292±28.1	292±29.2
PPO (W)	354±44.8:	356±47.5	355±40.5	346±41.2:	361±47.6	361±44.1
Sdec (%)	4.43±1.56	6.04±2.95	5.55±2.19	3.87±2.51	5.68±3.75	4.71±3.58
TW (J)	10656±1260	10274±1140	10357±1009	10084±1340	10385±1260	10497±1050

*Significantly different when compared with S2 in PL, $p < 0.05$

:Significantly different when compared with S3 independently of condition, $p < 0.05$

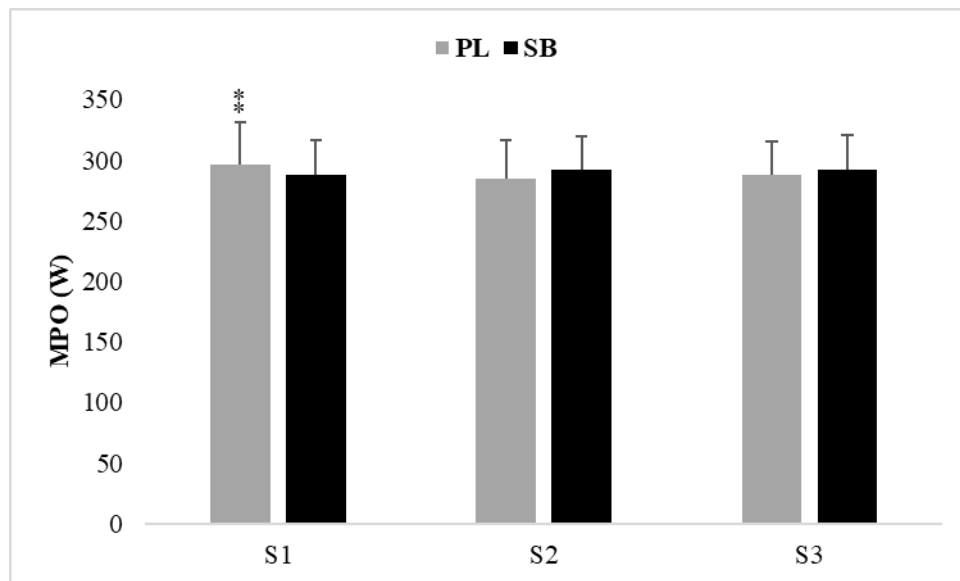


Figure 2. Results of performance in the RST: MPO.

Regarding performance, there was a significant interaction between time and condition for MPO ($F(1.162, 10.457) = 4.854, p = 0.047$). Specifically, at S2, mean values of MPO were significantly lower than at S1 in PL ($p = 0.012$), while in SB they did not change with time, suggesting that SB possibly allowed the athletes to maintain their MPO throughout the 3 sets.

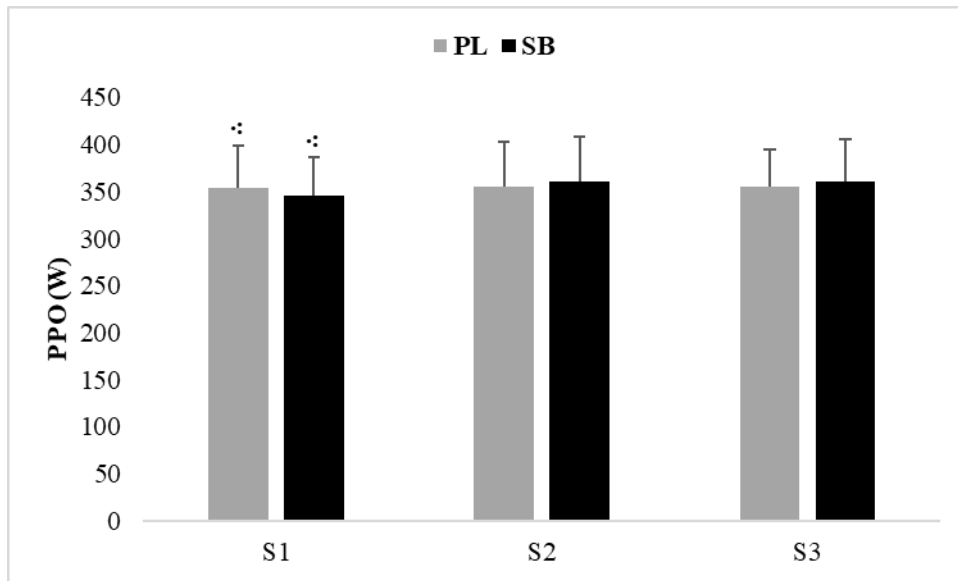


Figure 3. Results of performance in the RST: PPO.

For PPO, there was a significant main effect of time ($F(2, 18) = 5.563, p = 0.013$). It was observed that the mean value at S3 was significantly higher than at S1 ($p = 0.008$).

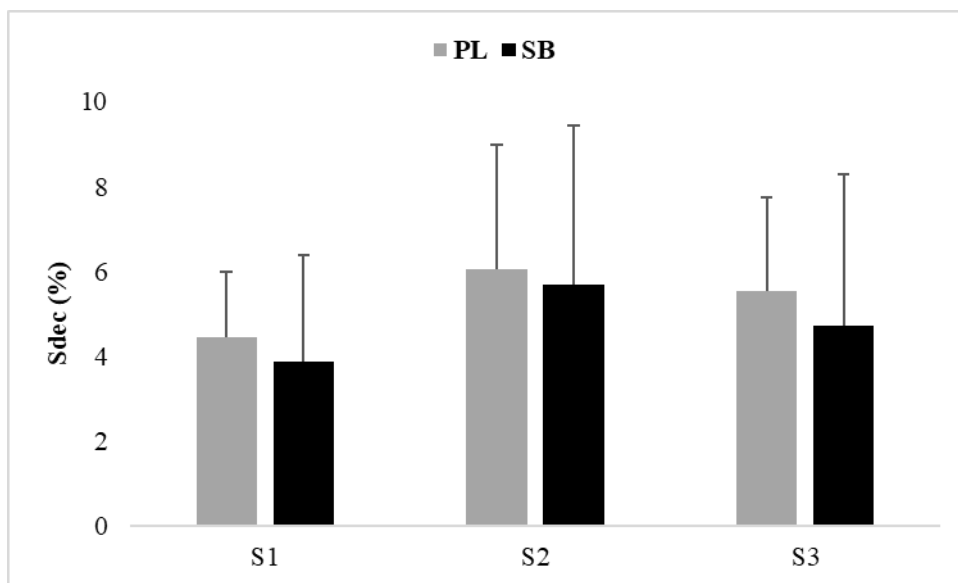


Figure 4. Results of performance in the RST: Sdec.

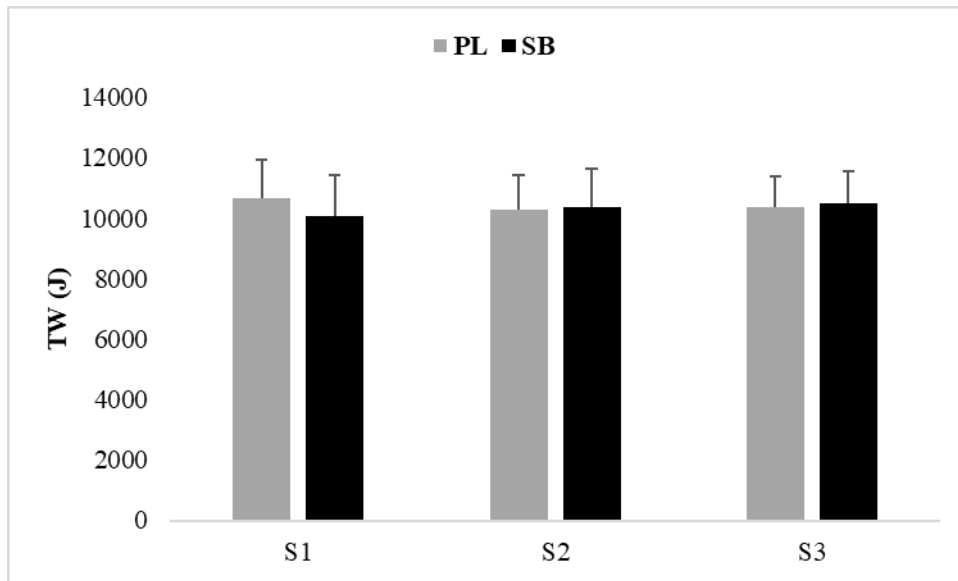


Figure 5. Results of performance in the RST: TW.

No significant main effect of time or condition nor interaction between time and condition was found for Sdec or TW.

4.3 RATED PERCEIVED EXERTION

The results for RPE reported after each series in both supplementation conditions are presented in Figure 6. The ∴ represents a significant difference compared with S3 independently of condition, for $p < 0.05$. Neither the interaction between time and condition nor the condition effect were significant but there was a significant effect of time ($F(1.312, \infty) = 27.049, p < 0.001$). More precisely, the values of RPE were statistically higher at S3 when compared with S1 for both conditions (PL: $p = 0.002$; SB: $p = 0.001$).

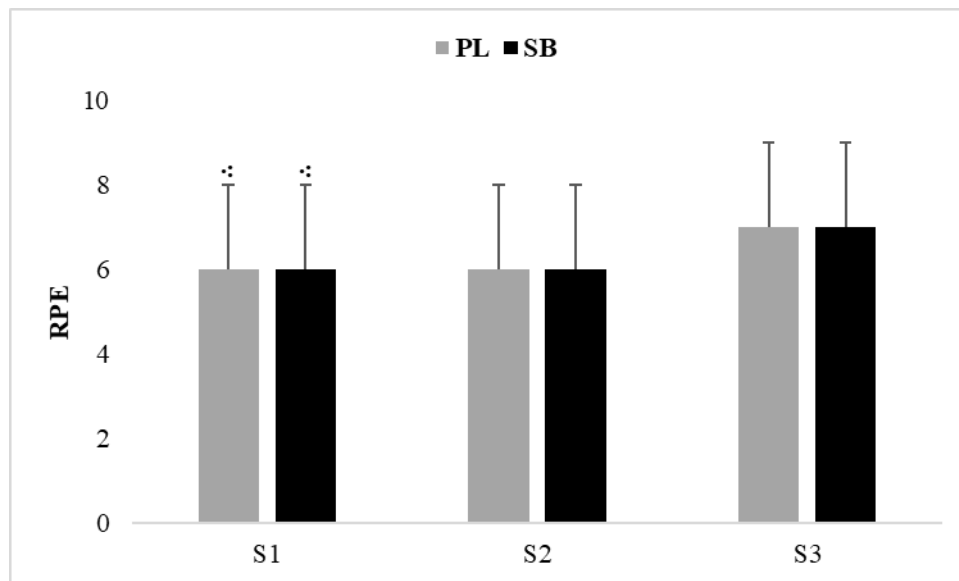


Figure 6. Results of RPE.

4.4 LACTATE ACCUMULATION

In regard to lactate accumulation, the results obtained before (Rest), after each set (S1, S2 and S3) and after the RST (Max) in both supplementation conditions are described in Table 3. Figure 7 contains a schematic representation of the results.

Table 3. Results of lactate accumulation.

Condition	Lactate (mmol/L)				
	Rest	S1	S2	S3	Max
PL	1.87±0.52***†	7.44±2.02***†	9.43±2.36•	10.1±2.92•	10.9±3.55•
SB	1.78±0.46***†	6.91±2.77***†	11.2±3.53†•	13.3±4.17•	14.0±4.32•

*Significantly different when compared with S1 in either PL or SB, $p < 0.05$

‡Significantly different when compared with S2 in either PL or SB, $p < 0.05$

‡Significantly different when compared with S3 in either PL or SB, $p < 0.05$

†Significantly different when compared with Max in either PL or SB, $p < 0.05$

•Significantly different between conditions, $p < 0.05$

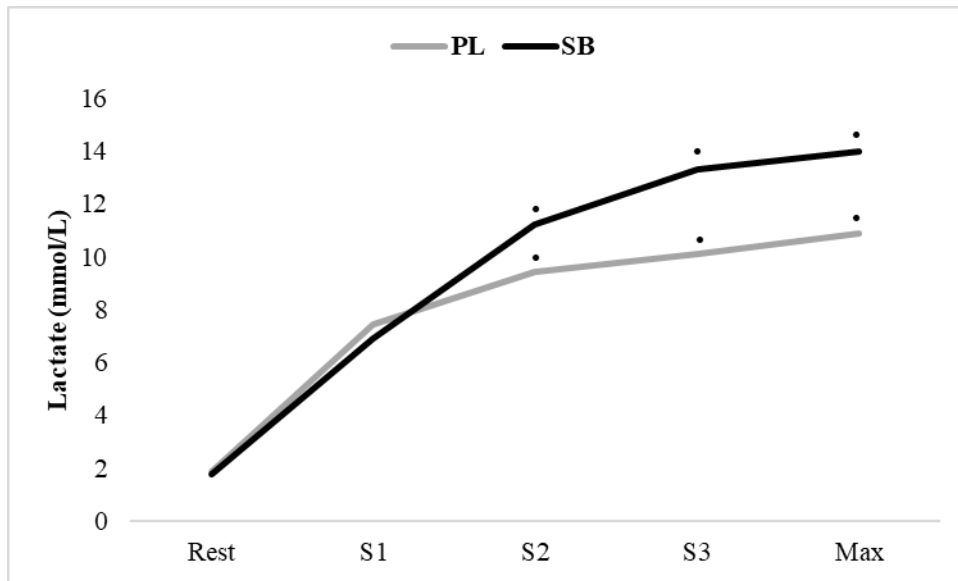


Figure 7. Results of lactate accumulation.

According to the results, there was a significant interaction between time and condition ($F(4, 40) = 7.741, p < 0.001$). Significant main effects of time ($F(1.801, 18.013) = 67.016, p < 0.001$) and condition ($F(1, 10) = 8.753, p = 0.014$) were also observed. Specifically, in PL, lactate was significantly lower at Rest when compared with at S1, S2, S3 and at Max ($p < 0.001$ in all cases). Also, it was statistically lower at S1 in comparison with S2 ($p = 0.011$), S3 ($p = 0.030$) and Max ($p = 0.048$). In SB, lactate was significantly lower at Rest when compared with at S1 ($p = 0.002$), S2, S3 and at Max ($p < 0.001$ in the 3 cases). Furthermore, it was lower at S1 than at S2 ($p = 0.005$), S3 ($p = 0.002$) and at Max ($p = 0.002$) and at S2 when compared with at Max ($p = 0.030$). In addition, the values of lactate were significantly higher in SB than in PL at S2 ($p = 0.019$), S3 ($p = 0.006$) and at Max ($p = 0.010$). This suggests that lactate increased more in SB throughout the RST.

4.5 PULMONARY GAS EXCHANGE AND HEART RATE

The results of pulmonary gas exchange, specifically the maximum relative and total amount of O₂ inhaled (VO₂) and total amount of CO₂ exhaled (VCO₂), and the maximum RER, VE and HR observed during each set in both supplementation conditions are presented in Table 4 and Figures 8, 9, 10, 11 and 12.

Table 4. Results of pulmonary gas exchange and HR.

Variable	PL			SB		
	S1	S2	S3	S1	S2	S3
VO₂ (mL/kg/min)	42.3±4.76	41.0±4.85	40.5±4.51	44.4±11.5	42.7±7.24	44.5±9.02
VO₂ (L)	314±29.3	315±30.6	305±53.8	313±33.0	322±34.3	324±40.9
VCO₂ (L)	343±34.0••:	312±29.7•	296±50.4•	354±43.3••:	331±32.3•	326±42.5•
RER	1.28±0.10	1.23±0.08•	1.23±0.13	1.34±0.11**	1.29±0.09•	1.21±0.09
VE (L/min)	93.6±9.67	95.9±9.39	97.1±14.1	90.5±8.63	91.4±7.04	96.2±11.6
HR (bpm)	169±11.3•:	173±11.6	177±12.2	169±10.7•:	173±10.7	178±11.2

**Significantly different when compared with S3 in SB, $p < 0.05$

:Significantly different when compared with S2 independently of condition, $p < 0.05$

•:Significantly different when compared with S3 independently of condition, $p < 0.05$

•Significantly different between conditions, $p < 0.05$

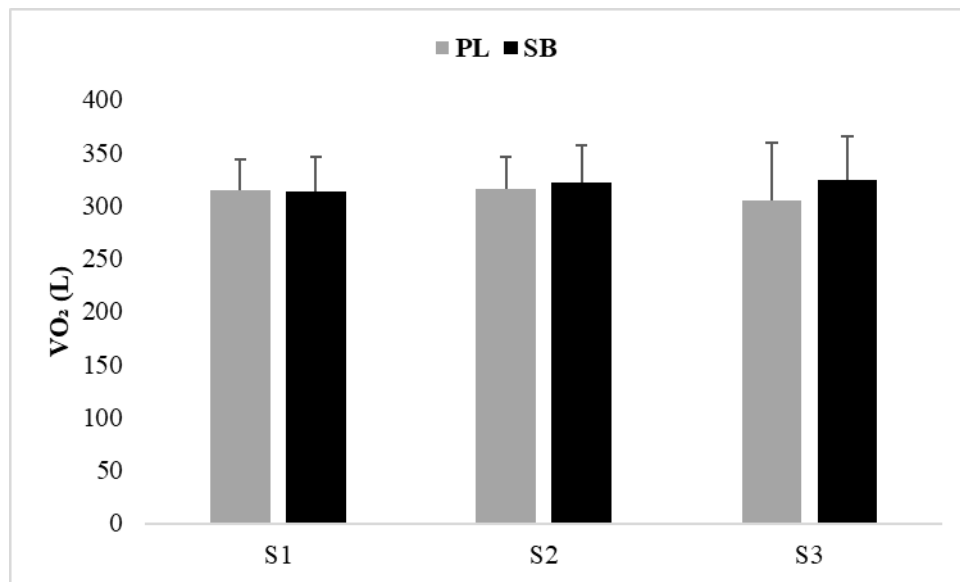


Figure 8. Results of pulmonary gas exchange: VO₂.

In relation to VO₂, it was not found any significant main effect of time or condition nor a significant interaction between time and condition both for the relative values and for the integral of this variable.

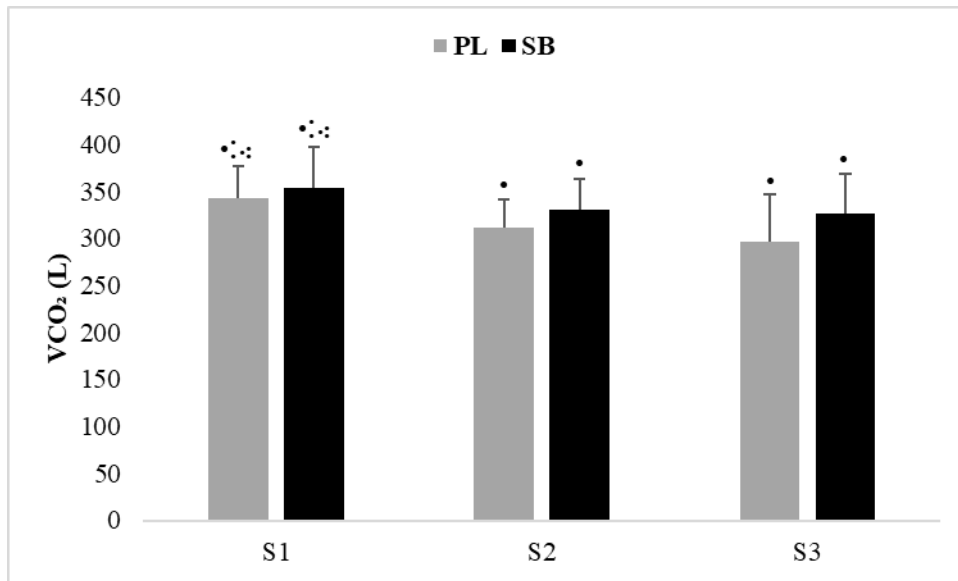


Figure 9. Results of pulmonary gas exchange: VCO₂.

On the other hand, for VCO₂, there was a significant main effect of time ($F(2, 20) = 34.454, p < 0.001$) since both at S2 and at S3 ($p < 0.001$ in both cases) the values were significantly lower than at S1. Furthermore, a significant main effect of condition was also observed ($F(1, 10) = 5.731, p = 0.038$) which relates to the fact that VCO₂ was significantly higher in SB than in PL, suggesting that SB allows a higher removal of CO₂ through breathing.

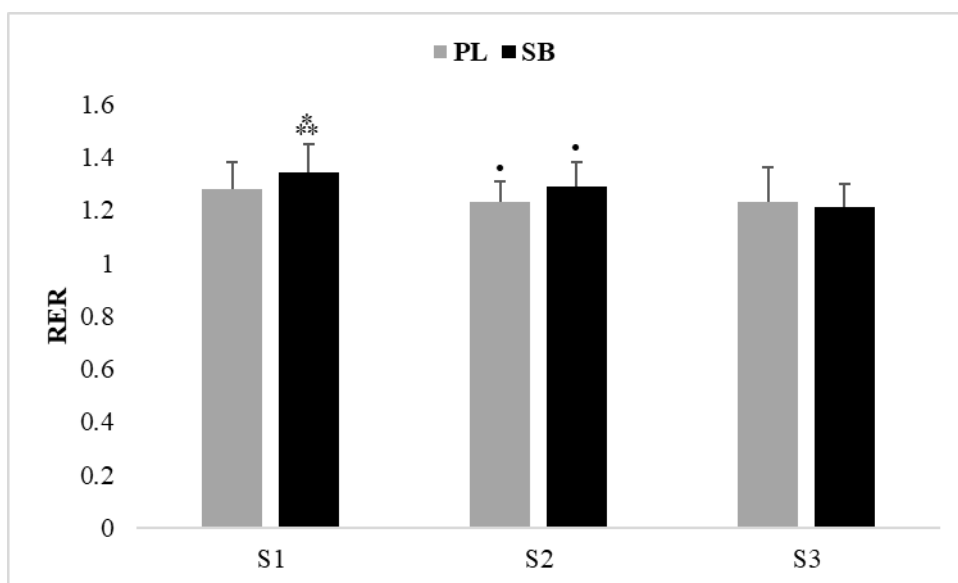


Figure 10. Results of pulmonary gas exchange: RER.

Regarding RER, an interaction between time and condition was observed ($F(1.867, \infty) = 3.635, p = 0.029$). No main effect of condition was detected but there was a significant main effect of time ($F(1.779, \infty) = 16.510, p < 0.001$). In fact, at S2, RER was significantly lower in PL than in SB ($p = 0.018$) and furthermore, in SB, it was significantly lower at S3 when compared with at S1 ($p < 0.001$).

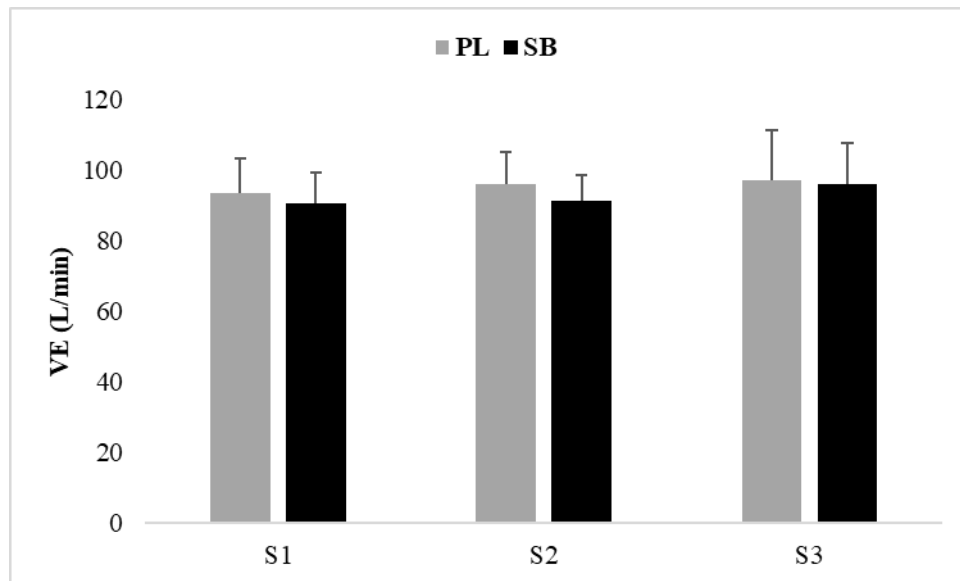


Figure 11. Results of pulmonary gas exchange: VE.

For VE, although a significant main effect of time was found ($F(2, 20) = 3.822, p = 0.039$), the Bonferroni post-hoc procedure was not able to identify any significant difference between sets.

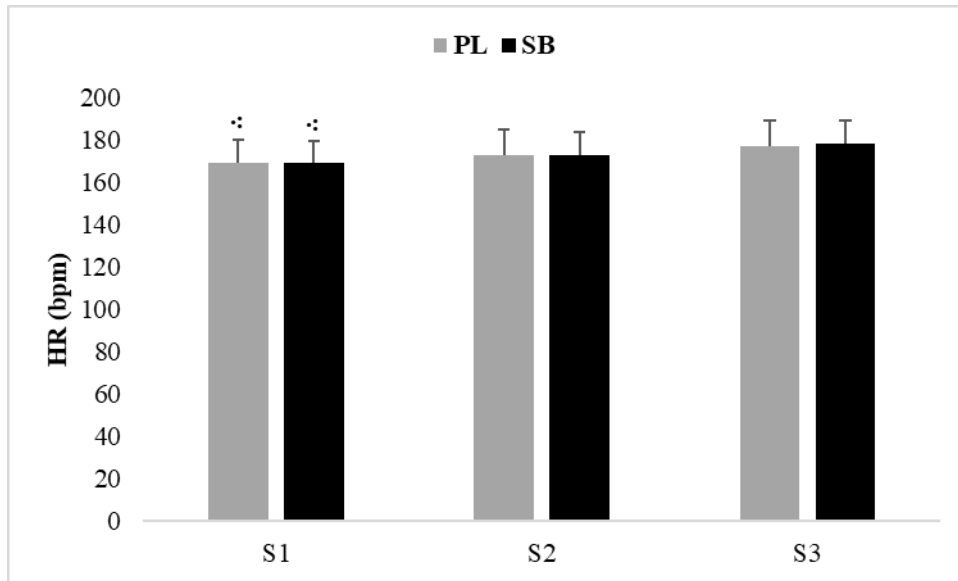


Figure 12. Results of HR.

Regarding HR, neither the condition effect nor the interaction between time and condition were significant but there was a significant effect of time ($F(1.547, \infty) = 88.143$, $p < 0.001$). The results of Friedman test show that HR was significantly higher at S3 when compared with S1 for both conditions ($p < 0.001$ in both cases).

4.6 MUSCLE OXYGENATION

The results obtained with near-infrared spectroscopy for O₂Hb, HHb and TOI are presented in Table 5 and Figures 13, 14 and 15.

Table 5. Results of muscle oxygenation: O₂Hb, HHb and TOI.

Variable	PL			SB		
	S1	S2	S3	S1	S2	S3
O₂Hb	-578±409: [:]	-450±456	-256±555	-547±586: [:]	-241±672	-54.3±642
HHb	336±388: [:]	528±473	454±571	361±266: [:]	581±248	518±325
TOI	72.1±2.51	70.6±3.10 ^{**}	72.7±3.16	72.7±3.08	72.0±2.85	72.4±3.26

[:]Significantly different when compared with S2 independently of condition, $p < 0.05$

[:]Significantly different when compared with S3 independently of condition, $p < 0.05$

^{**}Significantly different when compared with S3 within PL, $p < 0.05$

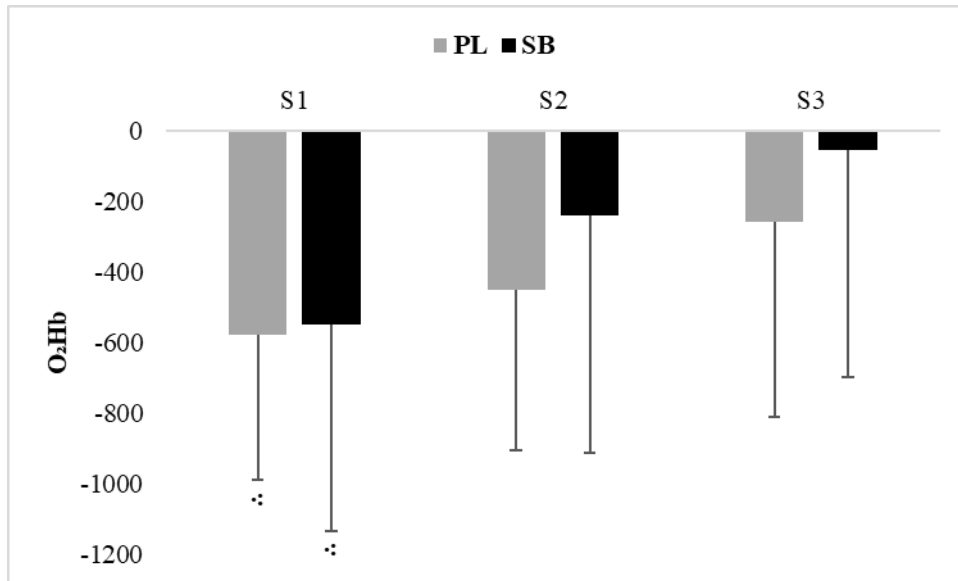


Figure 13. Results of muscle oxygenation: O₂Hb.

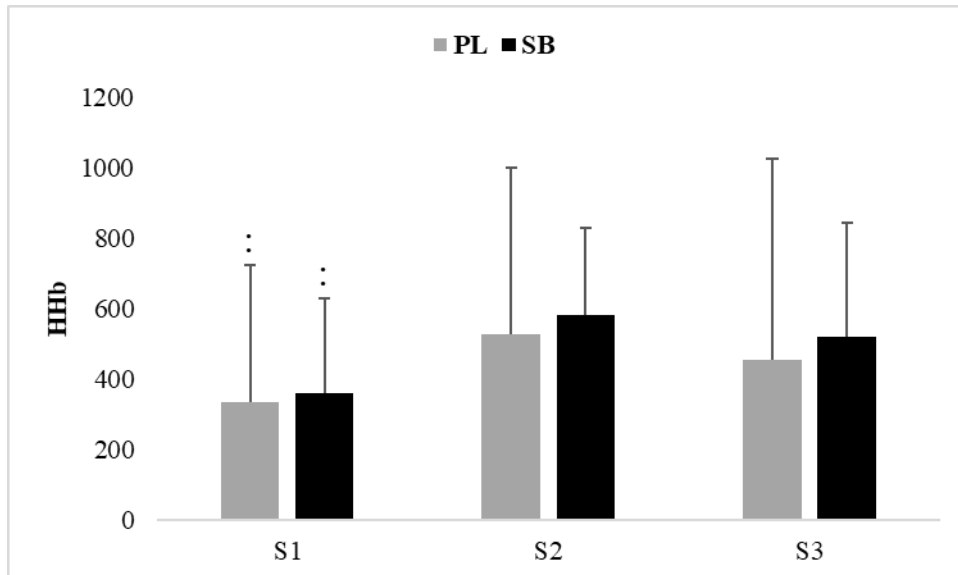


Figure 14. Results of muscle oxygenation: HHb.

Regarding O₂Hb and HHb, neither the interaction between time and condition nor the condition effect were significant but there was a significant effect of time (O₂Hb: $F(1.367, \infty) = 10.702, p < 0.001$; HHb: $F(1.471, \infty) = 13.192, p < 0.001$). Specifically, O₂Hb was significantly higher at S3 than at S1 in both PL ($p = 0.022$) and SB ($p = 0.001$). This suggests that at S3 values for O₂ availability in the muscle are coming closer to the resting values. HHb was significantly higher at S2 than at S1 in both conditions (PL: $p = 0.011$; SB: $p = 0.001$) suggesting a higher O₂ extraction in the muscle in the second set of the RST.

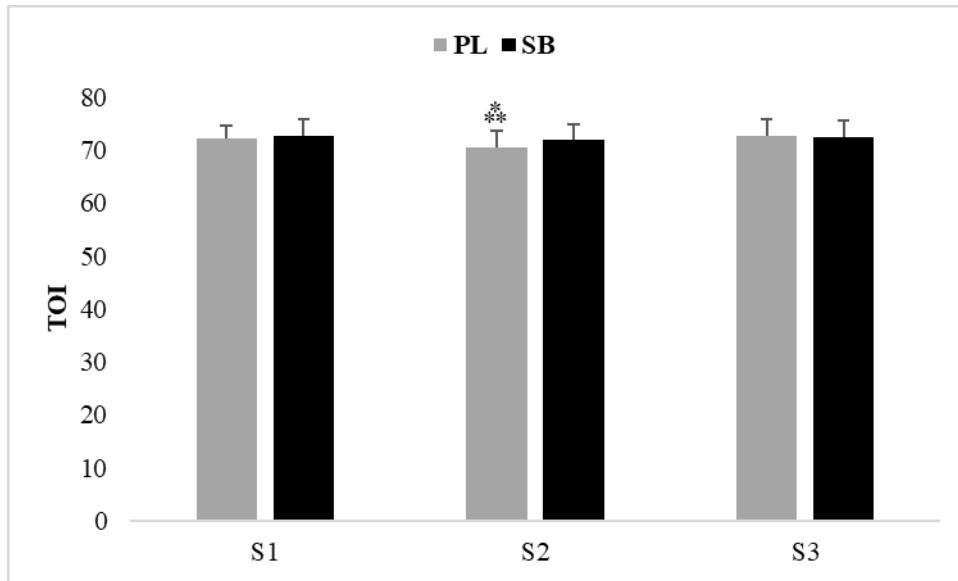


Figure 15. Results of muscle oxygenation: TOI.

In relation to TOI, a significant interaction between time and condition was found ($F(1.452, \infty) = 3.626, p = 0.041$). Also, the condition effect was not significant but there was a significant effect of time ($F(1.232, \infty) = 6.475, p = 0.007$). This was due to the fact that TOI was significantly higher at S3 than at S2 ($p = 0.001$) in PL, while in SB there were no differences between sets.

4.7 NEUROMUSCULAR FATIGUE

In regard to neuromuscular fatigue, the results of JH in the CMJ obtained before and after the RST in both conditions are presented in Table 6. Figure 16 contains a schematic representation of these results.

Table 6. Neuromuscular fatigue: JH in the CMJ.

Variable	PL		SB	
	Pre	Post	Pre	Post
JH (cm)	25.94±3.662‡•	24.02±3.992	25.23±3.783•	25.03±4.607

‡Significantly different when compared with Post in PL, $p < 0.05$

•Significantly different between conditions, $p < 0.05$

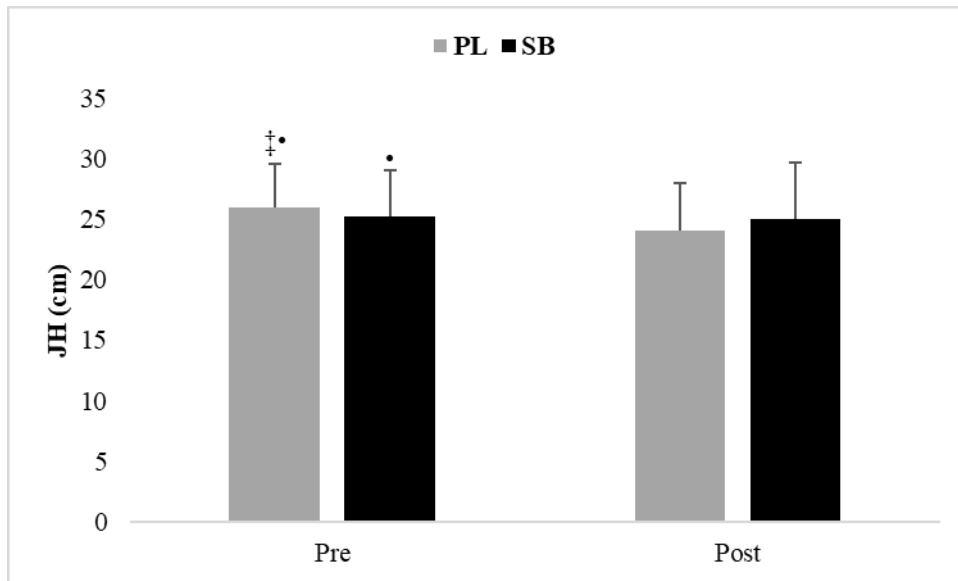


Figure 16. Results of neuromuscular fatigue: JH in the CMJ.

According to the results, there was a significant interaction between time and condition ($F(1, 9) = 5.455, p = 0.044$) and a significant main effect of time ($F(1, 9) = 9.043, p = 0.015$), with the values of JH being statistically lower in pre-exercise in SB than in PL ($p = 0.009$). Furthermore, in PL, the values of JH were significantly lower post-exercise than pre-exercise ($p = 0.003$) while in SB JH was maintained.

CHAPTER 5: DISCUSSION

As far as we are aware, this is the first research where the effects of acute sodium bicarbonate supplementation on RSA were studied in female football players. Furthermore, most studies regarding sodium bicarbonate supplementation focused its effects on performance with only a few exploring the effects of this supplement on physiological variables such as pulmonary gas exchange. The greatest novelty of this investigation relates to the fact that muscle oxygenation was evaluated with a near-infrared spectroscopy device in order to understand if an alkaline environment pre-exercise would influence muscular tissue oxygenation. The main finding of our research is that sodium bicarbonate allows a higher lactate accumulation, induces a greater elimination of CO₂ and shows a tendency to provide a larger muscle oxygenation, factors associated with a later onset of fatigue. Nevertheless, this positive metabolic effects did not traduce into major benefits between conditions in regard to performance other than preventing a decline in MPO in the second set. It was also found that sodium bicarbonate has benefits at the neuromuscular level, preventing a decline on JH in a CMJ.

The results of performance do not support major benefits of sodium bicarbonate supplementation. Our initial hypothesis (H1) was that sodium bicarbonate could benefit MPO and PPO, inducing a smaller power decrement along the exercise protocol, as well as allow a higher work capacity. In fact, there were no significant differences between conditions in the performance variables at the end of the trial. Nevertheless, the fact that MPO suffered a significant drop from the first to the second set in the placebo trial, while for the sodium bicarbonate trial it was maintained, meets this hypothesis. Regarding PPO, a significant increase from the first to the third set of the exercise protocol was observed independently of condition, but a tendency for higher values of this parameter in the sodium bicarbonate trial should be noted. In what regards to the Sdec, there were not significant differences to note but there was a tendency for lower values of this variable in the experimental condition, corroborating our hypothesis that it would induce a smaller power decrement.

After a RSA test in 10 recreational team-sport playing females, consisting of 5 x 6-s all-out cycle sprints every 30 s, Bishop et al. (2004) observed that, compared with placebo, the sodium bicarbonate treatment resulted in a significant increase in TW for the 5 sprints and in PPO for the last 3 sprints. Unlike these authors, we could not find a statistically significant increase in TW nor PPO with sodium bicarbonate in our study. Bishop et al. (2004) also calculated the percent peak power decrement to assess fatigue

and like in their study, we could not observe a treatment effect for this variable. Bishop and Claudius (2005) studied the effects of sodium bicarbonate supplementation in 7 female team-sport athletes recurring to an intermittent cycling sprint test (2 x 36-min “halves” of repeated ~2-min blocks: all-out 4-s sprint, 100 s of active recovery at 35% $\text{VO}_{2\text{peak}}$ and 20 s of rest). They did not find a significant difference between conditions in the TW completed during the first or second half of the exercise protocol but the work completed during 7 of the 18 second-half sprints was found to be significantly greater in the experimental condition. There was also no significant difference between conditions in the PPO achieved in both halves but this was significantly greater for sodium bicarbonate during 8 of the 18 second-half sprints. These results point to a beneficial effect of sodium bicarbonate as the exercise protocol progresses. Although without statistical significance, this was also observed in our study, with a tendency for higher values of PPO in the sodium bicarbonate trial in the last 2 sets of the exercise protocol.

From a more ecological perspective of studying the effects of sodium bicarbonate on performance, Delextrat et al. (2018) designed a basketball simulation test to evaluate the repeated sprint and jump performance of 15 female university basketball players and they could observe that sodium bicarbonate resulted in significant decreases in mean sprint times and mean circuit times, as well as lower ideal and total sprint times and sprint performance decrement. The results of these authors suggest a beneficial effect of this supplement on performance. On the other hand, Kozak-Collins et al. (1994) did not find a difference between interventions in the number of intervals completed by 7 female competitive cyclists performing 1-min cycling at 95% of $\text{VO}_{2\text{max}}$ followed by 1-min recovery at 60 W, repeated until exhaustion. In a study with 8 female elite hockey players consisting of 3 Field Hockey Skill Tests intersped with 4 sets of the Loughborough Intermittent Shuttle Test (FHST+2LIST+FHST+2LIST+FHST), Macutkiewicz and Sunderland (2018) could not find a beneficial effect of sodium bicarbonate on performance in any of the 2 types of test. Furthermore, in 12 female elite water polo players submitted to a water polo match simulation test (59-min protocol that included a task of 56 x 10-m maximal-sprint swims every 17 s), Tan et al. (2010) did not find a difference between conditions on mean sprint times. Additionally, in a study with 18 female high-level freestyle wrestlers performing 2 bouts of the Wingate Test intersped with a Dummy Throw Test, Durkalec-Michalski et al. (2020b) could not find a significant difference in mean power, peak power, power drop nor number of completed throws

between conditions. In summary, the results of Kozak-Collins et al. (1994), Macutkiewicz and Sunderland (2018), Tan et al. (2010) and Durkalec-Michalski et al. (2020b) point to the inexistence of performance benefits related to sodium bicarbonate supplementation. Overall, there is not a consensus regarding the effects of sodium bicarbonate supplementation on performance and the high methodological differences between studies may be responsible for this divergence.

In relation to the way athletes perceived the effort, we could only find a main effect of time for RPE, with statistically higher values in the last set than in the first one, independently of condition. These results are in accordance with Kozak-Collins et al. (1994) who could not find a significant difference between conditions in perceived exertion at any of the intervals RPE was tested nor at the last value recorded prior to the conclusion of exercise (6-20 scale). Tan et al. (2010) could also not find significant differences for perceived exertion between the two trials (0-10 scale). However, it should be noted that Macutkiewicz and Sunderland (2018), not only registered a higher RPE during the third and fourth sets of the Loughborough Intermittent Shuttle Test comparing with the first one, but also lower values of RPE during the bicarbonate condition (6-20 scale). This divergence between studies could possibly be explained by the different scale each group of authors used to assess RPE as well as the different exercise protocols.

Regarding lactate accumulation, we could observe that, in both trials, lactate was significantly lower at rest and at the end of the first set when compared with the following moments of measurement. However, the lactate accumulation profile was significantly different between conditions. In fact, for the sodium bicarbonate trial, we could also verify that lactate was statistically lower at the end of the second set with respect to its maximum value. The main finding in this domain relates to the fact that lactate accumulation was significantly higher in the sodium bicarbonate trial in the last 3 moments of measurement, corroborating the initial hypothesis where we proposed that this supplement would allow a higher lactate accumulation in the blood along the exercise protocol. We attribute the higher raise in lactate in the sodium bicarbonate trial to the elevation in extracellular HCO_3^- , enlarging the gradient of concentration between extracellular and intracellular H^+ , which stimulates the La^-/H^+ cotransporter and promotes a greater efflux of lactate from muscle cells to the extracellular fluid (Miller et al., 2016).

These results are in line with the study of Bishop et al. (2004) where blood lactate concentration increased in both treatments after the exercise protocol but this increase

was 28% higher in sodium bicarbonate than in placebo. Although Bishop and Claudius (2005) could not find a significant difference in lactate during either half of the intermittent sprint test, post-test plasma lactate was significantly higher in the sodium bicarbonate trial, which is in accordance with our results. In the same trend of results, Delextrat et al. (2018) did not find a significant difference between conditions in pre-exercise lactate concentration but this was significantly greater post-exercise in the sodium bicarbonate condition. Tan et al. (2010) observed a higher lactate accumulation from the second quarter of the exercise protocol onward in the sodium bicarbonate trial. Both the results of Delextrat et al. (2018) and Tan et al. (2010) are in accordance with the findings of our study. On the contrary, Macutkiewicz and Sunderland (2018) and Durkalec-Michalski et al. (2020b) did not find any differences between conditions regarding lactate accumulation. Despite the contradictory results of these 2 studies, the effects of sodium bicarbonate on lactate accumulation seem to be similar across studies, with higher values of this substance in the experimental condition.

In relation to pulmonary gas exchange, although for VO_2 we could not find any statistically significant difference, VCO_2 was statistically lower in the second and third sets in comparison with the first set of the exercise task for both conditions and, in addition, it was significantly higher in the sodium bicarbonate trial than in the placebo one. These results allow the confirmation of our hypothesis where we proposed that sodium bicarbonate would induce a greater elimination of CO_2 through breathing. We attribute this to the fact that, in addition to the elimination of the CO_2 produced by the muscles, which 60 to 70% is transported to the lungs as HCO_3^- , sodium bicarbonate ingestion increases the levels of this ion in the extracellular fluid, traducing into a higher elimination through VE (Calvo et al., 2021). For the RER, we could verify that it was significantly lower in the third set than in the first one for sodium bicarbonate and we could also note higher values in the experimental condition in the second set. This is in accordance with the results obtained for VO_2 and VCO_2 in this trial since there was a significant decrease in VCO_2 but no significant variation in VO_2 and the VCO_2 values were higher for the sodium bicarbonate condition at all moments of evaluation. Regarding VE, we could observe an increase along the exercise protocol for both conditions with a tendency for lower values in the sodium bicarbonate trial. This increase may be explained by the fact that VE is highly dependent on pH, increasing when pH decreases, and could explain the decrease in VCO_2 observed along the exercise protocol, inducing a higher

removal of the same. Also, since sodium bicarbonate prevents a decrease in pH, smaller values of VE were expected in this trial.

In the study of Bishop and Claudius (2005), no significant differences were found between conditions for either VO_2 or RER. The results of VO_2 of this authors are in accordance with our results although for RER we could observe a significant difference in the second set. Kozak-Collins et al. (1994) reported an increase in VE and RER during the exercise protocol for both interventions with a tendency for lower values in the sodium bicarbonate trial, although without statistical significance. Despite our results of VE meet the results of these authors, an opposite trend was observed for RER in our study. In a study with 12 male elite BMX cyclists performing 3 races of 400 m with 15 min intervals, Peinado et al. (2019) did not find a main effect of condition on VO_2 , VCO_2 nor VE, although all variables were significantly higher post-exercise compared with the pre-exercise measurements. Also, these authors observed a significant decrease in VCO_2 along the 3 races in both trials. These results tend to be in line with our research but in what concerns to VCO_2 , we could identify a significant difference between conditions. This may be related with the fact that these authors did not continuously measure the pulmonary gas exchange parameters along the exercise protocol like we did in our investigation but only pre- and post-race.

The results of HR, as it was expected, demonstrate that it was statistically higher in the last set than in the first one independently of condition. Our results are in line with the results of Bishop and Claudius (2005), Kozak-Collins et al. (1994) and Macutkiewicz and Sunderland (2018), who could not find differences between conditions for this variable. In addition, Wu et al. (2010) did not find a significant difference between conditions for HR in a tennis simulated match of 50 min duration in 9 male college tennis players. On the other hand, Tan et al. (2010) found a significant difference in this variable between conditions, with higher values for the placebo trial. Nevertheless, the results of most studies lead us to conclude that sodium bicarbonate does not seem to have a major influence in HR.

Regarding muscle oxygenation, our results showed that O_2Hb , traducing available O_2 in the muscle (both intra and extracellular) using as reference the resting state, significantly increased from the first to the third set in both conditions, which means it approached the resting values, and a tendency for higher values of this variable should be noted in the sodium bicarbonate trial. For HHb , we observed a significant increase from

the first to the second set of the exercise protocol in both conditions and the values of this variable tended to be higher in the sodium bicarbonate trial as well. In relation to TOI, we could not find any significant difference between conditions but we could observe statistically lower values in the second set than in the last one in the placebo condition. Overall, the results obtained in this domain point to a higher muscle oxygenation in the experimental condition, which tends to be in line with the hypothesis initially established. This was more pronounced in the second set of the exercise protocol suggesting a greater need for O₂ in this set.

As far as we are aware, no studies to date have explored the effects of acute sodium bicarbonate ingestion on muscle oxygenation which limits the discussion of our results in this topic. Jones et al. (2013) proposed to analyse the near-infrared spectroscopy parameters on 6 male university standard rugby players completing 9 repeated shuttle runs, which incorporated forward, backward and change of direction movements. The group averaged data showed a clear decrease in O₂Hb, increase in HHb and drop in TOI during sprints with reference to the resting state. In the study of Buchheit and Dufland (2011), to assess the effects of an 8-week endurance training program on RSA, 18 moderately trained men performed 2 x 15-s all-out 20-m shuttle runs intersped with 15 s of passive recovery before and after the program. The program was successful at improving RSA, which was due to an acceleration of post-sprints muscle reoxygenation rate. During sprints, a decrease in O₂Hb, increase in HHb and decrease in TOI in relation to the resting state was also observed. The results of these groups of authors show that, as expected, exercise induces a higher extraction of O₂ in the muscles and concomitant drop in the saturation index, but this analysis approach is quite different from that used in our study, limiting a direct comparison with our results.

The greatest novelty of this investigation was in fact the use of near-infrared spectroscopy to assess muscle oxygenation and although we could not find statistically significant differences between conditions, both O₂Hb and HHb tended to be higher in the sodium bicarbonate trial, pointing to a larger O₂ apport to the capillary bed of the muscle and concomitant higher extraction of O₂ in this condition. Given that O₂ affinity for hemoglobin decreases when pH decreases (Bohr effect), the higher pH induced by the ingestion of sodium bicarbonate could prevent the dissociation of O₂ from hemoglobin, decreasing O₂ extraction, but our results of HHb tend to show the opposite. However, it should be noted that this methodological approach cannot differentiate between

hemoglobin's heme and myoglobin's or other heme groups in the muscle tissue, limiting the conclusions we can take in this topic. Therefore, more studies in this domain are needed to corroborate our results, that overall suggest a positive influence of sodium bicarbonate on muscle oxygenation.

In what concerns to neuromuscular fatigue, the results obtained show that JH in the CMJ significantly decreased after the exercise protocol in placebo while in sodium bicarbonate it was maintained. Furthermore, it was statistically higher before the exercise protocol in placebo than in sodium bicarbonate. These results meet our initial hypothesis (H3) where we theorized that sodium bicarbonate would induce a smaller decrement on JH in the CMJ. Thus, it can be suggested that this supplement induces a faster recovery of the motor units recruited during the RSA test, allowing a higher jump performance. Nevertheless, we were not expecting a significant higher JH in the placebo trial before the exercise protocol in comparison with sodium bicarbonate.

Delextrat et al. (2018) observed that sodium bicarbonate supplementation resulted in a significantly greater JH in a CMJ compared to placebo, with no significant difference between conditions in jump percentage decrement. Unlike these authors, we could not detect a significant higher JH in the sodium bicarbonate trial, but sodium bicarbonate was able to prevent JH decrease after the RST, although it should be noted that these authors recorded JH for every circuit of the basketball simulated test and that we did not directly calculate a percentage decrement variable like these authors did. In a study with 15 male semiprofessional adolescent soccer players performing the Running Anaerobic Sprint Test (Guimarães et al., 2020), supplementation with sodium bicarbonate did not improve CMJ performance, with similar values of JH pre- and post-exercise for both conditions. In the study of Zabala et al. (2008), 9 male elite BMX riders undertook two trials involving repeated sprints (3 x Wingate Test (WT) separated by 30-min recovery). A significant enhancement in CMJ performance after WT1 was observed under the influence of sodium bicarbonate and CMJ performance also tended to be better in this condition after WT2 and WT3. However, a decrease in CMJ performance over the bouts was reported in both trials. Another study with 10 male elite BMX riders performing the same exercise protocol but with intervals of 15 min between bouts (Zabala et al., 2011), could not find significant differences in CMJ test after WT1, WT2 or WT3, but performance also tended to be better for the experimental trial. Overall, the results of

other studies, just like ours, suggest that sodium bicarbonate favours performance at the neuromuscular level.

The number of studies that aimed to explore the effects of acute sodium bicarbonate supplementation on performance in football is very reduced, especially in women players. Also, the majority of researchers have focused the effects of this supplement on performance, with few studies reporting the way sodium bicarbonate may affect pulmonary gas exchange and with no study to date (to our knowledge) exploring its influence on muscle oxygenation, greatly limiting the discussion of our results in this topic. Nevertheless, the results of our study allowed us to globally accept our hypothesis that sodium bicarbonate favours RSA in female football players, allowing a higher removal of lactate from the active musculature, greater elimination of CO₂ through breathing and with a tendency to provide higher levels of O₂ apport and extraction in the muscle, although this has not traduced into relevant performance benefits. This supplement is also beneficial at the neuromuscular level. Although our results point to an overall beneficial effect of sodium bicarbonate on RSA, more research with female football players is needed to prove this assumption.

CHAPTER 6: CONCLUSIONS

6.1 CONCLUSIONS

The main conclusion of our study is that acute ingestion of sodium bicarbonate before a RSA test in female football players allows a higher lactate accumulation, greater removal of CO₂ through breathing and tends to provide a larger O₂ apport to the capillary bed of the muscle with a concomitant higher O₂ extraction, factors associated with a later onset of fatigue. Nevertheless, this metabolic effects did not traduce into major benefits in terms of performance. Another conclusion of our research is that sodium bicarbonate also benefits athletes at a neuromuscular level, with smaller decrements on JH in a CMJ after the exercise protocol. In practical terms, the results of our investigation suggest that female football players can acutely supplement sodium bicarbonate before important matches during a football season, aiming at supporting the high physiological demands of the game, consequently improving their performance.

It should be noted that, attending the significant differences found for lactate accumulation, gas exchange and muscle oxygenation, we were expecting higher benefits on performance but only slight improvements were detected. Therefore, our results point to greater benefits of sodium bicarbonate at a physiological level rather than in terms of performance. Besides, since sodium bicarbonate also proved to be beneficial in terms of neuromuscular performance, perhaps if the exercise protocol had been longer, the differences obtained in power output and total work would have been more evident. More research regarding the effects of acute sodium bicarbonate supplementation on performance in female football players is needed to clarify our results.

6.2 LIMITATIONS

This study has some limitations that should be considered when assuming its main findings. First, the sample size was small. A larger sample would possibly allow the identification of more significant differences and/or consolidate those obtained. However, the recruitment of athletes for the study turned out to be something difficult not only due to the reduced number of female football players living near the university but also

because the study required the ingestion of a large number of capsules, making it impossible for some athletes to participate due to their difficulty in ingesting pills.

Second, having a sample of female athletes increased the variability of the results due to the specific morphologic and physiologic characteristics of women, for which also contributed the fact that no control of hormonal parameters was done (ex.: standardisation of the phase of the menstrual cycle in which athletes were). Nevertheless, this would have required a greater methodological complexity which was not compatible with the time established for data collection. Furthermore, the group was not homogeneous in terms of competitive level nor years of practice, although this is not considered a factor of great influence in the results since no substantial differences were observed between athletes in the different parameters analysed.

Third, the exercise task was not representative of the type of actions athletes are used to during practices and matches and although we included a familiarization session, the results would have been more precise if an ecological task was used. However, attending the equipment required to collect data, it would have not been possible to do an exercise protocol on the field and not even to use a treadmill, making the cycle ergometer the only available option.

Another limitation of this study relates to the fact that near-infrared spectroscopy is highly dependent on the FM in the zone where the probe was placed, which tends to be particularly higher in women in the femoral, hip and gluteal regions. Therefore, the results obtained for O₂Hb, HHb and TOI may be affected by this factor, although none of the athletes had a very large fat fold in the zone of the vastus lateralis.

6.3 FUTURE RESEARCH

The limitations of this study highlight the relevance of having an adequate sample size, especially because studies with supplementation tend to have a reduced number of participants. Therefore, future studies with sodium bicarbonate supplementation should consider this limitation and work to recruit a larger number of athletes. Furthermore, future studies with female athletes should consider their morphologic and physiologic specificities, namely in what concerns to menstrual cycle, searching for a standardisation

of the phase of the cycle in which data collection is done and preferably seeking a biochemical confirmation of that one.

Another aspect that would be of great interest to study is the influence of ingesting sodium bicarbonate on performance in acute hypoxic conditions. Recently, some authors have been investigating the effects of supplementation with sodium bicarbonate when combined with stimuli such as hypoxia (Gough et al., 2019) and this acquires special relevance in sports like football since some authors have been testing the efficacy of this training method on RSA in athletes from team sports (Girard et al., 2017). In this domain, the results of our study may be useful to evaluate the constraints and risks of using this supplement in hypoxic conditions, especially in what concerns to VE and HR. Thus, future studies could develop this topic of investigation and explore it in the female athlete.

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